Molecular Ecology

WILEY

MOLECULAR ECOLOGY

ORIGINAL ARTICLE OPEN ACCESS

Drivers of Microbiome Composition Among Helminth Parasites Sharing the Same Insectivorous Bat Host

Nallely G. Ruiz-Torres[1,2] | Shamayim Martínez-Sánchez[1,2] | Arit de León-Lorenzana[3,4] | Ricardo A. Mercado-Juárez[3] | Priscila M. Salloum[5] | Robert Poulin[5] | Osiris Gaona[3] | Luisa I. Falcón[3] | Oscar Rico-Chávez[2]

[1]Posgrado en Ciencias Biológicas, Universidad Nacional Autónoma de México, Ciudad Universitaria, México D.F, Mexico | [2]Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de, México, Ciudad Universitaria, México, Mexico | [3]Laboratorio de Ecología Bacteriana, Instituto de Ecología, Unidad Mérida, Universidad, Nacional Autónoma de México, Ucú, Yucatán, México | [4]Universidad Intercultural Maya de Quintana Roo-CONAHCYT, Presumida, Quintana Roo, Mexico | [5]Department of Zoology, University of Otago, Dunedin, New Zealand

Correspondence: Luisa I. Falcón (falcon@ecologia.unam.mx) | Oscar Rico-Chávez (orich@unam.mx)

Received: 17 December 2025 | **Revised:** 28 April 2026 | **Accepted:** 4 May 2026

Keywords: 16S rRNA | bat | helminths | microbiome | Nematoda | Trematoda

ABSTRACT

Parasitic metazoans are increasingly recognised to form close associations with microbial taxa. Under the holobiont concept, these associations are an eco-evolutionary unit under joint selection. However, for most parasitic helminth species and particularly those associated with wildlife, these interactions and their effect on parasite evolution remain unknown. Investigating the factors determining the composition of helminth microbiomes is the first step towards a better understanding of helminth holobionts. Using the insectivorous bat *Peropteryx kappleri* and its parasitic helminths as a model system, we characterised the microbiome of 41 helminth individuals of four trematode and one nematode species in various bat intestinal and biliary microhabitats, along with bat tissues and luminal fluids. Our results based on 16S rRNA metabarcoding revealed that the microbiome composition of the different helminth species is partly influenced by their microhabitat (bat tissue), but ultimately each helminth species exhibits a distinctive microbial signature. Microbiome composition among the four trematode species showed no phylogenetic signal (no correlation with genetic similarity). Compared to the bat host, each helminth species exhibited enriched microbial taxa with putative symbiotic potential, some of which are commonly found in arthropods (potential intermediate hosts of helminths) and may be conserved throughout the parasite's life cycle. We propose that helminth microbiomes are determined by ecologically relevant factors and provide a basis for future functional research with implications for parasite establishment, development, and transmission.

1 | Introduction

Animals do not live in isolation, and to survive and reproduce, most have evolved interactions with other species (Rosenberg and Zilber-Rosenberg 2013; Theis et al. 2016). Therefore, interspecific interactions constitute one of the most important processes influencing the patterns of adaptation and variation in species. One such interaction is symbiosis, a universal phenomenon that plays a key role in the maintenance of life (Gilbert et al. 2012). For example, it is well known that multicellular eukaryotes harbour a significant number of symbiotic microorganisms that inhabit and specialise in different regions of their bodies, collectively known as the microbiome (Theis et al. 2016; Bik 2019; McFall-Ngai et al. 2013). This biotic community comprising the organism and its symbiotic microbiome is recognised as a biological unit, the "holobiont," that has been the subject of intense study in recent years (Bordenstein and Theis 2015; Moran and Sloan 2015).

Parasitic metazoans are multicellular eukaryotes that harbour microorganisms nested within their bodies. Therefore, they should not be considered autonomous individuals but rather holobionts (Dheilly 2014; Dheilly et al. 2015, 2019). However, their study has been limited because historically research in parasitology has been conducted in host–parasite pairs, excluding the microbiomes of the parasites, which in turn are part of the host's microbiome (Dheilly 2014; Dheilly et al. 2015). In general, studies have focused on the host's response to parasitic infection or on the parasite's virulence mechanisms, offering a simplistic view of the interaction between the two associates (Dheilly et al. 2015). However, the holobiont-host and holobiont-parasite interaction could influence fitness among all associates (Dheilly et al. 2015; Simon et al. 2019).

In the case of helminth parasites, there is a lag in research on microbiome characterisation compared to the explosion of knowledge in other research areas (Salloum, Jorge, Dheilly, and Poulin 2023). Helminths are ubiquitous parasites with complex life cycles, and are especially interesting from an evolutionary perspective, with patterns of coevolution and cospeciation with their hosts and phylogenetically diverse characteristics that shape key host–parasite interactions, such as immune responses. (Dheilly et al. 2015; Brealey et al. 2022; Martinson et al. 2020) and phenotypic variation (Poulin et al. 2023). There is still much to be investigated regarding microbiome-helminth dynamics and their eco-evolutionary implications for the parasite and for each component of the microbiome. Perhaps one of the most pressing questions to be addressed is what factors influence the microbiome composition of helminths, as most microbiome studies in wild helminths have reported high variability in microbial taxa (Salloum, Jorge, Dheilly, and Poulin 2023; Brealey et al. 2022; Hasegawa et al. 2025; Trejo-Meléndez and Contreras-Garduño 2025). Helminth microbiomes have mostly been found to differ from those of their hosts, sometimes with intraspecific and geographic variation (Jorge et al. 2022), although certain components may be constant (core microbiome) throughout the stages of their ontogenetic cycle (Jorge et al. 2020).

Bats (Chiroptera: Mammalia) are a key group for understanding the processes that shape host–parasite–microbiome associations in a multi-partite context because they harbour very diverse macro and microsymbiotic communities (Ingala et al. 2021; Lutz et al. 2019; Santos and Gibson 2015). In addition, bats present a wide range of trophic guilds (Ingala et al. 2021; Fenton 1990; Peixoto et al. 2018) and access wide geographical ranges by flying, which results in unique physiological adaptations (Peixoto et al. 2018; Shen et al. 2010). To date, studies of the microbiome associated with bats focus on understanding its diversity (Ingala et al. 2021; Lutz et al. 2019, 2022), whereas knowledge of the microbiome of their endoparasitic helminths is nil, representing an important overlooked component in the eco-evolutionary dynamics involving bats, helminths, and their microbiomes (Dheilly et al. 2015, 2019).

Our study aims to identify the most influential determinants of the microbiome composition of helminth parasites in bats. We hypothesise that eukaryotic parasite species sharing the same hosts and microhabitats (host organ), as well as being closely phylogenetically related, will exhibit similar microbiome compositions. Conversely, helminth species that are distantly related phylogenetically and inhabit different microhabitats within the same host are predicted to vary in their microbiome composition and structure. With gastrointestinal helminths associated with the insectivorous bat *Peropteryx kappleri* as model organisms, we sought to answer the following questions: (1) Does the microbiome of helminth species resemble that of their immediate environment (host tissue and lumen fluid)? (2) Is there intra- and interspecific variation in the microbiome composition of helminths infecting the same bat? (3) Do different helminth species harbour a unique core microbiome? and (4) Does the evolutionary history of helminths influence the composition of their microbiome, i.e., do they show evidence of phylosymbiosis? Answering these questions will reveal whether phylogenetic relatedness or niche similarity (occupying the same or different host organ), or other factors, play the greatest role in shaping the composition of helminth microbiomes.

2 | Materials and Methods

2.1 | Field Sampling, Bat and Helminth Collection and Preservation

We conducted sampling in December 2021 at the beginning of the winter season in Boca de Chajul, Chiapas, Mexico (16°6′58″ N; 90°55′25″ W) (Figure 1A). All procedures were in accordance with the American Society of Mammalogists criteria for the capture, handling, and ethical euthanasia of bats (Sikes et al. 2019), and the Food and Agriculture Organization (El Masry et al. 2020), under the scientific collector's permit DGVS/04214/19 issued to ORC by the Secretaría del Medio Ambiente y Recursos Naturales de México (SEMARNAT). Bats were captured with mist nets and placed in sterile cotton bags prior to transferring them to the laboratory for processing. Individuals were identified at the species level following the field guide of Medellín et al. (2008) and the specialised keys of Reid (1997). We recorded each bat's sex, reproductive condition, forearm length, and body mass.

The bats were euthanised through an overdose of inhaled isoflurane. Following this, we conducted an in situ parasitological assessment to identify endoparasites. The instruments used for dissecting bats and collecting parasites were sterilised with two applications of 10% sodium hypochlorite, followed by rinsing with 96% ethanol after each use. To retrieve endoparasites, a ventral incision of the body (from mouth to anus) was made, and the body cavity of each bat was inspected with the help of a stereomicroscope. Then, we isolated each internal organ in a sterile Petri dish containing a 0.85% sterile saline solution. We thoroughly checked each organ for helminths using fine dissecting needles and brushes.

For microbiome characterisation, each adult helminth morphotype was first individually washed and vortexed four times with sterile phosphate-buffered solution (PBS) to remove visible contamination from gut contents and loosely adherent microbes from the tegument or cuticle. Washed parasitic worms were placed individually in sterile cryotubes and preserved in liquid

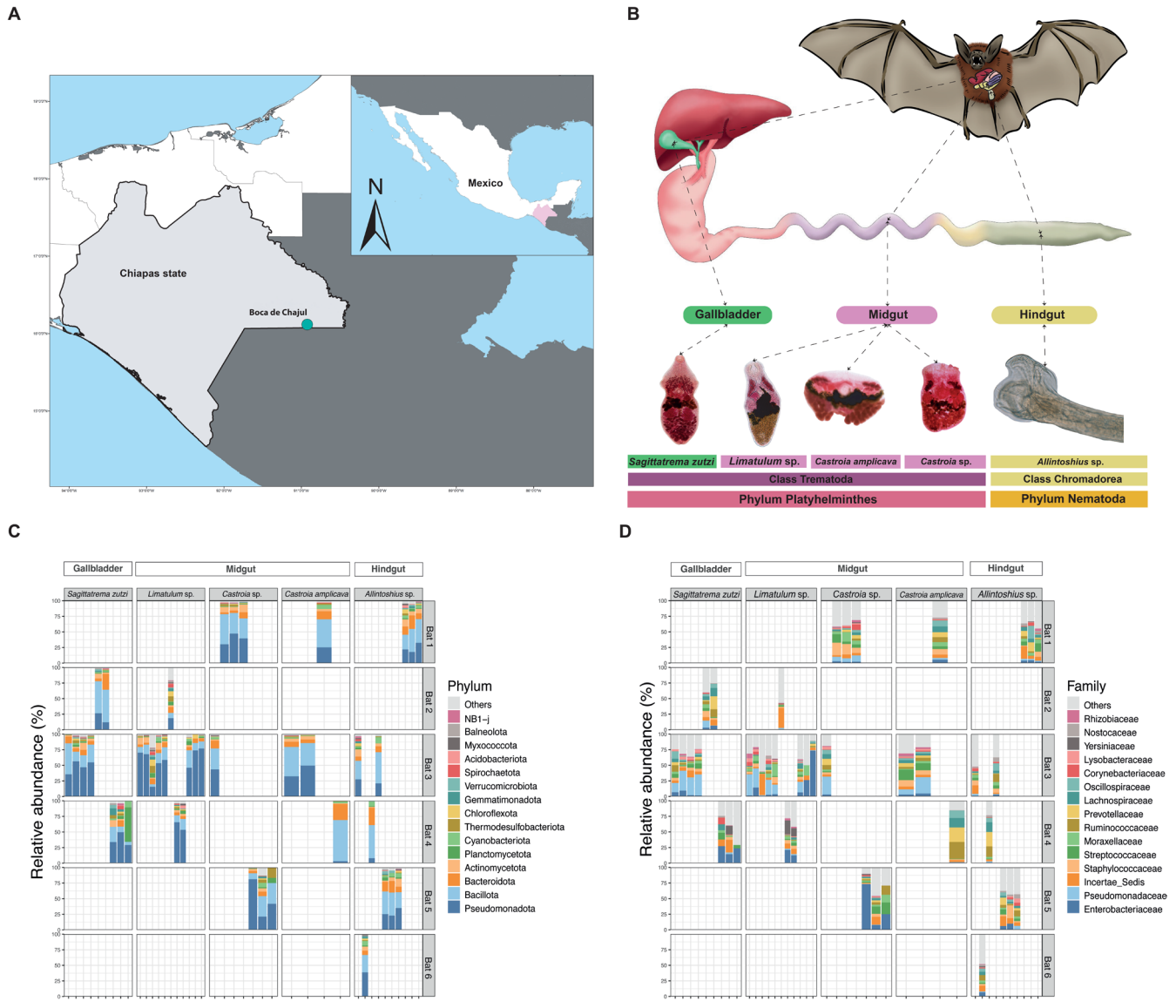

FIGURE 1 | (A) Collection site of the insectivorous bat *Peropteryx kappleri* in Boca de Chajul, Chiapas, Mexico. (B) Conceptual diagram illustrating the microhabitat where each of the helminth species investigated in this study was located in situ. Relative abundance of the 15 most abundant bacterial and archaeal taxa at the following taxonomic levels: (C) phylum and (D) family. Each bar per column represents an individual grouped by helminth species. Each row represents an individual bat host.

nitrogen. Individual surface washes of helminths were defined as the luminal fluid of the host. The four rinses per individual helminth were pooled for analyses, stored in an individual cryotube, and preserved in liquid nitrogen. The host tissue inhabited by each parasite morphotype (gallbladder, midgut and hindgut) was separated and individually preserved in sterile cryotubes stored in liquid nitrogen until its storage. All samples were kept at −20°C in the ultra-low temperature freezer until the extraction of genomic DNA.

