View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Repositorio INDICASAT AIP

PLOS ONE

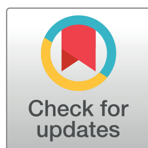
RESEARCH ARTICLE

Habitat disturbance and the organization of bacterial communities in Neotropical hematophagous arthropods

Kelly L. Bennett[1]*, **Alejandro Almanza**[1], **W. Owen McMillan**[1], **Kristin Saltonstall**[1], **Evangelina López Vdovenko**[1], **Jorge S. Vinda**[1], **Luis Mejia**[1,2], **Kaitlin Driesse**[3], **Luis F. De León**[4], **Jose R. Loaiza**[1,2,5]*

1 Smithsonian Tropical Research Institute, Balboa Ancon, República de Panamá, **2** Instituto de Investigaciones Científicas y Servicios de Alta Tecnología, Panamá, República de Panamá, **3** University at Albany, State University of New York, NY, United States of America, **4** Department of Biology, University of Massachusetts Boston, Boston, MA, United States of America, **5** Programa Centroamericano de Maestría en Entomología, Universidad de Panamá, Panamá, República de Panamá

* BennettK@si.edu (KLB); jloaiza@indicasat.org.pa(JRL)



OPEN ACCESS

Citation: Bennett KL, Almanza A, McMillan WO, Saltonstall K, Vdovenko EL, Vinda JS, et al. (2019) Habitat disturbance and the organization of bacterial communities in Neotropical hematophagous arthropods. PLoS ONE 14(9): e0222145. https://doi.org/10.1371/journal.pone.0222145

Editor: Arunachalam Ramaiah, University of California San Diego, UNITED STATES

Received: May 13, 2019

Accepted: August 22, 2019

Published: September 6, 2019

Copyright: © 2019 Bennett et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: Metagenomic sequence data is available under the BioProject PRJNA552605 in GenBank (https://www.ncbi.nlm.nih.gov/genbank/).

Funding: This work was financed in part by the Secretariat for Science, Technology and Innovation of Panama (SENACYT), through the research grant IDDS15-047 and the National System of Investigation (SNI), supported research activities by JRL. Research activity by KLB was supported

Abstract

The microbiome plays a key role in the biology, ecology and evolution of arthropod vectors of human pathogens. Vector-bacterial interactions could alter disease transmission dynamics through modulating pathogen replication and/or vector fitness. Nonetheless, our understanding of the factors shaping the bacterial community in arthropod vectors is incomplete. Using large-scale 16S amplicon sequencing, we examine how habitat disturbance structures the bacterial assemblages of field-collected whole-body hematophagous arthropods that vector human pathogens including mosquitoes (Culicidae), sand flies (Psychodidae), biting midges (Ceratopogonidae) and hard ticks (Ixodidae). We found that all comparisons of the bacterial community among species yielded statistically significant differences, but a difference was not observed between adults and nymphs of the hard tick, *Haemaphysalis juxtakochi*. While *Culicoides* species had the most distinct bacterial community among dipterans, tick species were composed of entirely different bacterial OTU's. We observed differences in the proportions of some bacterial types between pristine and disturbed habitats for *Coquillettidia* mosquitoes, *Culex* mosquitoes, and *Lutzomyia* sand flies, but their associations differed within and among arthropod assemblages. In contrast, habitat quality was a poor predictor of differences in bacterial classes for *Culicoides* biting midges and hard tick species. In general, similarities in the bacterial communities among hematophagous arthropods could be explained by their phylogenetic relatedness, although intraspecific variation seems influenced by habitat disturbance.

Introduction

Bacterial communities are important components of hematophagous arthropods (e.g., blood feeders) vectoring disease-causing pathogens to humans and wildlife, and they are likely to

by the Smithsonian Institution Fellowship Program, George Burch Fellowship, The Edward M. and Jeanne C. Kashian Family Foundation Inc., and Nicholas Logothetis of Chartwell Consulting. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors received funding from The Edward M. and Jeanne C. Kashian Family Foundation Inc., and Nicholas Logothetis of Chartwell Consulting. There are no patents, products in development or marketed products associated with this research to declare. This does not alter our adherence to PLOS ONE policies on sharing data and materials.

play a key role in vector ecology, evolution and transmission capacity [1–4]. Several important human and animal diseases result from bacterial infection transmitted through the bite of arthropod vectors [5,6]. Bacteria also interact with the arthropod host to reduce or increase the transmission of pathogens or indirectly alter disease dynamics through the modification of nutrition [7], development, reproduction or the immune response of arthropod vectors [8,9]. Our understanding of the factors shaping the organization of bacterial communities in hematophagous arthropods vectoring human diseases is still limited. Studies regarding the microbiome of disease vectors have attempted to describe the structure and bacterial composition of specific taxonomic groups of arthropods, and to understand how it varies according to particular ecological or physiological factors, with the most comprehensive studies focused on mosquitoes [10] and ticks [2]. Although some studies have considered the impact of habitat or environment type on arthropod microbiota in mosquitoes [11–16], ticks [17–20] and biting midges [21], none to date have investigated the role of habitat disturbance in shaping bacterial assemblages among co-distributed hematophagous arthropods.

Mosquitoes (Diptera: Culicidae), sand flies (Diptera: Psychodidae), biting midges (Diptera: Ceratopogonidae) and hard ticks (Acari: Ixodidae) are collectively responsible for numerous medically important diseases worldwide, including arthropod-borne viruses (e.g., arboviruses) transmitted to humans (Dengue–DENV, chikungunya—CHIKV, Zika—ZIKV, Yellow Fever–YFV, West Nile–WNV, Mayaro and Oropuche) and to agriculturally important livestock (Vesicular Stomatitis–VSV, Blue-Tongue–BTV, Epizootic Hemorrhagic Disease–EHDV and African Horse Sickness–AHSV) or to both (Venezuelan Equine Encephalitis–VEEV, Eastern Equine Encephalitis–EEEV and Rift Valley Fever) [22,23]. In addition, some species in these arthropod assemblages are involved in the transmission of parasites such as filarial nematodes (*Mansonella*—filariasis) [24], protozoan (*Leishmania*—Leishmaniasis) [25] and bacteria (*Rickettsia*—Lyme disease and babesiosis) [26].

The ability of hematophagous arthropods to carry and transmit pathogens biologically is given by their population dynamics and feeding behaviour in relation to that of their vertebrate host, plus their immune responses to infection [9,27]. Some bacterial commensals impact the capacity of arthropods as biological vectors, through diminishing pathogen replication and dissemination in the host tissues or by reducing vector fitness and lifespan [4,9,28,29]. Studies from members of the Culicidae demonstrate the importance of the microbiome in modulating disease transmission. For example, *Chromobacterium*, *Proteus* and *Paenibacillus* bacteria can inhibit DENV replication in mosquitoes while the resident bacteria are required for its establishment [28]. Furthermore, the intracellular bacterium *Wolbachia* is known to adversely influence the transmission of DENV, CHIKV, ZIKV, YFV and WNV [30–35]. Alternatively, some bacteria are associated with an increase in disease transmission by their arthropod vectors. For example, members of Enterobacteriae are correlated with higher *Plasmodium* infection rates in *Anopheles* mosquitoes, while *Serratia odorifera* can increase the replication of both DENV and CHIKV in the midgut of *Aedes aegypti* [28,36]. Although, studies have endeavored to characterize the core microbiome of members of Psychodidae sand flies, Ceratopogonidae biting midges and Ixodidae hard ticks, it is still generally unknown how similar or different their microbiomes are, and whether some bacteria may influence disease transmission dynamics in these arthropod assemblages [18,19,21,37–42]. Nonetheless, some studies have revealed that resident bacteria are essential for the development of *Leishmania* parasite in Psychodidae through antibiotic treatment [37,43].

Metagenomic studies of disease vectors in the Order Diptera have revealed that different genera including those with a distinct ecology generally share a core microbiome, but often exhibit differences in bacterial composition and structure that distinguish a species [1,13,38,44–46]. Conversely, tick species may exhibit a distinct taxonomic structure in their

microbiome, because they are associated with specific vertebrate hosts throughout their entire lifetime, including during the immature stages [47]. Core microbiota of Diptera are largely acquired from the environment during the immature stages, many of which persist until the adult stage [13,48–50]. Bacteria are also acquired during adult blood feeding, therefore the microbiome of arthropod vectors is likely impacted by both developmental stage and gender [1,19,49,51]. The core microbiota of ticks is either maternally-inherited, acquired from blood feeding on hosts or through the colonization of environmental microorganisms from vertebrate skin or the soil on physical contact [2,52].

Hematophagous arthropods can exhibit intra-specific variation in their bacterial associates between geographic locations, explained by differences in the quality of larval habitats or host preferences at sampling sites for both Diptera [11,37,46,53] and ticks [17,18]. Hence, it has been proposed that larval habitat conditions and geographic location are important factors shaping the bacterial community of some adult hematophagous arthropods. Conversely, some mosquitoes [44] and ticks [54] do not exhibit intra-specific variation in the bacterial community across geographical locations or habitats. This finding supports a more specific and long term association between some blood-feeding arthropods and their bacterial associates, which is likely mediated by the immune system of the host, rather than by their external environment [13,54].

Our goal herein is to test for variation in the diversity of bacteria among four epidemiologically discrete groups of hematophagous arthropods, and to identify the factors shaping this variation. Specifically, we address the following questions: (1) *How do patterns of bacterial diversity and composition differ among the microbiomes of mosquitoes, biting midges, sand flies and hard ticks*?, and (2) *Does habitat disturbance influence the organization of bacterial communities within these arthropod assemblages*? We posit that blood-feeding arthropod species in the Order Diptera will harbor comparable bacterial organizations, since they are more closely phylogenetically related, while hard ticks within the Order Ixodida are considered as an outgroup. We also postulate that intra-specific bacterial diversity and taxa composition will change owing to variation in habitat quality, but changes are only expected within Culicidae mosquitoes, Psychodidae sand flies and Ceratopogonidae biting midges. This is anticipated because host–tick interactions in obligated ectoparasites such as hard ticks are more likely to shape their microbiome than habitat disturbance. Although hard ticks can acquire surface microbiota from their environment, our study largely targets intracellular and gut bacteria colonized through vertical transmission or ingestation. We use a metabarcoding approach to compare inter-and intra- group bacterial communities among these arthropod assemblages, and also in relation to changes in habitat quality. If habitat disturbance is a significant predictor of bacterial assemblages, this could have ramifications for disease transmission through variation of the vector microbiome and correlated vectorial capacity.

