**CHAPTER 3**

**Vertebrate blood meal type and richness drive differences in the gut microbial communities of a Chagas disease vector across sampling region and habitat type**

**3.1 Abstract**

Anthropogenic land use change can impact vector-borne infectious disease transmission by influencing the spatial distribution of disease vectors and hosts and changes in vector and host community composition. However, the underlying mechanisms of changes are not well understood. One potential driver of increased infectious disease risk is the diversity and species composition of the vector gut microbiome. By focusing on how land use change impacts the gut microbiomes of disease vectors, we can further study how the landscape can influence the transmission of vector-borne diseases, such as Chagas disease.

In this study, we sequenced the gut bacterial diversity of infected and noninfected triatomines (N = 283) in 3 different land use types (forest remnants, pasture, peridomicile) across three regions in central Panama (Veraguas, Capira, and La Chorrera). The gut bacterial alpha diversity of samples collected in Veraguas was significantly greater than samples collected in the two regions near the Panama Canal (Capira and La Chorrera), and samples collected in forest remnant palms had greater gut bacterial diversity than samples collected in pasture and peridomestic palms. Our data also showed that samples collected from forest remnant palms in Veraguas displayed considerably higher mean relative abundances of the environmental bacterial genera *Dietzia* than samples collected elsewhere. Triatomine samples from Veraguas fed primarily on common opossums (*Didelphis*) and spiny pocket mice (*Heteromys*), while samples collected from the two regions near the Panama Canal fed primarily on four-eyed opossums (*Philander*). Our data indicate that blood meal sources primarily drive the differences in gut bacterial diversity across sampling regions and habitat types. These results highlight the importance of examining the gut microbial communities across biological scales and more fine-scale ecological characteristics. Furthermore, this study evaluates relationships between deforestation, landscape ecology, and gut bacterial communities, which may help to inform vector control and Chagas disease management programs.

**3.2 Introduction**

Anthropogenic land use change can influence infectious disease dynamics directly and indirectly by altering host and vector distribution, abundance, demography, behavior, immune response, contacts, and by shifting host community composition1,2,2–6. Habitat disturbance, in particular, is often associated with an increase in vector abundance, contact rates between vectors and hosts, and ultimately an increased risk of transmission. For example, increased temperatures and increased surface water due to land cover changes throughout western Kenyan highlands were associated with increased rates of several mosquito species4, and forest fragmentation and biodiversity loss were associated with increased risk for Lyme disease in the northeastern United States6. Furthermore, deforestation was associated with increased vector abundance and increased *T. cruzi* infection rates2,7, and another study found that deforested areas driven by cattle ranching may support between 2.5 and 5.1 times more palm-dwelling triatomines (*Rhodnius*) than less disturbed forests5. Although many studies have investigated the relationship between anthropogenic disturbance and disease transmission risk, further research is required to clarify the mechanisms driving this association.

Much less studied is the relationship between vector gut microbial communities, disturbed landscapes, and pathogen transmission risk. The diversity and composition of vector gut microbial communities can directly influence the invasion and establishment of human pathogens8–13; therefore, it is important to better understand the relationships between vector gut microbial communities and habitat disturbances. Anthropogenic environmental disturbances can result in shifts in the gut microbial community by modifying food sources14–17, altering abiotic conditions18, and changing parasite dynamics19,20. For example, researchers recently found that human-blood feeding in mosquitoes was significantly associated with a higher abundance of bacteria overall and that some bacteria taxa were exclusively associated with specific blood meal sources21. Furthermore, Onyango et al.18 found that an increase in mosquito rearing temperatures resulted in the enrichment of specific taxa, while Hussain et al.22 found a reduction in symbiont richness with increasing rearing temperatures, which demonstrates that altered abiotic conditions can shift vector gut bacterial profiles. And finally, parasite infections have been associated with modifying gut bacterial communities of triatomines23–26 and therefore changes to parasite dynamics in the field may subsequently shift vector gut microbial communities. Additional studies are necessary to untangle the interactions between anthropogenic disturbance and disease vector gut microbial communities. A more comprehensive understanding of these interactions may provide a basis for vector control and public health policies. By further understanding how anthropogenic environmental disturbances can shape and shift these gut microbial communities, we can better predict the impacts on vector-borne disease transmission.

While most studies of gut microbial communities of arthropod vectors of infectious disease have been conducted in controlled laboratories and experimental systems, there is limited research on how various environmental conditions affect vector gut microbial communities in the wild. In this study, we used a *16S* rRNA metabarcoding approach to explore the gut bacterial communities of wild-caught *R. pallescens* across a deforestation gradient in central Panama and analyzed the bacterial alpha (within sample) diversity and beta (between sample) composition and structure. The main objective of this study was to investigate associations between landscape-scale environmental variability, including habitat type and geographic region variations, on vector gut microbial composition and diversity.

**3.3 Materials and Methods**

**Sampling efforts and insect collection.**

*Rhodnius pallescens* (N = 288) were opportunistically collected from 41 *Attalea butyracea* palm crowns across central Panama using Noireau traps (Noireau et al., 2002) during the summer of 2017. The samples consisted of adults (N = 54), nymphal stage 5 (N = 27), nymphal stage 4 (N = 14), nymphal stage 3 (N = 60), nymphal stage 2 (N = 60), and nymphal stage 1 (N = 74). These samples were collected from 3 regions, La Chorrera (8°52′49″N 79°47′0″W) and Capira (8°45′0″N 79°52′12″W), which are located in Panama Oeste province, and Santa Fe district (8°30′N 81°04′W), located in Veraguas province (Fig. 1). Among these, samples were collected in habitat types categorized as peridomestic (Capira (N = 15), La Chorrera (N = 57), Veraguas (n= 37)), pasture (Capira (N = 54), La Chorrera (N = 59), Veraguas (N = 51)), and forest remnants (Veraguas (N = 28)) (Table S1). Peridomestic palms were classified as palms found in yards or areas within 100 meters from a lodging, pasture palms were classified as cleared areas for cattle grazing, and forest remnants were classified as mid to late secondary forest patches surrounded by land previously deforested for agriculture or cattle.

**Sample preparation and DNA extraction.**

After capture, each triatomine was placed in 70% ethanol and kept at 4˚C. The body surface of each individual was rinsed in sterile water, and then the midgut guts were dissected using sterile techniques. Between each dissection, forceps and scalpels were rinsed in 70% ethanol and then sterilized using a glass beads sterilizer. Each triatomine gut sample was macerated and digested overnight in digest ATL buffer with Proteinase K and extracted using phenol-chloroform-isoamyl alcohol. Extractions were reconstituted in TLE buffer (10 mM Tris, pH 8; 0.1 mM EDTA), and impurities were removed with Sera-Mag SpeedBeads™ (Thermo Fisher Scientific, Waltham, MA, USA; Faircloth and Glenn27) with a final reconstitution of 30 μL TLE. In addition, 14 negative samples were included during extractions, *16S* rRNA amplification, and library sequencing for a total of 307 samples.

**DNA amplification, *16S,* and *12S* rRNA sequencing.**

We amplified bacterial *16S* rRNA DNA using the S-D-Bact-0341-b-S-17 (5′-CCT ACG GGN GGC WGC AG-3′) forward and S-D-Bact-0785-a-A-21 (5′-GAC TAC HVG GGT ATC TAA TCC-3′) reverse primer pair following the protocol from Kieran et al.28. Blood meal DNA was amplified in the samples using *12S* rRNA gene-specific primers for vertebrates following the protocol from Kieran et al.29. All libraries were sent to the Georgia Genomics and Bioinformatics Core (http://dna.uga.edu) for sequencing on an Illumina MiSeq using a v3 PE300 kit (Illumina, San Diego, CA, USA).

**Bioinformatic processing.**

Amplicon indices were demultiplexedusing Mr. Demuxy 1.20 (https://pypi.org/project/Mr\_Demuxy/), and the resulting files were imported into QIIME2 v. 2022.230. Using the QIIME2 plugin *q2-cutadapt*31, primers were trimmed, paired, and reads were merged. The quality of the sequences was verified, the sequences were filtered, and any chimera sequences were removed using the QIIME2 plugin *q2-demux,* followed by denoising with DADA2 via the *q2-dada2* plugin32 (Callahan et al., 2016) to a final set of amplicon sequence variants (ASVs). All ASVs were aligned using mafft via the *q2-alignment* plugin33 and then used to construct a phylogeny with fasttree2 via the *q2-phylogeny* plugin34. Taxonomy was assigned the *q2-feature-classifier* plugin35 against the Naive Bayes classifier Geengenes 13\_8 99% OTUs36 that was trained on this sample dataset. We filtered out non-bacterial sequences (i.e., chloroplast and mitochondria) and Wolbachia sequences. QIIME v. 1.937 was used to assign operational taxonomic units (OTUs) for vertebrate genera identification with a custom reference database. We eliminated vertebrate genera hits receiving ≤10% of total read hits for the sample. Blood meals assigned to house mice (*Mus musculus*) were not included in any blood meal analyses as house mice were used in the triatomine Nouri traps, and humans were eliminated due to likely contamination during processing. Relative abundance plots were created using the R package *phyloseq*38 to calculate the bacterial community composition across sampling regions, habitat type, and blood meal type and visualized using *ggplot2*39 in R v. 4.2.040.

**Blood meal analyses**.

Across the triatomine samples, up to 5 distinct blood meal vertebrate genera were identified from *12S* rRNA gene sequencing (Table S1) (Fig. S1). However, in the alpha and beta analyses, the dominant blood meal only includes a single vertebrate animal per triatomine sample with the greatest number of reads.