2.2 | Morphological Identification and Ecological Descriptors of Helminths

A subsample of helminths (different from those used for DNA extraction; see below) was used for species determination. For morphological characterisation, trematodes were fixed in warm 4% formalin and preserved in 70% ethanol following the method described by Lamothe Argumedo (1997); they were subsequently stained with Meyer's Paracarmine and mounted in Canada Balsam for total preparations. Nematodes were fixed in warm 70% ethanol and preserved in 70% ethanol, then cleared in 100% glycerine for observation of internal organs. Additionally, some individuals were examined with scanning electron microscopy (SEM) at the Biodiversity Microscopy and Photography Laboratory I, Institute of Biology, National Autonomous University of Mexico (UNAM), Mexico City. Taxonomic determination for each group was based on specialised literature (Bray et al. 2008; Fernandes et al. 2021; Ruiz-Torres et al. 2024). Infections were characterised using prevalence (the percentage of bat hosts infected), mean abundance (the average number of parasites per host), and intensity range (the minimum and maximum number of parasites per host), as described by Bush et al. (1997).

2.3 | Molecular Methodologies

Genomic DNA extraction from 91 samples including parasites, host luminal fluid, and host tissue was performed using the DNeasy Blood & Tissue extraction kit (Qiagen, Valencia, CA), following the manufacturer's protocol after incubating samples (parasites, luminal fluid and host tissue) overnight at 56°C at 1500 rpm with proteinase K (10 mg·mL^{-1}). The DNA was eluted with 300 μL of AE buffer before being precipitated with 100% ethanol, 0.1 volumes of 3 M sodium acetate, and 2 μL of glycoblue (15 mg·mL^{-1}) (Gaona et al. 2020). The resulting DNA was quantified by spectrophotometry, and its quality was determined by agarose gel electrophoresis.

To confirm helminth identity, we performed PCRs of the nuclear 28S ribosomal RNA gene region (D1-D3) using the universal forward primer 391 (5′ AGCGGAGGAAAAAGAAACTAA-3′; Nadler and Hudspeth, 1998) and reverse primer 536 (5′-CAGCTATCCTGAGGGAAAC-3′; Stock et al., 2001). Additionally for nematodes, we amplified the COX1 region using the LCO1490/HCO2198 primer set, (Medellín et al. 2008). PCR and Sanger sequencing were carried out according to methods by (Ruiz-Torres et al. 2024; Panti-May et al. 2025). Consensus DNA sequences were assembled using Geneious Pro 4.8.4 (Biomatters; http://www.geneious.com/). To rule out contamination or bat host amplification, sequences underwent a BLAST search on the NCBI website (http://www.ncbi.nlm.nih.gov), and any sequences matching the bat host or non-helminths were excluded from the dataset. All DNA sequences generated were submitted to GenBank under accession numbers: COX1 (PX637274-77) and 28S (PX649025-42).

The 28S rRNA gene sequences dataset generated in this study was constructed in Mesquite 3.62 (http://www.mesquiteproject.org/) for Trematoda. The morphological characterisation of trematodes was confirmed based on a phylogenetic analysis following (Ruiz-Torres et al. 2024) and the phylogenetic position of the trematode species is shown in Figure S1. For the nematode taxon, COI sequences were used to support molecular placement of *Allinoshius* sp. within *Heligmonellidae* (Figure S2). Individuals that showed discrepancies between morphological and molecular analyses were discarded as a precautionary measure. This did not affect the conclusions of the microbiome analysis.

The V4 hypervariable region of the 16S rRNA gene was amplified through PCR targeting the V4 hypervariable region using the universal bacterial/archaeal primers F515 (5′-GTGCCAGCMGCCGCGGTAA-3′) and R806 (5′-GGACTACHACHVGGGGGTWTCTAAT-3′), in accordance with (Caporaso et al. 2018; Carrillo-Araujo et al. 2015). Extraction controls (which allow for the detection of contamination during DNA extraction), no-template controls (which allow for the detection of contaminating DNA introduced during library preparation), and a standard positive control (which ensures the reagents used during PCR are functional) were included. The negative and positive controls were processed and sequenced along with the biological samples. Four reactions per sample were carried out with Takara Ex Taq DNA Polymerase (Shiga, Japan), utilising the specified PCR protocol: initial denaturation at 94°C for 30 s, followed by 35 cycles consisting of 95°C for 30 s, 52°C for 40 s, and 72°C for 90 s, concluding with a final elongation step of 12 min at 72°C, and subsequently maintained at 4°C. The resulting PCR products were purified with the Agencourt AMPure XP PCR Purification System (Beckman Coulter, USA) according to the manufacturer's instructions. Purified 16S rRNA amplicons were quantified with a QUBIT fluorometer (Promega, USA), and approximately 20 ng per sample was used to construct the library that was sequenced with the Illumina MiSeq platform (Yale Center for Genome Analysis CT, USA), generating ~250 bp pairwise reads. All samples in this study were sequenced in a single run. Sequences generated are deposited in the NCBI under BioProject ID: PRJNA1381229.

2.4 | 16SrRNA Sequence Processing

Raw paired-end sequencing reads underwent quality control using fastp v0.23.2 (Chen et al. 2018) with the following parameters: automatic adapter detection and trimming (*--detect_adapter_for_pe*), base correction in overlapped regions (--correction), and quality filtering with a minimum Phred score threshold of Q30 (*--qualified_quality_phred 30*). Reads with >40% of bases below Q30, length <175 bp, or containing >5 N bases were discarded. Filtered sequences were processed using QIIME2 qiime2-amplicon-2024.10.1 (Bolyen et al. 2019) for downstream analysis. Sequence data were denoised with DADA2 and clustered into amplicon sequence variants (ASVs) using the q2-dada2 plugin (Callahan et al. 2016). All ASVs were aligned with MAFF (Katoh et al. 2002) with the complement q2-alignment, which was used to construct a phylogenetic tree with fasttree2 (Price et al. 2010) and the q2-phylogeny plugin. Taxonomy was assigned to ASVs using the SILVA 138.2 database (Quast et al. 2013) and the MBDP pathogen database (Yang et al. 2023). We compiled the ASVs, metadata, taxonomic information, and phylogenetic tree into a single object for subsequent analysis in R v. 4.5.0. We estimated accumulation curves to determine and evaluate the abundance and ASV distributions by sample type, using the *specaccum* function in the vegan package of R.

The R package Phyloseq v1.22.3 (McMurdie and Holmes 2013) was used for filtering the data as follows: (1) We removed unanalysed sequences (positive control); (2) We discarded ASVs that were "Unassigned" as well as chloroplasts and mitochondria; (3) We removed low-prevalence ASVs that did not appear more than five times in more than 10% of the samples; and (4) We removed taxa with zero sequences and negative control taxa from the complete data subset. Ultimately, we eliminated contaminants identified in the negative controls [luminal fluid (PBS controls) and extraction blank] that may have resulted from cross-contamination (0.5% of ASVs) using the *decontam* R package function (Davis et al. 2018) and according to Jorge et al. (2020).

2.5 | Microbiome Statistical Analysis

The dominant bacterial and archaeal taxa were visualised at the phylum and genus levels by helminth species in each bat host separately. The different dominant groups of bacteria and

archaea among the host tissue types (host lumen fluid, gallbladder, midgut and hindgut) were visualised separately.

We created family-level heat maps using *tax_glom*; that were transformed to relative abundance per sample and, for contrast, to $\log_{10}$ with a pseudo-count of 10^{-6}. For visualisation, we curated the 40 families with the highest global abundance using the function *pheatmap* in R. Shannon, Number of Observed Features, Simpson, and Faith's phylogenetic diversity indices were used as metrics to calculate alpha diversity for helminth species and samples from the same microhabitat at the ASV level.

Dissimilarity (beta diversity) between microbiome assemblages based on host microhabitat was calculated using the Bray–Curtis dissimilarity index, weighted and unweighted UniFrac at ASV level; multivariate analysis was visualised using non-metric multidimensional scaling (NMDS). We created Venn diagrams to visualise the number of ASVs shared between samples (bat tissues and helminths) per microhabitat (gallbladder, midgut, and hindgut) and between helminth species. The above analyses were performed with the microeco v0.15.0 package (Liu et al. 2021).

To test for multivariate effects, the PERMANOVA test was carried out using the adonis function with the vegan library version 2.7–1 (Oksanen et al. 2025). PERMANOVA assesses whether the Bray-Curtis (BC), weighted Unifrac (wUF) and unweighted Unifrac (uwUF) dissimilarity distance differs between the groups analysed; 9999 permutations were performed to ensure a robust statistical evaluation. Beta dispersion was calculated using the *betadisper* function in the vegan package.

The DESeq2 package version 1.44.0 (Love et al. 2014) was used to identify bacterial taxa with differential abundance between each parasite species and its surrounding bat tissue, using the Wald statistical test. An NCBI BLAST search confirmed the identity of all ASVs that were significantly differentially expressed between parasites and host tissue.

2.6 | Phylosymbiosis Analyses

To test the effect of phylogenetic distance between trematode species on their microbiome similarity, pairwise phylogenetic distances were calculated using the *cophenetic.phylo* function of the ape v5.0 package (Paradis and Schliep 2019), based on the partial 28S RNA gene sequences for four parasite species: *Sagittatrema zutzi* (Ruiz-Torres et al. 2024), and those we generated for *Limatulum* sp., *Castroia amplicava* and *Castroia* sp. Sequences were aligned using MAFFT v7.505 with default settings (Katoh et al. 2002) and used to create an unrooted neighbour-joining tree using the *nj* function of the ape package. Pairwise dissimilarities in the microbiota composition of different trematode species were calculated using the same metrics as for beta diversity analyses (Bray-Curtis, weighted and unweighted Unifrac). The Mantel test (Spearman rank correlations with 9999 permutations), a correlation test between phylogenetic distance and microbiota dissimilarity, was calculated in R with the vegan package (Oksanen et al. 2025).

3 | Results

3.1 | Composition and Population Variation in Helminths

Six *Peropteryx kaplerix* bats (3 females and 3 males) were examined, all parasitised by one or more helminth species. The 155 individual parasites collected belonged to five taxa of helminths, including the four trematodes *Sagittatrema zutzi, Limatulum* sp., *Castroia amplicava*, and *Castroia* sp., along with one nematode taxon, *Allintoshius* sp. (Figure 1B, Figures S1 and S2). Trematodes were most abundant, with a total of 133 individuals, representing 85.5% of the total. Trematodes infected five of the six hosts, while nematodes were found in all hosts examined (Table 1).

3.2 | Microbial Composition

Microbiome sequencing data from host tissues, host luminal fluid, and helminths returned a total of 3,766,419 sequences after quality filtering and chimera removal, with a total of 15,234 ASVs (196 min–1496 max ASVs per sample). Of these, 14,821 ASVs were bacterial, and 413 ASVs were archaeal. The final dataset consisted of a total of 73 samples, comprising 11 bat tissue samples, 5 faecal samples, 16 bat lumen samples, and 41 helminth samples corresponding to *Sagittatrema zutzi* ($n = 9$), *Limatulum* sp. ($n = 11$), *Castroia amplicava* ($n = 4$), *Castroia* sp. ($n = 7$), and *Allintoshius* sp. ($n = 10$).

Relative abundance graphs showed that the phylum Pseudomonadota was the most abundant bacterial phylum in all helminth samples, with a grouped relative abundance (RA) of 39%. It was followed by Bacillota with 28% and Bacteroidota with 8.92%. At the family level, Enterobacteriaceae had the highest RA with 10.35%, followed by Pseudomonadaceae (8.86%) and Streptococcaceae (8.86%) (Figure 1C,D). Heatmaps at the family level with hierarchical clustering showed that 0.5%–2.4% of the most abundant bacterial groups contributing to the microbiome composition are *Incertae_sedis* (taxonomic placement unresolved) in all helminth samples (Figure 2).

