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RESEARCH ARTICLE

# The relationship between pinworm (*Trypanoxyuris*) infection and gut bacteria in wild black howler monkeys (*Alouatta pigra*)

**Rodolfo Martínez-Mota**[1] | **Nicoletta Righini**[2] | **Elizabeth K. Mallott**[3] | **Thomas R. Gillespie**[4,5] | **Katherine R. Amato**[3]

[1]Centro de Investigaciones Tropicales (CITRO), Universidad Veracruzana, Xalapa, Veracruz, Mexico

[2]Instituto de Investigaciones en Comportamiento Alimentario y Nutrición (IICAN), Universidad de Guadalajara, Ciudad Guzmán, Jalisco, Mexico

[3]Department of Anthropology, Northwestern University, Evanston, Illinois, USA

[4]Department of Environmental Sciences, Program in Population Biology, Ecology, and Evolutionary Biology, Emory University, Atlanta, Georgia, USA

[5]Department of Environmental Health, Rollins School of Public Health, Emory University, Atlanta, Georgia, USA

**Correspondence**

Katherine R. Amato, Department of Anthropology, Northwestern University, 1810 Hinman Ave., Evanston, IL 60208, USA.
Email: katherine.amato@northwestern.edu

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## Abstract

Gut bacteria may coexist with other groups of organisms, such as nematode parasites, that inhabit the gastrointestinal tract of primates; however, the possible effects of endoparasites on bacterial communities are frequently overlooked. Here we explored whether infection with *Trypanoxyuris*, an oxyurid gastrointestinal parasite, is associated with changes in the gut bacterial community of wild black howler monkeys (*Alouatta pigra*), by comparing gut bacterial communities of consistently infected individuals and individuals that never tested positive for *Trypanoxyuris* throughout different months across the year. We additionally controlled for other sources of variation reported to influence the primate microbiome including individual identity, social group, and seasonality. *Trypanoxyuris* infection was not related to differences in gut bacterial alpha diversity, but was weakly associated with differences in gut bacterial community structure. In contrast, among the covariates considered, both individual identity and social group were more strongly associated with variation in the howler gut bacterial community. Our results suggest that gastrointestinal parasites may be associated, to some extent, with shifts in the gut bacterial communities hosted by free-ranging primates, although a causal link still needs to be established. Further studies of wild primate hosts infected with parasite species with different pathogenicity are needed to better elucidate health-related consequences from the parasite-microbiome interplay.

**KEYWORDS**

gut microbiome, nematodes, *Trypanoxyuris*, parasites, sociality, Mexico

## 1 | INTRODUCTION

The gastrointestinal (GI) tract of vertebrates, including primates, is inhabited by a diverse range of microorganisms, including bacteria, archaea, microbial eukaryotes, and viruses. Gut microbial communities play important roles in supporting host colonic health, degrading toxins and xenobiotics (Koppel et al., 2017), and regulating host inflammation and immune responses (Al Nabhani & Eberl, 2020; Hooper et al., 2012). Thus, microbial activity has important implications for host health, ecology, and evolution. However, our understanding of these dynamics is limited given that most studies focus on relationships between hosts and single groups of GI microbes (i.e., bacteria) and ignore their interactions.

Parasites have successfully colonized the vertebrate digestive tract where they reproduce and obtain nutrients (Lee et al., 2014). Currently, evidence indicates that GI parasite infections can alter the diversity, structure, and metabolic function of the mammalian gut bacterial community (Leung et al., 2018), suggesting an interaction between groups of organisms that have cohabited with their hosts over evolutionary time (Brosschot & Reynolds, 2018; Loke & Lim, 2015). For example, in humans, GI parasite infections have been

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found to regulate intestinal immune function and inflammation via changes in the composition and relative abundance of commensal bacteria such as lactobacilli (Berrilli et al., 2012; Reynolds et al., 2015). Similarly, controlled animal studies where individuals were experimentally infected with GI helminths have reported that infected subjects tend to exhibit bacterial community shifts and reduced relative abundances of bacterial metabolic pathways related to carbohydrate fermentation in the colon (Li et al., 2012). Although these associations do not necessarily imply a causal mechanistic link between these groups of microbes, further describing the parasite-bacteria association across contexts is a first step for unraveling their potential interactions.

The mechanisms by which shifts in the gut microbiota associated with GI parasites occur are not well-studied. Parasite infections induce physical and immune changes in the gut, such as increased production of gut epithelial mucus or secretion of antimicrobial peptides, which may create conditions favoring either proliferation or inhibition of microbial taxa (Brosschot & Reynolds, 2018; Leung et al., 2018). Additionally, parasites and other microbes may interact directly, either positively or negatively impacting each other. This interplay may have synergistic or antagonistic effects on the host that would not be observed if each organism acted independently (Leung et al., 2018).

Currently, studies of microbial interactions in the gut most commonly target the interplay between GI parasites and gut bacteria (Leung et al., 2018). Nevertheless, a limited subset of hosts and parasites are represented, and most studies use laboratory animal models. This dearth of data constrains our ability to effectively describe patterns and explore mechanisms since interactions are likely to vary depending on the type of parasite, the host response, and the structure of the bacterial community (Peachey et al., 2017). More data describing a diversity of parasite-gut bacteria co-occurrences and potential interactions, particularly in wild animals under selective pressure, are necessary to facilitate further mechanistic research in this area.