Materials and methods

Arthropod collection and sample preparation

Permission was obtained from MiAmbiente under permit identification ID 8-447-900-PAN. The study was conducted in the lowland tropical rainforest ecosystem of central Panama, a region formerly known as the Panama Canal Zone. Adult specimens of mosquitoes, sand flies, biting midges and hard ticks were gathered from three forested areas that varied in their levels of anthropogenic disturbance and original habitat quality. These included a pristine site, Barro Colorado Island (BCI), which is comprised of old-growth forest with low levels of disturbance (e.g., >65% forest cover). In addition, two disturbed forest sites, Achiote (ACH) and Las Pavas (PVAS), encompass patches of secondary-growth forest subject to intermediate and high levels

of disturbance (e.g., >35% and <65% forest cover) respectively [55,56]. Dipterans were collected using six Center for Disease Control (CDC) miniature light traps (John W. Hock Company, Gainesville, Florida), operating overnight in the understory (1.5 m height) and six in the canopy (> 25 m height), alternating each night. Each trap was situated along a transect and spaced at least 300 meters apart from each other to avoid pseudoreplication as in Loaiza *et al.* [55,57]. They were baited with 0.5 pounds of dry ice to attract blood-seeking dipterans. Adult specimens were retrieved from the traps at sunrise and taken to the laboratory in a portable freezer container holding dry ice. Individuals were sorted and identified using a chill table and taxonomic keys [58–62].

Ixodid ticks were collected with two methods at BCI and PVAS: the standard tick-dragging technique [63], and a pair of home-made cloth-pants, fabricated with white rustic fabric. Two human collectors traversed linear transects of up to 200 meters through the vegetation using either method. Adult specimens were removed from the cloth with entomological forceps, while immature stages (e.g., larvae and nymphs) were detached using transparent adhesive tape. Individuals were placed in separate cryo-vials, and subsequently transported to the laboratory. Taxonomic characters were used to identify ticks to the species level [64,65]. The samples were washed with 70% ethanol to remove surface contamination before storage in 95% ethanol. Details on the number of samples processed from each site and for each species are provided in S1 Table.

DNA extraction, 16S rRNA gene library and sequencing

Each arthropod species was processed using the following laboratory procedures independently. Each sample was rinsed in 70% ethanol before they were pooled. DNA was isolated from pools of adult female dipterans and both adults and immature ticks using a BioSprint 96 robot and associated BioSprint® 96 DNA Blood kit (Qiagen, Gaithersburg, MD, USA). Each pool was crushed individually in tissue lysis buffer using a high-speed shaking TissueLyser II and ceramic beads; the supernatant was placed in a well of a 96-well plate and followed by DNA isolation protocol from the manufacturer. DNA pools were made by combining 2 μl of DNA extract from 20 to 35 individuals of sand flies and biting midges, plus up to 5 individuals per pool of mosquitoes and ticks. Pooled DNA was used as a template to amplify the V4 region of the 16S rRNA locus using a two-step PCR protocol. The first PCR was composed of 5 μl of 2X Maxima HotStart PCR Master Mix (Thermo), 0.2 μl of each primer (which included an Illumina sequencing primer on the 5' end (10 mM)), and 1 μl of pooled DNA. Then 1 μl of the resulting PCR product was used to add on unique barcodes and Illumina sequencing adaptors in a second PCR of six cycles. The PCR cycling conditions had an initial denaturation step of 3 min at 94˚ C proceeding 25 cycles of 94˚C for 45 sec, 50˚C for 60 sec, and 72˚C for 90 sec, followed by 10 min at 72˚C extension. Resulting reactions were cleaned using PCR Normalization plates (Charm Biotech, San Diego, CA, USA) and samples pooled into a library which we concentrated using Kapa magnetic beads. The DNA concentration of each library was verified with the Qubit HS assay (Invitrogen, Waltham, MA, USA) and quality checked with a Bioanalyzer dsDNA High Sensitivity assay before sequencing on an Illumina MiSeq in a 2x250 paired end run. In the Culicidae family (mosquitoes), 40 pools of adult *Culex* including 20 pools of each *Culex coronator* and *Culex declarator* plus 20 pools of *Coquillettidia venezuelensis* were sequenced. Within the Ceratopogonidae (biting midges) and Psychodidae (sand flies), 94 pools of adult *Culicoides* including 34 pools of *Culicoides batesi*, 30 of *Culicoides foxi*, and 30 of *Culicoides heliconiae*, plus 75 pools of adult *Lutzomyia* including 30 pools of *Lutzomyia panamensis*, 23 of *Lutzomyia gomezi* and 22 of *Lutzomyia trapidoi* were sequenced and analyzed. Sequences within the hard tick family Ixodidae were obtained from 37 pools in total, including

6 pools of adults and 12 pools of nymphs of *Haemaphysalis juxtakochi*, 12 pools of adult *Amblyomma tapirellum* and 7 pools of adult of *Amblyomma oblongoguttatum* (S1 Table).

Analysis of 16S metadata

Analysis of sequence reads was performed using the Quantitative Insights Into Microbial Ecology (QIIME) software package versions 1.9.1 and 2.0. The DADA2 data quality filtering pipeline implemented in QIIME 2.0 was used to trim sequences with base quality scores lower than 20. Operational taxonomic units (OTU's) were assigned with a Naive Bayes classifier trained on the Greengenes 99% sequence similarity database v13.8 with sequences bound by the 515F and 806R primer pair [66]. Low abundance OTU's (0.005%) were filtered from the resulting relative abundance table to reduce bias by sequencing error.

The feature table was rarefied to a sequencing depth of 7 000 reads before alpha and beta diversity values were calculated. The statistical test PERMANOVA was applied to the resulting UNIFRAC distance matrixes to test for significant differences between the beta diversity of metadata groups. Principle coordinates analysis (PCoA) plots were generated from unweighted UNIFRAC distance matrixes. In addition, taxonomic summary plots of the relative abundance of bacteria were generated to depict the bacterial orders with an overall proportion of $> 0.1\%$ in at least one species. Indicator species analysis was applied to identify the OTU's unique to each species group.

Results

In total, 11 435 639 sequence reads of the bacterial 16S gene were captured from 265 sample pools, encompassing 4 916 individuals from four different hematophagous arthropod families, six genera and 12 species. After quality filtering and rarefaction to a depth of 7 000 reads, 10 838 632 sequences remained from 229 sample pools with an average of 40 900 sequences per pool (SE ± 1,209) and a total of 1 404 OTU's composed of 13 phyla, 30 classes, 55 orders, 106 families and 137 genera. Rarefaction curves revealed that the majority of bacterial diversity for all the species of arthropods was captured with subsampling of 7 000 sequences per sample pool (S1 Fig).

Bacterial diversity and composition in mosquitoes, biting midges, sand flies and hard ticks

Among dipterans, members of the genera *Culex*, *Coquillettidia*, *Culicoides* and *Lutzomyia* had comparable proportions of bacterial OTU's, bacterial diversity and community evenness index. In contrast, two tick species in the genus *Amblyomma* (i.e., *Amblyomma tapirellum* and *Amblyomma oblongoguttatum*) had higher number of OTU's, and bacterial diversity, and the least even community composition. A third tick species, *Haemaphysalis juxtakochi*, had the highest overall bacterial phylogenetic diversity, although it had a lower number of OTU's per tick pool and values of Shannon's diversity compared to *Amblyomma* species (Table 1 and S1 Fig).

All arthropod species were dominated by the phylum Proteobacteria with proportions ranging from 48 to 72%. Other major bacteria phyla that were shared among all arthropod species included Firmicutes, Bacteriodetes and Actinobacteria. Bacterial Orders and families were generally shared among arthropod genera in the Order Diptera, although they also exhibited notable differences in their relative proportions, which are visualized to the level of Order in Fig 1 and summarised to the genus level in S2 Table. Within the bacterial phyla shared between *Culex* and *Coquillettidia* mosquitoes, *Culicoides* biting midges, and *Lutzomyia* sand flies, the

Table 1. Average measures of bacterial alpha diversity for 12 species of blood-feeding arthropods at a rarefaction depth of 7 000 16S sequences.

Taxonomy	Species		Observed OTU's	Shannon's diversity	Faith's phylogenetic diversity	Evenness
Acari:Ixodidae	*H. juxtakochi*		43.12	3.51	14.12	0.66
		Adults	55.71	3.39	11.65	0.69
		Nymphs	34.3	7.12	11.28	0.94
	A. tapirellum		194.82	7.15	11.43	0.95
	A. oblongoguttatum		144.75	5.64	11.5	0.81
Diptera:Culicidae	*Coq. venezuelensis*		53.35	3.67	5.83	0.65
	Cux. coronator		49.7	3.64	5.91	0.65
	Cux. declarator		49.2	3.29	6.39	0.59
Diptera:Ceratopogonidae	*C. batesi*		59.38	4.03	7.13	0.69
	C. foxi		61.73	3.93	7.18	0.67
	C. heliconiae		56.15	3.63	6.81	0.63
Diptera:Psychodidae	*Lu. gomezi*		55.04	2.99	7.1	0.52
	Lu. panamensis		62.97	3.59	6.85	0.61
	Lu. trapidoi		43.59	2.86	5.76	0.54

https://doi.org/10.1371/journal.pone.0222145.t001

major classes consisted of Gammaproteobacteria, Betaproteobacteria, Alphaproteobacteria, Bacilli, and Actinobacteria.