3.3 | Differences in Microbiome Diversity Among Helminths and Microhabitats

The alpha diversity indices (Shannon, Observed Features and Simpson) showed differences in microbial communities among the helminth species. In all cases, *Castroia amplicava* presented the highest microbial diversity. No significant association was observed between alpha diversity and microhabitat (i.e., bat tissue, Figure 3A). Finally, Faith's phylogenetic diversity (PD) did not differ significantly among the helminth species (Kruskal-Wallis test, $\chi^2 = 3.29$, $df = 4$, $p = 0.51$), suggesting similar levels of phylogenetic microbial diversity across helminth species.

The ASV-level NMDS plot showed that microbial communities of helminths inhabiting the gallbladder (Figure 3B.1) and midgut (Figure 3B.2) partially overlap with the microbiota of the respective bat organ tissue. In the hindgut, however, the nematode

TABLE 1 | Infection parameters of helminths parasitising *Peropteryx kappleri* in Boca de Chajul, Chiapas, Mexico.

Host		Helminth species					
		Microhabitat of the parasitised host					
		Gallbladder	Midgut			Hindgut	Total no. of helminths per host
# Host ID	Sex	*Sagittatrema zutzi*	*Castroia amplicava*	*Castroia* sp.	*Limatulum* sp.	*Allintoshius* sp.	
1	F	0	0	0	0	2	2
2	M	8	0	6	2	3	19
3	F	12	2	3	13	3	33
4	M	0	3	0	0	5	8
5	M	1	10	16	23	8	58
6	F	6	3	13	12	1	35
Individuals per species of helminths		27	18	38	50	22	155
%		66.6	66.6	66.6	66.6	100	
MA		4.5	3	6.33	8.33	3.5	
IR		1–12	2–10	2–16	2–23	1–8	

Abbreviations: %, Prevalence; F, Female; IR, Intensity range; M, Male; MA, Mean abundance.

Allintoshius sp. exhibits a different microbiota than the corresponding bat host tissue (Figure 3B.3) and other trematode species (Figure 3B.4). The results of the PERMANOVA analysis (Table 2) support these findings, indicating that comparisons of the midgut [*p*-value = BC (0.014) and wUF (0.032)] and hindgut [*p*-value = BC (0.01) and wUF (0.005)] were statistically significant. The differences in microbiota between helminth species were also statistically significant [*p*-value = BC (0.035) and wUF (0.023)]. The paired PERMANOVA analysis showed a significant difference only between the nematode *Allintoshius* sp. and the trematode *Limatulum* sp. (*p*-values of BC = 0.02) (Table 3).

Venn diagrams revealed many unique ASVs among helminths that are not shared with bat tissues (Figure 3C.1-C.3), suggesting that the bat's internal microenvironment does not entirely determine the microbiome composition of these parasitic helminth species. Similarly, when comparing all parasite species, only 71 ASVs were shared (1.9%–5.6% of the total), and notably, *Limatulum* sp. was the species that contained the most unique ASVs (Figure 3C.4).

3.4 | Host and Helminth Microbiomes Are Enriched With Different Bacterial Groups

Differential abundance analysis (DESeq2) was performed to determine whether helminth species associated with the bat *Peropteryx kappleri* exhibited lower or higher abundance of specific microorganisms relative to the host tissue they inhabited. In every helminth species analysed, associated microorganisms showed positive and high $\log_2$FoldChange (LFC) values when compared to the bat tissue they parasitised. This pattern shows a consistent difference between microbial taxa associated with helminths and those found in the host microhabitat.

Sagittatrema zutzi worms inhabiting the gallbladder presented 36 ASVs (padj < 0.01) with differential abundance compared to the tissue; the genera with the highest LFC values (> 22.46) were *Pseudomonas*, *Corynebacterium*, and *Stenotrophomonas* (Figure 4A). *Limatulum* sp., *Castroia amplicava*, and *Castroia* sp. showed significantly different abundance from midgut tissue in 7, 10, and 12 ASVs (padj < 0.01), respectively. For *Limatulum* sp., the genera *Brucella*, *Streptococcus*, *Stenotrophomonas*, *Pseudomonas*, *Corynebacterium*, and *Haemophilus* showed LFC > 21.12 (Figure 4B); for *Castroia amplicava*, the genera CAG-352, *Xylanibacter*, *Streptococcus*, UCG-005, *Xylanibacter*, *Brucella*, *Pseudomonas*, *Stenotrophomonas*, and *Haemophilus* had LFC values > 22.55 (Figure 4C). In *Castroia* sp., the following genera *Streptococcus*, *Stenotrophomonas*, *Brucella*, CAG-352, *Pseudomonas*, *Corynebacterium*, *Haemophilus*, and *Xylanibacter* had LFC > 21.10 (Figure 4D).

The nematode *Allintoshius* sp. presented the lowest number of ASVs in differential abundance when compared to parasitised tissue samples, with only 4 ASVs detected as significant (p.adj < 0.01). However, these ASVs showed LFC e values ranging from 8.55 to 21.95, indicating a marked over-representation in the helminth microbiome. Two of these ASVs were assigned to the taxonomic order level (Actinomarinales and Bacteroidales), while the remaining ones could be determined to the genus level, *Treponema* (Spirochaetales) and *Scytonema* (Cyanobacteriales) (Figure 4E).

3.5 | Lack of Detectable Phylosymbiosis Signal

In this study model, we found no evidence of phylosymbiosis, suggesting that helminth species that are phylogenetically closely related do not share more similar microbiomes than

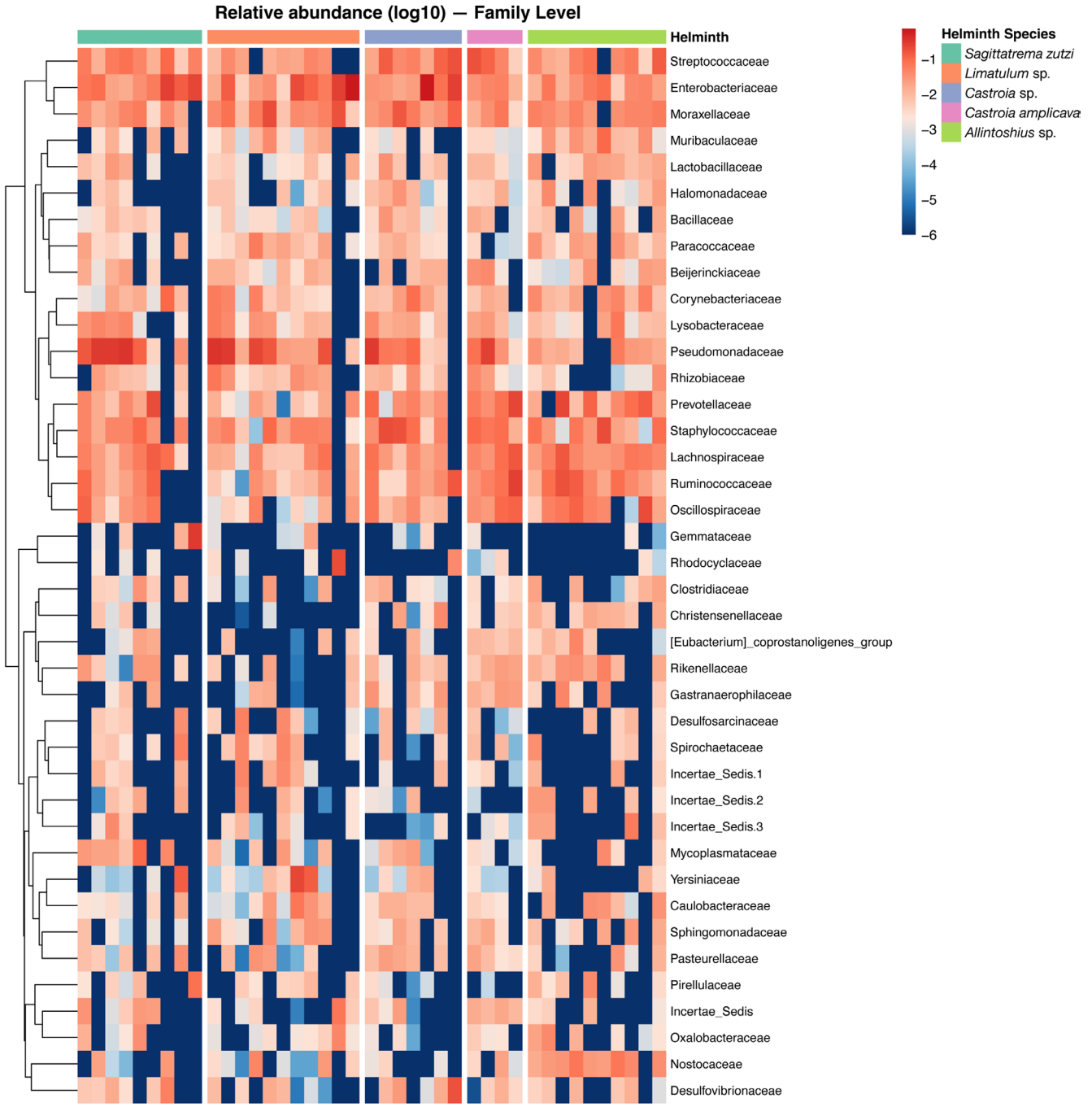

FIGURE 2 | Heat map of relative abundances ($\log_{10}$ transformed) of bacterial taxa associated with each helminth species. Rows: Bacterial taxa (e.g., families); columns: Grouped samples by helminth species and host tissue (microhabitat). Colour intensity represents relative abundance ($\log_{10}$ transformed); rows are ordered by increasing similarity and columns are ordered by helminth species.

those that are more distantly related (Figure 5A). The Mantel test showed a low positive correlation ($r = 0.072$, $p = 0.153$), while the Spearman correlation was similar ($\rho = 0.072$, $p = 0.132$). This lack of correlation between genetic-microbiota distances is also visually reflected in Figure 5A. Additionally, we assessed interspecies microbiome variability using the *beta_disper* method. Each helminth species centroid position showed marked differences (Figure 5B). Some helminth species displayed highly variable microbiomes among individuals, while others were more homogeneous, ranging from 0.499 to 0.727.

4 | Discussion

Despite growing interest in investigating the composition and structure of microbial communities associated with parasitic metazoans, especially helminths, few studies have investigated the factors driving compositional differences, i.e., the determinants of microbial community assembly across different parasitic species, particularly across biological scales (Hasegawa et al. 2025; Koellsch et al. 2024; Salloum, Jorge, and Poulin 2023). In this study, we characterised the microbiome associated with different helminth species which share the same