To increase our knowledge of the parasite-gut bacteria system in wild primate hosts, we explored the relationship of GI parasite infection with the gut bacteria of free-ranging black howler monkeys (*Alouatta pigra*), an endangered Mesoamerican primate species. Although *A. pigra* may harbor different GI parasites, such as trematodes that inhabit the bile ducts (Kowalzik et al., 2010; Pastor-Nieto, 2015), it is also frequently infected by GI nematodes of the genus *Trypanoxyuris* (Nematoda: Oxyuridae), which are found in the large intestine (Hugot et al., 1996; Solórzano-García & Pérez-Ponce de León, 2017; Solórzano-García et al., 2016). This feature makes *Trypanoxyuris* a suitable model to explore associations between parasite infection and gut bacterial community composition. *Trypanoxyuris* is characterized by a direct life cycle, in which egg ingestion is the primary transmission mechanism (Felt & White, 2005; Hugot et al., 1996; Solórzano-García et al., 2016). In general, the eggs of oxyurid parasites such as *Trypanoxyuris* are deposited in the perianal region of the host, which sets the conditions for reinfection (Adamson, 1994). The time from egg ingestion to oviposition by adult females is 4–6 weeks. It is estimated that oxyurids may live up to 13 weeks inside the vertebrate host (Anderson, 2000; Burkhart & Burkhart, 2005). Some evidence exists to indicate that oxyurid parasites can alter gut bacterial community structure. GI helminth infections tend to affect gut homeostasis as well as the physical properties of the gut with direct repercussions on bacterial communities, independent of host and parasite species (Kodio et al., 2020; Peachey et al., 2017). Moreover, school children with recurrent oxyurid parasite infection exhibit increased intestinal microbial diversity (Yang et al., 2017).

Given our understanding of oxyurids and their impacts on gut bacterial communities more broadly, we hypothesized that *Trypanoxyuris* infection would alter the howler monkey gut bacterial community. Specifically, we predicted that black howler monkeys infected by *Trypanoxyuris* would have different gut bacterial community composition and structure and increased bacterial diversity compared with individuals never deemed to be infected. To test this hypothesis, we compared gut bacterial community composition of wild black howler monkeys in which infections were not detected over a period of 15 months to gut bacterial community composition of wild black howler monkeys that were found to be consistently infected with *Trypanoxyuris* sp. over the same period. Previous research indicates that the gut bacterial community can be influenced by social group interactions and seasonality (Amato et al., 2016, 2017; Hicks et al., 2018; Sarkar et al., 2020). Thus, to account for such potential effects we included these two factors as covariates in our analysis.

## 2 | METHODS

This study complied with the legal requirements of Mexico (SEMARNAT-DGVS/09084/10) and was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Illinois at Urbana-Champaign (protocols #10054 and #10062). The research complied with the American Society of Primatology principles for the ethical treatment of non-human primates.

### 2.1 | Study site, subjects, and sample collection

We studied wild black howler monkeys (*A. pigra*) living in six social groups in the municipality of Escárcega, State of Campeche, Mexico (18°36′ N, 90°48′ W). These groups live in fragments of semi-deciduous tropical forest ranging in size from less than 3 ha to 2100 ha (Table 1); the groups were part of a longer-term behavioral and ecological research project in which all the study subjects were individually recognized and monitored for more than a year (January 2011–March 2012, a period that encompassed rainy and dry seasons) (Martínez-Mota et al., 2017).

Over the course of this period, we collected a total of 390 fecal samples that were screened for GI parasite infection (see below) from howlers living in these six groups; 178 fecal samples belonged to 11 individuals that were consistently negative for *Trypanoxyuris* infection

**TABLE 1** Information on the study groups and characteristics of the fragments inhabited by black howler monkeys (*Alouatta pigra*) in the Municipality of Escárcega, State of Campeche, Mexico

| Group ID | Group size | Sampled individuals | Fragment area (ha) | Basal area of feeding trees ($m^2$/ha) | Shannon index of feeding-trees | Site name |
|---|---|---|---|---|---|---|
| G1 | 12 | 6 | 2100.0 | 24.7 | 2.4 | El Tormento |
| G2 | 6 | 3 | 2100.0 | 11.8 | 2.2 | El Tormento |
| G3 | 6 | 2 | 2.6 | 2.5 | 1.9 | División del Norte |
| G4 | 8 | 3 | 2.4 | 1.8 | 1.7 | División del Norte |
| G5 | 6 | 4 | 5.0 | 6.6 | 2.2 | División del Norte |
| G6 | 11 | 4 | 9.0 | 5.0 | 1.7 | División del Norte |

(Figure S1), while 212 fecal samples belonged to another 11 individuals that were consistently positive for *Trypanoxyuris* infection (Figure S2). Fecal samples were collected from each individual at least once a month. *Trypanoxyuris* prevalence among groups and between seasons did not differ significantly; this information is provided in Table S1. Due to funding limitations, we selected a subset of fecal samples ($N = 56$) from 22 individuals collected during February–October 2011 for gut bacterial analyses, resulting in paired parasite and bacterial community composition data from two seasons (a dry season: February–May, and a rainy season August–November). Based on individual profiles of infection (Figures S1 and S2), 25 fecal samples from 11 individuals for which *Trypanoxyuris* was never detected across the whole study period were compared with 31 fecal samples from another 11 individuals that showed recurrent *Trypanoxyuris* infections throughout different months across the year. For gut bacterial analysis, we used an aliquot of the same fecal samples that were tested for *Trypanoxyuris*. The numbers of analyzed samples per individual in each condition are provided in Table S2.