**Statistical Analyses.**

***Alpha (within-sample) diversity.*** Statistical analyses were completed using QIIME2 and R. For alpha and beta diversity analyses, samples were rarefied to 2133 sequences. Four alpha diversity metrics (ASV observed richness, Shannon diversity41, Faith’s phylogenetic diversity42, and Pielou’s evenness43) were calculated using *phyloseq* and QIIME2. Each test was compared among variables using Pairwise Wilcoxon Rank Sum Tests with Benjamini-Hochberg adjustments (for groups of two) or a Kruskal-Wallis analysis of variance (for more than two groups) followed by Pairwise Wilcoxon Rank Sum Tests with Benjamini-Hochberg adjustments44 to test for pairwise significance. Boxplots were visualized using *ggplot2*. We used linear mixed models (LMMs) that were implemented using the *lme4*45 and *lmerTest*46 packages in R to evaluate the associations between sampling region, habitat type, dominant blood meals, and blood meal richness on changes in bacterial alpha diversity. Log-transformed alpha diversity metrics were used as the response variables (ASV richness, Shannon diversity, Faith’s phylogenetic diversity, and Pielou’s evenness), and model predictors included sampling region, habitat type, dominant blood meal, and blood meal richness, and palm identity was treated as a random effect. *R. pallescens* developmental stage, split into two groups by early nymphs (N1-N3) and older nymphs and adults (N4-adult), was included as a covariate to account for the known effects on triatomine bacterial communities47. R2 values were calculated using *partR2* in R48.

***Beta (between-sample) diversity.*** Beta diversity calculations were used to measure community structure and composition differences across sampling regions, habitat type, blood meal richness, and dominant blood meal. Differences in beta diversity between samples were estimated using a permutational multivariate analysis of variance (PERMANOVA) on unweighted and weighted UniFrac distance matrices with 999 permutations using the QIIME2 *q2-diversity* plugin and with Benjamini-Hochberg adjustments and were supported with beta-dispersion. Unweighted UniFrac (community composition) measures the presence/absence of ASVs that incorporates phylogenetic distances, while weighted UniFrac (community structure) considers the phylogenetic distances between observed ASVs in addition to the relative abundance of ASVs. Palm identity and *R. pallescens* triatomine age groups were included as model predictors, as they contributed most to beta diversity in the full models (Tables S2) and were included first in each full model to specifically test the effects of sampling region, habitat type, blood meal type, and blood meal richness. Principal coordinate analysis ordinations (PCoA) were calculated based on unweighted and weighted UniFrac distance matrices with 999 permutations using the *q2-diversity* plugin in QIIME2 and visualized using *ggplot2*.

**3.4 Results**

A total of 288 *R. pallescens* samples were sequenced, yielding a total of 3,124,000 raw sequencing reads. After filtering and denoising the samples with DADA2, the remaining read total was 1,444,104. The samples were then rarefied to a sampling depth of 2,133 based on rarefaction curves, which resulted in 603,639 reads, 283 samples, and 3543 unique ASVs. In total, 452 vertebrate blood meals across eight orders and 19 genera of vertebrate blood meals were identified across all sampled *R. pallescens* (Table S1). Figures S2-S3 show the microbial community composition and structure across palm identity, age group, sampling region, and habitat type.

**Palm identity strongly influences gut microbial communities of triatomines.**

Across all samples, palm identity, which is each individual palm we sampled from, had the greatest effect on gut microbial community composition and structure. We found that palm significantly contributed to 31-41% of the variations in community composition and 31-51% of the variation in community structure (Tables 9,11,18,19-20, Fig. S4)

**Developmental stage influences gut bacteria communities.**

Across all samples, older nymphs and adults (N4-adults, N = 91) displayed greater alpha diversity for all four metrics than younger nymphs (N1-N3, N = 191) (Fig. S5, Table S3). In the linear mixed models, triatomine age group was only a significant predictor of Shannon diversity (LMM: R2 = 0.019, *p* = 0.006) and Pielou’s evenness (R2 = 0.018, *p* = 0.0101) (Table S3). Across pasture palms, triatomine age group was only a significant predictor of Faith’s phylogenetic diversity (LMM: R2 = 0.018, *p* = 0.02032) (Table 1), across the peridomestic palms triatomine age group was a significant predictor of Shannon diversity (LMM: R2 = 0.042, *p* = 0.03293) (Table 2), and finally, across Veraguas palms, triatomine age group was a significant predictor across Shannon diversity (LMM: R2 = 0.0279, *p* = 0.0255) (Table 3). Triatomine age group did not affect any alpha diversity metrics across Capira or La Chorrera palms (Tables 4-5).

Triatomine age group was also a significant predictor of the bacterial composition and structure of the triatomines across all sampling regions and habitat types, and it explained 1.5% of the variation in bacterial composition and 2.5% of the variation in bacterial structure (UniFrac: R2 = 0.015, *p* = 0.001, wUniFrac: R2 = 0.025, *p* = 0.001) (Table S2).

**Gut microbial composition and structure across sampling regions.**

***Relative bacterial abundance.*** Across all samples, *Sphingomonas* was the dominant genera at 74.5% mean relative abundance. After *Sphingomonas,* the most abundant genera were *Erwinia* (6.1% mean relative abundance), *Stenotrophomonas* (5.4% relative abundance), and *Dietzia* (3.6% relative abundance). Across the three sampling regions, *Sphingomonas* was the dominant genera (mean relative abundance at Capira: 70.6%, La Chorrera: 74.4%, and Veraguas: 58.8%) (Fig. 2). At Veraguas, *Sphingomonas* displayed a lower abundance than at the other two regions, with *Erwinia* and *Dietzia* having greater mean relative abundance than at the other two sampling regions (13.3% and 7.6%, respectively).

***Alpha (within-sample).*** Alpha diversity was greatest at Veraguas as measured by ASV richness, Shannon diversity, and Faith’s phylogenetic diversity (Fig. S10) and was significantly different compared to samples from both La Chorrera (Richness: *p* < 0.001; Shannon diversity: *p* < 0.001; Faith’s phylogenetic diversity: *p* < 0.001; Pielou’s evenness: *p* = 0.92) and Capira (Richness: *p* = 0.0025; Shannon diversity index: *p* < 0.001; Faith’s phylogenetic diversity: *p* = 0.0059; Pielou’s evenness: *p* = 0.92) (Table S4). Between the two Panama Canal sites, Capira had greater ASV richness and Shannon diversity (Richness: *p* = 0.017; Shannon diversity: *p* = 0.044) (Fig. S10) compared to La Chorrera; however, there was no difference in Faith’s phylogenetic diversity or Pielou’s evenness (*p* = 0.501 and *p* = 0.917, respectively) (Fig. S10; Table S4).

The dataset was split by habitat type to examine associations between sampling region and gut bacterial diversity more closely. When only examining pasture palms, a slightly different pattern was found. Vector gut microbial diversity in Veraguas was significantly greater than La Chorrera in ASV richness (*p* = 0.0019) and Shannon diversity (*p* < 0.001) and only significantly greater than Capira in Shannon diversity (*p* = 0.019) (Table 6). Additionally, Capira had greater richness (*p* = 0.017) and Shannon diversity (*p* = 0.019) when compared to La Chorrera samples (Fig. 3; Table 6). Across all peridomestic palms, samples collected at Veraguas were also significantly greater than samples collected at Capira and La Chorrera in richness (*p* < 0.001; *p* < 0.001, respectively), Shannon diversity (*p* = 0.0015; *p* < 0.001), and Faith’s phylogenetic diversity (*p* < 0.001; *p* < 0.001). However, at peridomestic palms, there was no difference in alpha diversity between Capira and La Chorrera samples (Fig. 3, Table 7). Forest remnant palms were only sampled in Veraguas and were therefore not compared to any other sampling regions.

***Mixed linear models for alpha diversity.*** Sampling region affected the Shannon diversity (R2 = 0.0762, *p* = 0.0338) across all triatomine samples, however, after splitting the data by habitat type, sampling region did not affect the alpha diversity within the pasture or peridomestic palms (Tables 1-2).

***Beta (between-sample) diversity.*** Without accounting for other factors, community composition varied across sampling regions (UniFrac PERMANOVA: pseudo-F statistic = 13.033199, *p* = 0.001, with non-significant beta-dispersion on 999 permutations: *p* = 0.261) (Table S6), whereas community structure (wUniFrac) showed considerably more overlap across all regions, and Veraguas had significantly greater variance than the other two regions (wUniFrac PERMANOVA: pseudo-F statistic = 25.136, *p* = 0.001, with significant beta-dispersion on 999 permutations: *p* = 0.001) (Table S6). Across only pasture palms, community composition clustered by sampling region with Capira samples more distinct than the other regions and no differences in variances (UniFrac: pseudo-F statistic = 9.195085, *p* = 0.001 with non-significant beta-dispersion on 999 permutations: p = 0.127) (Table 8) while community structure showed more overlap across all regions and Veraguas had significantly greater variance than the other two regions (wUniFrac: pseudo-F statistic = 13.223361, *p* = 0.001, beta-dispersion on 999 permutations: *p* = 0.019) (Fig. 5, Table 8). These results suggest variance is driven, in part, by less abundant taxa. However, palm identity and triatomine age group explained 38% of the variation in community composition and 52% of the variation in community structure (Table 9). After accounting for palm identity and triatomine age groups, sampling region alone did not explain any variation (Table 9).

At peridomestic palms, there was significant clustering across sampling region with some overlap for both measures of beta diversity and differences in variances using weighted UniFrac distances (UniFrac: pseudo-F statistic = 6.491517, *p* = 0.001, with non-significant beta-dispersion on 999 permutations: *p* = 0.051; wUniFrac: pseudo-F statistic = 12.048014, *p* = 0.001, with significant beta-dispersion on 999 permutations: *p* = 0.028) (Table 10). Similar to pasture palms, after accounting for palm identity and triatomine age groups (42% of total variation in community composition and 48% of total variation in community structure), sampling region alone did not contribute to variation in beta diversity amongst peridomestic palm samples (Table 11).

**Gut microbial composition and structure across palm habitat type.**

***Relative abundance.*** Across habitat types for all samples, *Sphingomonas* was the dominant bacterial genera. Similar to samples collected across Veraguas, bugs captured from forest remnants displayed lowered mean relative abundances of *Sphingomonas* (pasture palms: 69.8%, peridomestic palms: 68.1%, and forest remnant palms: 55.6%) and greater relative mean abundance of *Erwinia* (pasture palms: 2.9%, peridomestic palms: 4.6%, forest remnant palms: 20.1%) than bugs collected from the other two habitat types.