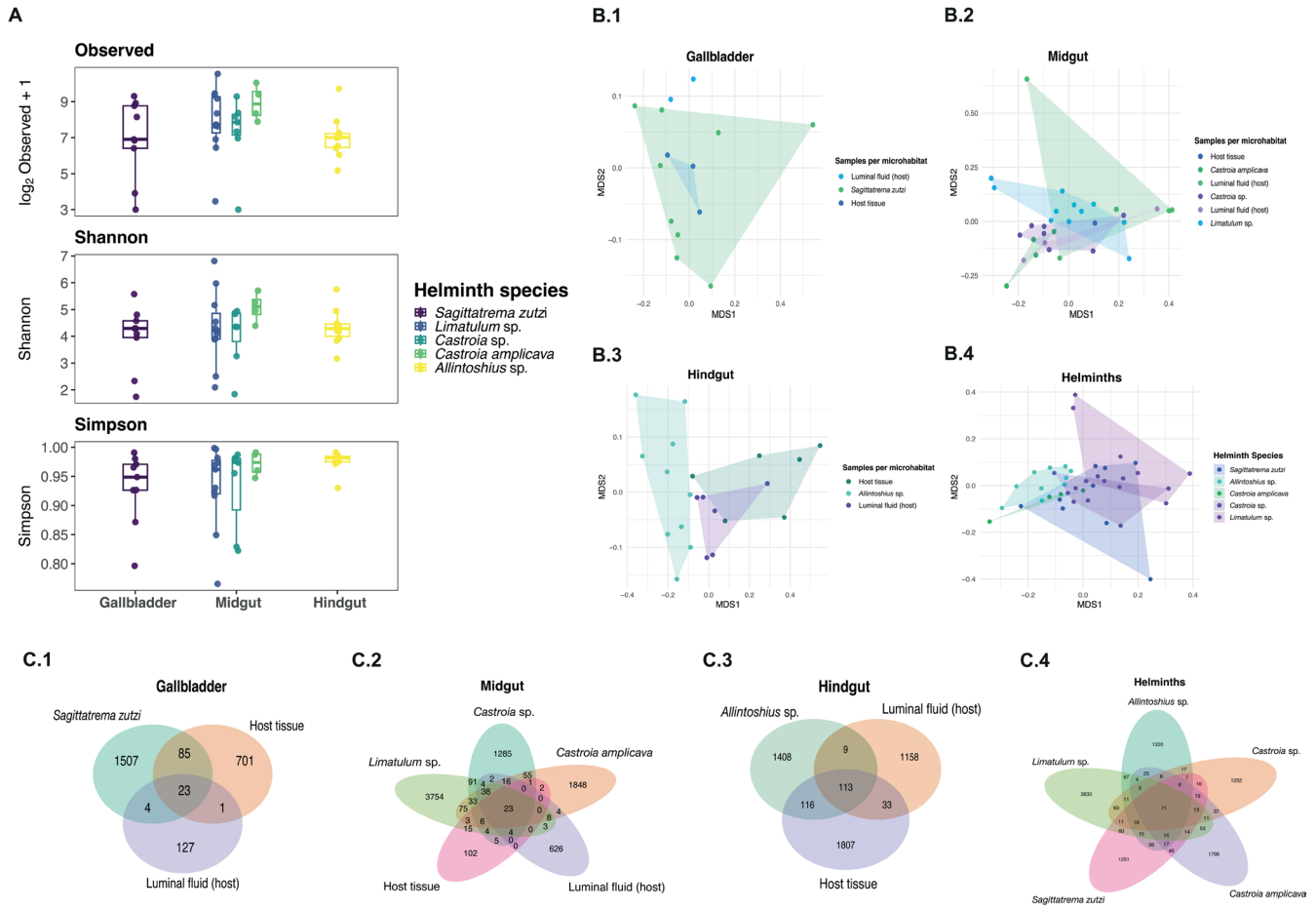

FIGURE 3 | (A) Alpha diversity, measured as the Observed richness, Shannon and Simpson index, for each helminth species. Each point represents an individual helminth, grouped by helminth species (x-axis), with different colours corresponding to different microhabitats within the host. (B) NMDS plots of microbial diversity based on Weighted UniFrac dissimilarity: (B.1) distance between samples from the gallbladder microhabitat; (B.2) distance between samples from the midgut microhabitat; and (B.3) distance between samples from the hindgut microhabitat and (B.4) among helminth species. The colour scale represents the sample type per microhabitat; these metrics were calculated at the ASV level. (C) Venn diagrams showing the number of unique and shared ASVs of the total microbiota per microhabitat: (C.1) Gallbladder, (C.2) Midgut and (C.3) Hindgut and among the five-helminth species (C.4) *Sagittatrema zutzi*, *Limatulum* sp., *Castroia amplicava*, *Castroia* sp. *Allintoshus* sp.

TABLE 2 | Statistical comparison using PERMANOVA (Adonis) of microbiome variability between samples by microhabitat type and between helminth species, using the 16s rRNA gene.

Variable	Metrics	Df	SumOfSqs	R^2	F	p (Pr(>F))
Gallbladder	Bray-Curtis	2	0.915	0.154	1.005	0.403
	Weighted UniFrac	2	0.08	0.172	1.149	0.261
	Unweighted UniFrac	2	0.809	0.156	1.019	0.382
Midgut	Bray-Curtis	5	2.657	0.196	1.222	*0.014
	Weighted UniFrac	5	0.01	0.091	0.505	0.988
	Unweighted UniFrac	5	2.435	0.174	1.054	*0.032
Hindgut	Bray-Curtis	2	1.279	0.135	1.487	*0.001
	Weighted UniFrac	2	0.082	0.194	2.3	*0.01
	Unweighted UniFrac	2	0.94	0.119	1.283	*0.005
Helminth species	Bray-Curtis	4	2.007	0.111	1.125	*0.035
	Weighted UniFrac	4	0.013	0.084	0.831	0.8
	Unweighted UniFrac	4	1.979	0.106	1.076	*0.023

*Statistically significant results.

TABLE 3 | Pairwise statistical comparison of microbiome composition of *Peropteryx kappleri* associated helminth species using 16 s rRNA gene sequencing results.

		Bray Curtis			UniFrac			WuniFrac		
Group1	**Group2**	R^2	*p*	p_adjusted	R^2	*p*	p_adjusted	R^2	*p*	p_adjusted
Allintoshius sp.	*Castroia* sp.	0.069	0.115	0.286	0.061	0.446	0.974	0.067	0.073	0.146
Allintoshius sp.	*Castroia amplicava*	0.088	0.045	0.225	0.054	0.903	0.974	0.086	0.015	0.1333
Allintoshius sp.	*Sagittatrema zutzi*	0.063	0.124	0.286	0.062	0.138	0.974	0.066	0.027	0.133
Allintoshius sp.	*Limatulum* sp.	0.073	0.002	*0.02	0.049	0.395	0.974	0.054	0.065	0.146
Castroia sp.	*Castroia amplicava*	0.092	0.722	0.722	0.081	0.66	0.974	0.104	0.17	0.283
Castroia sp.	*Sagittatrema zutzi*	0.064	0.455	0.568	0.056	0.784	0.974	0.071	0.326	0.362
Castroia sp.	*Limatulum* sp.	0.056	0.568	0.631	0.029	0.918	0.974	0.056	0.755	0.755
Castroia amplicava	*Sagittatrema zutzi*	0.081	0.431	0.568	0.042	0.974	0.974	0.088	0.319	0.362
Castroia amplicava	*Limatulum* sp.	0.079	0.193	0.321	0.027	0.957	0.974	0.079	0.04	0.133
Sagittatrema zutzi	*Limatulum* sp.	0.060	0.143	0.286	0.064	0.312	0.974	0.056	0.269	0.362

*Statistically significant results.

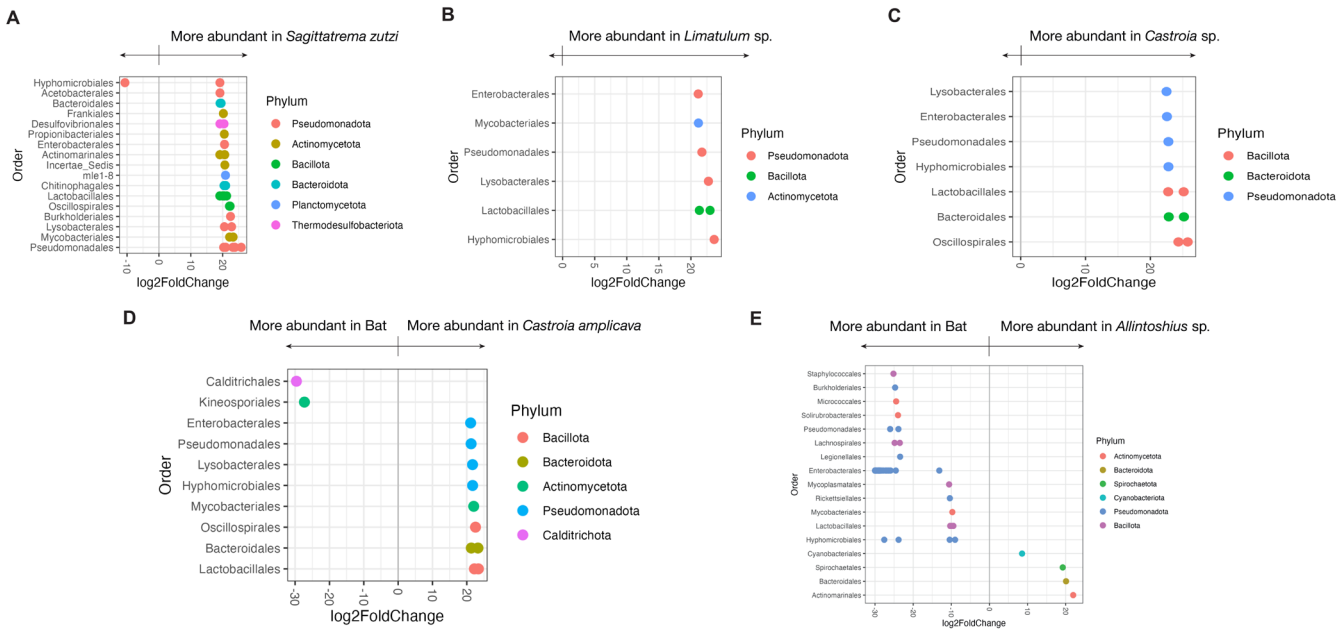

FIGURE 4 | ASVs (including all samples) showing significant differences in abundance (between bat host tissue and helminth species): (A) Gallbladder vs. *Sagittatrema zutzi*, (B) Midgut vs. *Limatulum* sp., (C) Midgut vs. *Castroia ampliclava*, (D) Midgut vs. *Castroia* sp., and (E) Hindgut vs. *Allintoshus* sp. The orders (vertical axis) and corresponding phyla (colours) are shown. Positive values indicate higher ASV abundance in helminth samples, while negative values indicate lower ASV abundance in bat host tissue samples. Only statistically significant values (padj < 0.05) are included.

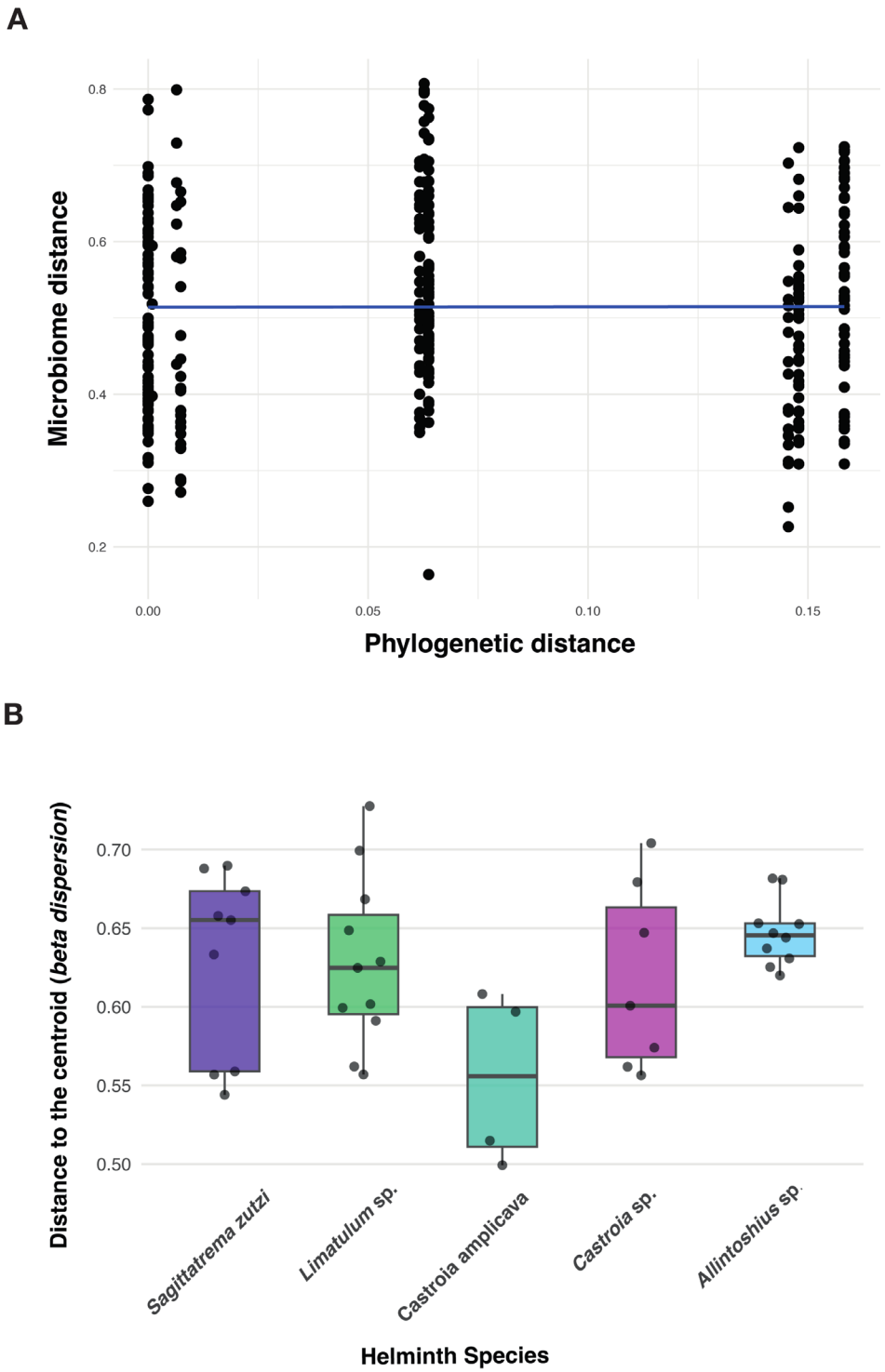

FIGURE 5 | (A) The figure shows Bray-Curtis results. Correlation between helminths' genetic distance (based on 28S) and microbiome dissimilarity (Bray-Curtis) for trematodes only. (B) Beta dispersal of microbiome composition for each helminth species, measured as the distance of the microbiota in each helminth individual to the centroid for that species (*betadisper*). Figure S1. Phylogenetic hypothesis of the superfamily Microphalloidea. Tree inferred using Bayesian consensus inference (BI), based on 28S rRNA gene sequences. Numbers next to internal nodes show the percentage values of Bayesian posterior probabilities (BPP). Highlighted clades indicate the position of the species studied in this work. Scale bars represent the branch length Figure S2. Bayesian inference phylogeny based on mitochondrial DNA sequences (COI). Numbers on branches indicate posterior (Bayesian) probability values for the main nodes. Tips are labelled with species names, followed by their corresponding GenBank accession numbers, as applicable. The clade highlighted in blue indicates the position of the nematode *Allintoshius* sp. (Heligmonellidae) studied in this work. Scale bars represent the branch length.

bat hosts. Furthermore, we compared helminth microbiomes with that of their immediate environment, the host microhabitat (tissue and luminal fluid) of the bat *Peropteryx kappleri*. Results indicate that the microbiome associated with helminth species exhibits a distinct composition compared to the host bat microbiome. Each helminth species exhibited a strong affinity for specific microbial groups, indicating a microbial signature, even when they coexist in the same microhabitat and belong to the same bat-specific trematode taxonomic lineage (Lecithodendridae: Microphalloidea). We also observed notable differences in microbial community composition between the two main helminth taxonomic groups studied: trematodes (phylum Platyhelminthes) and nematodes (phylum Nematoda), which may be derived from the anatomical, dietary, physiological, and life cycle differences between these parasitic taxa.