## 2.2 | Parasite detection, sequence processing, and microbial analyses

Fecal samples were collected, labeled according to each study subject, and divided into two aliquots: 3 g of each sample were homogenized and fixed in 10% buffered-formalin solution and kept in a domestic refrigerator at 4°C until shipping to the United States for parasitological analysis; and another 5 g were stored in 96% ethanol and kept in a refrigerator at 4°C until shipping to the United States for microbiome analysis. Once in the United States, samples used for gut bacterial analysis were stored at −80°C until processing.

The parasitological analysis took place at Emory University in the Gillespie lab. Parasite eggs were recovered from feces using flotation ($NaNO_3$ solution) and fecal sedimentation techniques as described in Gillespie (2006). Flotation techniques have been found to be a reliable method for recovering pinworm eggs from howler monkey feces (Alvarado-Villalobos et al., 2017; Solórzano-García & Pérez-Ponce de León, 2017) while polymerase chain reaction (PCR)-based analyses have thus far given mixed results (Dole et al., 2011; Leblanc et al., 2014). Due to logistical limitations, only one slide for each technique per fecal sample was systematically scanned using a compound microscope. As a standard procedure, parasite eggs were counted under the ×10 objective lens and measured under the ×40 objective lens to the nearest 0.1 µm with an ocular micrometer. One drop of Lugol's iodine solution was added to facilitate identification. Given previously reported concerns with using egg counts to infer parasite burden (Anderson & Schad, 1985; Gillespie, 2006), egg counts were used only to determine the presence or absence of *Trypanoxyuris* as a conservative measure of parasitic infection. Based on this approach, we compared individuals in which *Trypanoxyuris* was not detected in any sample across the study period with individuals in which multiple samples were positive for *Trypanoxyuris* throughout the study. Individuals for which only one or two samples were positive during the study period were discarded from the analysis.

Microbiome analysis took place at Northwestern University in the Amato lab. DNA was extracted from samples using a MOBio PowerSoil kit. The V4 hypervariable region of the bacterial/archaeal 16S rRNA gene was amplified using a two-step PCR (Earth Microbiome Project primers 515F/926R and Fluidigm Access Array primers containing sample-specific barcodes) as previously described (Mallott & Amato, 2018). PCR products were purified and normalized using a SequalPrep Normalization Plate and sequenced on the Illumina MiSeq V3 platform to produce 2 × 250 bp paired-end reads at the University of Illinois Chicago DNA Services Facility.

Microbial sequences were processed in the Quantitative Insights into Microbial Ecology 2 (QIIME 2) bioinformatics platform, version qiime2-2020.2 (Estaki et al., 2020). We first removed primers using the q2-cutadapt plugin. Later, single-end sequences were subjected to quality control including denoising, merging, and chimera removal using the DADA2 pipeline (Callahan et al., 2016) implemented in QIIME 2. Microbial reads were then assigned as amplicon sequence variants (ASVs; Callahan et al., 2017) using the q2-dada2 plugin. Sequence taxonomic assignment was performed using the naive Bayes classifier trained on the SILVA 132 99% OTUs sequence reference (Quast et al., 2013). Additionally, we performed filtering of chloroplast, mitochondria, and singletons. Samples were rarefied to 13,300 sequences before downstream alpha and beta diversity analyses. No samples were excluded by the rarefaction step.

To determine changes in bacterial diversity associated with *Trypanoxyuris* infection status three metrics of microbial alpha diversity were estimated for each sample in QIIME 2. These included Faith's phylogenetic diversity index, the Shannon diversity index, and Pielou's evenness index. Each measure of alpha diversity was compared between samples deemed noninfected and infected with generalized linear mixed models (GLMMs) using the glmmADMB package (Skaug et al., 2018) in the R software (R Core Team, 2017). We controlled for the social group, seasonality (dry vs. rainy season), and read counts by including these covariates in all models. Faith's phylogenetic diversity index and the Shannon index followed a normal distribution (Shapiro–Wilk test: $p > 0.05$), therefore GLMMs were fitted with a Gaussian distribution and identity function. Values of the evenness index range from 0 to 1, thus a GLMM on this index was fitted with a beta distribution and a logit function. The individual ID was set as a random factor. A likelihood ratio test was used to compare the fit of each full model against a reduced model which lacked the infection status term. Analysis of variance (ANOVA) tables showing predictor effects were generated using the car package (Fox & Weisberg, 2019). $p$ values were adjusted with Benjamini–Hochberg false discovery rate.