Culicoides species share OTU's with the other genera of dipterans, but PCA and taxonomic analysis revealed that they have a more distinct bacterial community than *Lutzomyia*, *Culex* and *Coquillettidia* together with unique bacterial types including a disease-causing agent in the genus Arcobacte*r* (proteobacterial class Epsilonproteobacteria, Order Campylobacterales) [67], and *Candidatus cardinium* (phylum of Bacteriodetes, class Cytophagia), which is known

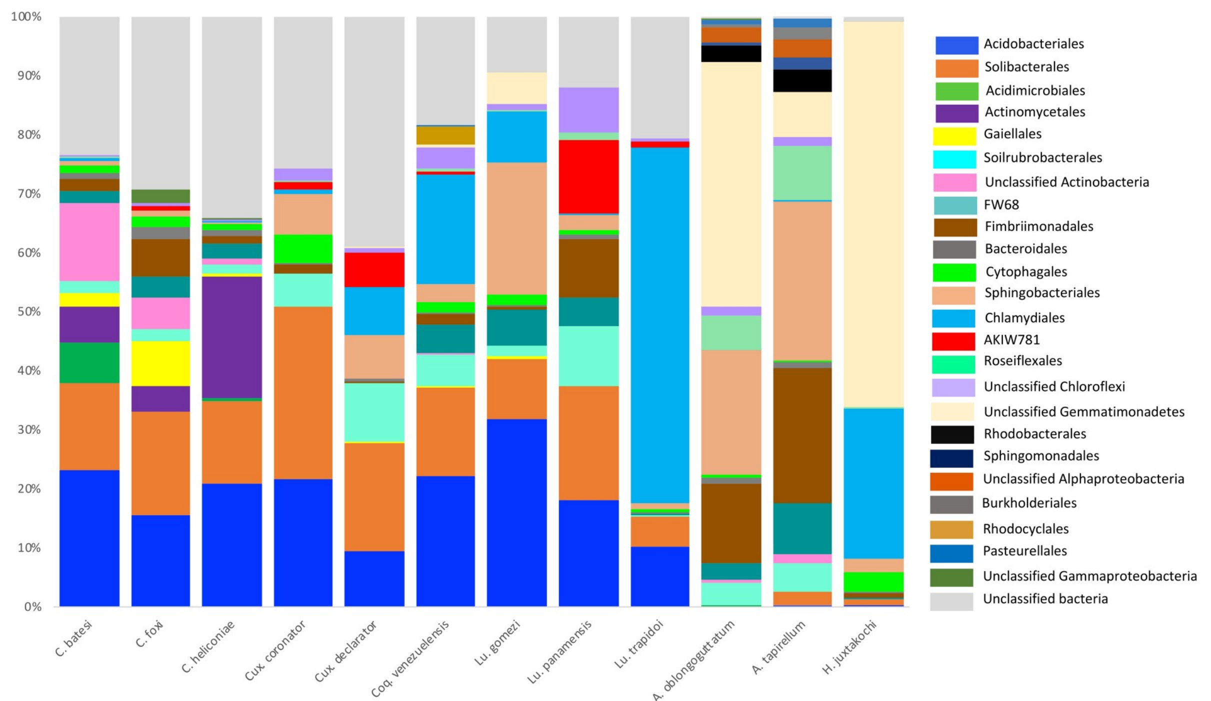


Fig 1. Relative abundances of bacterial orders above 0.1% summarized for each blood-feeding arthropod species.

https://doi.org/10.1371/journal.pone.0222145.g001

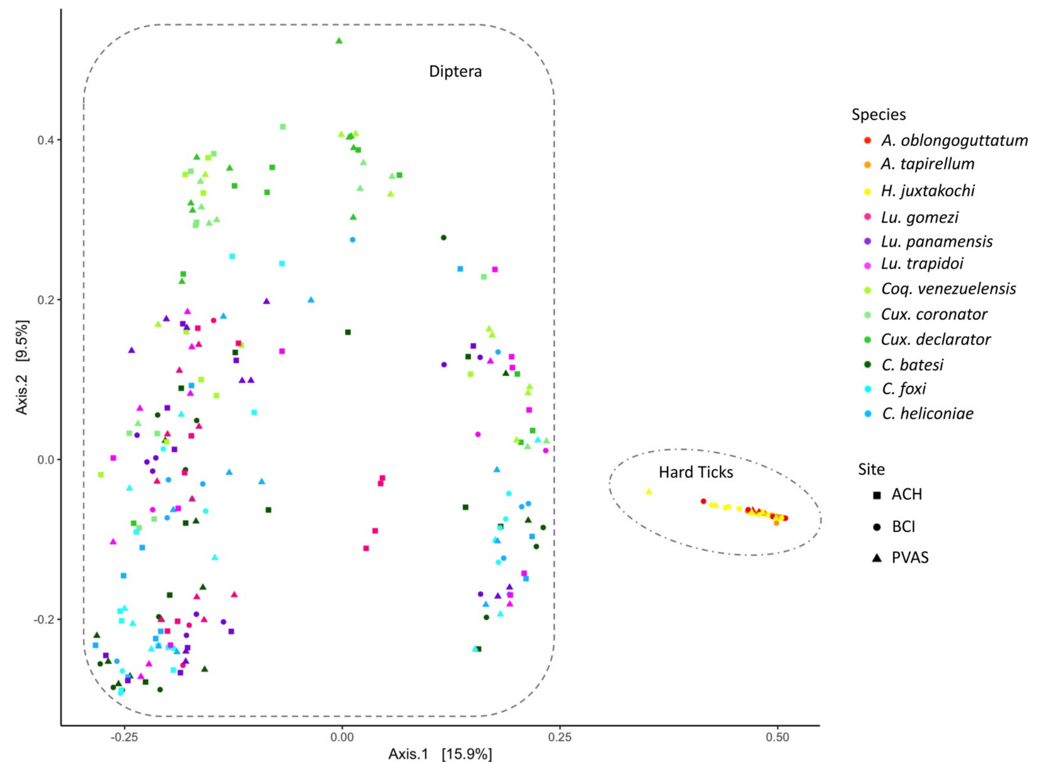


Fig 2. PCoA ordination analysis based on UNIFRAC distances with 16S gene sequence variation of the bacterial communities from six blood-feeding arthropod genera.

https://doi.org/10.1371/journal.pone.0222145.g002

to alter arthropod reproduction [68]. Moreover, *Culicoides batesi*, *Culicoides foxi* and *Lutzomyia trapidoi* had unique OTU's in the phyla Chlamydiae.

The bacterial phyla and classes of all tick species were composed of entirely different OTU's than the other arthropod assemblages, hence they were the most distinct in terms of bacterial composition (Fig 2). Ticks in the genus *Amblyomma* had bacterial phyla that were not found in any other arthropod genus, including Chloroflexi, Acidobacteria, Gemmatimonadetes, Armatimonadetes and TM7. Likewise, *Amblyomma* ticks had a number of classes unique to this genus, including the Protobacterium Deltaproteobacteria, Saprospirae, Cytophagia within the phylum of Bacteriodetes, and the Actinobacteria Thermoleophilia and Acidimicrobiia. *A*. *tapirellum* had the largest proportion (14.7%) of OTU's unique to its species (Fig 3).

All comparisons of the bacterial community among the different genera and species of adult hematophagous arthropods through PERMANOVA tests yielded statistically significant differences (Table 2). Additionally, there were no statistically significant differences between the adults and nymphs of *H*. *juxtakochi* based on UNIFRAC distances of bacterial OTU's (PERMANOVA, pseudo-F = 1.38, P = 0.247), although they share only 80 OTU's (25%). Variation in the number of OTU's shared among the different arthropod species are visualized in Fig 3. Arthropods within the same genus shared between ~33 to 50% of OTU's while a smaller proportion were unique to each species (between 2 to 20%). The taxonomy of indicator OTU's for each arthropod species in this study identified as significant and with an indicator value over 0.25 are provided in S3 Table.