***Alpha diversity***. Across habitat types, samples from forest remnant palms were consistently different from samples collected in both pasture palms (Richness: *p* = 0.0204; Shannon diversity: *p* < 0.001; Faith’s phylogenetic diversity: *p* = 0.00270; Pielou’s evenness *p* < 0.001) and peridomestic palms (Richness: *p* < 0.001; Shannon diversity: *p* < 0.001; Faith’s phylogenetic diversity: *p* = 0.00631, Pielou’s evenness: *p* = 0.001) (Table S7). Pasture and peridomestic palm samples, however, were only significantly different across ASV richness (Richness: *p* = 0.045; Shannon diversity: *p* = 0.880; Faith’s phylogenetic diversity: *p* = 0.377; Pielou’s evenness: *p* = 0.534) (Fig. S10, Table S7).

To further examine the relationships between within-sample bacterial communities and habitat type, we split the dataset by sampling region. At Capira, pasture and peridomestic samples were statistically similar as measured by Shannon diversity, Faith’s phylogenetic diversity, and Pielou’s evenness; however, pasture palms displayed greater ASV richness compared to peridomestic palms (*p* = 0.0074) (Fig. 4, Table 12). At La Chorrera, pasture and peridomestic palms were not significantly different across any alpha diversity metrics (Fig. 4, Table 13). Similarly, at Veraguas, pasture palms were also not significantly different across any alpha diversity metric than peridomestic palms (Fig. 5, Table 14). However, at Veraguas, forest remnant palm samples were significantly greater than pasture palms when measured by Shannon diversity (*p* = 0.0087) and Pielou’s evenness (*p* = 0.00043), but not Faith’s phylogenetic diversity or ASV richness, and forest remnant palms were significantly greater than peridomestic palms as measured by Shannon diversity (*p* = 0.0159) and Pielou’s evenness (*p* = 0.00533), but again not Faith’s phylogenetic diversity or ASV richness (Table 14).

***Mixed linear models for alpha diversity across habitat types.*** Habitat type was marginally associated with Shannon diversity across La Chorrera samples (R2 = 0.04, *p* = 0.047) (Table 5) but was not associated with alpha diversity across Capira or Veraguas samples (Tables 3-4).

***Beta diversity.*** Using a PCoA for visualization, both beta diversity metrics demonstrated that the samples clustered by habitat type (UniFrac: pseudo-F statistic: 4.385148, *p* = 0.001, with non-significant beta-dispersion on 999 permutations: *p* = 0.464; wUniFrac: pseudo-F statistic= 6.847971, *p* = 0.001, with non-significant beta-dispersion on 999 permutations: *p* = 0.097) (Fig. S11; Table S8).

At Capira, pasture and peridomestic palms had significantly different beta diversity variances using unweighted UniFrac distances (pseudo-F statistic: 5.146824, *p* = 0.001, with marginal beta-dispersion on 999 permutations: *p* = 0.049, Table 15) but showed no difference in clustering or variance using weighted UniFrac (pseudo-F statistic: 3.046044, *p* = 0.061, with non-significant beta-dispersion on 999 permutations: *p* = 0.631) (Table 15). After accounting for palm identity and triatomine age groups (about 44% of the variation in community composition and about 54% of the variation in community structure), habitat type alone did not contribute to any variation in beta diversity (Table 18).

Amongst La Chorrera samples there were some differences in composition across pasture and peridomestic using unweighted UniFrac distances (pseudo-F statistic: 2.754805, *p* = 0.004, with non-significant beta-dispersion on 999 permutations: *p* = 0.152) (Table 16) but no differences using weighted UniFrac (pseudo-F statistic: 1.909, *p* = 0.058, with non-significant beta-dispersion on 999 permutations: *p* = 0.129) (Table 16). At Veraguas, the forest and peridomestic samples clustered together, indicating little difference in composition between the two habitat types, while pasture palms only slightly overlapped using unweighted UniFrac distances (pseudo-F statistic: 2.732, *p* = 0.001, with non-significant beta-dispersion on 999 permutations, *p* = 0.427) (Table 17), demonstrating some differences in composition across habitat types. Similarly, although there was some overlap between the habitat types using weighted UniFrac distances, the community structure differs across habitat types (pseudo-F statistic: 3.98348, *p* = 0.001, with non-significant beta-dispersion on 999 permutations, *p* = 0.222) (Table 17). After accounting for palm identity and triatomine age groups (about 33-44% of the variation in community composition and 37-54% of the variation in community structure), habitat type only did not significantly contribute to variation in composition or structure across any of the sampling regions (Tables 18-20).

**Associations between gut microbial diversity and vertebrate blood meals**.

***Relative abundance****.* After specifically examining dominant blood meal genera across sampling regions, *Didelphis­*- and *Heteromys*-fed individuals were primarily collected at Veraguas, and *Philander*-fed individuals were primarily only found at Capira and La Chorrera (Fig. S10). We only considered individuals that fed on *Didelphis*, *Heteromys*, and *Philander* as their dominant blood meal for the relative abundance figures and the alpha diversity analyses. Compared to *Heteromys*-fed and *Philander*-fed triatomines, individuals that fed on *Didelphis* displayed lower mean relative abundance of *Sphingomonas* (*Didelphis*: 46.0%, *Heteromys*: 66.1%, *Philander*: 75.0%) but a greater mean relative abundance of *Erwinia* (*Didelphis*: 21.4%, *Heteromys*: 8.7%, *Philander*: 0.96%) and *Dietzia* (Didelphis: 15.3%, Heteromys: 3.5%, Philander: 0.54%) (Figure 14). Conversely, the mean relative of bacteria genera across blood meal richness was similar (Fig. S11)

***Alpha diversity.*** Compared to *Heteromys*-fed and *Philander*-fed individuals, *Didelphis-fed* individuals displayed the greatest alpha diversity, and *Philander*-fed individuals had the lowest alpha diversity (Figure 7). Compared to *Heteromys*-fed individuals, *Didelphis*-fed individuals had significantly greater alpha diversity when measured by Shannon diversity (*p* < 0.001), Faith’s phylogenetic diversity (*p* = 0.023), and Pielou’s evenness (*p* < 0.001) and compared to *Philander*-fed individuals, *Didelphis*-fed individuals displayed the greatest alpha diversity across all metrics (Richness: *p* < 0.001, Shannon diversity: *p* < 0.001, Faith’s phylogenetic diversity: *p* < 0.001, Pielou’s evenness: *p* < 0.001) (Table 21). And finally, *Heteromys*-fed individuals displayed significantly greater alpha diversity across all metrics compared to *Philander*-fed individuals (Richness: *p* < 0.001, Shannon: *p* < 0.001, Faith’s phylogenetic diversity *p* < 0.001, and Pielou’s evenness: *p* = 0.005) (Fig. 7).

The total number of blood meals identified for each sample corresponds to blood meal richness. For all pairwise comparisons across blood meal richness, the only significant associations were found between individuals with one identified blood meal versus individuals with two blood meals as measured by Pielou’s evenness (*p* = 0.004) and between individuals with two blood meals versus three blood meals as measured by Shannon diversity (*p* = 0.026) (Fig. S10, Table S9).

***Mixed linear models for alpha diversity across blood meals.*** Across all samples**,** dominant blood meal genera affected both Faith’s phylogenetic diversity (R2 = 0.1542, *p* = 0.002727) and Pielou’s evenness (R2 = 0.0825, *p* = 0.046) while blood meal richness only affected Pielou’s evenness (R2 = 0.0454, *p* = 0.01246) (Table S5). Across pasture palms, blood meal richness and dominant blood meals affected Pielou’s evenness (R2 = 0.0468, *p* = 0.0127; R2 = 0.15, *p* = 0.0280, respectively) (Table 1), and across peridomestic palms dominant blood meals affected the phylogenetic diversity (R2 = 0.3417, *p* < 0.001) (Table 2).

***Beta diversity***. After accounting for palm identity and triatomine age group, blood meal richness and dominant blood meal did not explain any significant between sample variation across all pasture palm samples, however, both factors contributed to about 8% of the variation in community composition (UniFrac: R2 = 0.0583, *p* = 0.165 for dominant blood meal; R2 = 0.0201, *p* = 0.395 for blood meal richness) and about 6% of the variation in structure (wUniFrac: R2 = 0.040, *p* = 0.322 for dominant blood meal; R2 = 0.024, *p* = 0.109 for blood meal richness (Table 9). Similarly, while dominant blood meal and blood meal richness did not significantly contribute to beta diversity across peridomestic palm samples after accounting for palm identity and age groups, blood meals accounted for about 10% of variation in community composition (UniFrac: R2 = 0.0656, *p* = 0.121 for dominant blood meal; R2 = 0.0317, *p* = 0.255 for blood meal richness) (Table 11) and about 8% of variation in community structure (wUniFrac: R2 = 0.059, *p* = 0.273 for dominant blood meal, R2 = 0.0205, *p* = 0.663 for blood meal richness) (Table 11).

Across Capira samples, blood meals contributed to about 18% of variation in community composition (R2 = 0.0901, *p* = 0.354 for dominant blood meal, R2= 0.0860, *p* = 0.08 for blood meal richness) and about 8% of variation in community structure (R2 = 0.0376, *p* = 0.47, for dominant blood meal, R2 = 0.0385, *p* = 0.613 for blood meal richness) (Table 18). Across La Chorrera palms, blood meals contributed to about 11% of variation in community composition (UniFrac R2 = 0.076422, *p* = 0.269 for dominant blood meal, R2 = 0.033249, *p* = 0.307 for blood meal richness) and about 10% of variation in community structure (wUniFrac: R2 = 0.05577, *p* = 0.366 for dominant blood meal, R2 = 0.043824, *p* = 0.051 for blood meal richness) (Table 19). And finally, across Veraguas samples, blood meals contributed to about 5% of variation in community composition (UniFrac: R2 = 0.018797, *p* = 0.152 for dominant blood meal, R2 = 0.035174, *p* = 0.182 for blood meal richness) and about 9% of variation in community structure (wUniFrac: R2 = 0.045345, *p* = 0.006 for dominant blood meal, R2 = 0.044081, *p* = 0.075 for blood meal richness) (Table 20).

