CURRENT STATUS OF METATRANSCRIPTOMIC AND RELATED STUDIES IN HEMATOPHAGOUS DISEASE-TRANSMITTING VECTORS

Christina B. McCarthy[1,2]

[1]Centro Regional de Estudios Genómicos (CREG), Universidad Nacional de La Plata (UNLP), (1900) La Plata, Buenos Aires, Argentina.

[2]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), (C1425FQB) CABA, Argentina.

Correspondence author: Dr Christina B. McCarthy, E-mail: mccarthychristina@gmail.com

Subject editor: Rui-De Xue

ABSTRACT

The incidence of numerous vector-borne diseases (VBDs) has recently increased alarmingly due to various widespread factors, including unplanned urbanization, greater human mobility, environmental changes, vector resistance to insecticides, and evolving pathogens. In this context, the World Health Organization (WHO) has repositioned effective and sustainable vector control as a key approach to prevent and eliminate VBDs. It has been shown that the microbiome influences development, nutrition, and pathogen defense in disease-transmitting vectors such as mosquitoes, sandflies, tsetse flies, triatomine bugs, and ticks. Consequently, understanding the endogenous regulation of vector biology can aid in developing effective approaches for vector control. In this respect, a metatranscriptomic approach analyzes all the expressed RNAs in an environmental sample (meta-RNAs) and can thus reveal how the metabolic activities of the microbiome influence vector biology. This review includes an extensive analysis of available literature on microbial and viral studies for some of the major hematophagous disease-transmitting arthropods, with a focus on studies that used next generation sequencing (NGS) approaches. Since a consensus terminology for these "meta-sequencing analyses" has not yet been established, a definition of these terms is presented here to provide the framework for systematically sorting the available information for each of the VBDs analyzed here to single out metatranscriptomic analyses. Finally, key gaps in knowledge were identified for some of these hematophagous disease-transmitting arthropods which will prove very useful for driving future studies.

Key words: Microbiota, Microbiome, Metabarcoding, Metagenomics, Metatranscriptomics, Vector-borne diseases, Mosquitoes, Sandflies, Tsetse flies, Triatomines, Kissing bugs, Ticks

INTRODUCTION

The importance of vector-borne diseases (VBDs) is indisputable. They represent more than 17% of all infectious diseases and cause more than 700,000 deaths each year (WHO 2017). Many of these vectors are bloodsucking arthropods that transmit disease-causing pathogens when taking a blood meal (WHO 2023a). Mosquitoes head the list with three main disease-transmitting genera: *Anopheles* (malaria), *Aedes* (mainly arboviruses, including dengue, Zika, chikungunya, yellow fever, Rift Valley and others), and *Culex* (West Nile virus and Japanese encephalitis virus); all three genera can vector Lymphatic filariasis (WHO 2023a). Sandflies (mainly leishmaniasis and sandfly fever), tsetse flies (African trypanosomiasis), kissing bugs (Chagas disease), and ticks (Lyme disease, relapsing fever or borreliosis, and Rickettsial diseases such as spotted fever and others), are also responsible for high levels of morbidity and mortality (WHO 2023a, 2017). Effective vaccines and medicines exist for a few of these VBDs, such as vaccines against yellow fever, Japanese encephalitis, and tick-borne encephalitis, medicines against lymphatic filariasis, human onchocerciasis and malaria, but they are currently lacking for most other VBDs. Consequently, vector control is still the most effective preventive approach against the majority of VBDs, and interventions that reduce human-vector contact and vector survival can suppress and even halt transmission (WHO 2017).

In this context, it is now accepted that all eukaryotes are meta-organisms and must be considered together with their microbiome as an inseparable functional unit (Jones 2013). The microbiome forms a dynamic and interactive micro-ecosystem that is integrated to the eukaryotic host and, as such, is crucial for its correct functioning and health (Berg et al. 2020). Consequently, an extensive understanding of the microbiome is pivotal for developing

effective vector control approaches. A significant progress in this respect has been witnessed for the past decade, particularly since the development of high throughput sequencing platforms which have transformed the field of microbial community analysis. Nevertheless, as usually happens when a certain field grows exponentially, consensus terminology is lacking. The misuse of terms such as microbiota, microbiome, metagenome, metagenomics, metabarcoding, metataxonomics and metatranscriptomics, among others, has contributed to the misinterpretation of many study results (Marchesi and Ravel 2015). In this review these terms are clearly defined (see below) based on different previous proposals, to establish a baseline and avoid confusion when interpreting the data and drawing conclusions.