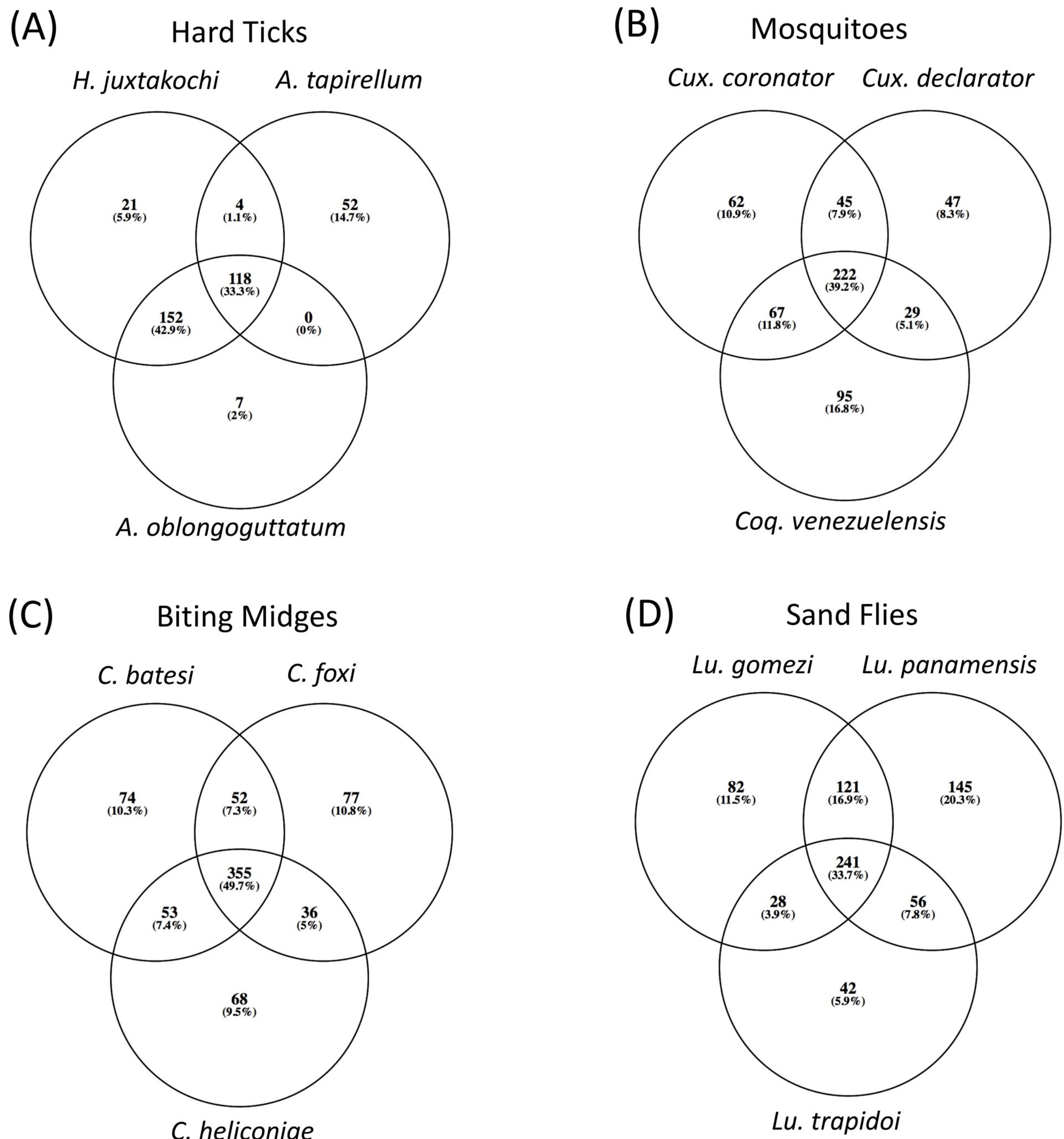


Fig 3. Venn diagram of shared and unique bacterial OTU's among (a) three different species of Ixodidae; (b) two species of *Culex* (Culicidae) and one species of *Coquillettidia* (Culicidae); (c) three species of *Culicoides* (Ceratopogonidae); (d) three species of *Lutzmyia* (Psychodidae).

https://doi.org/10.1371/journal.pone.0222145.g003

Effect of habitat disturbance on the organization of bacterial communities

Intra-specific variation in the bacterial community was observed between sampling areas depicting different degrees of habitat disturbance for *Coquillettidia*, one *Culex* species, and all but one comparison of *Lutzomyia*, while another comparison between *Culex coronator* was close to significant (Table 3). Although bacterial diversity was comparable across pristine and disturbed habitats for most groups overall (S4 Table), we observed differences in the

Table 2. Results of PERMANOVA test for the comparison of bacterial OTU's among pools of six different genera and 10 different species (with within genera comparisons) of blood-feeding arthropods based on unweighted UNIFRAC distances.

Genera comparisons		No.	No. of sample pools	pseudo-F	p-value	q-value
Amblyomma	*Coquilettidia*	195	39	77.076	0.001	0.001
Amblyomma	*Culex*	295	59	95.687	0.001	0.001
Amblyomma	*Culicoides*	2669	113	126.282	0.001	0.001
Amblyomma	*Haemaphysalis*	180	36	82.764	0.001	0.001
Amblyomma	*Lutzomyia*	2137	94	94.831	0.001	0.001
Coquilettidia	*Culex*	300	60	4.267	0.002	0.002
Coquilettidia	*Culicoides*	2674	114	17.305	0.001	0.001
Coquilettidia	*Haemaphysalis*	185	37	69.818	0.001	0.001
Coquilettidia	*Lutzomyia*	2142	95	10.184	0.001	0.001
Culex	*Culicoides*	2774	134	39.82	0.001	0.001
Culex	*Haemaphysalis*	285	57	86.494	0.001	0.001
Culex	*Lutzomyia*	2242	115	23.658	0.001	0.001
Culicoides	*Haemaphysalis*	2574	111	113.82	0.001	0.001
Culicoides	*Lutzomyia*	4616	169	25.43	0.001	0.001
Haemaphysalis	*Lutzomyia*	2127	92	85.509	0.001	0.001
Species comparisons						
A. oblongoguttatum	*A. tapirellum*	85	19	10.489	0.001	0.001
Cux. coronator	*Cux. declarator*	200	40	2.099	0.025	0.026
C. batesi	*C. foxi*	1825	64	1.499	0.102	0.102
C. batesi	*C. heliconiae*	1715	64	2.159	0.012	0.013
C. foxi	*C. heliconiae*	1608	60	1.611	0.064	0.065
Lu. gomezi	*Lu. panamensis*	1419	53	9.881	0.001	0.001
Lu. gomezi	*Lu. trapidoi*	1253	45	8.12	0.001	0.001
Lu. panamensis	*Lu. trapidoi*	1412	52	7.3	0.001	0.001
Life Stage comparisons						
Haemaphysalis adults	*Haemaphysalis* nymphs	85	17	1.38	0.247	0.247

https://doi.org/10.1371/journal.pone.0222145.t002

proportions of a number of bacterial types between pristine and disturbed habitats, although their associations differed within and among arthropod genera and species (Fig 4). For example, there was a high proportion of *Cyanobacteria* in both *Coquillettidia* and *Lutzomyia* from the disturbed sites at ACH and PVAS as well as an increased proportion of *Chlamydiae* for both *Culex* and *Lutzomyia* from the most disturbed site at PVAS. Similarly, there was an increased proportion of *Betaproteobacteria*, Order *Burkholderias* and the *Flavobacteriia*, family *Blattabacteriaceae* in pools of *Culex* from PVAS. Proportions of *Actinobacteria*, *Bacteriodetes*, *Flavobacteria* and *Bacteroidia* increased in *Luztomyia* from disturbed sites, whereas the proportion of *Deltaproteobacteria* increased from the pristine site BCI. Moreover, a number of bacterial classes including Nostococidae, Deltaproteobacteria, Deincoccci, Cytophagia and Chloroplast were found in *Coquillettidia* from the intermediately disturbed site at ACH, but not in the most disturbed site at PVAS. In contrast, within the three species of ticks, there was no difference in the proportion of bacterial classes between sampling areas or sampling method (Table 3, S4 Table and Fig 4). Similarly, no strong differences were detected in the bacterial classes of *Culicoides* among sampling areas or between vertical strata (i.e., forest understory or canopy) (Table 3 and S5 Table).

Table 3. Results of PERMANOVA test for the comparison of bacterial communities in pools of twelve different blood-feeding arthropod species among sampling areas based on unweighted UNIFRAC distances and 999 permutations. Significant results are highlighted in bold.

Taxonomy	Species	Site comparison		No. of sample pools	pseudo-F	p-value	q-value
Diptera:Culicidae	*Coq. venezuelensis*	ACH	PVS	20	6.920	**0.001**	**0.001**
	Cux. coronator	ACH	PVS	20	2.042	0.055	0.055
	Cux. declarator	ACH	PVS	20	3.061	**0.001**	**0.001**
Diptera:Ceratopogonidae	*C. batesi*	ACH	BCI	23	1.797	0.092	0.100
		ACH	PVS	22	2.229	0.027	0.081
		BCI	PVS	23	1.529	0.100	0.100
	C. foxi	ACH	BCI	20	1.627	0.087	0.131
		ACH	PVS	20	1.363	0.196	0.196
		BCI	PVS	20	1.882	0.041	0.123
	C. heliconiae	ACH	BCI	15	1.120	0.356	0.534
		ACH	PVS	15	2.104	0.041	0.123
		BCI	PVS	10	0.868	0.630	0.630
Diptera:Psychodidae	*Lu. gomezi*	ACH	BCI	13	5.341	**0.005**	**0.006**
		ACH	PVS	20	2.709	**0.002**	**0.006**
		BCI	PVS	13	3.311	**0.006**	**0.006**
	Lu. panamensis	ACH	BCI	20	3.033	**0.002**	**0.005**
		ACH	PVS	20	1.581	0.070	0.070
		BCI	PVS	20	2.645	**0.003**	**0.005**
	Lu. trapidoi	ACH	BCI	13	2.936	**0.004**	**0.005**
		ACH	PVS	19	3.107	**0.002**	**0.005**
		BCI	PVS	12	3.458	**0.005**	**0.005**
Acari:Ixodidae	*H. juxtakochi*	BCI	PVAS	17	0.254	0.918	0.918
	A. tapirellum	BCI	PVAS	12	1.584	0.157	0.157
	A. oblongoguttatum	BCI	PVAS	7	2.006	0.133	0.133

https://doi.org/10.1371/journal.pone.0222145.t003

Discussion

Habitat disturbance resulting from land use change can alter arthropod-borne disease transmission dynamics by modifying the habitat characteristics, community composition, behaviour, and patterns of dispersal and distribution of vectors or hosts [55,69,70]. Furthermore, habitat disruption can also modify the bacterial composition of natural environments, such as in the case of soil microbiota [71]. Yet, to our knowledge, no study has looked at the influence of habitat disturbance on the microbiome of human disease vectors, especially those that develop and interact with bacteria in the water, leaf litter, and soil or are acquired through animal host feeding in ecologically altered areas.

We tackled this issue by assessing bacterial communities associated with blood-feeding arthropods across sites with different degrees of habitat disturbance in the lowland tropical rainforest of central Panama. Specifically, we applied a 16S gene bacterial metagenomic approach to evaluate whether variation in the microbiome is associated with taxonomic relatedness, habitat disturbance or a combination of both. We focused on adults of Culicidae mosquitoes (i.e., *Culex* and *Coquillettidia*), Psychodidae sand flies (i.e., *Lutzomyia*) and Ceratopogonidae biting midges (i.e, *Culicoides*), which share ecological similarities in their development and adult life stages. Both *Culex* and *Coquillettidia* mosquitoes develop in aquatic sites associated with the roots of floating plants, while members of *Culicoides* develop in damp soil, water and organic matter [13,72–74]. All species of *Lutzomyia* develop in the soil within dark and humid places such as burrows and crevices associated with abundant leaf-litter or

decomposing organic matter [75]. The males of *Culex*, *Coquillettidia*, *Culicoides* and *Lutzomyia* feed on nectar while the females take blood from a wide range of bird and mammal hosts. In addition, we sampled both nymphs and adult Ixodidae (i.e., *Amblyomma*, *Ixodes*, *Haemaphysalis*), which are distinct in their ecology compared to dipterans. Both the nymphs and adults of hard ticks adhere to and feed on vertebrate hosts throughout their lifetime [76]. Although they spend time off their host to molt through the different life stages and "quest" for a new host, they do not depend on these environments for feeding.