**4.5 Discussion**

Host-associated microbes perform a myriad of beneficial functions within their host. Across arthropod disease vectors, gut symbionts are associated with increasing the vector’s tolerance to environmental perturbations, priming the immune system, pathogen resistance, and aiding in digestion and nutrition13,47,49–52. Triatomines, in particular, have co-evolved with specific gut-associated bacteria to supplement their nutrient-poor diet (Jiménez-Cortés et al., 2018; Vogel and Coon, 2020). Research suggests that ontogeny, vector species identity, and the environment are the primary factors that shape the gut bacterial communities of triatomines47,52. The present study was designed to closely examine the influence of environmental factors on the gut communities of field-caught *R. pallescens* after controlling for variations across ontogeny. Studies have shown strong associations between collection site and gut microbial diversity of disease vectors in mosquitoes53–57, ticks17,58,59 and triatomines28,47, however, few studies have closely examined differences in the microhabitat of these collection sites. As *R. pallescens* primarily live in palm tree crowns, it is crucial to understand whether their gut bacterial community is influenced by microhabitat characteristics, particularly as land conversion and deforestation continue in areas of high vector prevalence. In this study, we demonstrate that dominant blood meal sources drive some differences in bacterial communities of triatomines across sample regions and habitat types in central Panama.

**Palm identity and triatome age groups drive variations in gut microbiota.** Previous studies have established that triatomine age and habitat location primarily drive variations in gut microbiota, and our observations support this pattern. We found that palm identity contributed to 31-41% of the variation in community composition and 31-51% of the variation in community structure, and age significantly contributed to 2-4% of the variation in community composition and about 2-10% of the variation in community structure. In our dataset, 41 palms were sampled, and up to 26 triatomines were sampled from a single palm. The significant contribution of palm identity may reflect the mode of initial gut symbiont acquisition. Triatomines initially pick up their gut microbiome via coprophagy from conspecifics after hatching, from specific maternally inherited bacteria, and from environmental microbes51,52. For these reasons, their gut symbionts will likely closely resemble other triatomines that live in the same palm. Additionally, triatomines from the same palm will feed from the same collection of palm-dwelling hosts, which may further drive similarities in their gut microbial communities.

Furthermore, the contribution of the developmental stage to gut bacteria variation was expected. Current research has shown that developmental stage, particularly between instars N1-N3 and N4-adult, is a major factor that shapes microbiome composition47,60,61, and our results are consistent with these conclusions. However, our results contradict findings from Rodríguez-Ruano et al.61 and Brown et al.47, such that our early-stage instars displayed lower bacterial richness and diversity than late-stage instars and adults. This difference may be an artifact of the vector species identity, as other studies sampled North American *Triatoma* species, and there are many species-specific differences across triatomine gut microbial communities23,47,61,62.

**There are differences in the alpha diversity and composition of the gut bacterial communities of triatomines collected across sampling regions.** Per the linear mixed models, sampling region is a factor that most explains the alpha diversity among all individuals (Tables S4). Figure S3 demonstrates that the greatest differences in alpha gut microbial diversity were found between samples collected in Veraguas and samples collected around the Panama Canal (i.e., Capira and La Chorrera). Alpha richness and diversity were greatest in Veraguas samples, indicating a greater number of ASVs present in the guts of triatomines (Fig. S1). However, evenness is the same across all regions, meaning sampling region is not differentially selecting for greater or fewer amounts of each taxon. Similarly, Veraguas samples displayed the greatest Faith’s phylogenetic diversity measurements, therefore indicating that not only is there an increase in amplicon diversity, but phylogenetic diversity as well.

Furthermore, after controlling for habitat type, we could closely examine diversity patterns across sampling regions within all pasture palms and all peridomestic palms. Similar alpha diversity patterns emerged between sampling regions when only examining peridomestic palms. Veraguas samples displayed greater alpha richness and diversity than both the Panama Canal regions; however, samples from Capira and La Chorrera were not significantly different from each other (Fig. 4). Conversely, when only examining pasture palms, Veraguas samples had greater ASV richness compared to both Capira and La Chorrera, but only displayed greater Shannon diversity compared to the La Chorrera samples (Fig. 3). Furthermore, the linear mixed models shows that habitat type was significantly associated with phylogenetic diversity at Capira and Shannon diversity La Chorrera samples (Tables 4-5). These results indicate that ecological differences at the habitat-level may drive these patterns between sampling regions.

Compositionally, samples from Veraguas displayed a great deviation from samples collected at the two canal sites. The mean relative abundances of *Sphingomonas*, the dominant genera across all samples, were slightly displaced by *Erwinia* and *Dietzia* in samples collected at Veraguas*.* *Dietzia* has been described in other triatomine species23,61,63–65 and other hematophagous insects, including *Aedes albopictus*66 and *Glossina pallidipes*67, which suggests *Dietzia* might be an essential mutualist for hematophagous vectors. However, Brown et al.47 did not identify *Dietzia* in their N1 samples across several species of *Triatoma* and concluded that *Dietzia* might not be transmitted vertically. *Erwinia* has also been found across several species of triatomines62,68. The bacterial genus *Erwinia* contains mostly plant pathogenic species and is most commonly found only in the guts of insects that feed on plant tissue. These results likely indicate that *Erwinia*, like *Dietzia*, is acquired from the environment rather than exposure to or ingesting maternal feces, which suggests a link between environmental microbes and triatomine gut microbial communities.

Many other studies have demonstrated a close association between biogeography and gut microbial diversity. Mosquitoes acquire their initial gut microbiota as larvae from their aquatic habitats, which results in distinct gut composition between sites53–55. Other studies found shared soil and other environmental bacteria between ticks and their habitats58,59, and finally, the gut microbiota of triatomines have also been found to be associated with locality, with distinct compositions of microbes that differed across sites28,47. However, any differences in the environmental factors between these areas were not closely examined to identify any ecological drivers of these patterns. As demonstrated thus far, there may be factors explicitly associated with Veraguas, such as greater precipitation or available vertebrate host species, aside from forest remnant samples, that may be driving some of the differences in gut bacterial composition and structure, and some marginal differences associated with Capira that may be driving differences between those samples and samples from La Chorrera.

**There are differences in the alpha diversity and composition between samples collected in different palm habitat types.** In addition to microbial differences between samples collected across Veraguas and the Panama Canal regions, there were also significant differences in within-sample gut bacterial composition and diversity across palm habitat types. As the environment may primarily influence the gut microbial communities, it is crucial to consider the microhabitat of the palms. Figure S5 shows that samples collected in forest remnant palms displayed greater microbial richness and diversity than those collected at pasture and peridomestic palms. Importantly, all of the samples from forest remnant palms were collected from the Veraguas region, so it was expected for the forest samples to have greater richness and diversity based on our previous results. For this reason, our data were split by sampling region to better assess any associations with palm microhabitat irrespective of region. There were little to no differences between pasture and peridomestic palms in each of the three sampling regions (Figs. 8-10), however, at Veraguas, forest remnant samples had greater alpha diversity measurements than pasture and peridomestic palms. These results again suggest that samples collected at Veraguas are different from samples collected around the Panama Canal, specifically, samples collected in forest remnant palms, and that pasture and peridomestic palms are very similar.

As previously discussed, environmental variables have been found to primarily shape gut microbial communities. *R. pallescens* live primarily in the crowns of *Attalea* palms, and the amount of dead organic matter, surrounding canopy cover, and the number of connected trees can influence temperature and humidity fluctuations within the palm crown. Padukone69 found that temperature and humidity fluctuations stabilized as these environmental variables increased by providing a buffer between the *Attalea* palm crown’s microclimate and the changing ambient climate conditions throughout the day. Another study that sampled the same regions and similar palms as this project found that the microclimate conditions of the palm crowns stayed within an ideal range for triatomines (25-28° C and 79 – 98% relative humidity), although pasture palm crowns experienced larger microclimate fluctuations within 24 hours compared to palms in forest remnants and peridomestic areas70. These stabilized microclimates within the palm crowns may have contributed to the marginal differences between pasture and peridomestic palms seen in this project across each sampling region.

**Dominant blood meal source and blood meal richness primarily drive differences in gut microbial communities across sampling regions and habitat types.** In addition to the microclimate of the palm crowns, vertebrate blood meals may have influenced the gut microbial communities of the triatomines. Dominant blood meals and blood meal richness were significantly associated with within-sample diversity across all samples (Table S5). Dominant blood meal source had a significant effect on the community structure of the samples collected at Veraguas (Table 20) and contributed to 4.5% of the community variation between samples collected across Veraguas. Furthermore, after palm identity and triatomine age group, dominant blood meals accounted for up to 18% of the variation in gut microbial community composition across samples and up to 9% of the variation in gut microbial community structure across samples (Tables 9,11,18-20).

The samples collected at Veraguas had fed on a larger number of blood meal species and had significantly more rodent blood meals than the other two sampling regions. Capira, on the other hand, had the fewest detected blood meal species which may have influenced the marginal differences in gut bacterial diversity between Capira and La Chorrera. There were some associations between blood meal identity and alpha bacterial composition and diversity, with individuals that fed on *Didelphis* displaying the greatest Shannon diversity. Furthermore, *Didelphis*-fed individuals exhibited a greater relative abundance of *Erwinia* and *Dietzia*. In contrast, *Heteromys*-fed individuals showed a similar, but smaller, difference in relative abundances of these bacteria compared to other triatomines (Fig. 4). As discussed previously, *Erwinia* and *Dietzia* are likely acquired from leaf litter in their palm habitats.

Previous studies have found that blood meal source strongly correlates with gut bacterial diversity. One study showed a strong impact of host blood meal identity on tick (*Ixodes* *pacificus*) bacterial species richness and composition17. These researchers found that lizard-fed ticks exhibited lowered bacterial diversity than mammal-fed ticks of the same species. In mosquitoes, a recent study showed a significantly greater alpha diversity of the gut bacteria of laboratory-reared individuals fed on either sugar, rabbit blood, or a mixture of chicken and rabbit blood than that of chicken-fed mosquitoes16. And finally, another study found that gut bacterial diversity shifts after ingestion of a blood meal but will return to its original structure within six days Muturi et al.16. If this remains true for *R. pallescens*, a blood meal may temporarily alter the composition and structure and significantly shape the gut microbial communities. It is important to note, however, while multiple blood meals were detected from each triatomine, the results from this project only incorporated a single blood meal for each sample (i.e., dominant blood meal identity). And notably, *Didelphis*-fed and *Heteromys*-fed individuals were only sampled in Veraguas, which may have strongly influenced the differences seen between Veraguas and the Panama Canal regions.