Patterns in bacterial community composition were visualized through nonmetric multidimensional scaling (NMDS). Here, Jaccard (presence – absence of microbes) and Bray–Curtis (microbial relative abundance) distance matrices were used as measures of beta diversity (Knight et al., 2018). We tested for an association between *Trypanoxyuris* infection status of howler monkeys and overall bacterial community composition, using permutational multivariate analysis of variance (PERMANOVA). In these analyses we also controlled for individual ID, the social group, and seasonality. These analyses were run in R, using the function *adonis2* implemented in the vegan (Oksanen et al., 2019) and qiimer (Bittinger, 2016) packages. We further validated and detected effects by means of PERMANOVAs using a sub-set of data which included only one sample per individual during one season (rainy).

We used a series of linear mixed-effects models to test the relationship between *Trypanoxyuris* infection and the relative abundance of individual bacterial taxa at the ASV and genus level. For these analyses, we used the nlme and car packages (Fox & Weisberg, 2019; Pinheiro et al., 2020). In all models, infection status and group were included as fixed effects and the individual ID as random effect. Model results also were corrected for false discovery rate (fdrtool package; Klaus & Strimmer, 2015).

## 3 | RESULTS

We detected three parasite taxa in our parasite scanning: *Trypanoxyuris* sp. (Oxyuridae), *Controrchis biliophilus* (Dicrocoeliidae), and an unidentified trematode (Dicrocoeliidae). As previously described, of these three parasites, only *Trypanoxyuris* inhabits the large intestine where direct interactions with bacteria can occur. Therefore, we did not consider the other parasites as factors in our subsequent analyses. Our 16S rRNA sequencing effort yielded 1,272,780 sequence reads with an average of 22,728 ± SD 5168 sequences per sample. After filtering we obtained a total of 1648 bacterial/archaeal ASVs with an average of 188 ± SD 28 ASVs per sample.

### 3.1 | Associations of *Trypanoxyuris* infection with microbial diversity

We found that *Trypanoxyuris* infection status was not significantly associated with differences in bacterial diversity as estimated using three alpha diversity metrics (Table S3). The GLMMs revealed that there were no significant differences between samples deemed noninfected and infected for the Faith's phylogenetic diversity index ($F_{1,45} = 0.02$, $p = 0.88$), the Shannon index ($F_{1,45} = 0.00$, $p = 0.95$), or the evenness index ($F_{1,45} = 0.01$, $p = 0.90$). The covariate seasonality was not associated with differences in bacterial diversity either (Table S3). However, the covariate social group was significantly associated with differences in the three alpha diversity metrics (phylogenetic diversity: $F_{5,45} = 10.4$, $p < 0.001$; Shannon index: $F_{5,45} = 11.0$, $p < 0.001$; evenness index: $F_{5,45} = 6.3$, $p < 0.001$; Table S3 and Figure S3). Per sample read counts did not influence the Shannon or evenness indices (Table S3) but were associated with the phylogenetic diversity index ($F_{1,45} = 13.1$, $p < 0.001$) despite rarefaction.

PERMANOVA tests using Jaccard and Bray–Curtis distance matrices revealed that *Trypanoxyuris* infection status was significantly associated with differences in gut bacterial community composition of black howler monkeys (Jaccard: pseudo-$F = 2.5$, $p < 0.001$; Bray–Curtis: pseudo-$F = 3.6$, $p < 0.001$), although the effect sizes were weak (Jaccard $R^2 = 0.033$; Bray–Curtis $R^2 = 0.044$; Figure 1). These effects held even when a sub-set of single timepoint samples from each group of individuals (consistently infected and never found to be infected) were used for validation (Figure S4). PERMANOVA tests showed that covariates were differentially associated with bacterial community composition; while seasonality did not have a strong relationship with bacterial community composition, major differences in the bacterial communities of howler monkeys were visible among social groups (Table S4; Figure S5). Bacterial communities were also significantly different among individuals (Table S4).

### 3.2 | Microbial taxonomic composition

At the phylum level, the gut bacterial community of howler monkeys from this population was mainly composed of Firmicutes, Bacteroidetes, Cyanobacteria, Tenericutes, Proteobacteria, and Actinobacteria (Figure 2a). Linear mixed-effects models showed that *Trypanoxyuris* infection had a significant relationship with the relative abundance of bacterial taxa belonging to Prevotellaceae and Gastranaerophilales (Figure 2b). While the abundance of two ASVs from Prevotellaceae (ASV1: $\chi^2 = 15.2$, $p < 0.001$, $q = 0.01$; ASV2: $\chi^2 = 19.9$, $p < 0.001$, $q = 0.003$) was up to 5.8-fold higher in *Trypanoxyuris*-infected samples, the relative abundance of bacteria from