The **microbiota** encompasses the living prokaryotic (Bacteria, Archaea) and eukaryotic (*e.g.*, Protozoa, Fungi, and Algae) microorganisms present in a defined environment (Marchesi and Ravel 2015). Viruses, plasmids, prions, viroids, and free (or "relic") DNA are not part of the microbiota because they are not living microorganisms (Dupré and O'Malley 2013).

On the other hand, the **microbiome** includes the community of microorganisms and their "theatre of activity" (Whipps et al. 1988). The latter encompasses all molecules produced by microorganisms (structural elements [nucleic acids, proteins, lipids, polysaccharides], metabolites [signaling molecules, toxins, organic, and inorganic molecules]), as well as viruses and "relic" DNA. Consequently, microorganisms, viruses, plasmids, prions, viroids, and free DNA, are all part of the microbiome (Berg et al. 2020).

Metataxonomics covers the large-scale analysis of sequencing data (DNA or RNA) to identify microorganisms and/or viruses from complex environmental samples. Metataxonomic studies can be undertaken using two main approaches (see below): 1) metabarcoding and 2) metagenomic/metatranscriptomic shotgun DNA/RNA sequencing as modified from (Cox et al. 2017).

Metabarcoding is the large-scale analysis of biodiversity (*i.e.*, species composition within a sample) through the amplification and sequencing of homologous genes (Creer et al. 2010), such as the mitochondrial protein-coding gene cytochrome c oxidase subunit I (COI) for animal specimens (Hebert et al. 2003), the 16S ribosomal DNA gene region for bacteria and archaea (Tringe and Hugenholtz 2008), and the 18S ribosomal DNA gene region for microbial eukaryotes (Creer et al. 2010). Thus, barcoding of environmental DNA/RNA (or eDNA/eRNA) enables the simultaneous identification of many taxa within the same sample (Wikipedia 2023), and eRNA metabarcoding also provides a measure of the active or viable community (Mengoni et al. 2005).

A **metagenome** is the collection of genomes and genes of the microbiota, and is obtained through shotgun sequencing of DNA extracted from a sample (**metagenomics**). The sequencing data is first assembled and mapped, or directly mapped, to a reference database, and finally annotated. It thus provides information on the functional *potential* of the microbiota.

Since a metabarcoding analysis is based on the amplification and sequencing of taxonomic marker genes, it is not metagenomics (Marchesi and Ravel 2015).

While metagenomics provides information on the putative activities of a microbial community, it cannot reveal the activities that are occurring at a specific time and place, nor how those activities change in response to the environment or to biotic interactions. The challenge is to discover which of those potential functions (metagenome) are happening at a particular point in time (metatranscriptome) and, ultimately, to identify what causes the difference (Moran 2009).

Thus, **metatranscriptomics** analyses all the expressed RNAs in an environmental sample (meta-RNAs) by next generation sequencing (NGS) of the corresponding meta-cDNAs (Marchesi and Ravel 2015). Consequently, it has the potential to identify all the taxa within an environment (and not just those within a targeted lineage, as is the case with metabarcoding) and also provides a comprehensive overview of the loci that are being transcribed and of their expression levels (Galen et al. 2020). Therefore, metatranscriptomics is a more informative approach compared to metagenomics, because it not only characterizes the genetic content (as in a metagenomic analysis) but also identifies the populations that are transcriptionally active (Bashiardes et al. 2016). Metatranscriptomics has been used for determining the functional profile of the microbiome, but it also has the potential to detect and classify RNA from different lineages (metataxonomics), and this latter aspect has been exploited to detect and characterize viruses (Batson et al. 2021; Marcelino et al. 2019; Ortiz-Baez et al. 2020; Westreich et al. 2019).

METHODS

To attain an exhaustive review of published literatures on microbial and viral studies for the hematophagous disease-transmitting arthropods included in this review (mosquitoes, sand flies, tsetse flies, triatomines, and ticks), with a focus on studies that used NGS approaches, a literature search was conducted on English databases,

mainly PubMed and Google up to 28 September 2023, using a set of terms without language or publication-type restrictions.