**The samples from Veraguas differed significantly from samples collected at the two Panama Canal regions, which may be influenced by dominant blood meal source, vector taxonomy, and landscape features.** The samples collected in Veraguas were significantly different across most alpha diversity metrics than those collected from the two Canal regions, while the two Canal sites were similar. There may be several reasons for this phenomenon, including biogeography, blood meal diversity, and vector taxonomy. Veraguas is 136.82 km from the middle of the two Panama Canal regions, while the two Panama Canal regions are only approximately 16.56 km away from each other. Researchers recently found that there was high migration of *R. pallescens* between sampling regions around the Panama Canal but limited migration rates from Veraguas to sites around the Canal71. These researchers concluded that the differences in migration are likely due to the greater distance between the Panama Canal sites and Santa Fe, Veraguas (~ 150 km), topographical/elevation effects, and proximity and similarity in biogeographic features between the Panama Canal sampling regions. The similar gut bacterial patterns between triatomines collected in Capira and La Chorrera may be the result of microbial dispersal dynamics across small spatial scales that may, in part, drive similar community patterns between close populations of insects54.Our data also show that Veraguas samples fed primarily on *Didelphis* and *Heteromys* while samples from the Canal primarily fed on *Philander.* As noted previously, many studies have shown significant associations between blood meal sources and gut microbial diversity.

Lastly, there is evidence that the triatomines found in Veraguas are genetically dissimilar from those found around the Panama Canal. The samples found in Veraguas have a darker pigmentation and are slightly larger but are otherwise morphologically similar to the Canal triatomines72. In addition to different migration patterns across central Panama, Kieran et al.71 found that triatomines in Veraguas were genetically differentiated from the triatomines collected around the Canal, while samples from various regions and sites around the Panama Canal had little genetic differentiation. Several studies have shown that host taxonomy is a significant driver of gut bacterial diversity within arthropods, even within closely related species47,73–75. Although we cannot pinpoint the exact factors that drive the gut bacterial communities of triatomines collected in pasture and peridomestic palms to be more similar to each other than triatomines collected in forest remnants, nor specific factors that drive Veraguas samples to differ from the different sampling regions, these results motivate further research into the influence of microhabitats and landscape features on gut communities.

In conclusion, we evaluated the gut bacteria *R. pallescens*, the primary vector of Chagas disease in Panama. We found differences between sampling regions, with samples collected from Veraguas that were distinctly different alpha diversity. These patterns likely reflect limited migration patterns of triatomines between Veraguas and the Canal regions and greater migration patterns amongst the Canal regions and genetically isolated Veraguas triatomines. We also found differences between samples collected at Veraguas across habitat types, with forest samples distinctly different from those collected in peridomestic and pasture palms. Furthermore, samples collected in pasture and peridomestic palms had very similar gut bacteria communities. And finally, we found that the dominant blood meal source was associated with some variation in beta diversity between samples across sampling regions and habitat types, with *Didelphis*-fed and *Heteromys*-fed individuals from Veraguas likely driving these patterns. These patterns across habitat types are probably the result of greater biogeographic similarities between pasture and peridomestic palms compared to forest remnant palms.

Overall, our research suggests that there are regional differences in diversity and composition. Particularly, our results show that the gut bacterial diversity of *R. pallescens* is greatest in the less disturbed forest remnant palms of Veraguas, which is likely driven by the vertebrate blood meal species, vector taxonomy, and landscape features such as higher precipitation. Previous research in Panama has shown strong associations between lower disturbance and lowered *T. cruzi* infections. Although this present study did not include infection data, these results together showcase essential insights into the relationships between vector-borne disease transmission and anthropogenic land use change, which is imperative for vector control and vector-borne disease management.

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**3.7 Tables**

**Table 3.1. Linear mixed models testing the effects of sampling region, dominant blood meal, and blood meal richness on alpha diversity across pasture palms after accounting for triatomine age groups.**

Alpha diversity values were log-transformed prior to fitting and palm identity was included as a random effect.

Marginal R2 values were reported for full models and partial R2 values were reported for each covariate. Significant relationships are shown in bold.

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **R^2** | | **Sum SQ** | | **Mean SQ** | | **NumDF** | | **DenDF** | | **F Statistic** | **p value** |
| **ASV Richness** |  | |  | |  | |  | |  | |  |  |
| Age group | 0.1009 | | 0.19707 | | 0.197074 | | 1 | | 90.846 | | 3.4099 | 0.06806 |
| Sampling region | 0.2811 | | 0.34123 | | 0.170613 | | 2 | | 29.379 | | 2.952 | 0.06788 |
| Blood meal richness | 0.121 | | 0.37833 | | 0.126112 | | 3 | | 94.276 | | 2.182 | 0.09526 |
| Dominant blood meal | 0.019 | | 0.56492 | | 0.070615 | | 8 | | 85.822 | | 1.2218 | 0.29617 |
|  |  | |  | |  | |  | |  | |  |  |
|  |  | |  | |  | |  | |  | |  |  |
| **Shannon** |  | |  | |  | |  | |  | |  |  |
| Age group | 0.0115 | | 0.13187 | | 0.131867 | | 1 | | 92.052 | | 3.8411 | 0.05304 |
| Sampling region | 0.1138 | | 0.17851 | | 0.089256 | | 2 | | 30.784 | | 2.5999 | 0.09051 |
| Blood meal richness | 0.0197 | | 0.23372 | | 0.077907 | | 3 | | 95.121 | | 2.2693 | 0.08544 |
| Dominant blood meal | 0.1447 | | 0.3929 | | 0.049113 | | 8 | | 85.164 | | 1.4306 | 0.19559 |
| Full Model | 0.3604 | |  | |  | |  | |  | |  |  |
|  |  | |  | |  | |  | |  | |  |  |
| **Faith's PD** |  | |  | |  | |  | |  | |  |  |
| Age group | 0.0185 | | 0.39308 | | 0.39308 | | 1 | | 86.734 | | 5.5876 | **0.02032** |
| Sampling region | 0.0706 | | 0.18852 | | 0.09426 | | 2 | | 26.308 | | 1.3399 | 0.27917 |
| Blood meal richness | 0 | | 0.14015 | | 0.04672 | | 3 | | 90.977 | | 0.6641 | 0.57627 |
| Dominant blood meal | 0.0812 | | 0.68597 | | 0.08575 | | 8 | | 88.493 | | 1.2189 | 0.29742 |
| Full Model | 0.2369 | |  | |  | |  | |  | |  |  |
|  |  | |  | |  | |  | |  | |  |  |
| **Evenness** |  | |  | |  | |  | |  | |  |  |
| Age group | 0.0232 | | 0.009128 | | 0.009128 | | 1 | | 94.228 | | 2.5452 | 0.11398 |
| Sampling region | 0.0108 | | 0.002366 | | 0.001183 | | 2 | | 29.493 | | 0.3298 | 0.72169 |
| Blood meal richness | 0.0468 | | 0.040871 | | 0.013624 | | 3 | | 96.609 | | 3.7987 | **0.0127** |
| Dominant blood meal | 0.15 | | 0.066235 | | 0.008279 | | 8 | | 79.171 | | 2.3085 | **0.02798** |
| Full Model | 0.2775 | |  | |  | |  | |  | |  |  |
|  |  | |  | |  | |  | |  | |  |  |
| **Table 3.2 Linear mixed models testing the effects of sampling region, dominant blood meal, and blood meal richness on alpha diversity across peridomestic palms after accounting for triatomine age groups.** | | | | | | | | | | | | | |
| Alpha diversity values were log-transformed prior to fitting and palm identity was included as a random effect. | | | | | | | | | | | | | |
| Marginal R2 values were reported for full models and partial R2 values were reported for each covariate. Significant relationships are shown in bold. | | | | | | | | | | | | | |
|  | | **R^2** | | **Sum SQ** | | **Mean SQ** | | **NumDF** | | **DenDF** | **F Statistic** | **p value** |
| **ASV Richness** | |  | |  | |  | |  | |  |  |  |
| Age group | | 0.0035 | | 0.02167 | | 0.021671 | | 1 | | 60 | 0.365 | 0.54801 |
| Sampling region | | 0.0063 | | 0.03949 | | 0.019747 | | 2 | | 60 | 0.3326 | 0.71837 |
| Blood meal richness | | 0.0162 | | 0.10126 | | 0.033754 | | 3 | | 60 | 0.5685 | 0.6379 |
| Dominant blood meal | | 0.1377 | | 0.811 | | 0.115858 | | 7 | | 60 | 1.9514 | 0.07701 |
| Full Model | | 0.3054 | |  | |  | |  | |  |  |  |
|  | |  | |  | |  | |  | |  |  |  |
| **Shannon** | |  | |  | |  | |  | |  |  |  |
| Age group | | 0.0421 | | 0.164273 | | 0.164273 | | 1 | | 60 | 4.7671 | **0.03293** |
| Sampling region | | 0.0108 | | 0.042157 | | 0.021078 | | 2 | | 60 | 0.6117 | 0.54579 |
| Blood meal richness | | 0.0299 | | 0.116717 | | 0.038906 | | 3 | | 60 | 1.129 | 0.34459 |
| Dominant blood meal | | 0.0534 | | 0.20817 | | 0.029739 | | 7 | | 60 | 0.863 | 0.54082 |
| Full Model | | 0.3548 | |  | |  | |  | |  |  |  |
|  | |  | |  | |  | |  | |  |  |  |
| **Faith's PD** | |  | |  | |  | |  | |  |  |  |
| Age group | | 0.004 | | 0.03 | | 0.03 | | 1 | | 61 | 0.6338 | 0.429 |
| Sampling region | | 0.0112 | | 0.08346 | | 0.04173 | | 2 | | 61 | 0.8817 | 0.4193 |
| Blood meal richness | | 0.0327 | | 0.24454 | | 0.081513 | | 3 | | 61 | 1.7222 | 0.1718 |
| Dominant blood meal | | 0.3417 | | 2.13313 | | 0.304733 | | 7 | | 61 | 6.4383 | **1.10E-05** |
| Full Model | | 0.531 | |  | |  | |  | |  |  |  |
|  | |  | |  | |  | |  | |  |  |  |
| **Evenness** | |  | |  | |  | |  | |  |  |  |
| Age group | | 0.0264 | | 0.010821 | | 0.010821 | | 1 | | 61 | 2.4959 | 0.1193 |
| Sampling region | | 0.0034 | | 0.001399 | | 0.000699 | | 2 | | 61 | 0.1613 | 0.8514 |
| Blood meal richness | | 0.0253 | | 0.010387 | | 0.003462 | | 3 | | 61 | 0.7986 | 0.4994 |
| Dominant blood meal | | 0.1038 | | 0.042598 | | 0.006085 | | 7 | | 61 | 1.4037 | 0.2203 |
| Full Model | | 0.218 | |  | |  | |  | |  |  |  |

**Table 3.3 Linear mixed models testing the effects of habitat type, dominant blood meal, and blood meal richness on alpha diversity across Veraguas samples after accounting for triatomine age groups.**

Alpha diversity values were log-transformed prior to fitting and palm identity was included as a random effect.