Our results are generally similar to those obtained in previous studies, where arthropod vectors species were dominated by Proteobacteria, including *Gammaproteobacteria*, *Betaproteobacteria*, *Alphaproteobacteria*, and to a lesser extent by Firmicutes, commonly *Bacilli* and *Actinobacteria* [13,18,44–46,77,78], These groups included bacterial genera previously described for *Culex* [13,44,46,77], *Culicoides* [21,38], *Lutzomyia* [37,79], *Haemaphysalis* [78,80] and *Amblyomma* [42,81].

We found that mosquitoes, biting midges and sand flies share a large proportion of their bacteria but statistical analysis also revealed significant differences in the OTU composition of each genera and species. It should be noted that variability at the 16S rRNA region, primer affinity and composition of the bacterial database will influence the resolution of the between-species comparisons based on OTU's [82]. However, this finding suggests that these arthropods might encounter distinct bacterial types associated with differences in their habitat use or diet. Also, the colonization success of these bacterial types could differ among the arthropod hosts. We found that all tick species shared some bacterial OTU's, but that this association did not extend to the dipteran assemblages. This is likely to reflect both their degree of taxonomic relatedness, since phylogenetically related species tend to share similar functional microbiomes [83], but also their distinct ecology. For instance, while all dipteran genera undergo larval development in either aquatic sites or organic soil before blood feeding as adults, hard ticks are largely associated with their host throughout their lifetime. Ticks undergo a series of molting events after each blood meal, which could be obtained from a series of animal hosts, from which they are expected to acquire much of their microbiome [80], while some symbiotic bacteria are also maternally inherited [2]. In contrast, dipteran genera also acquire bacteria through blood feeding, but their microbial community maintained through to adulthood is largely acquired during larval feeding and contact with the physical environment [13,48,49].

We observed significant differences in the bacterial community among areas with different degrees of habitat disturbance for two ecologically similar mosquito species within *Culex* and *Coquillettidia*, and three *Lutzomyia* sand fly species. These differences could be related to changes in the mammal or bird communities that served as feeding choices for adult arthropods as a result of habitat disruption. Alternatively, intra-specific differences could also result from changes to the pool of environmental bacteria, which might be associated with habitat disturbance. In support of these assumptions, we observed differences in a number of environmentally associated bacteria between primary forest, secondary forest and agricultural land, although changes in specific bacterial types generally vary among the different arthropod assemblages. For instance, the *Cyanobacteria nostococidae*, which has previously been associated with aquatic environments inhabited by mosquito larvae [13], was present in both *Culex* and *Lutzomyia* collected from secondary forest and disturbed habitats, but not from pristine forest sites. In addition, it was more common for *Culex* and *Lutzomyia* to be associated with *Chlamydia* in secondary forest and disturbed pastureland than in pristine forest, suggesting either differences in the mammal host reservoir or increased infection of mammals associated with changes in habitat quality.

We did not observe a significant difference in the bacterial community for any *Culicoides* species as a function of habitat disturbance. A potential explanation for this outcome is that

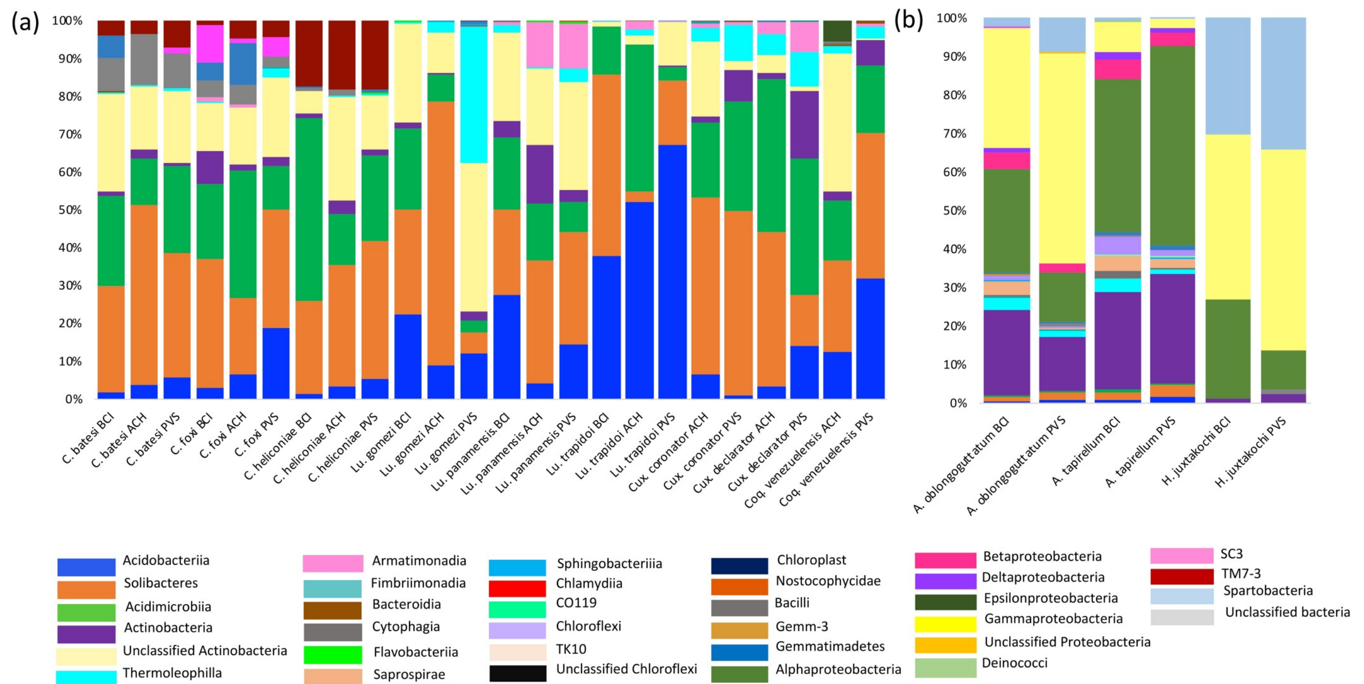


Fig 4. Relative abundances of bacterial classes summarized for (A) dipteran species and (B) hard ticks gathered from BCI (i.e., Pristine), ACH (i.e., intermediately disturbed) and PVAS (i.e., highly disturbed).

https://doi.org/10.1371/journal.pone.0222145.g004

Culicoides species either share a narrow ecological niche or because their optimal breeding habitats are not impacted by habitat disturbance. *Cuilicoides* regularly develop in areas with a high degree of organic matter known to modulate bacterial diversity [84], and are sensitive to temperature and humidity [85]. Nonetheless, the bacterial community of *Culicoides* in their preferred breeding sites has thus far been poorly characterized. Characterization of the differences in microhabitat features in *Culicoides* between land use types is required to confirm whether their breeding habitats and associated microbiota remain stable despite habitat disturbance. Furthermore, the host preferences of *Culicoides*, including the species in the current study are poorly classified and generally unknown within natural habitats, but some studies showed that most *Culicoides* species are opportunistic feeders, while others specialize on birds or mammals [86,87]. Another explanation for the lack of differences in the bacterial community of *Culicoides* between sites could be a stricter association of bacteria with the insect host than for other dipterans. That we did not see significant intra-specific differences in the bacterial community among tick species across areas with different habitat quality is not surprising given their specialized ecology [88].

We identified OTUs of several disease-causing bacteria as well as bacteria thought to alter life history characteristics and/or viral replication in all the arthropod genera, although these could not be identified to species. For example, we amplified *Coxiella*, whose members cause Q fever from all three tick species, *Ehrlichia* which causes ehrlichiosis infection from *A*. *tapirellum* and *Rickettsia* from *A*. *oblongoguttatum* and *H*. *juxtakochi*, which causes a variety of bacterial infections in humans and animals [89]. In addition, *Rickettsia* was also identified from *Lu*. *trapidoi* while *Bartonella* was detected from *Lu*. *panamensis* and *Lu*. *gomezi* plus all three species of *Culicoides*.

Rickettsia rickettsii, known to cause Rocky Mountain spotted fever in Panama has been previously isolated from *Amblyomma mixtum*, *Dermacentor nitens* and *Haemaphysalis*

leporispalustris. In addition, two other *Rickettsia* species have been isolated from ticks in Panama including *Rickettsia bellii* from *Amblyomma rotundatum* and *Rickettsia amblyommii* from *A*. *mixtum* [90]. Although identification of the *Rickettsia* OTU's were not to species level in this study, to our knowledge, this is first record of *Rickettsia* isolated from *A*. *oblongoguttatum* and *H*. *juxtakochi* in Central America as well as from *Lutzomyia spp*. However, agents causing bartonellosis have not yet been described from *Culicoides* biting midges. The ability of *Culicoides* to vector *Bartonella* requires further confirmation, but its presence in all three species is suggestive of a likely transmission role in Panama.

Congruently, we found several genera of bacteria with the potential to impact vector pathogen transmission. For instance, the genus *Paenibacillus*, which can inhibit DENV replication in *Aedes* mosquitoes was present in all *Culicoides* species as well as in *Lu*. *panamensis* [28]. Similarly, *Serratia* which can increase DENV and CHIKV in *Ae*. *aegypti* mosquitoes was present in all species of biting midges, mosquitoes and sand flies [28]. The family *Enterobacteriae*, which has been known to increase *Plasmodium* parasite infection in *Anopheles* mosquitoes was present in all, but *A*. *oblongoguttatum* [36]. Moreover, the bacteria *Wolbachia*, which impacts on vectors of arboviruses, *Plasmodium* infection and life history traits such as reproductive fitness and adult lifespan [91–94] was found from all Diptera.