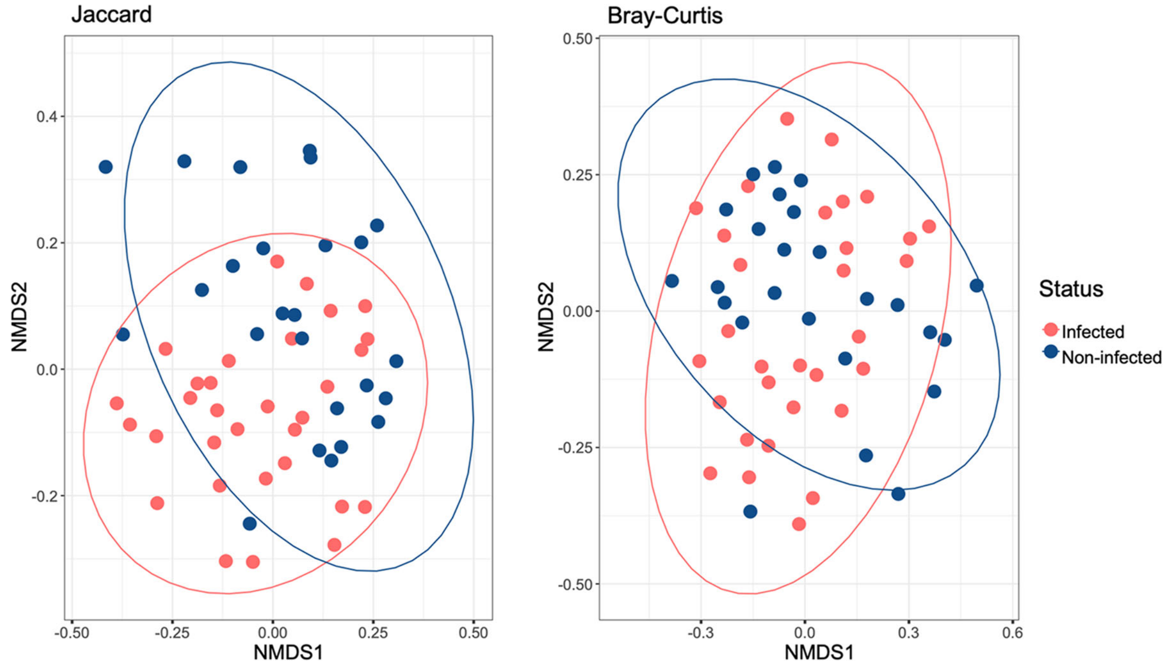


**FIGURE 1** Gut bacterial community changes between samples deemed noninfected and infected with *Trypanoxyuris* in black howler monkeys (*Alouatta pigra*) at Escárcega, State of Campeche, Mexico. NMDS plots are based on Jaccard and Bray–Curtis distance matrices; 95% confidence interval ellipses are shown

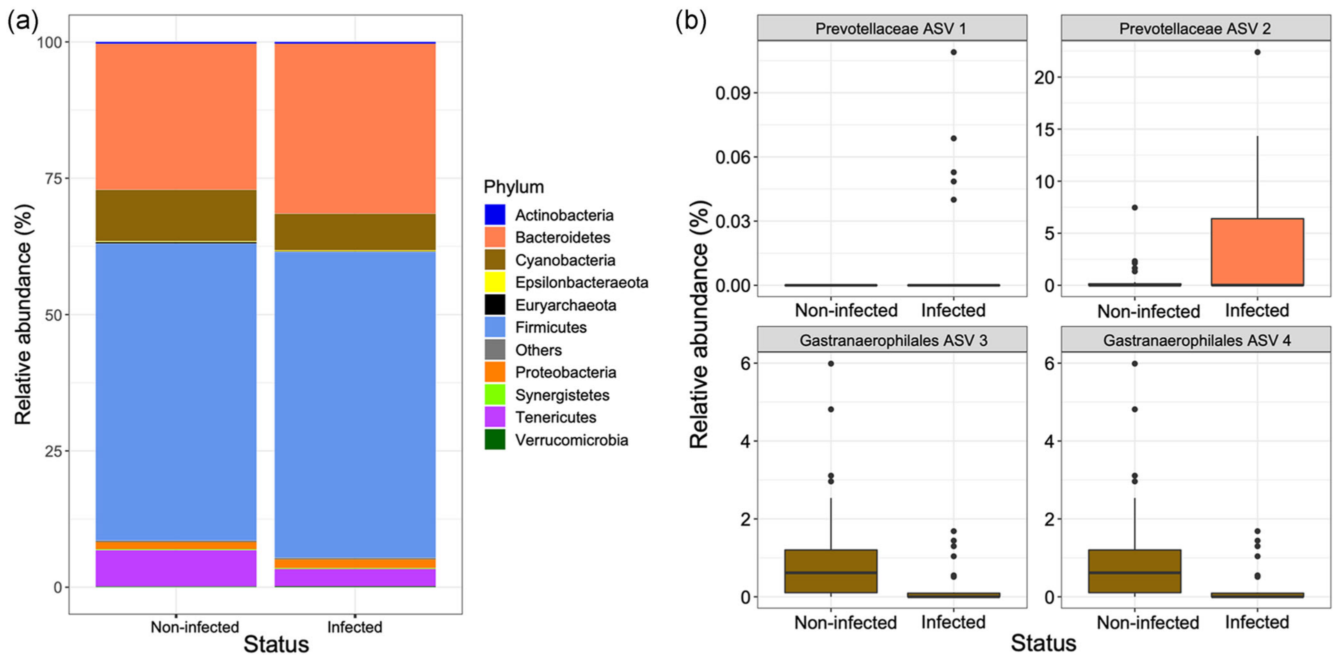


**FIGURE 2** Major microbial phyla observed in black howler monkeys (*Alouatta pigra*) (a) and significant changes in relative abundances of four bacterial ASVs between samples deemed noninfected and infected with *Trypanoxyuris* at Escárcega, State of Campeche, Mexico (b). ASVs belonging to the family Prevotellaceae (ASV 1: *0e15f4b60779adeb015e539702a322c8*; ASV 2: *fb92a3310cd973280aa6aeb6cc5433c7*) and the order Gastranaerophilales (ASV 3: *a6de24e2a4e4887db40dbbd0fa941de1*; ASV 4: *89a90d6d377b4fbbed790cbae0496a2b*) were significantly affected by infection status

Gastranaerophilales (ASV3: $\chi^2 = 12.9$, $p < 0.001$, $q = 0.03$; ASV4: $\chi^2 = 13.3$, $p < 0.001$, $q = 0.03$) decreased between 2.8 and 5.2-fold.