The first and necessary step to provide the framework for systematically sorting the available information for each of the vectors analyzed here, and then singling out the metatranscriptomic analyses, consisted in defining the terms used in this review (microbiota, microbiome, metagenome, metagenomics, metabarcoding, metataxonomics and metatranscriptomics). For this, a database search was performed using keywords that included: "Microbiota", "Microbiome", "Metabarcoding", "Metagenomic", "Metatranscriptomic", "Virome", "Metavirome", "next-generation sequencing", "high-throughput sequencing", "Vector-borne diseases". Following this database search, the title and abstract of the retrieved publications were screened to identify studies and reviews that were potentially eligible for inclusion.

Next, the full texts of likely suitable studies were retrieved and i) further assessed for eligibility, and ii) screened for other relevant studies that may not have been found in the previous step.

In this way, the full text of 26 eligible publications were thoroughly assessed to define the mentioned terms, and thus provide the necessary baseline for interpreting the data that was retrieved in the following step.

The search for each vector was performed separately. Once again, keywords were used for the database searches, that included: "Microbiota", "Microbiome", "Metabarcoding", "Metagenomic", "Metatranscriptomic", "Virome", "Metavirome", "next-generation sequencing", "high-throughput sequencing", and "Mosquitoes", "Sandflies", "Ticks", "Triatomines", "Kissing bugs", "Tsetse flies", depending on the vector.

Following this, the title and abstract of the retrieved publications were screened to identify studies and reviews that were potentially eligible for inclusion.

Next, the full texts of suitable reviews were retrieved and screened to confirm: i) the eligibility of the studies that were selected and retrieved from the database search, and ii) to search for other relevant studies that may not have been found in the database searches.

Subsequently, the full texts of potentially suitable studies were retrieved and i) thoroughly assessed for eligibility, ii) screened for other relevant studies that may not have been found in the previous step, and iii) classified according to the type of analysis (e.g., metabarcoding, metavirome, metatranscriptomic) following the terminology proposed in this review. With respect to this last point, it is important to note that a thorough screening of the full texts was paramount to correctly assign the type of analysis because, due to the mentioned lack of consensus terminology, titles can be misleading. For example, titles of metavirome studies have used the terms "metatranscriptomic" (Feng et al. 2022) or "Shotgun metagenomics" (Aragão et al. 2023). Similarly, the titles of culture -dependent and -independent studies have used the terms "microbial" (Clay et al. 2008) or "microbiota" (Yadav et al. 2015), and thus had to be screened in detail to determine eligibility.

Finally, the full text of 144 suitable publications on microbial and viral studies (including studies and reviews) for mosquitoes, sandflies, tsetse flies, triatomines, and ticks, were thoroughly assessed and used for this review.

The publications that were included in this analysis are mentioned in the text and have been incorporated in the references list.

METATRANSCRIPTOMIC STUDIES IN HEMATOPHAGOUS DISEASE-TRANSMITTING ARTHROPODS

In accordance with the previously defined terms, in this review a metatranscriptomic study was considered as such if it used NGS to analyze all the expressed RNAs (*i.e.*, not only taxonomic marker genes) in the microbiome (or from at least two lineages *e.g.*, prokaryotes and viruses). Consequently, studies that used environmental RNA (eRNA) sequencing to identify only one lineage (*e.g.*, viruses) were considered metataxonomic analyses, and are only mentioned as background information. Furthermore, due to space constraints, this review focuses on the (main) hematophagous disease-transmitting arthropods, *i.e.*, mosquitoes, sandflies, tsetse flies, triatomines, and ticks, which include obligate and non-obligate blood feeders (Beaty and Marquardt 1996). Obligate blood feeders feed exclusively on vertebrate blood during all life stages (*e.g.*, triatomine bugs and ticks) or only as adults (*e.g.*, tsetse flies). Non-obligate blood feeders (*e.g.*, mosquitoes and sandflies) consume organic materials during immature stages and, during adulthood, in addition to blood ingest sugars to obtain energy (Song et al. 2022).

In the following sections the mentioned hematophagous disease-transmitting arthropods are considered individually, and in each case, a brief introduction is included on the pathogens they transmit, vector biology, and available microbial and viral studies. Following that, a specific subsection briefly describes the metatranscriptomic studies for that vector (if there are any).