Conclusion

Habitat disturbance has been shown to increase the likelihood of disease outbreaks of zoonotic (e.g., animal origin) infections through modifying the vector or host communities, or impacting their life history characteristics. However, the epidemiological role of bacteria associated with blood-feeding arthropods in relation to habitat disturbance is still poorly understood. Here, we observed that variation in the bacterial communities across a diverse array of hematophagous arthropods is likely to be explained by host phylogenetic relatedness, while intraspecific changes in community composition and prevalence are influenced by habitat quality. We found that the proportions of known disease-causing agents in infected arthropod species were comparable across sampling areas with different levels of habitat disturbance. However, further work is needed to determine whether the changes to the bacterial community with habitat disruption could influence disease transmission to humans. We argue further that changes in the microbiome of disease vectors should be considered when assessing the impact of habitat disturbance on disease transmission risk and emergence.

Supporting information

S1 Fig. Rarefaction results based on Faith's Phylogenetic Diversity of 12 species of blood-feeding arthropods at a rarefaction depth of 7 000 16S rRNA sequences per species.
(TIF)

S1 Table. Collection and processing information for each pool of arthropod samples.
(XLSX)

S2 Table. Relative proportions of bacteria for each arthropod species and composite individuals.
(XLSX)

S3 Table. The bacteria identified by indicator species analysis as significant for each arthropod species.
(XLSX)

S4 Table. Results of PERMANOVA test for the comparison of bacterial OTU's in pools of three Ixodid species between sampling methods based on unweighted UNIFRAC distances and 999 permutations.
(XLSX)

S5 Table. Results of PERMANOVA test for the comparison of bacterial communities in pools of three *Culicoides* species originating from the ground or canopy level collections based on UNIFRAC distances.
(XLSX)

Acknowledgments

We are grateful to Panama's Ministry of Environment (*Mi Ambiente*) for supporting scientific collecting of insects in Panama.

Author Contributions

Conceptualization: Kelly L. Bennett, Jose R. Loaiza.

Data curation: Kelly L. Bennett.

Formal analysis: Kelly L. Bennett, Jorge S. Vinda.

Funding acquisition: Kelly L. Bennett, W. Owen McMillan, Jose R. Loaiza.

Investigation: Kelly L. Bennett, Alejandro Almanza, Evangelina López Vdovenko, Kaitlin Driesse, Luis F. De León, Jose R. Loaiza.

Methodology: Alejandro Almanza, Kristin Saltonstall, Evangelina López Vdovenko.

Project administration: Kristin Saltonstall.

Supervision: W. Owen McMillan, Jose R. Loaiza.

Visualization: Kelly L. Bennett.

Writing – original draft: Kelly L. Bennett.

Writing – review & editing: Alejandro Almanza, W. Owen McMillan, Kristin Saltonstall, Evangelina López Vdovenko, Jorge S. Vinda, Luis Mejia, Kaitlin Driesse, Luis F. De León, Jose R. Loaiza.

References

1. Minard G, Mavingui P, Moro CV. Diversity and function of bacterial microbiota in the mosquito holobiont. Parasit Vectors. 2013; 6: 146. https://doi.org/10.1186/1756-3305-6-146 PMID: 23688194
2. Bonnet SI, Binetruy F, Hernández-Jarguín AM, Duron O. The tick microbiome: Why non-pathogenic microorganisms matter in tick biology and pathogen transmission. Front Cell Infect Microbiol. 2017; 7: 236. https://doi.org/10.3389/fcimb.2017.00236 PMID: 28642842
3. Telleria EL, Martins-da-Silva A, Tempone AJ, Traub-Csekö YM. Leishmania, microbiota and sand fly immunity. Parasitology. 2018; 145: 1336–1353. https://doi.org/10.1017/S0031182018001014 PMID: 29921334
4. Cirimotich CM, Ramirez JL, Dimopoulos G. Native microbiota shape insect vector competence for human pathogens. Cell Host Microbe. 2011; 10: 307–310. https://doi.org/10.1016/j.chom.2011.09.006 PMID: 22018231
5. Gubler DJ. Resurgent vector-borne diseases as a global health problem. Emerg Infect Dis. 1998; 4: 442–450. https://doi.org/10.3201/eid0403.980326 PMID: 9716967
6. Cantas L, Suer K. Review: The important bacterial zoonoses in "One Health" concept. Frontiers in Public Health. 2014; 2: 144. https://doi.org/10.3389/fpubh.2014.00144 PMID: 25353010

7. Weger-Lucarelli J, Auerswald H, Vignuzzi M, Dussart P, Karlsson EA. Taking a bite out of nutrition and arbovirus infection. PLoS Negl Trop Dis. 2018; 12: e0006247. https://doi.org/10.1371/journal.pntd.0006247 PMID: 29596427

8. Engel P, Moran NA. The gut microbiota of insects–diversity in structure and function. FEMS Microbiol Rev. 2013; 37: 699–735. https://doi.org/10.1111/1574-6976.12025 PMID: 23692388

9. Baxter RHG, Contet A, Krueger K. arthropod innate immune systems and vector-borne diseases. Biochemistry. 2017; 56: 907–918. https://doi.org/10.1021/acs.biochem.6b00870 PMID: 28072517

10. Guégan M, Zouache K, Démichel C, Minard G, Tran Van V, Potier P, et al. The mosquito holobiont: fresh insight into mosquito-microbiota interactions. Microbiome. 2018; 6: 49. https://doi.org/10.1186/s40168-018-0435-2 PMID: 29554951

11. Coon KL, Brown MR, Strand MR. Mosquitoes host communities of bacteria that are essential for development but vary greatly between local habitats. Mol Ecol. 2016; 25: 5806–5826. https://doi.org/10.1111/mec.13877 PMID: 27718295

12. Buck M, Nilsson LKJ, Brunius C, Dabiré RK, Hopkins R, Terenius O. Bacterial associations reveal spatial population dynamics in *Anopheles gambiae* mosquitoes. Sci Rep. 2016; 6: 22806. https://doi.org/10.1038/srep22806

13. Duguma D, Rugman-Jones P, Kaufman MG, Hall MW, Neufeld JD, Stouthamer R, et al. bacterial communities associated with *Culex* mosquito larvae and two emergent aquatic plants of bioremediation importance. PLoS One. 2013; 8: e72522. https://doi.org/10.1371/journal.pone.0072522 PMID: 23967314

14. Thongsripong P, Chandler JA, Green AB, Kittayapong P, Wilcox BA, Kapan DD, et al. Mosquito vector-associated microbiota: Metabarcoding bacteria and eukaryotic symbionts across habitat types in Thailand endemic for dengue and other arthropod-borne diseases. Ecol Evol. 2017; 8: 1352–1368. https://doi.org/10.1002/ece3.3676 PMID: 29375803

15. Yun J-H, Roh SW, Whon TW, Jung M-J, Kim M-S, Park D-S, et al. Insect gut bacterial diversity determined by environmental habitat, diet, developmental stage, and phylogeny of host. Appl Environ Microbiol. 2014; 80: 5254 LP– 5264. https://doi.org/10.1128/AEM.01226-14 PMID: 24928884

16. Tchioffo MT, Boissière A, Abate L, Nsango SE, Bayibéki AN, Awono-Ambéné PH, et al. Dynamics of bacterial community composition in the malaria mosquito's epithelia. Front Microbiol. 2015; 6: 1500. https://doi.org/10.3389/fmicb.2015.01500 PMID: 26779155

17. van Overbeek L, Gassner F, van der Plas CL, Kastelein P, Nunes–da Rocha U, Takken W. Diversity of *Ixodes ricinus* tick-associated bacterial communities from different forests. FEMS Microbiol Ecol. 2008; 66: 72–84. https://doi.org/10.1111/j.1574-6941.2008.00468.x PMID: 18355299

18. Carpi G, Cagnacci F, Wittekindt NE, Zhao F, Qi J, Tomsho LP, et al. Metagenomic profile of the bacterial communities associated with *Ixodes ricinus* ticks. PLoS One. 2011; 6: e25604. https://doi.org/10.1371/journal.pone.0025604 PMID: 22022422

19. Menchaca AC, Visi DK, Strey OF, Teel PD, Kalinowski K, Allen MS, et al. Preliminary assessment of microbiome changes following blood-feeding and survivorship in the *Amblyomma americanum* nymph-to-adult transition using semiconductor sequencing. PLoS One. 2013; 8: e67129. https://doi.org/10.1371/journal.pone.0067129 PMID: 23826210

20. Thapa S, Zhang Y, Allen MS. Effects of temperature on bacterial microbiome composition in *Ixodes scapularis* ticks. Microbiologyopen. 2018; 8: e00719–e00719. https://doi.org/10.1002/mbo3.719 PMID: 30239169

21. Díaz-Sánchez S, Hernández-Jarguín A, Torina A, Fernández de Mera IG, Estrada-Peña A, Villar M, et al. Biotic and abiotic factors shape the microbiota of wild-caught populations of the arbovirus vector *Culicoides imicola*. Insect Mol Biol. 2018; 27: 847–861. https://doi.org/10.1111/imb.12526 PMID: 30058755

22. Weaver SC, Charlier C, Vasilakis N, Lecuit M. Zika, Chikungunya, and other emerging vector-borne viral diseases. Annu Rev Med. 2018; 69: 395–408. https://doi.org/10.1146/annurev-med-050715-105122 PMID: 28846489

23. Sick F, Beer M, Kampen H, Wernike K. *Culicoides* biting midges-underestimated vectors for arboviruses of public health and veterinary importance. Viruses. 2019; 11: 376. https://doi.org/10.3390/v11040376 PMID: 31022868

24. Mediannikov O, Ranque S. Mansonellosis, the most neglected human filariasis. New microbes new Infect. Elsevier; 2018; 26: S19–S22. https://doi.org/10.1016/j.nmni.2018.08.016 PMID: 30402239

25. Courtenay O, Peters NC, Rogers ME, Bern C. Combining epidemiology with basic biology of sand flies, parasites, and hosts to inform leishmaniasis transmission dynamics and control. PLOS Pathog. 2017; 13: e1006571. https://doi.org/10.1371/journal.ppat.1006571 PMID: 29049371

26. Wikel SK. Ticks and tick-borne infections: Complex ecology, agents, and host interactions. Vet Sci. 2018; 5: 60. https://doi.org/10.3390/vetsci5020060 PMID: 29925800

27. Altizer S, Dobson A, Hosseini P, Hudson P, Pascual M, Rohani P. Seasonality and the dynamics of infectious diseases. Ecol Lett. 2006; 9: 467–484. https://doi.org/10.1111/j.1461-0248.2005.00879.x PMID: 16623732