Marginal R2 values were reported for full models and partial R2 values were reported for each covariate. Significant relationships are shown in bold.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | R^2 | Sum SQ | Mean SQ | NumDF | DenDF | F Statistic | p value |
| **ASV Richness** |  |  |  |  |  |  |  |
| Age group | 0.0058 | 0.052136 | 0.052136 | 1 | 84.829 | 0.862 | 0.3558 |
| Habitat type | 0 | 0.011168 | 0.005584 | 2 | 9.695 | 0.0923 | 0.9126 |
| Blood meal richness | 0.0279 | 0.227848 | 0.075949 | 3 | 84.858 | 1.2557 | 0.2948 |
| Dominant blood meal | 0.0178 | 0.101257 | 0.033752 | 3 | 38.055 | 0.5581 | 0.6459 |
| Full Model | 0.0602 |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |
| **Shannon** |  |  |  |  |  |  |  |
| Age group | 0.0279 | 0.1637 | 0.163696 | 1 | 85.021 | 5.1699 | **0.0255** |
| Habitat type | 0.0294 | 0.0412 | 0.0206 | 2 | 7.941 | 0.6506 | 0.54745 |
| Blood meal richness | 0.0627 | 0.19862 | 0.066205 | 3 | 85.078 | 2.0909 | 0.10743 |
| Dominant blood meal | 0.0706 | 0.21851 | 0.072837 | 3 | 36.822 | 2.3004 | 0.09332 |
| Full Model | 0.233 |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |
| **Faith's PD** | |  |  |  |  |  |  |
| Age group | 0.0318 | 0.270109 | 0.270109 | 1 | 86.21 | 4.0335 | **0.04774** |
| Habitat type | 0.0119 | 0.027241 | 0.013621 | 2 | 8.683 | 0.2034 | 0.81974 |
| Blood meal richness | 0.0257 | 0.173688 | 0.057896 | 3 | 86.105 | 0.8645 | 0.46276 |
| Dominant blood meal | 0.0025 | 0.049922 | 0.016641 | 3 | 39.501 | 0.2485 | 0.86192 |
| Full Model | 0.1135 |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |
| **Evenness** |  |  |  |  |  |  |  |
| Age group | 0.0148 | 0.097843 | 0.097843 | 1 | 84.791 | 5.1738 | **0.02545** |
| Habitat type | 0.0636 | 0.040263 | 0.020131 | 2 | 6.81 | 1.0645 | 0.39602 |
| Blood meal richness | 0.0713 | 0.138233 | 0.046078 | 3 | 84.617 | 2.4365 | 0.07026 |
| Dominant blood meal | 0.0816 | 0.150616 | 0.050205 | 3 | 29.537 | 2.6548 | 0.06677 |
| Full Model | 0.2681 |  |  |  |  |  |  |

**Table 3.4 Linear mixed models testing the effects of habitat type, dominant blood meal, and blood meal richness on alpha diversity across Capira samples after accounting for triatomine age groups.**

Alpha diversity values were log-transformed prior to fitting and palm identity was included as a random effect.

Marginal R2 values were reported for full models and partial R2 values were reported for each covariate. Significant relationships are shown in bold.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | R^2 | Sum SQ | Mean SQ | NumDF | DenDF | F value | p value |
| **ASV Richness** |  |  |  |  |  |  |  |
| Age group | 0.1002 | 0.007225 | 0.007225 | 1 | 20.9804 | 0.1027 | 0.7518 |
| Habitat type | 0.0155 | 0.012241 | 0.012241 | 1 | 5.1252 | 0.174 | 0.6935 |
| Blood meal richness | 0.0816 | 0.209666 | 0.069889 | 3 | 22.0301 | 0.9935 | 0.4142 |
| Dominant blood meal | 0.0041 | 0.131392 | 0.032848 | 4 | 19.9735 | 0.4669 | 0.7593 |
| Full Model | 0.1975 |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |
| **Shannon** |  |  |  |  |  |  |  |
| Age group | 0 | 0.001439 | 0.001439 | 1 | 22.2591 | 0.0314 | 0.861 |
| Habitat type | 0.0967 | 0.04613 | 0.04613 | 1 | 3.7175 | 1.0056 | 0.3767 |
| Blood meal richness | 0.1777 | 0.250108 | 0.083369 | 3 | 22.3606 | 1.8174 | 0.173 |
| Dominant blood meal | 0.2034 | 0.199932 | 0.049983 | 4 | 16.7601 | 1.0896 | 0.3934 |
| Full Model | 0.272 |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |
| **Faith's PD** | |  |  |  |  |  |  |
| Age group | 0.0015 | 0.03592 | 0.035919 | 1 | 19.9301 | 0.3851 | 0.5419 |
| Habitat type | 0 | 0.00028 | 0.000281 | 1 | 5.8551 | 0.003 | 0.958 |
| Blood meal richness | 0 | 0.23505 | 0.078349 | 3 | 21.4408 | 0.8401 | 0.4868 |
| Dominant blood meal | 0.0612 | 0.35491 | 0.088727 | 4 | 21.1454 | 0.9514 | 0.4542 |
| Full Model | 0.1759 |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |
| **Evenness** | |  |  |  |  |  |  |
| Age group | 0.0009 | 0.000645 | 0.000645 | 1 | 24 | 0.0411 | 0.84102 |
| Habitat type | 0.0887 | 0.071176 | 0.071176 | 1 | 24 | 4.5396 | **0.04357** |
| Blood meal richness | 0.0978 | 0.068058 | 0.022686 | 3 | 24 | 1.4469 | 0.25395 |
| Dominant blood meal | 0.1195 | 0.083137 | 0.020784 | 4 | 24 | 1.3256 | 0.28903 |
| Full Model | 0.2563 |  |  |  |  |  |  |

**Table 3.5 Linear mixed models testing the effects of habitat type, dominant blood meal, and blood meal richness on alpha diversity across La Chorrera samples after accounting for triatomine age groups.**

Alpha diversity values were log-transformed prior to fitting and palm identity was included as a random effect.

Marginal R2 values were reported for full models and partial R2 values were reported for each covariate. Significant relationships are shown in bold.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | R^2 | Sum SQ | Mean SQ | NumDF | DenDF | F value | p value |
| **ASV Richness** |  |  |  |  |  |  |  |
| Age group | 0.0346 | 0.086869 | 0.086869 | 1 | 65.638 | 1.8046 | 0.1838 |
| Habitat type | 0.0151 | 0.043544 | 0.043544 | 1 | 5.381 | 0.9046 | 0.3823 |
| Blood meal richness | 0.02 | 0.14958 | 0.04986 | 3 | 64.695 | 1.0358 | 0.3827 |
| Dominant blood meal | 0.0681 | 0.282256 | 0.040322 | 7 | 42.667 | 0.8376 | 0.5624 |
| Full Model | 0.1018 |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |
| **Shannon** |  |  |  |  |  |  |  |
| Age group | 0.013 | 0.038741 | 0.038741 | 1 | 67 | 1.2375 | 0.26992 |
| Habitat type | 0.04 | 0.12742 | 0.12742 | 1 | 67 | 4.0703 | **0.04765** |
| Blood meal richness | 0.0246 | 0.073291 | 0.02443 | 3 | 67 | 0.7804 | 0.50904 |
| Dominant blood meal | 0.0492 | 0.146512 | 0.02093 | 7 | 67 | 0.6686 | 0.69774 |
| Full Model | 0.1703 |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |
| **Faith's PD** | |  |  |  |  |  |  |
| Age group | 0.0043 | 0.07532 | 0.075317 | 1 | 61.015 | 1.5714 | 0.21478 |
| Habitat type | 0.0711 | 0.24595 | 0.245952 | 1 | 4.062 | 5.1316 | 0.08515 |
| Blood meal richness | 0.0521 | 0.44489 | 0.148297 | 3 | 61.734 | 3.0941 | **0.03331** |
| Dominant blood meal | 0.1908 | 1.49533 | 0.213619 | 7 | 42.333 | 4.457 | **0.00087** |
| Full Model | 0.3351 |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |
| **Evenness** |  |  |  |  |  |  |  |
| Age group | 0.001 | 0.000742 | 0.000742 | 1 | 67 | 0.0892 | 0.7661 |
| Habitat type | 0.0139 | 0.010763 | 0.010763 | 1 | 67 | 1.2941 | 0.2593 |
| Blood meal richness | 0.0481 | 0.037206 | 0.012402 | 3 | 67 | 1.4913 | 0.2249 |
| Dominant blood meal | 0.0841 | 0.065115 | 0.009302 | 7 | 67 | 1.1185 | 0.3621 |
| Full Model | 0.1514 |  |  |  |  |  |  |

**Table 3.6 Pairwise Wilcoxon rank-sum test comparisons of four different alpha diversity metrics across samples from pasture palms**

Results highlighted in boldface indicate p-values of less than 0.05. All p-values were corrected with Benjamini-Hochberg adjustments.

|  |  |  |  |
| --- | --- | --- | --- |
| Pasture Samples | Capira:Chorrera | Capira:Veraguas | Veraguas:Chorrera |
| Richness | **0.0173** | 0.2253 | **0.00190** |
| Shannon | **0.019** | **0.019** | **0.000380** |
| Faith | 0.267 | 0.783 | 0.083 |
| Evenness | 0.353 | 0.103 | 0.074 |

**Table 3.7 Pairwise Wilcoxon rank-sum test comparisons of four different alpha diversity metrics across samples from peridomestic palms**

Results highlighted in boldface indicate p-values of less than 0.05. All p-values were corrected with Benjamini-Hochberg adjustments.