4.1 | Microbial Composition: Nesting in Trematodes (Moderate Environmental Filtering) Versus Dissimilarity in Nematodes

Given the careful protocol applied to avoid external contamination of the parasites with host tissue (washing the parasite and filtering out low-abundance and low-prevalence ASVs), it was expected that the majority of the parasite microbiota characterised was internal to the parasite specimens. Therefore, the partial overlap between trematode-associated and host-associated microbial communities observed in the NMDS and PERMANOVA analyses suggests that the host may act as a microbial source from which the helminths acquire part of their microbiota. This pattern indicates that the assembly of the trematode-associated microbiome could result from the interaction between deterministic and stochastic processes. The latter could favour the exchange of nonspecific, low-abundance microbes with the bat tissues and luminal fluid, suggesting the presence of a possible transient microbiome acquired through horizontal transfer from the host microbiome. Similar findings have been documented in adult flatworms, in which microbial communities nested within the host microbiome have been identified, thus suggesting a hierarchical system of microbial colonisation (Brealey et al. 2022; Jorge et al. 2020; Kashinskaya et al. 2020). The functional role of this transient microbiome in helminth biology is still unknown (Dheilly et al. 2019, 2017; Salloum, Jorge, Dheilly, and Poulin 2023; Formenti et al. 2020; Hodžić et al. 2023; Rinaldi et al. 2024).

The observed partial overlap is also consistent with environmental filtering processes; a deterministic community assembly mechanism whereby intestinal environmental conditions restrict which microbial taxa can colonise the parasites' microhabitats (Jovel et al. 2016; Zhou and Ning 2017; Kohl 2020; Nemergut et al. 2013). In this context, the "helminth holobiont" perspective suggests that helminths, their microbiota, and the host constitute a nested unit in which microorganisms can maintain or favour the parasites' ecology (Poulin et al. 2023; Hodžić et al. 2023; Rinaldi et al. 2024).

These patterns must be interpreted with caution due to the limited sample size, as parasite samples were collected from six bats, and multiple helminth individuals were derived from the same host. Consequently, the individual identity of the host was not explicitly modelled, and potential non-independence among parasites from the same bat could not be addressed. Some of the observed variation within and between species may indicate host-specific effects, rather than solely reflecting stochastic assembly processes (Jovel et al. 2016; Zhou and Ning 2017; Kohl 2020; Nemergut et al. 2013). The current sample design makes it difficult to distinguish host individual effects from ecological drift, priority effects, or random colonisation of the host microbial reservoir.

The nematode *Allintoshius* sp. is an exception, exhibiting compositional differences relative to its immediate environment (host tissue and luminal fluid) in the bat hindgut, quantified by beta diversity. These results are similar to those observed in previous studies on nematode microbiomes (Bhat et al. 2025; Midha et al. 2022; San Juan et al. 2025; Wang et al. 2025). However, in terms of alpha diversity, the nematodes (*Allintoshius* sp.) presented higher values compared to their bat host with an insect-dominated diet, in contrast to other studies on nematodes that parasitise hosts with a plant diet (Bhat et al. 2025; Midha et al. 2022; San Juan et al. 2025; Wang et al. 2025; Cain et al. 2022, 2023; Castañeda et al. 2025; Muslim et al. 2024; Paz et al. 2024). The diet of bats may therefore affect the microbiome of their worms. Insectivorous bats have been reported to have higher microbial diversity and function compared to frugivores and nectarivores (Ingala et al. 2021; Carrillo-Araujo et al. 2015). Their high insect consumption, between 61% and 80% of their body mass (Bateman and Vaughan 1974; Kalka and Kalko 2006) due to the high metabolic demand of flight, promotes an environment rich in proteins, fats, and nutrients ideal for bacterial growth, thus contributing to the high microbial diversity observed (Kalka and Kalko 2006; Morni et al. 2025) and consequently partially reflected in the nematodes investigated here. Additionally, species in the nematode family Heligmosomidae, to which the genus *Allintoshius* sp. belongs, have been reported to have mixed feeding habits: hematophagous and/or mucophagous (blood and intestinal secretions), which would contribute to greater microbial diversity. Parasites with specific diets tend to harbour microbiomes with lower diversity, as is the case with hematophagous animals (e.g., leeches, isopods, and copepods), versus those with trophic plasticity (Goffredi et al. 2023; Marden et al. 2016; McClure et al. 2021).

4.2 | Absence of Phylosymbiotic Signal in Trematodes

Recently, the term phylosymbiosis has been proposed to describe the eco-evolutionary relationship linking microbial communities with the evolutionary history of their hosts. This phenomenon has been suggested as the driver of microbial structure among various host clades within the evolutionary tree of metazoans, particularly mammals and arthropods (Brooks et al. 2016; Lim and Bordenstein 2020). Essentially, phylosymbiosis is understood as the congruence between the phylogeny of the hosts and the structure of their associated microbial communities; that is, it emerges as a correlation between the phylogenetic distances among host taxa and the dissimilarity in the composition of their associated communities of microorganisms (Brooks et al. 2016; Lim and Bordenstein 2020; Mazel et al. 2018).

In our data focusing on a monophyletic group of bat-restricted trematodes of the superfamily Microphalloidea (Bray et al. 2008; Ruiz-Torres et al. 2024), phylosymbiotic analysis indicated a lack of phylogenetic signal in microbiome composition. This lack of phylogenetic signal may be due to the interaction of multiple processes underlying the assembly of microbial communities (Zhou and Ning 2017; Kohl 2020; Nemergut et al. 2013). In particular, microbial dispersal (horizontal transmission) between coexisting trematode species might lead to a convergence of their microbiomes, regardless of their taxonomic identity (Hasegawa et al. 2025; Chai et al. 2025). This result is supported by the partial overlap of microbiomes among helminth species in the NMDS and PERMANOVA analyses, especially among species that cohabit in the midgut, including *Limatulum* sp., *Castroia amplicava*, and *Castroia* sp. In this context, physical proximity and high microbial connectivity between species could favour the homogenisation of their microbiomes (Sprockett et al. 2018; Maritan et al. 2024).

In addition to these factors, the intraspecific microbiome heterogeneity observed in each of the investigated species, supported by *betadisper* analysis, could be masking any phylogenetic signal, thus contributing to the absence of a phylosymbiotic pattern. This high variability suggests a significant role for stochastic processes in microbiome assembly, particularly for low-abundance microorganisms. In this regard, ecological drift, along with colonisation priority effects and random colonisation from a shared microbial reservoir in the host, can generate substantial differences between individuals of the same species and between species (Zhou and Ning 2017; Kohl 2020). Intraspecific variability in microbiome composition within helminth species has been previously documented (Dheilly et al. 2015; Jorge et al. 2022; Chai et al. 2025), although the factors that determine it remain unclear. One potentially determining factor is the immediate environment of the endoparasites, especially when the host exhibits high variability in its own microbiome, as occurs in bats (Ingala et al. 2021; Perofsky et al. 2017; Tung et al. 2015). Due to their high metabolic rate and fast intestinal transit associated with flight, bats show rapid microbial turnover (Phillips et al. 2012; Song et al. 2020; Brun et al. 2019), which could directly influence the variability of the parasites' microbiome.

Moreover, in parasites with complex life cycles, such as trematodes, transitions between intermediate hosts and free-living stages can involve losses and gains of microorganisms, contributing to the recurrent reconfiguration of their microbiome (Jorge et al. 2020; Hahn et al. 2022). Furthermore, the absence of an adaptive immune system in trematodes (Loker et al. 2004; Schulenburg et al. 2007) suggests that the host's capacity for targeted selection of its microbiome is limited, which could favour more open and dynamic communities. In contrast, in vertebrates, adaptive immunity allows for the specific recognition and regulation of a more stable microbiome, contributing to more consistent patterns over evolutionary time (Figueiredo and Kramer 2020; Lee and Mazmanian 2010; Mallott and Amato 2021; Müller et al. 2018). Finally, the lack of microbiome characterisation in specific body regions in our trematodes, resulting from the complexity of small organisms and the consequent analysis of the total microbiome, may dilute phylosymbiotic signals. This absence of phylogenetic signals is consistent with other studies in small-bodied invertebrates (Colston and Jackson 2016). Research in free-living macroscopic metazoans has focused on highly differentiated tissue samples (e.g., the digestive system), which harbour more stable microbiomes over evolutionary time (Groussin et al. 2017; Sharpton 2018; Zilber-Rosenberg and Rosenberg 2021). Therefore, further studies of the helminth associated microbiome focusing on specific organs and microbial symbiotic associations (e.g., the core microbiome) throughout the larval stages, and comparisons with the microbiomes of intermediate and definitive hosts, may provide a better understanding of the eco-evolutionary dynamics of phylosymbiosis in these systems.

4.3 | Microbial Signature by Helminth Species

It is now recognised that helminths possess a phylogenetically diverse and species-specific microbiome, distinct from that of their host or the external environment (Salloum, Jorge, and Poulin 2023). This supports the idea that animals, including helminth parasites, form integrated entities or holobionts (Moran and Sloan 2015; Dheilly 2014; Hodžić et al. 2023; Bottone and Zhang 1995). Results presented here are consistent with this idea; for each helminth species, we identified significantly enriched bacterial and archaeal ASVs (pad $< 10^{-6}$) with minimal overlap (as shown in the Venn diagram) when compared to bat tissue and luminal fluid, as well as among the studied helminth species. This suggests that the parasite does not merely passively sample the host's microhabitat; its microbiome consists of both a core microbiome and a component acquired horizontally from the host, supporting the existence of a microbiome fingerprint for each helminth species. We observed a pattern of coexistence between bacterial families and genera, despite differences in their microhabitats. Recurrent taxonomic groups include Pseudomonadota (e.g., *Pseudomonas*, *Stenotrophomonas*), Pasteurellaceae (*Haemophilus*), Corynebacteriaceae, and common intestinal families such as Oscillospirales/Ruminococcaceae, Prevotellaceae, and Streptococcaceae.

Given that the four trematode species belong to a bat-associated lineage and occupy the digestive tract, we propose that these results reflect two mechanisms: (1) ecological, involving the construction of microniches by manipulation of the host microbiome (Poulin et al. 2023), and (2) the local physicochemical conditions imposed by bile and oxygen availability (Flynn et al. 2019; Gipson et al. 2020; Dohet et al. 2016).

However, this should not be interpreted as evidence of phylosymbiosis, since we found no association between phylogenetic distance and microbiome dissimilarity. Instead, common ancestry may shape traits relevant to microbiome assembly without producing a detectable phylogenetic signal in microbiome composition. To further support these ideas, validation of these mechanisms will require metagenomic and metatranscriptomic studies, cophylogenetic studies, and ontogenetic comparisons with studies of the helminths' microbiomes in their intermediate hosts.

Building on this, the life cycle of Microphalloidea (Bray et al. 2008) is known to include insects as intermediate hosts; thus, it is plausible that some of the microbial taxa observed in trematodes reflect the persistence or retention of bacterial

lineages from earlier life stages. For example, *Pantoea* (recorded in *Sagittarema zutzi* in our study) is a recurrent genus that forms part of the bacterial core of several bark beetles (*Dendroctonus*) (Hernández-García et al. 2017; Briones-Roblero et al. 2017), with a high capacity for persistence between generations (Gómez-Govea et al. 2024). Similarly, in trematodes, the transmission of bacterial endosymbionts through the different phases of their ontogenetic cycle has been demonstrated, indicating that this retention is biologically possible in these parasites (Jorge et al. 2022, 2020; Chai et al. 2025). In addition to *Pantoea*, several groups of Proteobacteria (*Pseudomonas*, *Stenotrophomonas*, *Acinetobacter*, and *Rhodococcus*), as well as *Methylobacterium*/*Massilia* (Sieng et al. 2023), have been described in the insect microbiome; this further corroborates the idea of retention from intermediate hosts.