Microbial phyla composition was similar across study groups (Figure 3a), and bacterial taxa such as Lachnospiraceae and Ruminococcaceae (UCG_014) were represented in similar proportions in all howler groups (Figure 3b). However, other taxa (e.g., Anaerostipes, Erysipelotrichaceae, Mollicutes_RF39_rumen_bacterium_F9) did vary in their relative abundance across groups (Figure 3b). Overall, bacterial relative abundance varied little between seasons, with only eight bacteria from Bacteroidales, Clostridiales, Gastranaerophilales, Desulfovibrionales, and Mollicutes RF3 showing significant changes (Figure S6).

## 4 | DISCUSSION

In this study, we explored whether GI parasite infection is associated with differences in the gut bacteria of wild black howler monkeys. Our parasite screening revealed that black howlers were infected by two trematodes reported to inhabit the bile ducts, and *Trypanoxyuris* sp., a common nematode harbored in the intestine. Using *Trypanoxyuris* sp. as a model for macroparasite infection (i.e., parasitic helminths), we found that *Trypanoxyuris* infections are associated with weak changes in the gut bacterial communities of these primates. However, individual identity and the social group were more strongly associated with howler monkey gut bacterial community composition, as previously observed in other *A. pigra* populations (Amato et al., 2017). Although we also accounted for seasonality, we found that this covariate had a negligible relationship with gut bacterial community structure in our study groups. Our findings highlight the importance of considering the co-occurrence of GI macroparasites and gut bacterial communities to better understand the broad effects of the gut microbiome on wild primate physiology and health.

In our study, howler monkeys repeatedly infected by *Trypanoxyuris* across several months showed negligible differences in their gut microbiota compared to individuals considered noninfected. *Trypanoxyuris* parasites are most commonly found in the large intestine of the host, where they coexist and potentially interact with bacterial members of the gut microbiome. Contrary to our expectations, *Trypanoxyuris* infection was not associated with alterations in bacterial alpha diversity, and the presence of these parasites was weakly associated with alterations in gut bacterial community structure. For example, *Trypanoxyuris* infected individuals showed increased relative abundances of Prevotellaceae, a family of microbes that have been positively associated with colitis and mucosal inflammation recently (Iljazovic et al., 2020). Although causation should not be inferred from this relationship, our results suggest that the presence of these parasites might be an additional factor that contributes to subtle modifications in the gut microbiome of black howler monkeys. To what extent these microbial changes affect howler health is still unclear and further research to elucidate these effects is needed.

Our results also agree with previous findings showing that the effects of parasites on the animal gut bacterial community may be influenced by the parasite pathogenic potential and associated host immunological responses (Aivelo & Norberg, 2018; Kreisinger et al., 2015; Morton et al., 2015). For example, alterations in bacterial abundance have been observed in mammalian hosts that were infected with GI parasites that exert health costs such as diarrhea, abdominal pain, anorexia, or deficient nutrient absorption (Li et al., 2012). In contrast, our data revealed that the relationship between *Trypanoxyuris* infection in black howler monkeys and gut bacterial community composition was considerably weak relative to