|  |  |  |  |
| --- | --- | --- | --- |
| Peridomestic Samples | Capira:Chorrera | Capira:Veraguas | Veraguas:Chorrera |
| Richness | 0.45746 | **2.50E-04** | **2.60E-05** |
| Shannon | 0.3904 | **0.0015** | **1.40E-05** |
| Faith | 0.2 | **1.10E-05** | **2.70E-05** |
| Evenness | 0.32 | 0.32 | 0.51 |

**Table 3.8 PERMANOVA and beta-dispersion results of pasture palms with unweighted and weighted UniFrac distance matrix**

Results highlighted in boldface indicate p-values of less than 0.05.

|  |  |  |
| --- | --- | --- |
| **PERMANOVA results** |  |  |
| **Distance matrix** | Unweighted UniFrac | Weighted UniFrac |
| **test statistic name** | pseudo-F | pseudo-F |
| **sample size** | 164 | 164 |
| **number of groups** | 3 | 3 |
| **test statistic** | 9.195085 | 13.223361 |
| **PERMANOVA p-value** | 0.001 | **0.001** |
| **Beta-dispersion p-value** | 0.127 | **0.019** |
| **number of permutations** | 999 | 999 |

**Table 3.9 PERMANOVAs testing the effects of region, blood meal type, and blood meal richness on community composition (unweighted) and structure (weighted) across pasture palms after accounting for palm identity and developmental stage**

Results highlighted in boldface indicate p-values of less than 0.05.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Unweighted UniFrac** |  |  |  |  |  |  |
|  | **Df** | **SumsOfSqs** | **MeanSqs** | **F.Model** | **R2** | **p-value** |
| **Palm identity** | 20 | 8.576488 | 0.428824 | 2.76765 | 0.363399 | **0.001** |
| **Age group** | 1 | 0.622197 | 0.622197 | 4.015681 | 0.026363 | **0.001** |
| **Sampling region** | 2 | 0 | 0 | 0 | 0 | NaN |
| **Dominant blood meal** | 8 | 1.377352 | 0.172169 | 1.111186 | 0.058361 | 0.165 |
| **Blood meal richness** | 3 | 0.474423 | 0.158141 | 1.020648 | 0.020102 | 0.395 |
| **Residuals** | 81 | 12.550279 | 0.154942 | NaN | 0.531775 | NaN |
| **Total** | 113 | 23.600739 | NaN | NaN | 1 | NaN |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
| **Weighted UniFrac** |  |  |  |  |  |  |
|  | **Df** | **SumsOfSqs** | **MeanSqs** | **F.Model** | **R2** | **p-value** |
| **Palm identity** | 20 | 1.404589 | 0.070229 | 5.007237 | 0.501377 | **0.001** |
| **Age group** | 1 | 0.081188 | 0.081188 | 5.788569 | 0.028981 | **0.001** |
| **Sampling region** | 2 | 0 | 0 | 0 | 0 | NaN |
| **Dominant blood meal** | 8 | 0.111524 | 0.01394 | 0.993931 | 0.039809 | 0.322 |
| **Blood meal richness** | 3 | 0.068087 | 0.022696 | 1.618152 | 0.024304 | 0.109 |
| **Residuals** | 81 | 1.136073 | 0.014026 | NaN | 0.405529 | NaN |
| **Total** | 113 | 2.801461 | NaN | NaN | 1 | NaN |
|  |  |  |  |  |  |  |

**Table 3.10 PERMANOVA and beta-dispersion results of peridomestic palms with unweighted and weighted UniFrac distance matrix**

Results highlighted in boldface indicate p-values of less than 0.05.

|  |  |  |
| --- | --- | --- |
| **PERMANOVA results** |  |  |
| **Distance matrix** | **Unweighted UniFrac** | **Weighted UniFrac** |
| **test statistic name** | pseudo-F | pseudo-F |
| **sample size** | 104 | 104 |
| **number of groups** | 3 | 3 |
| **test statistic** | 6.491517 | 12.048014 |
| **PERMANOVA p-value** | **0.001** | **0.001** |
| **Beta-dispersion p-value** | 0.051 | **0.001** |
| **number of permutations** | 999 | 999 |

**Table 3.11 PERMANOVAs testing the effects of region, blood meal type, and blood meal richness on community composition (unweighted) and structure (weighted) across peridomestic palms after accounting for palm identity and developmental stage**

Results highlighted in boldface indicate p-values of less than 0.05.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Unweighted UniFrac** |  |  |  |  |  |  |
|  | **Df** | **SumsOfSqs** | **MeanSqs** | **F.Model** | **R2** | **p-value** |
| **Palm identity** | 13 | 6.160405 | 0.473877 | 3.291909 | 0.401659 | **0.001** |
| **Age group** | 1 | 0.343783 | 0.343783 | 2.388178 | 0.022415 | **0.003** |
| **Sampling region** | 2 | 0 | 0 | 0 | 0 | NaN |
| **Dominant blood meal** | 6 | 1.005242 | 0.16754 | 1.163861 | 0.065542 | 0.121 |
| **Blood meal richness** | 3 | 0.486432 | 0.162144 | 1.126374 | 0.031715 | 0.255 |
| **Residuals** | 51 | 7.341558 | 0.143952 | NaN | 0.47867 | NaN |
| **Total** | 74 | 15.337419 | NaN | NaN | 1 | NaN |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Weighted UniFrac** |  |  |  |  |  |  |
|  | **Df** | **SumsOfSqs** | **MeanSqs** | **F.Model** | **R2** | **p-value** |
| **Palm identity** | 13 | 0.727384 | 0.055953 | 3.758583 | 0.407883 | **0.001** |
| **Age group** | 1 | 0.154646 | 0.154646 | 10.388255 | 0.086718 | **0.001** |
| **Sampling region** | 2 | 0 | 0 | 0 | 0 | NaN |
| **Dominant blood meal** | 6 | 0.105508 | 0.017585 | 1.181233 | 0.059164 | 0.273 |
| **Blood meal richness** | 3 | 0.036562 | 0.012187 | 0.818668 | 0.020502 | 0.663 |
| **Residuals** | 51 | 0.759218 | 0.014887 | NaN | 0.425734 | NaN |
| **Total** | 74 | 1.783318 | NaN | NaN | 1 | NaN |

**Table 3.12 Pairwise Wilcoxon rank-sum test comparisons of four different alpha diversity metrics across samples from Capira**

Results highlighted in boldface indicate p-values of less than 0.05. All p-values were corrected with Benjamini-Hochberg adjustments.

|  |  |
| --- | --- |
| Capira Samples | Pasture:Peridomestic |
| Richness | **0.0074** |
| Shannon Diversity | 0.088 |
| Faith's PD | 0.14 |
| Pielou's Evenness | 0.16 |

**Table 3.13 Pairwise Wilcoxon rank-sum test comparisons of four different alpha diversity metrics across samples from La Chorrera**

Results highlighted in boldface indicate p-values of less than 0.05. All p-values were corrected with Benjamini-Hochberg adjustments.

|  |  |
| --- | --- |
| La Chorrera Samples | Pasture:Peridomestic |
| Richness | 0.98 |
| Shannon | 0.36 |
| Faith's PD | 0.44 |
| Pielou's Evenness | 0.51 |

**Table 3.14 Pairwise Wilcoxon rank-sum test comparisons of four different alpha diversity metrics across samples from Veraguas**

Results highlighted in boldface indicate p-values of less than 0.05. All p-values were corrected with Benjamini-Hochberg adjustments

|  |  |  |  |
| --- | --- | --- | --- |
| Veraguas Samples | Forest:Pasture | Forest:Peridomestic | Pasture:Peridomestic |
| Richness | 0.71 | 0.71 | 0.71 |
| Shannon | **0.0087** | 0.0159 | 0.4311 |
| Faith's PD | 0.26 | 0.46 | 0.15 |
| Pielou's Evenness | **0.00043** | **0.00533** | 0.36566 |

**Table 3.15 PERMANOVA and beta-dispersion results of Capira samples with unweighted and weighted UniFrac distance matrix**

Results highlighted in boldface indicate p-values of less than 0.05.

|  |  |  |
| --- | --- | --- |
| **PERMANOVA results** |  |  |
| **Distance matrix** | **Unweighted UniFrac** | **Weighted UniFrac** |
| **test statistic name** | pseudo-F | pseudo-F |
| **sample size** | 68 | 68 |
| **number of groups** | 2 | 2 |
| **test statistic** | 5.146824 | 3.046044 |
| **PERMANOVA p-value** | **0.001** | 0.068 |
| **Beta-dispersion p-value** | 0.49 | 0.061 |
| **number of permutations** | 999 | 999 |

**Table 3.16 PERMANOVA and beta-dispersion results of La Chorrera samples with unweighted and weighted UniFrac distance matrix**

Results highlighted in boldface indicate p-values of less than 0.05.

|  |  |  |
| --- | --- | --- |
| **PERMANOVA results** |  |  |
| **Distance matrix** | Unweighted UniFrac | Weighted UniFrac |
| **test statistic name** | pseudo-F | pseudo-F |
| **sample size** | 116 | 116 |
| **number of groups** | 2 | 2 |
| **test statistic** | 2.754805 | 1.909703 |
| **PERMANOVA p-value** | **0.004** | 0.07 |
| **Beta-dispersion p-value** | 0.152 | 0.129 |
| **number of permutations** | 999 | 999 |

**Table 3.17 PERMANOVA and beta-dispersion results of Veraguas samples with unweighted and weighted UniFrac distance matrix**

Results highlighted in boldface indicate p-values of less than 0.05.

|  |  |  |
| --- | --- | --- |
| **PERMANOVA results** |  |  |
| **Distance matrix** | Unweighted UniFrac | weighted UniFrac |
| **test statistic name** | pseudo-F | pseudo-F |
| **sample size** | 100 | 100 |
| **number of groups** | 3 | 3 |
| **test statistic** | 2.73291 | 3.98348 |
| **PERMANOVA p-value** | 0.001 | 0.001 |
| **Beta-dispersion p-value** | 0.427 | 0.222 |
| **number of permutations** | 999 | 999 |

**Table 3.18 PERMANOVAs testing the effects of habitat type, blood meal type, and blood meal richness on community composition (unweighted) and structure (weighted) across Capira palms after accounting for palm identity and developmental stage**