The nematode *Allinthoshius* sp. exhibits only three enriched ASVs: *Enterococcus*, *Treponema*, and *Scytonema*. *Enterococcus* (Enterococcaceae) is the most prevalent Bacillus group in the nematode microbiome, suggesting that its presence could be a key factor in these worms (Nemergut et al. 2013; Bhat et al. 2025; Wang et al. 2025). It has been documented that infection of nematodes with a high abundance of *Enterococcus* (Bacillota) decreases butyrate-producing bacteria (e.g., Pseudomonadota) in the digestive tract of their vertebrate host; this effect is associated with greater infection success and increased long-term survival (Ghareeb et al. 2022). In the case of cyanobacteria, they have only been recorded in free-living nematodes from the seabed (McQueen et al. 2023) and Antarctic streams (Li et al. 2018), with their function being unknown. However, in tests in which the nematode *Caenorhabditis elegans* were exposed to cyanobacteria of the genus *Nostoc* (Nostocaceae), the high production of polysaccharides resulted in a higher survival rate of the nematodes, due to the bacteria's effects on stress, longevity, and age-related symptoms (Zhong et al. 2021; Noda et al. 2018).

Finally, this study reports for the first time the genus *Treponema* in nematodes, whether free-living or parasitic in vertebrates. This genus has been identified primarily in the gut of termites (Noda et al. 2018), but also in pigs (Pringle et al. 2009). Some species are implicated in infectious diseases such as syphilis, gingivitis, and periodontitis in humans (LaFond and Lukehart 2006; Zeng et al. 2021). *Treponema* exhibits virulence characteristics, such as high mortality and the ability to adhere to and invade the host; however, given the characteristics of the genomic region studied (16S rRNA), its biological function in the system is unknown. In addition to *Treponema*, taxa previously reported as potentially zoonotic were detected. Their presence in helminths suggests a possible use of helminths as vectors or a symbiotic relationship, though this study did not aim to evaluate such links. Consequently, metagenomic studies are necessary to clarify their ecological relevance in helminths.

5 | Conclusions

The microbiome associated with the five coexisting helminth species of the insectivorous bat *Peropteryx kappleri* exhibited differences from one another, despite the fact that the parasites inhabited the same host and, in certain instances, the same microhabitat. We demonstrate that microbial selection is not entirely random, but rather that multiple parallel pathways are involved in the formation of their microbiota. We suggest that a small portion of their microbiome is influenced by their immediate environment, the host, through environmental filters specific to each trematode species. In contrast, we found no evidence of phylosymbiosis among the examined trematode species, suggesting that phylogenetic relatedness did not serve as a predictor for microbiome similarity in this system. On the other hand, the microbiome of the nematode *Allintoshius* sp. shows independence from the host; this phenomenon could be due to morphological and physiological characteristics specific to this group.

The findings indicate that helminths possess unique microbial signatures, rather than merely mirroring the microbiota of the host tissue in which they reside. Simultaneously, our sampling design restricts our capacity to separate the individual effects of hosts from stochastic assembly processes, as multiple parasites were collected from only six bats and host identity was not explicitly modelled.

This study enhances the existing understanding of helminth-associated microbiomes in wildlife and proposes testable hypotheses regarding microbial acquisition, persistence, and function, ultimately advancing our understanding of host–parasite interactions. Future research that incorporates functional approaches and sampling across both intermediate and definitive hosts will be essential for assessing the ecological significance of these microbial associations.

Author Contributions

N.G.R.-T., O.R.-C., and L.I.F. conceived and designed the study; O.R.-C. and L.I.F. obtained funding. N.G.R.-T., S.M.-S., and O.R.-C. performed fieldwork and collected samples. N.G.R.-T., A.L.-L., O.G., and L.I.F. conducted molecular laboratory work. R.A.M.-J., P.M.S., R.P., O.R.-C. and L.I.F. advised analyses; N.G.R.-T. and S.M.-S. performed bioinformatic and statistical analyses. N.G.R.-T. wrote the manuscript; all authors contributed to revisions and approved the final version.

Acknowledgments

This article is part of the degree requirement of N.G.R.-T. in the Posgrado en Ciencias Biológicas, UNAM. N.G.R.-T. (583329) and S.M.-S. (697152) received graduate studies fellowships from SECIHTI, México. L.I.F. (UNAM, PAPIIT IB200421) and O.R. (UNAM, PAPIIT IN218020) received funding for this research. P.M.S. was supported by a Marsden Fund grant (Royal Society of New Zealand, contract UOO2113) awarded to R.P. We are deeply grateful to the bats and parasites that provided the samples used in this study. We thank the Lombera family and Karen Martínez for their field support. We also thank Fernanda Sánchez-Soto and Alfredo Yañéz-Montalvo for laboratory support. We thank Luis García-Prieto, David I. G. Hernández-Mena, and Sara A. Ramírez-Caña for their assistance with the taxonomic identification of the helminths. We are grateful to Diana García-Cortez and Bernardo Aguila Salgado for teaching N.G.R.-T. to use bioinformatics software during data analysis. We also thank the International Society for Microbial Ecology (Academic Mobility Fund) and the UNAM Graduate Studies Support Program (PAEP) for international research grants awarded to N.G.R.-T. We thank Octavio Zavala for creating the drawing of the bat. We are also very grateful to two anonymous reviewers for constructive comments on an earlier version of the manuscript.

Funding

This research was supported by Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (PAPIIT-DGAPA, UNAM; project Nos. BV200421 and IN218020) awarded to L.I.F. and O.R.-C. S.M.-S. and N.R.T. were supported by SECIHTI graduate scholarships.

Ethics Statement

Research followed the ethical standards of Universidad Nacional Autónoma de México and was conducted under collection permit DGVS/04214/19 issued by SEMARNAT, Mexico.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All 16S rRNA raw sequence data are publicly available in NCBI under BioProject accession number ID: PRJNA1381229. (https://dataview.ncbi.nlm.nih.gov/object/PRJNA1381229?reviewer=5eu7ok5c374a8qmv4k1o72r4fq).

References

Bateman, G. C., and T. A. Vaughan. 1974. "Nightly Activities of Mormoopid Bats." *Journal of Mammalogy* 55: 45–65. https://doi.org/10.2307/1379256.

Bhat, A. H., I. M. Malik, H. Tak, B. A. Ganai, and P. Bharti. 2025. "Host, Parasite, and Microbiome Interaction: *Trichuris ovis* and Its Effect on Sheep Gut Microbiota." *Veterinary Parasitology* 333: 110356.

Bik, H. M. 2019. "Microbial Metazoa Are Microbes Too." *mSystems* 4: e0010919. https://doi.org/10.1128/msystems.00109-19.

Bolyen, E., J. R. Rideout, M. R. Dillon, et al. 2019. "Reproducible, Interactive, Scalable and Extensible Microbiome Data Science Using QIIME 2." *Nature Biotechnology* 37: 852–857.

Bordenstein, S. R., and K. R. Theis. 2015. "Host Biology in Light of the Microbiome: Ten Principles of Holobionts and Hologenomes." *PLoS Biology* 13: e1002226. https://doi.org/10.1371/journal.pbio.1002226.

Bottone, E. J., and D. Y. Zhang. 1995. "*Haemophilus parainfluenzae* Biliary Tract Infection: Rationale for an Ascending Route of Infection From the Gastrointestinal Tract." *Journal of Clinical Microbiology* 33: 3042–3043. https://doi.org/10.1128/jcm.33.11.3042-3043.1995.

Bray, R. A., D. I. Gibson, and A. Jones. 2008. "Keys to the Trematoda, Volume 3."

Brealey, J. C., L. A. Lecaudey, M. Kodama, et al. 2022. "Microbiome "Inception": An Intestinal Cestode Shapes a Hierarchy of Microbial Communities Nested Within the Host." *MBio* 13: e0067922. https://doi.org/10.1128/mbio.00679-22.

Briones-Roblero, C. I., J. A. Hernández-García, R. Gonzalez-Escobedo, L. V. Soto-Robles, F. N. Rivera-Orduña, and G. Zúñiga. 2017. "Structure and Dynamics of the Gut Bacterial Microbiota of the Bark Beetle, *Dendroctonus rhizophagus* (Curculionidae: Scolytinae) Across Their Life Stages." *PLoS One* 12: e0175470. https://doi.org/10.1371/journal.pone.0175470.

Brooks, A. W., K. D. Kohl, R. M. Brucker, E. J. van Opstal, and S. R. Bordenstein. 2016. "Phylosymbiosis: Relationships and Functional Effects of Microbial Communities Across Host Evolutionary History." *PLoS Biology* 14: e2000225. https://doi.org/10.1371/journal.pbio.2000225.

Brun, A., G. Fernandez Marinone, E. R. Price, et al. 2019. "Morphological Bases for Intestinal Paracellular Absorption in Bats and Rodents." *Journal of Morphology* 280, no. 9: 1359–1369. https://doi.org/10.1002/jmor.2103.

Bush, A. O., K. D. Lafferty, J. M. Lotz, and A. W. Shostak. 1997. "Parasitology Meets Ecology on Its Own Terms: Margolis Et al. Revisited." *Journal of Parasitology* 83: 575–583.

Cain, J. L., J. K. Norris, N. E. Ripley, et al. 2022. "The Microbial Community Associated With *Parascaris* spp. Infecting Juvenile Horses." *Parasites & Vectors* 15: 408. https://doi.org/10.1186/s13071-022-05533-y.

Cain, J. L., J. K. Norris, M. P. Swan, and M. K. Nielsen. 2023. "A Diverse Microbial Community and Common Core Microbiota Associated With the Gonad of Female *Parascaris* Spp." *Parasitology Research* 123: 56. https://doi.org/10.1007/s00436-023-08086-w.

Callahan, B. J., P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson, and S. P. Holmes. 2016. "DADA2: High-Resolution Sample Inference From Illumina Amplicon Data." *Nature Methods* 13: 581–583.

Caporaso, J. G., G. Ackermann, A. Apprill, et al. 2018. "EMP 16S Illumina Amplicon Protocol v1.1." *protocols.io*. https://doi.org/10.17504/protocols.io.nuudeww.

Carrillo-Araujo, M., N. Taş, R. J. Alcántara-Hernández, et al. 2015. "Phyllostomid Bat Microbiome Composition Is Associated to Host Phylogeny and Feeding Strategies." *Frontiers in Microbiology* 6: 447. https://doi.org/10.3389/fmicb.2015.00447.

Castañeda, S., C. Poveda, C. Suarez-Reyes, et al. 2025. "Microbiota Dynamics During *Ascaris suum* Larval Migration: Implications for Host Microbial Communities in a Murine Model." *Microbial Pathogenesis* 198: 107122.

Chai, X., P. M. Salloum, and R. Poulin. 2025. "Does the Host Matter? Testing the Impact of Host Identity on the Microbiome of a Trematode Parasite." *Parasitology Research* 124, no. 3: 38. https://doi.org/10.1007/s00436-025-08486-0.

Chen, S., Y. Zhou, Y. Chen, and J. Gu. 2018. "Fastp: An Ultra-Fast All-In-One FASTQ Preprocessor." *Bioinformatics* 34: i884–i890. https://doi.org/10.1093/bioinformatics/bty560.

Colston, T. J., and C. R. Jackson. 2016. "Microbiome Evolution Along Divergent Branches of the Vertebrate Tree of Life: What Is Known and Unknown." *Molecular Ecology* 25: 3776–3800. https://doi.org/10.1111/mec.13730.

Davis, N. M., D. M. Proctor, S. P. Holmes, D. A. Relman, and B. J. Callahan. 2018. "Simple Statistical Identification and Removal of Contaminant Sequences in Marker-Gene and Metagenomics Data." *Microbiome* 6: 226. https://doi.org/10.1186/s40168-018-0605-2.

Dheilly, N. M. 2014. "Holobiont–Holobiont Interactions: Redefining Host–Parasite Interactions." *PLoS Pathogens* 10: e1004093. https://doi.org/10.1371/journal.ppat.1004093.

Dheilly, N. M., D. Bolnick, S. Bordenstein, et al. 2017. "Parasite Microbiome Project: Systematic Investigation of Microbiome Dynamics Within and Across Parasite–Host Interactions." *mSystems* 2: e0005017. https://doi.org/10.1128/msystems.00050-17.