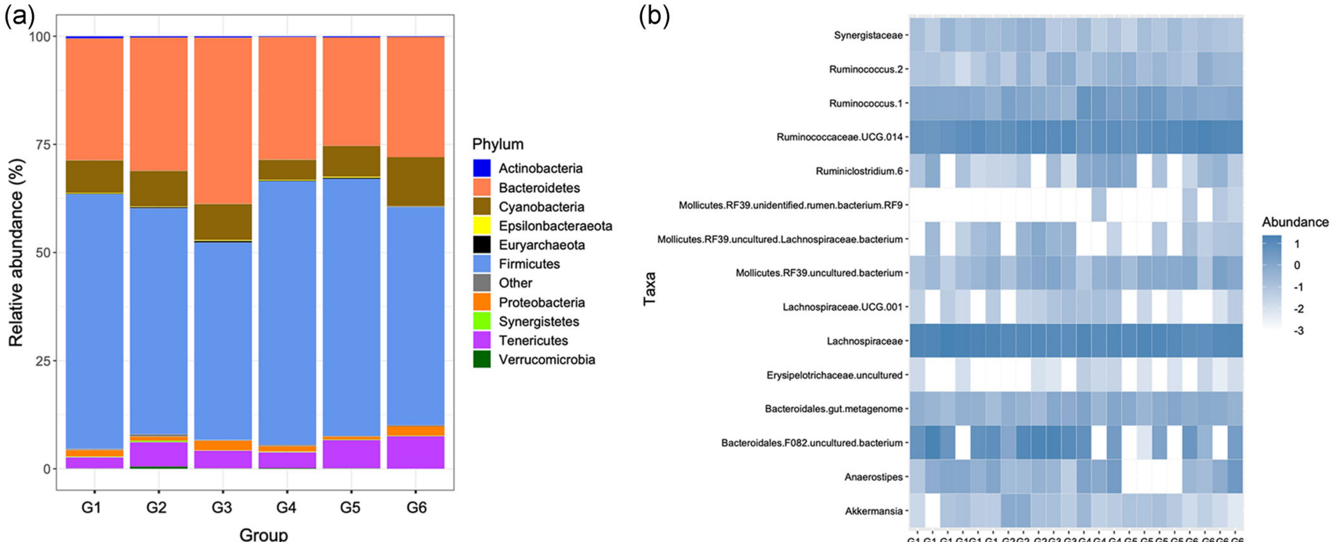


**FIGURE 3** Major microbial phyla observed in six groups (G1–G6) of black howler monkeys (*Alouatta pigra*) at Escárcega, State of Campeche, Mexico (a), and significant changes in bacterial relative abundances at genus level among six groups of black howler monkeys (b). In the heatmap, each column represents an individual, and darker and lighter colors represent higher and lower relative abundances, respectively. Log-scale abundance is shown (b)

other factors. *Trypanoxyuris* parasites belong to the group of oxyurid nematodes, which are organisms that have developed strong coevolutionary relationships with their hosts (Brooks & Glen, 1982; Solórzano-García et al., 2019). Because of this, serious damage from *Trypanoxyuris* to their host is uncommon, though a death related to *Trypanoxyuris* infection has been reported in a wild brown howler monkey (Amato et al., 2002). In our study, we did not record any instance of individuals showing enteric disease symptoms (e.g., diarrhea). Either adaptation and tolerance of howler monkeys to oxyurid nematodes, or the minimal pathogenic potential of these parasites, might have resulted in the modest association between *Trypanoxyuris* and howler gut bacterial community structure.

Our findings add to the premise that the relationship between parasites and the animal gut bacterial community is host–parasite specific (Aivelo & Norberg, 2018; Kreisinger et al., 2015). Previous studies have shown that different micro and macroparasite taxa distinctly affect bacterial communities in human and nonhuman primates. For example, the presence of the protozoan *Entamoeba* spp. is associated with differences in the abundance of bacterial taxa that are likely involved in nutrition and metabolism (e.g., fiber degradation and fermentation) in western lowland gorillas (*Gorilla gorilla gorilla*) (Vlčková et al., 2018). In mouse lemurs (*Microcebus rufus*), the presence of intracellular parasites is related to increased bacterial diversity (Aivelo & Norberg, 2018). Similarly, the presence of nematodes (*Callistoura* sp.) and other enteric parasites (*Blastocystis* sp., *Entamoeba* sp., *Ascaris lumbricoides*, *Necator americanus*, *Trichuris trichiura*, and *Strongyloides stercoralis*) has also been reported to modify the gut bacterial structure in lemurs (*Eulemur* spp., de Winter et al., 2020) and humans, respectively (Morton et al., 2015; Rubel et al., 2020; Tito et al., 2019). As exemplified in these studies and ours, variation in the expression and magnitude of microbial change seems to be related to the specific parasite infecting the host; nevertheless, the current evidence suggests that the primate microbiome differs to some degree when individuals are infected with GI parasites.

Parasitism is a widespread ecological phenomenon in the animal kingdom where virtually all wildlife harbor parasites (Combes, 2001; Seguel & Gottdenker, 2017; Thompson et al., 2009). There is an ecological dynamic between parasites and hosts in which infection, clearance, and reinfection occur over time. Given this established interaction, the gut microbiome of primate hosts might be primed for the periodic presence of GI parasites; therefore, fluctuations in bacterial diversity and community composition could signal normal changes in the microbiome, provoked by the interplay between organisms that takes place in a common arena (i.e., digestive tract). However, bacterial diversity has also been observed to be stable in free-living animal populations, where the majority of individuals coexist with parasites and infection is considered as the norm (Kreisinger et al., 2015). The study of the complex parasite–bacteria system in nonhuman primates and other animals is in its infancy and faces many challenges: one of the most important is understanding the mechanisms by which parasites interact—directly or indirectly, beneficially or detrimentally—with the rest of the host microbiome, including the evolutionary processes acting on different microbe–host–parasite systems (Dheilly et al., 2019). Thankfully, recent initiatives such as the Parasite Microbiome Project (Dheilly et al., 2019) are promoting transdisciplinary research in the field of host–parasite–microbe interactions and represent an encouraging step towards a better understanding of the complex dynamics among parasites, hosts, and their respective microbiota.

Some important methodological considerations must be integrated into studies of host–parasite–microbe interactions moving forward. First, although coproscopy has been the gold standard for scanning fecal samples to assess GI parasite communities in disciplines such as veterinary research (Ballweber et al., 2014), coproscopy does not provide certainty regarding the absolute absence of pinworms, that is, false negatives can occur. We tried to minimize the likelihood of false negatives in our study using longitudinal sampling; however, some uncertainty about infection status remains. DNA-based methods for detecting parasites are now becoming more common and could help solve some of these problems (O'Connell & Nutman, 2016). Nevertheless, similar shortcomings have been described for these approaches (Leblanc et al., 2014). We suggest that multiple parasite detection methods be integrated into studies moving forward to improve certainty regarding the presence/absence of a wider variety of parasites.