Results highlighted in boldface indicate p-values of less than 0.05.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Unweighted UniFrac** |  |  |  |  |  |  |
|  | **Df** | **SumsOfSqs** | **MeanSqs** | **F.Model** | **R2** | **p-value** |
| **Palm identity** | 7 | 2.799913 | 0.399988 | 2.81218 | 0.410588 | **0.001** |
| **Age group** | 1 | 0.258253 | 0.258253 | 1.81569 | 0.037871 | **0.046** |
| **Habitat type** | 1 | 0 | 0 | 0 | 0 | NaN |
| **Dominant blood meal** | 4 | 0.614722 | 0.15368 | 1.080476 | 0.090145 | 0.354 |
| **Blood meal richness** | 3 | 0.586181 | 0.195394 | 1.373748 | 0.085959 | 0.08 |
| **Residuals** | 18 | 2.560212 | 0.142234 | NaN | 0.375437 | NaN |
| **Total** | 33 | 6.81928 | NaN | NaN | 1 | NaN |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
| **Weighted UniFrac** |  |  |  |  |  |  |
|  | **Df** | **SumsOfSqs** | **MeanSqs** | **F.Model** | **R2** | **p-value** |
| **Palm identity** | 7 | 0.615071 | 0.087867 | 3.335282 | 0.495473 | 0.102 |
| **Age group** | 1 | 0.057596 | 0.057596 | 2.186225 | 0.046396 | 0.175 |
| **Habitat type** | 1 | 0 | 0 | 0 | 0 | NaN |
| **Dominant blood meal** | 4 | 0.046655 | 0.011664 | 0.442731 | 0.037583 | 0.47 |
| **Blood meal richness** | 3 | 0.047855 | 0.015952 | 0.60549 | 0.038549 | 0.613 |
| **Residuals** | 18 | 0.474206 | 0.026345 | NaN | 0.381999 | NaN |
| **Total** | 33 | 1.241382 | NaN | NaN | 1 | NaN |

**Table 3.19 PERMANOVAs testing the effects of habitat type, blood meal type, and blood meal richness on community composition (unweighted) and structure (weighted) across La Chorrera palms after accounting for palm identity and developmental stage**

Results highlighted in boldface indicate p-values of less than 0.05.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Unweighted UniFrac** |  |  |  |  |  |  |
|  | **Df** | **SumsOfSqs** | **MeanSqs** | **F.Model** | **R2** | **p-value** |
| **Palm identity** | 16 | 5.022065 | 0.313879 | 2.134166 | 0.344193 | **0.001** |
| **Age group** | 1 | 0.320773 | 0.320773 | 2.181037 | 0.021984 | **0.009** |
| **Habitat type** | 1 | 0 | 0 | 0 | 0 | **NaN** |
| **Dominant blood meal** | 7 | 1.115065 | 0.159295 | 1.083099 | 0.076422 | 0.269 |
| **Blood meal richness** | 3 | 0.485139 | 0.161713 | 1.099538 | 0.033249 | 0.307 |
| **Residuals** | 52 | 7.647818 | 0.147073 | NaN | 0.524151 | NaN |
| **Total** | 79 | 14.590859 | NaN | NaN | 1 | NaN |
|  |  |  |  |  |  |  |
| **Weighted UniFrac** |  |  |  |  |  |  |
|  | **Df** | **SumsOfSqs** | **MeanSqs** | **F.Model** | **R2** | **p-value** |
| **Palm identity** | 16 | 0.347019 | 0.021689 | 3.306797 | 0.40529 | **0.001** |
| **Age group** | 1 | 0.082872 | 0.082872 | 12.635205 | 0.096788 | **0.001** |
| **Habitat type** | 1 | 0 | 0 | 0 | 0 | NaN |
| **Dominant blood meal** | 7 | 0.047752 | 0.006822 | 1.040075 | 0.05577 | 0.366 |
| **Blood meal richness** | 3 | 0.037523 | 0.012508 | 1.906994 | 0.043824 | 0.051 |
| **Residuals** | 52 | 0.341058 | 0.006559 | NaN | 0.398329 | NaN |
| **Total** | 79 | 0.856223 | NaN | NaN | 1 | NaN |
|  |  |  |  |  |  |  |

**Table 3.20 PERMANOVAs testing the effects of habitat type, blood meal type, and blood meal richness on community composition (unweighted) and structure (weighted) across Veraguas palms after accounting for palm identity and developmental stage**

Results highlighted in boldface indicate p-values of less than 0.05.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Unweighted UniFrac** |  |  |  |  |  |  |
|  | **Df** | **SumsOfSqs** | **MeanSqs** | **F.Model** | **R2** | **p-value** |
| **Palm identity** | 12 | 6.042874 | 0.503573 | 3.329097 | 0.307012 | **0.001** |
| **Age group** | 1 | 0.627792 | 0.627792 | 4.150307 | 0.031895 | **0.001** |
| **Habitat type** | 2 | 0 | 0 | 0 | 0 | **NaN** |
| **Dominant blood meal** | 2 | 0.369981 | 0.18499 | 1.222963 | 0.018797 | 0.152 |
| **Blood meal richness** | 4 | 0.692317 | 0.173079 | 1.144219 | 0.035174 | 0.182 |
| **Residuals** | 79 | 11.949863 | 0.151264 | NaN | 0.607121 | NaN |
| **Total** | 98 | 19.682827 | NaN | NaN | 1 | NaN |
|  |  |  |  |  |  |  |
| **Weighted UniFrac** |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  | **Df** | **SumsOfSqs** | **MeanSqs** | **F.Model** | **R2** | **p-value** |
| **Palm identity** | 12 | 0.817136 | 0.068095 | 3.866874 | 0.312843 | **0.001** |
| **Age group** | 1 | 0.170083 | 0.170083 | 9.658482 | 0.065117 | **0.001** |
| **Habitat type** | 2 | 0 | 0 | 0 | 0 | NaN |
| **Dominant blood meal** | 2 | 0.118441 | 0.05922 | 3.362932 | 0.045345 | **0.006** |
| **Blood meal richness** | 4 | 0.115138 | 0.028785 | 1.634584 | 0.044081 | 0.075 |
| **Residuals** | 79 | 1.39117 | 0.01761 | NaN | 0.532614 | NaN |
| **Total** | 98 | 2.611968 | NaN | NaN | 1 | NaN |

**Table 3.21 Pairwise Wilcoxon rank-sum test comparisons of four different alpha diversity metrics across top three dominant blood meals across all samples**

Results highlighted in boldface indicate p-values of less than 0.05. All p-values were corrected with Benjamini-Hochberg adjustments.

|  |  |  |  |
| --- | --- | --- | --- |
| Dominant blood meals | Heteromys:Didelphis | Didelphis:Philander | Heteromys:Philander |
| Richness | 0.056 | **5.50E-06** | **7.80E-05** |
| Shannon | **6.70E-06** | **1.60E-10** | **5.00E-06** |
| Faith's PD | **0.0099** | **4.90E-10** | **3.80E-05** |
| Pielou's Evenness | **1.60E-05** | **0.00045** | **0.00532** |

**3.8 Figures**

**Figure 3.1 Map of Panama with sampling effort and administrative districts**

Map

Description automatically generated

**Figure 3.2 Average relative abundance across the three sampling regions.** Average relative abundances of the top 10 bacterial genera for all sampled *R. pallescens* (N = 283) split by sampling region as determined by *16S* rRNA metabarcoding.

Chart, bar chart

Description automatically generated

**Figure 3.3 Alpha diversity across pasture and peridomestic palms.** Four alpha diversity metrics (ASV richness and Shannon Diversity) for pasture and peridomestic palms at each of the three sampling regions. n = Number of bugs sampled. Horizontal bar indicates two regions that were compared via Pairwise Wilcoxon Rank Sum Tests to test for pairwise significance. Asterisks designates Benjamini-Hochberg-corrected *p*-values (\* = *p* < 0.05; \*\* = *p* < 0.01; \*\*\* = *p* < 0.001; ns, not significant).

Diagram, timeline

Description automatically generated

**Figure 3.4 Alpha diversity across Capira and La Chorrera samples.** Four alpha diversity metrics (ASV richness, and Shannon diversity) for samples collected at Capira and La Chorrera. n = Number of bugs sampled. Horizontal bar indicates two regions that were compared via Pairwise Wilcoxon Rank Sum Tests to test for pairwise significance. Asterisks designates Benjamini-Hochberg-corrected *p*-values (\* = *p* < 0.05; \*\* = *p* < 0.01; \*\*\* = *p* < 0.001; ns, not significant).

Diagram, timeline

Description automatically generated

Chart, box and whisker chart

Description automatically generated**Figure 3.5 Alpha diversity across Veraguas samples.** Four alpha diversity metrics (ASV richness, Pielou’s Evenness, Shannon diversity, and Faith’s phylogenetic diversity) for samples collected at Veraguas. n = Number of bugs sampled. Horizontal bar indicates two regions that were compared via Pairwise Wilcoxon Rank Sum Tests to test for pairwise significance. Asterisks designates Benjamini-Hochberg-corrected *p*-values (\* = *p* < 0.05; \*\* = *p* < 0.01; \*\*\* = *p* < 0.001; ns, not significant).

**Figure 3.6 Beta diversity across Veraguas samples.** Principle coordinate analysis (PCoA) using (a) Unweighted UniFrac and (b) weighted UniFrac distances for gut bacterial community composition and structure, respectively. Points colored by habitat types show clustering in coordinate space with 95% confidence interval ellipses.

Chart, diagram, bubble chart

Description automatically generated

**Figure 3.7 Alpha diversity across top three dominant blood meal sources across samples.** Four alpha diversity metrics (ASV richness, Pielou’s Evenness, Shannon diversity, and Faith’s phylogenetic diversity) for samples that fed on the top 3 dominant vertebrate genera that were identified by analysis of 12S rRNA sequencing across all sampling regions. n = number of bugs sampled. Horizontal bar indicates two regions that were compared via Pairwise Wilcoxon Rank Sum Tests to test for pairwise significance. Asterisks designates Benjamini-Hochberg-corrected p-values (\* = p < 0.05; \*\* = p < 0.01; \*\*\* = p < 0.001; ns, not significant).

Diagram, box and whisker chart

Description automatically generated