Dheilly, N. M., J. Martínez Martínez, K. Rosario, et al. 2019. "Parasite Microbiome Project: Grand Challenges." *PLoS Pathogens* 15: e1008028. https://doi.org/10.1371/journal.ppat.1008028.

Dheilly, N. M., R. Poulin, and F. Thomas. 2015. "Biological Warfare: Microorganisms as Drivers of Host–Parasite Interactions." *Infection, Genetics and Evolution* 34: 251–259.

Dohet, L., J.-C. Grégoire, A. Berasategui, M. Kaltenpoth, and P. H. W. Biedermann. 2016. "Bacterial and Fungal Symbionts of Parasitic *Dendroctonus* Bark Beetles." *FEMS Microbiology Ecology* 92: fiw129. https://doi.org/10.1093/femsec/fiw129.

El Masry, I., S. von Dobschuetz, L. Plee, et al. 2020. "Exposure of Humans or Animals to SARS-CoV-2 From Wild, Livestock, Companion and Aquatic Animals: Qualitative Exposure Assessment." FAO, Rome. (FAO Animal Production and Health Paper 181).

Fenton, M. B. 1990. "The Foraging Behaviour and Ecology of Animal-Eating Bats." *Canadian Journal of Zoology* 68: 411–422. https://doi.org/10.1139/z90-061.

Fernandes, T. F., F. T. de Vasconcelos Melo, and J. N. dos Santos. 2021. "A New Species of *Allintoschius* (Nematoda: Heligmosomoidea) From Two Species of Bats in Brazil." *Journal of Parasitology* 107: 703–709.

Figueiredo, A. R. T., and J. Kramer. 2020. "Cooperation and Conflict Within the Microbiota and Their Effects on Animal Hosts." *Frontiers in Ecology and Evolution* 8: 132. https://doi.org/10.3389/fevo.2020.00132.

Flynn, S., F. J. Reen, and F. O'Gara. 2019. "Exposure to Bile Leads to the Emergence of Adaptive Signaling Variants in the Opportunistic Pathogen *Pseudomonas aeruginosa*." *Frontiers in Microbiology* 10: 2013. https://doi.org/10.3389/fmicb.2019.02013.

Formenti, F., A. Cortés, P. J. Brindley, C. Cantacessi, and G. Rinaldi. 2020. "A Bug's Life: Delving Into the Challenges of Helminth Microbiome Studies." *PLoS Neglected Tropical Diseases* 14: e0008446. https://doi.org/10.1371/journal.pntd.0008446.

Gaona, O., D. Cerqueda-García, A. Moya, X. Neri-Barrios, and L. I. Falcón. 2020. "Geographical Separation and Physiology Drive Differentiation of Microbial Communities of Two Discrete Populations of the Bat *Leptonycteris yerbabuenae*." *MicrobiologyOpen* 9: e1022. https://doi.org/10.1002/mbo3.1022.

Ghareeb, R. Y., N. R. Abdelsalam, D. M. El Maghraby, M. H. Ghozlan, E. EL-Argawy, and R. A. I. Abou-Shanab. 2022. "*Oscillatoria* sp. as a Potent Anti-Phytopathogenic Agent and Plant Immune Stimulator Against Root-Knot Nematode of Soybean cv. Giza 111." *Frontiers in Plant Science* 13: 870518. https://doi.org/10.3389/fpls.2022.870518.

Gilbert, S. F., J. Sapp, and A. I. Tauber. 2012. "A Symbiotic View of Life: We Have Never Been Individuals." *Quarterly Review of Biology* 87: 325–341.

Gipson, K. P., K. P. Nickerson, E. Drenkard, et al. 2020. "The Great ESKAPE: Exploring the Crossroads of Bile and Antibiotic Resistance in Bacterial Pathogens." *Infection and Immunity* 88: e0086519. https://doi.org/10.1128/iai.00865-19.

Goffredi, S. K., R. G. Appy, R. Hildreth, and J. deRogatis. 2023. "Marine Vampires: Persistent, Internal Associations Between Bacteria and Blood-Feeding Marine Annelids and Crustaceans." *Frontiers in Microbiology* 13: 1113237. https://doi.org/10.3389/fmicb.2022.1113237.

Gómez-Govea, M. A., K. I. Peña-Carillo, G. Ruiz-Ayma, et al. 2024. "Unveiling the Microbiome Diversity in *Telenomus* (Hymenoptera: Scelionidae) Parasitoid Wasps." *Insects* 15: 468.

Groussin, M., F. Mazel, J. G. Sanders, et al. 2017. "Unraveling the Processes Shaping Mammalian Gut Microbiomes Over Evolutionary Time." *Nature Communications* 8: 14319. https://doi.org/10.1038/ncomms14319.

Hahn, M. A., A. Piecyk, F. Jorge, R. Cerrato, M. Kalbe, and N. M. Dheilly. 2022. "Host Phenotype and Microbiome Vary With Infection Status, Parasite Genotype, and Parasite Microbiome Composition." *Molecular Ecology* 31: 1577–1594. https://doi.org/10.1111/mec.16344.

Hasegawa, R., R. Poulin, and P. M. Salloum. 2025. "Testing for Consistency in Co-Occurrence Patterns Among Bacterial Taxa Across the Microbiomes of Four Different Trematode Parasites." *Microbial Ecology* 88: 45. https://doi.org/10.1007/s00248-025-02545-w.

Hernández-García, J. A., C. I. Briones-Roblero, F. N. Rivera-Orduña, and G. Zúñiga. 2017. "Revealing the Gut Bacteriome of *Dendroctonus* Bark Beetles (Curculionidae: Scolytinae): Diversity, Core Members and Co-Evolutionary Patterns." *Scientific Reports* 7: 13864. https://doi.org/10.1038/s41598-017-14031-6.

Hodžić, A., N. M. Dheilly, A. Cabezas-Cruz, and D. Berry. 2023. "The Helminth Holobiont: A Multidimensional Host–Parasite–Microbiota Interaction." *Trends in Parasitology* 39: 91–100.

Ingala, M. R., N. B. Simmons, M. Dunbar, C. Wultsch, K. Krampis, and S. L. Perkins. 2021. "You Are More Than What You Eat: Potentially Adaptive Enrichment of Microbiome Functions Across Bat Dietary Niches." *Animal Microbiome* 3: 82. https://doi.org/10.1186/s42523-021-00139-8.

Jorge, F., N. M. Dheilly, C. Froissard, E. Wainwright, and R. Poulin. 2022. "Consistency of Bacterial Communities in a Parasitic Worm: Variation Throughout the Life Cycle and Across Geographic Space." *Microbial Ecology* 83: 724–738. https://doi.org/10.1007/s00248-021-01774-z.

Jorge, F., N. M. Dheilly, and R. Poulin. 2020. "Persistence of a Core Microbiome Through the Ontogeny of a Multi-Host Parasite." *Frontiers in Microbiology* 11: 954. https://doi.org/10.3389/fmicb.2020.00954.

Jovel, J., J. Patterson, W. Wang, et al. 2016. "Characterization of the Gut Microbiome Using 16S or Shotgun Metagenomics." *Frontiers in Microbiology* 7: 459. https://doi.org/10.3389/fmicb.2016.00459.

Kalka, M., and E. K. V. Kalko. 2006. "Gleaning Bats as Underestimated Predators of Herbivorous Insects: Diet of *Micronycteris microtis* (Phyllostomidae) in Panama." *Journal of Tropical Ecology* 22: 1–10.

Kashinskaya, E. N., E. P. Simonov, G. I. Izvekova, A. N. Parshukov, K. B. Andree, and M. M. Solovyev. 2020. "Composition of the Microbial Communities in the Gastrointestinal Tract of Perch (*Perca fluviatilis*) and Cestodes Parasitizing the Perch Digestive Tract." *Journal of Fish Diseases* 43: 23–38. https://doi.org/10.1111/jfd.13096.

Katoh, K., K. Misawa, K. Kuma, and T. Miyata. 2002. "MAFFT: A Novel Method for Rapid Multiple Sequence Alignment Based on Fast Fourier Transform." *Nucleic Acids Research* 30: 3059–3066. https://doi.org/10.1093/nar/gkf436.

Koellsch, C., R. Poulin, and P. M. Salloum. 2024. "What Shapes a Microbiome? Differences in Bacterial Communities Associated With Helminth–Amphipod Interactions." *International Journal for Parasitology* 54: 733–742.

Kohl, K. D. 2020. "Ecological and Evolutionary Mechanisms Underlying Patterns of Phylosymbiosis in Host-Associated Microbial Communities." *Philosophical Transactions of the Royal Society, B: Biological Sciences* 375, no. 1798: 20190251. https://doi.org/10.1098/rstb.2019.0251.

LaFond, R. E., and S. A. Lukehart. 2006. "Biological Basis for Syphilis." *Clinical Microbiology Reviews* 19: 29–49. https://doi.org/10.1128/cmr.19.1.29-49.2006.

Lamothe Argumedo, R. 1997. *Manual de Técnicas Para Preparar y Estudiar Los ParáSitos de Animales Silvestres*. AGT Editor.

Lee, Y. K., and S. K. Mazmanian. 2010. "Has the Microbiota Played a Critical Role in the Evolution of the Adaptive Immune System?" *Science* 330: 1768–1773. https://doi.org/10.1126/science.1195568.

Li, H., L. Su, S. Chen, et al. 2018. "Physicochemical Characterization and Functional Analysis of the Polysaccharide From the Edible Microalga *Nostoc sphaeroides*." *Molecules* 23: 508.

Lim, S. J., and S. R. Bordenstein. 2020. "An Introduction to Phylosymbiosis." *Proceedings of the Royal Society B: Biological Sciences* 287: 20192900. https://doi.org/10.1098/rspb.2019.2900.

Liu, C., Y. Cui, X. Li, and M. Yao. 2021. "Microeco: An R Package for Data Mining in Microbial Community Ecology." *FEMS Microbiology Ecology* 97: fiaa255. https://doi.org/10.1093/femsec/fiaa255.

Loker, E. S., C. M. Adema, S.-M. Zhang, and T. B. Kepler. 2004. "Invertebrate Immune Systems—Not Homogeneous, Not Simple, Not Well Understood." *Immunological Reviews* 198: 10–24. https://doi.org/10.1111/j.0105-2896.2004.0117.x.

Love, M. I., W. Huber, and S. Anders. 2014. "Moderated Estimation of Fold Change and Dispersion for RNA-Seq Data With DESeq2." *Genome Biology* 15: 550. https://doi.org/10.1186/s13059-014-0550-8.

Lutz, H. L., J. A. Gilbert, and C. W. Dick. 2022. "Associations Between Afrotropical Bats, Eukaryotic Parasites, and Microbial Symbionts." *Molecular Ecology* 31: 1939–1950. https://doi.org/10.1111/mec.16044.

Lutz, H. L., E. W. Jackson, P. W. Webala, et al. 2019. "Ecology and Host Identity Outweigh Evolutionary History in Shaping the Bat Microbiome." *mSystems* 4: e0051119. https://doi.org/10.1128/msystems.00511-19.

Mallott, E. K., and K. R. Amato. 2021. "Host Specificity of the Gut Microbiome." *Nature Reviews Microbiology* 19: 639–653. https://doi.org/10.1038/s41579-021-00562-3.

Marden, J. N., E. A. McClure, and L. Beka. 2016. "Host Matters: Medicinal Leech Digestive-Tract Symbionts and Their Pathogenic Potential." *Frontiers in Microbiology* 7: 1569. https://doi.org/10.3389/fmicb.2016.01569.

Maritan, E., A. Quagliariello, E. Frago, T. Patarnello, and M. E. Martino. 2024. "The Role of Animal Hosts in Shaping Gut Microbiome Variation." *Philosophical Transactions of the Royal Society, B: Biological Sciences* 379, no. 1901: 20230071. https://doi.org/10.1098/rstb.2023.0071.

Martinson, V. G., R. M. R. Gawryluk, B. E. Gowen, C. I. Curtis, J. Jaenike, and S. J. Perlman. 2020. "Multiple Origins of Obligate Nematode and Insect Symbionts by a Clade of Bacteria Closely Related to Plant Pathogens." *Proceedings of the National Academy of Sciences USA* 117: 31979–31986. https://doi.org/10.1073/pnas.2000860117.

Mazel, F., K. M. Davis, A. Loudon, W. K. Kwong, M. Groussin, and L. W. Parfrey. 2018. "Is Host Filtering the Main Driver of Phylosymbiosis Across the Tree of Life?" *mSystems* 3: e0009718. https://doi.org/10.1128/msystems.00097-18.

McClure, E. A., M. C. Nelson, A. Lin, and J. Graf. 2021. "*Macrobdella decora*: Old World Leech Gut Microbial Community Structure Conserved in a New World Leech." *Applied and Environmental Microbiology* 87: e02082. https://doi.org/10.1128/AEM.02082-20.