28. Hegde S, Rasgon JL, Hughes GL. The microbiome modulates arbovirus transmission in mosquitoes. Curr Opin Virol. 2015; 15: 97–102. https://doi.org/10.1016/j.coviro.2015.08.011 PMID: 26363996

29. Weiss B, Aksoy S. Microbiome influences on insect host vector competence. Trends Parasitol. 2011; 27: 514–522. https://doi.org/10.1016/j.pt.2011.05.001 PMID: 21697014

30. Dodson BL, Hughes GL, Paul O, Matacchiero AC, Kramer LD, Rasgon JL. Wolbachia enhances West Nile Virus (WNV) infection in the mosquito *Culex tarsalis*. PLoS Negl Trop Dis. 2014; 8: e2965. https://doi.org/10.1371/journal.pntd.0002965 PMID: 25010200

31. Glaser RL, Meola MA. The native Wolbachia endosymbionts of *Drosophila melanogaster* and *Culex quinquefasciatus* increase host resistance to West Nile virus infection. PLoS One. 2010; 5. https://doi.org/10.1371/journal.pone.0011977 PMID: 20700535

32. Aliota MT, Walker EC, Uribe Yepes A, Dario Velez I, Christensen BM, Osorio JE. The wMel strain of Wolbachia reduces transmission of Chikungunya Virus in *Aedes aegypti*. PLoS Negl Trop Dis. 2016; 10: e0004677. https://doi.org/10.1371/journal.pntd.0004677 PMID: 27124663

33. Walker T, Johnson PH, Moreira LA, Iturbe-Ormaetxe I, Frentiu FD, McMeniman CJ, et al. The wMel Wolbachia strain blocks dengue and invades caged *Aedes aegypti* populations. Nature. 2011; 476: 450. https://doi.org/10.1038/nature10355 PMID: 21866159

34. van den Hurk AF, Hall-Mendelin S, Pyke AT, Frentiu FD, McElroy K, Day A, et al. Impact of Wolbachia on infection with Chikungunya and Yellow Fever Viruses in the mosquito vector *Aedes aegypti*. PLoS Negl Trop Dis. 2012; 6: e1892. https://doi.org/10.1371/journal.pntd.0001892 PMID: 23133693

35. Novakova E, Woodhams DC, Rodríguez-Ruano SM, Brucker RM, Leff JW, Maharaj A, et al. Mosquito microbiome dynamics, a background for prevalence and seasonality of West Nile Virus. Frontiers in Microbiology. 2017; 8: 526. https://doi.org/10.3389/fmicb.2017.00526 PMID: 28421042

36. Boissière A, Tchioffo MT, Bachar D, Abate L, Marie A, Nsango SE, et al. Midgut microbiota of the malaria mosquito vector *Anopheles gambiae* and interactions with *Plasmodium falciparum* infection. PLoS Pathog. 2012; 8. https://doi.org/10.1371/journal.ppat.1002742 PMID: 22693451

37. Kelly PH, Bahr SM, Serafim TD, Ajami NJ, Petrosino JF, Meneses C, et al. The gut microbiome of the vector *Lutzomyia longipalpis* is essential for survival of *Leishmania infantum*. MBio. 2017; 8: e01121–16. https://doi.org/10.1128/mBio.01121-16 PMID: 28096483

38. Campbell CL, Mummey DL, Schmidtmann ET, Wilson WC. Culture-independent analysis of midgut microbiota in the arbovirus vector *Culicoides sonorensis* (Diptera: Ceratopogonidae). J Med Entomol. 2004; 41. https://doi.org/10.1603/0022-2585-41.3.340 PMID: 15185934

39. Zhang X-C, Yang Z-N, Lu B, Ma X-F, Zhang C-X, Xu H-J. The composition and transmission of microbiome in hard tick, *Ixodes persulcatus*, during blood meal. Ticks Tick Borne Dis. 2014; 5: 864–870. https://doi.org/10.1016/j.ttbdis.2014.07.009 PMID: 25150725

40. Lalzar I, Harrus S, Mumcuoglu KY, Gottlieb Y. Composition and seasonal variation of *Rhipicephalus turanicus* and *Rhipicephalus sanguineus* bacterial communities. Appl Environ Microbiol. 2012; 78: 4110–4116. https://doi.org/10.1128/AEM.00323-12 PMID: 22467507

41. Gall CA, Reif KE, Scoles GA, Mason KL, Mousel M, Noh SM, et al. The bacterial microbiome of *Dermacentor andersoni* ticks influences pathogen susceptibility. Isme J. 2016; 10: 1846. https://doi.org/10.1038/ismej.2015.266 PMID: 26882265

42. Williams-Newkirk AJ, Rowe LA, Mixson-Hayden TR, Dasch GA. Characterization of the bacterial communities of life stages of free living lone star ticks (*Amblyomma americanum*). PLoS One. 2014; 9: e102130. https://doi.org/10.1371/journal.pone.0102130 PMID: 25054227

43. Louradour I, Monteiro CC, Inbar E, Ghosh K, Merkhofer R, Lawyer P, et al. The midgut microbiota plays an essential role in sand fly vector competence for *Leishmania major*. Cell Microbiol. 2017; 19: e12755. https://doi.org/10.1111/cmi.12755 PMID: 28580630

44. Muturi EJ, Ramirez JL, Rooney AP, Kim C-H. Comparative analysis of gut microbiota of mosquito communities in central Illinois. PLoS Negl Trop Dis. 2017; 11: e0005377. https://doi.org/10.1371/journal.pntd.0005377 PMID: 28245239

45. Bennett KL, Gómez-Martínez C, Chin Y, Saltonstall K, McMillan WO, Rovira JR, et al. Dynamics and diversity of bacteria associated with the biological competitors and disease vectors *Aedes aegypti* and *Aedes albopictus*. Sci Rep. 21;9:12160. https://doi.org/10.1038/s41598-019-48414-8 PMID: 31434963

46. Osei-Poku J, Mbogo CM, Palmer WJ, Jiggins FM. Deep sequencing reveals extensive variation in the gut microbiota of wild mosquitoes from Kenya. Mol Ecol. 2012;21. https://doi.org/10.1111/j.1365-294X.2012.05759.x PMID: 22988916

47. Kurilshikov A, Livanova NN, Fomenko N V, Tupikin AE, Rar VA, Kabilov MR, et al. Comparative metagenomic profiling of symbiotic bacterial communities associated with *Ixodes persulcatus*, *Ixodes pavlovskyi*and and *Dermacentor reticulatus* ticks. PLoS One. 2015; 10: e0131413. https://doi.org/10.1371/journal.pone.0131413 PMID: 26154300

48. Gimonneau G, Tchioffo MT, Abate L, Boissière A, Awono-Ambéné PH, Nsango SE, et al. Composition of *Anopheles coluzzii* and *Anopheles gambiae* microbiota from larval to adult stages. Infect Genet Evol. 2014; 28: 715–724. https://doi.org/10.1016/j.meegid.2014.09.029 PMID: 25283802

49. Coon KL, Vogel KJ, Brown MR, Strand MR. Mosquitoes rely on their gut microbiota for development. Mol Ecol. 2014; 23: 2727–2739. https://doi.org/10.1111/mec.12771 PMID: 24766707

50. Dickson LB, Jiolle D, Minard G, Moltini-Conclois I, Volant S, Ghozlane A, et al. Carryover effects of larval exposure to different environmental bacteria drive adult trait variation in a mosquito vector. Sci Adv. 2017; 3: e1700585. https://doi.org/10.1126/sciadv.1700585 PMID: 28835919

51. Monteiro CC, Villegas LEM, Campolina TB, Pires ACMA, Miranda JC, Pimenta PFP, et al. Bacterial diversity of the American sand fly *Lutzomyia intermedia* using high-throughput metagenomic sequencing. Parasit Vectors. 2016; 9: 480. https://doi.org/10.1186/s13071-016-1767-z PMID: 27581188

52. Narasimhan S, Fikrig E. Tick microbiome: the force within. Trends Parasitol. 2015; 31: 315–323. https://doi.org/10.1016/j.pt.2015.03.010 PMID: 25936226

53. Zouache K, Raharimalala FN, Raquin V, Tran-Van V, Raveloson LHR, Ravelonandro P, et al. Bacterial diversity of field-caught mosquitoes, *Aedes albopictus* and *Aedes aegypti*, from different geographic regions of Madagascar. FEMS Microbiol Ecol. 2011;75. https://doi.org/10.1111/j.1574-6941.2010.01012.x PMID: 21175696

54. Hawlena H, Rynkiewicz E, Toh E, Alfred A, Durden LA, Hastriter MW, et al. The arthropod, but not the vertebrate host or its environment, dictates bacterial community composition of fleas and ticks. ISME J. 2013; 7: 221–223. https://doi.org/10.1038/ismej.2012.71 PMID: 22739493

55. Loaiza JR, Dutari LC, Rovira JR, Sanjur OI, Laporta GZ, Pecor J, et al. Disturbance and mosquito diversity in the lowland tropical rainforest of central Panama. Sci Rep. 2017; 7: 7248. https://doi.org/10.1038/s41598-017-07476-2 PMID: 28775261

56. Eastwood G, Loaiza JR, Pongsiri MJ, Sanjur OI, Pecor JE, Auguste AJ, et al. Enzootic arbovirus surveillance in forest habitat and phylogenetic characterization of novel isolates of Gamboa Virus in Panama. Am J Trop Med Hyg. 2016; 94: 786–793. https://doi.org/10.4269/ajtmh.15-0445 PMID: 26834200

57. Loaiza JR, Dutari LC, Rovira JR, Sanjur OI, Laporta GZ, Pecor J, et al. Disturbance and mosquito diversity in the lowland tropical rainforest of central Panama. Sci Rep. 2017; 7: 7248. https://doi.org/10.1038/s41598-017-07476-2 PMID: 28775261

58. Pecor JE, Mallampalli VL, Harbach RE, Peyton EL. Catalog and Illustrated Review of the Subgenus Melanoconion of Culex (Diptera: Culicidae). Contrib Am Entomol Inst 27: 1–228.