We also must continue to explore the impacts of other covarying factors on the gut microbiome of primates. In our study, among the covariates considered, individual identity was strongly associated with differences in the howler gut bacterial community, confirming that intrinsic factors (e.g., host genetics, health) are associated with a certain degree of variability in bacterial community composition and functionality, as shown in human populations and other mammals (Blekhman et al., 2015; Martínez-Mota et al., 2020; Zhernakova et al., 2016). Interestingly, our results agree with a study in chimpanzees (*Pan troglodytes schweinfurthii*; Degnan et al., 2012) which found that different individuals possess distinct gut bacterial communities maintained across years, but they contrast with two other longitudinal studies in baboons (*Papio cynocephalus*) and mouse lemurs (*Microcebus rufus*) that revealed a high dynamism and intraindividual variation over time in the gut bacterial communities of these primates (Aivelo et al., 2016; Ren et al., 2016). These patterns suggest that host ecology and evolution influence patterns of microbiome stability over time. Comparative data should continue to be leveraged to further explore these dynamics.

Additionally, the howler monkeys in this study showed distinct bacterial communities according to their social group, which confirms that at least in this species of arboreal primates, sociality can affect gut bacterial community composition more than parasite infections. Attributes inherent to primate societies, such as group connectivity, individual networks, and social contact, promote sharing of microbial taxa in social primates, as exemplified in wild baboons (Trosvik et al., 2018; Tung et al., 2015). Although allogrooming is infrequent in howler monkeys, members of a howler group are highly cohesive and engage in activities such as resting, feeding, and traveling together (Pavelka, 2011). These activities performed in close proximity may

represent opportunities for bacterial exchange among individuals within a group. These dynamics have been described for the same species at a different site (Amato et al., 2017) and may explain differences between social groups, particularly when geographical separation limits intergroup contact. Furthermore, differences in diet across home ranges are likely to contribute to differences in the gut microbiome across social groups of the same host species, as documented for black howler monkeys at another site (Amato et al., 2013). In this study, some social groups occupied distinct habitats with different levels of anthropogenic influence (Martínez-Mota et al., 2017; Righini et al., 2017). Although we do not have quantitative dietary data, the differences in forest structure that are associated with this gradient of anthropogenic influence are qualitatively associated with different howler diets (Martínez-Mota, 2015; Righini et al., 2017).

Finally, seasonality was only weakly related to gut bacterial community composition, which contrasts with previous results for some other howler populations living in different habitat types in Mexico and Central America (Amato et al., 2015, 2016). It is possible that significant effects of seasonality as a covariate in our model cannot be detected due to our relatively small sample size. Although this field site is seasonal and we sampled across seasons, the sample size within any given season for a single individual is relatively low, and some individuals were only sampled in a single season.

In conclusion, we used wild black howler monkeys and *Trypanoxyuris* sp. as a natural system to explore the co-occurrence between parasites and the gut bacterial community, and found that the presence of *Trypanoxyuris* sp. was weakly associated with differences in howler monkey gut bacterial community composition. The gut is a complex environment, but given that *Trypanoxyuris* was the only parasite detected from the large intestine of our focal animals, this system allowed us to avoid confounding factors related to the presence of other parasites occupying the same niche. Few studies have examined parasite–bacteria relationships in wild primates. Thus, further research of wild primate hosts infected with micro and macroparasite species with different pathogenicity will help elucidate a large array of effects of GI parasites on the primate gut bacterial community. Although observational data like those presented here are useful, controlled experiments where individuals are subjected to parasite removal and reinfection will be essential for establishing causality and mechanism. Combining these data will be key to further understanding the impact of host-gut bacteria interactions on host health in wild primate populations.

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## CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

## AUTHOR CONTRIBUTIONS

**Rodolfo Martínez-Mota**: conceptualization (lead); data curation (equal); formal analysis (equal); methodology (lead); visualization (lead); writing original draft (lead); writing review & editing (equal). **Nicoletta Righini**: formal analysis (equal); investigation (supporting); methodology (supporting); writing original draft (supporting); writing review & editing (equal). **Elizabeth K. Mallott:** data curation (equal); formal analysis (supporting); methodology (supporting); writing-review & editing (equal). **Thomas R. Gillespie**: data curation (supporting); funding acquisition (equal); methodology (supporting); resources (supporting); supervision (supporting); writing review & editing (supporting). **Katherine R. Amato**: conceptualization (equal); data curation (equal); formal analysis (equal); funding acquisition (lead); methodology (supporting); project administration (equal); resources (supporting); writing original draft (equal); writing review & editing (equal).

## DATA AVAILABILITY STATEMENT

Sequences are available in SRA-NCBI (BioProject ID PRJNA645623).

## ORCID

*Rodolfo Martínez-Mota* https://orcid.org/0000-0001-7881-4598
*Nicoletta Righini* https://orcid.org/0000-0003-4327-6544
*Elizabeth K. Mallott* http://orcid.org/0000-0001-5446-8563
*Thomas R. Gillespie* https://orcid.org/0000-0002-7901-7248
*Katherine R. Amato* https://orcid.org/0000-0003-2722-9414

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## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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