McFall-Ngai, M., M. G. Hadfield, T. C. G. Bosch, et al. 2013. "Animals in a Bacterial World, a New Imperative for the Life Sciences." *Proceedings of the National Academy of Sciences USA* 110: 3229–3236. https://doi.org/10.1073/pnas.1218525110.

McMurdie, P. J., and S. Holmes. 2013. "Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data." *PLoS One* 8: e61217. https://doi.org/10.1371/journal.pone.0061217.

McQueen, J. P., K. Gattoni, E. M. S. Gendron, S. K. Schmidt, P. Sommers, and D. L. Porazinska. 2023. "External and Internal Microbiomes of Antarctic Nematodes Are Distinct, but More Similar to Each Other Than the Surrounding Environment." *Journal of Nematology* 55: 20230004.

Medellín, R. A., H. T. Arita, and O. Sánchez. 2008. *Identificación de Los Murciélagos de México, Clave de Campo*. 2nd ed. Instituto de Ecología, UNAM/CONABIO.

Midha, A., V. H. Jarquín-Díaz, F. Ebner, et al. 2022. "Guts Within Guts: The Microbiome of the Intestinal Helminth Parasite *Ascaris suum* Is Derived but Distinct From Its Host." *Microbiome* 10: 229. https://doi.org/10.1186/s40168-022-01399-5.

Moran, N. A., and D. B. Sloan. 2015. "The Hologenome Concept: Helpful or Hollow?" *PLoS Biology* 13: e1002311. https://doi.org/10.1371/journal.pbio.1002311.

Morni, M. A., J. William-Dee, E. R. Jinggong, et al. 2025. "Gut Microbiome Community Profiling of Bornean Bats With Different Feeding Guilds." *Animal Microbiome* 7: 21. https://doi.org/10.1186/s42523-025-00389-w.

Müller, V., R. J. de Boer, S. Bonhoeffer, and E. Szathmáry. 2018. "An Evolutionary Perspective on the Systems of Adaptive Immunity." *Biological Reviews* 93: 505–528. https://doi.org/10.1111/brv.12355.

Muslim, A., S. Aazmi, Y. X. Er, S. Shahrizal, and Y. A. L. Lim. 2024. "*Ascaris lumbricoides* Harbors a Distinct Gut Microbiota Profile From Its Human Host: Preliminary Insights." *Food and Waterborne Parasitology* 34: e00223.

Nemergut, D. R., S. K. Schmidt, T. Fukami, et al. 2013. "Patterns and Processes of Microbial Community Assembly." *Microbiology and Molecular Biology Reviews* 77, no. 3: 342–356. https://doi.org/10.1128/mmbr.00051-12.

Noda, S., D. Shimizu, M. Yuki, O. Kitade, and M. Ohkuma. 2018. "Host–Symbiont Cospeciation of Termite-Gut Cellulolytic Protists of the Genera *Teranympha* and *Eucomonympha* and Their *Treponema* Endosymbionts." *Microbes and Environments* 33: 26–33.

Oksanen, J., G. Simpson, F. Blanchet, et al. 2025. "Vegan: Community Ecology Package." https://vegandevs.github.io/vegan/.

Panti-May, J. A., G. M. C. Macswiney, J. Ortega, and W. I. Moguel-Chin. 2025. "First Record of *Seuratum* sp. (Nematoda: Seuratidae) in *Chrotopterus auritus* (Chiroptera: Phyllostomidae) From Mexico." *Therya Notes* 6: 144–150.

Paradis, E., and K. Schliep. 2019. "Ape 5.0: An Environment for Modern Phylogenetics and Evolutionary Analyses in R." *Bioinformatics* 35: 526–528. https://doi.org/10.1093/bioinformatics/bty633.

Paz, E. A., E. G. Chua, D. G. Palmer, et al. 2024. "Revealing the Associated Microflora Hosted by the Globally Significant Parasite *Trichostrongylus colubriformis*." *Scientific Reports* 14: 3723. https://doi.org/10.1038/s41598-024-53772-z.

Peixoto, F. P., P. H. P. Braga, and P. Mendes. 2018. "A Synthesis of Ecological and Evolutionary Determinants of Bat Diversity Across Spatial Scales." *BMC Ecology* 18: 18. https://doi.org/10.1186/s12898-018-0174-z.

Perofsky, A. C., R. J. Lewis, L. A. Abondano, A. Di Fiore, and L. A. Meyers. 2017. "Hierarchical Social Networks Shape Gut Microbial Composition in Wild Verreaux's Sifaka." *Proceedings of the Royal Society B: Biological Sciences* 284, no. 1868: 2017227. https://doi.org/10.1098/rspb.2017.227.

Phillips, C. D., G. Phelan, S. E. Dowd, et al. 2012. "Microbiome Analysis Among Bats Describes Influences of Host Phylogeny, Life History, Physiology and Geography." *Molecular Ecology* 21, no. 11: 2617–2627. https://doi.org/10.1111/j.1365-294X.2012.05568.x.

Poulin, R., F. Jorge, and P. M. Salloum. 2023. "Inter-Individual Variation in Parasite Manipulation of Host Phenotype: A Role for Parasite Microbiomes?" *Journal of Animal Ecology* 92: 807–812. https://doi.org/10.1111/1365-2656.13764.

Price, M. N., P. S. Dehal, and A. P. Arkin. 2010. "FastTree 2—Approximately Maximum-Likelihood Trees for Large Alignments." *PLoS One* 5: e9490. https://doi.org/10.1371/journal.pone.0009490.

Pringle, M., A. Backhans, F. Otman, M. Sjölund, and C. Fellström. 2009. "Isolation of Spirochetes of Genus *Treponema* From Pigs With Ear Necrosis." *Veterinary Microbiology* 139: 279–283.

Quast, C., E. Pruesse, P. Yilmaz, et al. 2013. "The SILVA Ribosomal RNA Gene Database Project: Improved Data Processing and Web-Based Tools." *Nucleic Acids Research* 41: D590–D596. https://doi.org/10.1093/nar/gks1219.

Reid, F. 1997. *A Field Guide to the Mammals of Central America and Southeast Mexico*. OUP USA. https://books.google.com.mx/books?id=TXQe9P5Z_7UC.

Rinaldi, G., C. Paz Meseguer, C. Cantacessi, and A. Cortés. 2024. "Form and Function in the Digenea, With an Emphasis on Host–Parasite and Parasite–Bacteria Interactions." In *Digenetic Trematodes*, edited by R. Toledo and B. Fried, vol. 1454, 3–45. Digenetic Trematodes. Springer. https://doi.org/10.1007/978-3-031-60121-7_1.

Rosenberg, E., and I. Zilber-Rosenberg. 2013. "Role of Microorganisms in Adaptation, Development, and Evolution of Animals and Plants: The Hologenome Concept." In *The Prokaryotes: Prokaryotic Biology and Symbiotic Associations*, edited by E. Rosenberg, E. F. DeLong, S. Lory,

E. Stackebrandt, and F. Thompson, 347–358. Springer. https://doi.org/10.1007/978-3-642-30194-0_117.

Ruiz-Torres, N. G., D. I. Hernandez-Mena, L. García-Prieto, L. I. Falcón, J. A. Panti-May, and O. Rico-Chávez. 2024. "A New Genus and Species of Microphalloidea (Digenea), Parasite of *Peropteryx* Spp. (Chiroptera: Emballonuridae) From the Neotropical Region of Mexico Revealed by Morphological and Phylogenetic Analyses." *Journal of Helminthology* 98: e63.

Salloum, P. M., F. Jorge, N. M. Dheilly, and R. Poulin. 2023. "Eco-Evolutionary Implications of Helminth Microbiomes." *Journal of Helminthology* 97: e22.

Salloum, P. M., F. Jorge, and R. Poulin. 2023. "Different Trematode Parasites in the Same Snail Host: Species-Specific or Shared Microbiota?" *Molecular Ecology* 32: 5414–5428. https://doi.org/10.1111/mec.17111.

San Juan, P. A., L. Palma, and K. C. Bell. 2025. "Pinworm Microbiomes Are Distinct From Their Chipmunk Host Gut Microbiota." *Journal of Mammalogy* 107, no. 1: 17–26. https://doi.org/10.1093/jmammal/gyae156.

Santos, C. P., and D. I. Gibson. 2015. "Checklist of the Helminth Parasites of South American Bats." *Zootaxa* 3937, no. 3: 471.

Schulenburg, H., C. Boehnisch, and N. K. Michiels. 2007. "How Do Invertebrates Generate a Highly Specific Innate Immune Response?" *Molecular Immunology* 44: 3338–3344.

Sharpton, T. J. 2018. "Role of the Gut Microbiome in Vertebrate Evolution." *mSystems* 3: e0017417. https://doi.org/10.1128/msystems.00174-17.

Shen, Y.-Y., L. Liang, Z.-H. Zhu, W.-P. Zhou, D. M. Irwin, and Y.-P. Zhang. 2010. "Adaptive Evolution of Energy Metabolism Genes and the Origin of Flight in Bats." *Proceedings of the National Academy of Sciences* 107: 8666–8671. https://doi.org/10.1073/pnas.0912613107.

Sieng, S., P. Chen, N. Wang, J. Y. Xu, and Q. Han. 2023. "*Toxocara canis*-Induced Changes in Host Intestinal Microbial Communities." *Parasites & Vectors* 16: 462. https://doi.org/10.1186/s13071-023-06072-w.

Sikes, R. S., T. A. Thompson, and J. A. Bryan II. 2019. "American Society of Mammalogists: Raising the Standards for Ethical and Appropriate Oversight of Wildlife Research." *Journal of Mammalogy* 100: 763–773. https://doi.org/10.1093/jmammal/gyz019.

Simon, J.-C., J. R. Marchesi, C. Mougel, and M.-A. Selosse. 2019. "Host–Microbiota Interactions: From Holobiont Theory to Analysis." *Microbiome* 7: 5. https://doi.org/10.1186/s40168-019-0619-4.

Song, S. J., J. G. Sanders, F. Delsuc, et al. 2020. "Comparative Analyses of Vertebrate Gut Microbiomes Reveal Convergence Between Birds and Bats." *MBio* 11, no. 1: 101128. https://doi.org/10.1128/mbio.02901-19.

Sprockett, D., T. Fukami, and D. A. Relman. 2018. "Role of Priority Effects in the Early-Life Assembly of the Gut Microbiota." *Nature Reviews Gastroenterology & Hepatology* 15, no. 4: 197–205.

Theis, K. R., N. M. Dheilly, J. L. Klassen, et al. 2016. "Getting the Hologenome Concept Right: An Eco-Evolutionary Framework for Hosts and Their Microbiomes." *mSystems* 1: e0002816. https://doi.org/10.1128/mSystems.00028-16.

Trejo-Meléndez, V. J., and J. Contreras-Garduño. 2025. "Master of Puppets: How Microbiota Drive the Nematoda Ecology and Evolution?" *Ecology and Evolution* 15: e71549. https://doi.org/10.1002/ece3.71549.

Tung, J., L. B. Barreiro, M. B. Burns, et al. 2015. "Social Networks Predict Gut Microbiome Composition in Wild Baboons." *eLife* 4: e05224. https://doi.org/10.7554/eLife.05224.

Wang, N., S. Sieng, T. Liang, P. Chen, J. Xu, and Q. Han. 2025. "Effect of *Toxocara canis* Infection on Liver and Lung Microbial Flora Diversity and Composition in Dogs." *Parasite* 32: 17. https://doi.org/10.1051/parasite/2025011.

Yang, X., G. Jiang, Y. Zhang, et al. 2023. "MBPD: A Multiple Bacterial Pathogen Detection Pipeline for One Health Practices." *iMeta* 2: e82. https://doi.org/10.1002/imt2.82.

Zeng, H., Y. Chan, W. Gao, W. K. Leung, and R. M. Watt. 2021. "Diversity of *Treponema denticola* and Other Oral Treponeme Lineages in Subjects With Periodontitis and Gingivitis." *Microbiology Spectrum* 9: e0070121. https://doi.org/10.1128/Spectrum.00701-21.

Zhong, G., W. Pan, Z. Huang, et al. 2021. "Physicochemical and Geroprotective Comparison of *Nostoc sphaeroides* Polysaccharides Across Colony Growth Stages and With Derived Oligosaccharides." *Journal of Applied Phycology* 33: 939–952. https://doi.org/10.1007/s10811-021-02383-6.

Zhou, J., and D. Ning. 2017. "Stochastic Community Assembly: Does It Matter in Microbial Ecology?" *Microbiology and Molecular Biology Reviews* 81, no. 4: 10–1128. https://doi.org/10.1128/mmbr.00002-17.

Zilber-Rosenberg, I., and E. Rosenberg. 2021. "Microbial-Driven Genetic Variation in Holobionts." *FEMS Microbiology Reviews* 45: fuab022.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** mec70389-sup-0001-FigureS1.pdf. **Figure S2:** mec70389-sup-0002-FigureS2.pdf.