59. Wilkerson RC, Peyton EL. Standardized nomenclature for the costal wing spots of the genus *Anopheles* and other spotted-wing mosquitoes (Diptera: Culicidae). J Med Entomol. 1990; 27: 207–224.

60. Sallum MA, Forattini OP. Revision of the Spissipes section of *Culex* (Melanoconion)(Diptera: Culicidae). J Am Mosq Control Assoc. 1996; 12: 517–600. PMID: 8887711

61. Chaniotis BN, Correa MA. comparative flying and biting activity of Panamanian Phlebotomine sandflies in a mature forest and adjacent open space. J Med Entomol. 1974; 11: 115–116. https://doi.org/10.1093/jmedent/11.1.115 PMID: 4828344

62. Young DG, Duran MA. Guide to the identification and geographic distribution of *Lutzomyia* sand flies in Mexico, the West Indies, Central and South America (Diptera: Psychodidae). Mem Am Entomol Inst. 1994. 54:881.

63. Gray JS. A carbon dioxide trap for prolonged sampling of *Ixodes ricinus* L. populations. Exp Appl Acarol. 1985; 1: 35–44. PMID: 3939717

64. Fairchild GB, Kohls GM, Tipton VJ. The ticks of Panama (Acarina: Ixodoidea). Ectoparasites of Panama. Field Museum Nat Hist, Chicago 1966. 167–219.

65. Mesa EO. Las garrapatas de la República de Colombia. Rev Fac Nac Agron. 1942; 5: 57–103.

66. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, et al. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc Natl Acad Sci U S A. 2011; 108: 4516–4522. https://doi.org/10.1073/pnas.1000080107 PMID: 20534432

67. Collado L, Figueras MJ. Taxonomy, epidemiology, and clinical relevance of the genus *Arcobacter*. Clin Microbiol Rev. 2011; 24: 174–192. https://doi.org/10.1128/CMR.00034-10 PMID: 21233511

68. Chigira A, Miura K. Detection of '*Candidatus cardinium*' bacteria from the haploid host *Brevipalpus californicus* (Acari: Tenuipalpidae) and effect on the host. Exp Appl Acarol. 2005; 37: 107–116. https://doi.org/10.1007/s10493-005-0592-4 PMID: 16180076

69. Gottdenker NL, Streicker DG, Faust CL, Carroll CR. anthropogenic land use change and infectious diseases: A review of the evidence. Ecohealth. 2014; 11: 619–632. https://doi.org/10.1007/s10393-014-0941-z PMID: 24854248

70. Keesing F, Belden LK, Daszak P, Dobson A, Harvell CD, Holt RD, et al. Impacts of biodiversity on the emergence and transmission of infectious diseases. Nature. 2010; 468: 647. https://doi.org/10.1038/nature09575 PMID: 21124449

71. Rampelotto PH, de Siqueira Ferreira A, Barboza ADM, Roesch LFW. Changes in diversity, abundance, and structure of soil bacterial communities in Brazilian savanna under different land use systems. Microb Ecol. 2013; 66: 593–607. https://doi.org/10.1007/s00248-013-0235-y PMID: 23624541

72. Serandour J, Willison J, Thuiller W, Ravanel P, Lemperiere G, Raveton M. Environmental drivers for *Coquillettidia* mosquito habitat selection: A method to highlight key field factors. Hydrobiologia. 2010. https://doi.org/10.1007/s10750-010-0372-y

73. Reisen WK. The contrasting bionomics of *Culex* mosquitoes in western North America. J Am Mosq Control Assoc. 2012; 28: 82–91. https://doi.org/10.2987/8756-971X-28.4.82 PMID: 23401947

74. Carpenter S, Groschup MH, Garros C, Felippe-Bauer ML, Purse B V. *Culicoides* biting midges, arboviruses and public health in Europe. Antiviral Res. 2013; 100: 102–113. https://doi.org/10.1016/j.antiviral.2013.07.020 PMID: 23933421

75. Vivero RJ, Torres-Gutierrez C, Bejarano EE, Peña HC, Estrada LG, Florez F, et al. Study on natural breeding sites of sand flies (Diptera: Phlebotominae) in areas of Leishmania transmission in Colombia. Parasit Vectors. 2015; 8: 116. https://doi.org/10.1186/s13071-015-0711-y PMID: 25888915

76. Mccoy KD, Léger E, Dietrich M. Host specialization in ticks and transmission of tick-borne diseases: A review. Front Cell Infect Microbiol. 2013; 3: 57. https://doi.org/10.3389/fcimb.2013.00057 PMID: 24109592

77. Sant'anna MR, Darby AC, Brazil RP, Montoya-Lerma J, Dillon VM, Bates PA, et al. Investigation of the bacterial communities associated with females of *Lutzomyia* sand fly species from South America. PLoS One. 2012; 7. https://doi.org/10.1371/journal.pone.0042531 PMID: 22880020

78. Khoo J-J, Chen F, Kho KL, Ahmad Shanizza AI, Lim F-S, Tan K-K, et al. Bacterial community in *Haemaphysalis* ticks of domesticated animals from the Orang Asli communities in Malaysia. Ticks Tick Borne Dis. 2016; 7: 929–937. https://doi.org/10.1016/j.ttbdis.2016.04.013 PMID: 27132518

79. Pires ACAM Villegas LEM, Campolina TB Orfanó AS, Pimenta PFP Secundino NFC. Bacterial diversity of wild-caught *Lutzomyia longipalpis* (a vector of zoonotic visceral leishmaniasis in Brazil) under distinct physiological conditions by metagenomics analysis. Parasit Vectors. 2017; 10: 627. https://doi.org/10.1186/s13071-017-2593-7 PMID: 29284535

80. Qiu Y, Nakao R, Ohnuma A, Kawamori F, Sugimoto C. Microbial population analysis of the salivary glands of ticks; A possible strategy for the surveillance of bacterial pathogens. PLoS One. 2014; 9: e103961. https://doi.org/10.1371/journal.pone.0103961 PMID: 25089898

81. Budachetri K, Browning RE, Adamson SW, Dowd SE, Chao C-C, Ching W-M, et al. An insight into the microbiome of the *Amblyomma maculatum* (Acari: Ixodidae). J Med Entomol. 2014; 51: 119–129. https://doi.org/10.1603/me12223 PMID: 24605461

82. Poretsky R, Rodriguez-R LM, Luo C, Tsementzi D, Konstantinidis KT. Strengths and limitations of 16S rRNA gene amplicon sequencing in revealing temporal microbial community dynamics. PLoS One. 2014; 9: e93827. https://doi.org/10.1371/journal.pone.0093827 PMID: 24714158

83. Degli Esposti M, Martinez Romero E. The functional microbiome of arthropods. PLoS One. 2017; 12: e0176573–e0176573. https://doi.org/10.1371/journal.pone.0176573 PMID: 28475624

84. Neumann D, Heuer A, Hemkemeyer M, Martens R, Tebbe CC. Importance of soil organic matter for the diversity of microorganisms involved in the degradation of organic pollutants. ISME J. 2014; 8: 1289–1300. https://doi.org/10.1038/ismej.2013.233 PMID: 24430482

85. Parras MA, Rosa JR, Szelag EA, Salomón OD. Identification of the natural breeding sites of sandflies (Diptera: Psychodidae: Phlebotominae), potential vectors of leishmaniasis, in the province of Chaco, Argentina. Memórias do Instituto Oswaldo Cruz. 2012. 107: 550–552.

86. Santiago-Alarcon D, Havelka P, Schaefer HM, Segelbacher G. bloodmeal analysis reveals avian Plasmodium infections and broad host preferences of *Culicoides* (Diptera: Ceratopogonidae) vectors. PLoS One. 2012; 7: e31098. https://doi.org/10.1371/journal.pone.0031098 PMID: 22363557

87. Lassen SB, Nielsen SA, Kristensen M. Identity and diversity of blood meal hosts of biting midges (Diptera: Ceratopogonidae: Culicoides Latreille) in Denmark. Parasit Vectors. 2012; 5: 143. https://doi.org/10.1186/1756-3305-5-143 PMID: 22824422

88. Esser HJ, Herre EA, Blüthgen N, Loaiza JR, Bermúdez SE, Jansen PA. Host specificity in a diverse Neotropical tick community: an assessment using quantitative network analysis and host phylogeny. Parasit Vectors. 2016; 9: 372. https://doi.org/10.1186/s13071-016-1655-6 PMID: 27357506

89. Parola P, Raoult D. Ticks and tickborne bacterial diseases in humans: An emerging infectious threat. Clin Infect Dis. 2001; 32: 897–928. https://doi.org/10.1086/319347 PMID: 11247714

90. Lopes MG, May Junior J, Foster RJ, Harmsen BJ, Sanchez E, Martins TF, et al. Ticks and rickettsiae from wildlife in Belize, Central America. Parasit Vectors. 2016; 9: 62. https://doi.org/10.1186/s13071-016-1348-1 PMID: 26831147

91. Hedges LM, Brownlie JC, O'Neill SL, Johnson KN. Wolbachia and virus protection in insects. Science. 2008; 322. https://doi.org/10.1126/science.1162418 PMID: 18974344

92. Kitrayapong P, Baimai V, O'Neill SL. Field prevalence of Wolbachia in the mosquito vector *Aedes albopictus*. Am J Trop Med Hyg. 2002; 66: 108–111. https://doi.org/10.4269/ajtmh.2002.66.108 PMID: 12135259

93. Almeida F de, Moura AS, Cardoso AF, Winter CE, Bijovsky AT, Suesdek L. Effects of Wolbachia on fitness of *Culex quinquefasciatus* (Diptera; Culicidae). Infect Genet Evol. 2011; 11: 2138–2143. https://doi.org/10.1016/j.meegid.2011.08.022 PMID: 21907309

94. Iturbe-Ormaetxe I, Walker T, O' Neill SL. Wolbachia and the biological control of mosquito-borne disease. EMBO Rep. 2011; 12. https://doi.org/10.1038/embor.2011.84 PMID: 21546911