Mosquitoes (*Diptera: Culicidae*)

There are thousands of mosquito species, but the main disease-transmitting vectors with the greatest threat to public health, belong to the genera *Anopheles*, *Aedes*, and *Culex* (Beaty and Marquardt 1996; Clements 1992; WHO 2023a). Mosquitoes are responsible for transmitting some of the most dangerous pathogens, including protozoa (most importantly *Plasmodium*), filarial nematodes, and viruses (Gabrieli et al. 2021). In 2021 nearly half of the world's population was at risk of malaria, with an estimated 247 million cases and 619,000 deaths worldwide (WHO 2023b). *Culex* spp. mosquitoes transmit both arboviruses, such as West Nile virus (*Flaviviridae*: *Flavivirus*), and filarial parasites, and *Aedes* spp. (mainly *Aedes aegypti* and *Ae. albopictus*) transmit arboviruses of medical importance to animals and humans, including dengue (*Flaviviridae*: *Flavivirus*), *Zika* (*Flaviviridae*: *Flavivirus*) and chikungunya (*Togaviridae*: *Togavirus*) viruses (Weaver et al. 2018; WHO 2017). Some of these pathogens have been wreaking havoc for a long time, and others are emerging or resurging, and have a very real devastating potential (Weaver et al. 2018).

The mosquitoes' immature stages (larvae and pupae) are aquatic, and larvae feed on organic materials. On the other hand, adults are terrestrial and feed on plant saps and nectars, whereas females also ingest animal blood for egg development (Clements 1992). Consequently, the mosquito microbiome is (at least partly) environmentally acquired, and can be found in the midgut, salivary glands and reproductive tracts (Gao et al. 2020). The microbiome affects vector competence, host immune system signaling, and longevity, among others, and as such, is critical for mosquito development (Caragata et al. 2019; Guégan et al. 2018; Strand 2018). Due to its influence on vector-borne pathogen transmission, and potential for vector control, the mosquito microbiome has attracted increasing attention over the past decade. With this escalating interest, analyses of the mosquito microbiome using NGS approaches are generating hundreds of scientific publications every year (Dada et al. 2021b). Of these, the vast majority correspond to metataxonomic analyses that have used either DNA metabarcoding or RNA shotgun sequencing to study the different components of the microbiome separately. The metabarcoding analyses have mainly focused on bacteria (*e.g.*, Boissière et al. 2012; Buck et al. 2016; Coon et al. 2016, 2014; Dada et al. 2021a, 2019; Díaz et al. 2021; Dickson et al. 2017; Duguma et al. 2019; Gimonneau et al. 2014; Hegde et al. 2018; Mancini et al. 2018; Muturi et al. 2016; Osei-Poku et al. 2012; Sharma et al. 2014; Trzebny et al. 2023; Villegas et al. 2018; Wang et al. 2011), but a couple have analyzed the fungal (Tawidian et al. 2021) and eukaryotic (Belda et al. 2017) components, and one metabarcoding study included both prokaryotes and eukaryotes (Thongsripong et al. 2018).

The viral component of the microbiome has also been extensively studied by means of metataxonomic approaches that used meta-RNA shotgun sequencing (*e.g.*, Aragão et al. 2023; Fauver et al. 2016; Feng et al. 2022; Hameed et al. 2021; Li et al. 2023; Liu et al. 2023; Ramírez et al. 2020; Sadeghi et al. 2018; Shi et al. 2015, 2017; Thongsripong et al. 2021; Wu et al. 2023; X. Yang et al. 2023).

Metatranscriptomic studies in mosquitoes

Four metatranscriptomic studies in mosquitoes have been published to date (see Table 1), however, neither of these studies analyzed the metatranscriptomic data to determine the expression profile of the microbiomes. The first one was designed as a proof of concept to characterize the members of the mosquito microbiome (Chandler et al. 2015). The authors used meta-RNA shotgun sequencing on seven individual field-collected female mosquitoes from three species, *Culex pipiens* (Farajollahi et al. 2011), *Culiseta incidens* and *Ochlerotatus sierrensis* (Ledesma and Harrington 2011). Sequences from viruses, bacteria, and fungi were identified in each individual, and mosquito species identities were also verified using the sequencing data. Single stranded RNA viruses of the *Bunyaviridae* and *Rhabdoviridae* were identified, along with an unclassified genus of double-stranded RNA viruses. Further, sequences related to 8 bacterial and 13 fungal families were found across the seven samples. *Bacillus* and *Escherichia/Shigella* were identified in all samples and *Wolbachia* was identified in all *Cx. pipiens* samples, while no single fungal genus was found in more than two samples. This study underscores the advantage of using this approach to characterize the mosquito microbiome and, especially, the value of identifying all the components associated with a specific host (Chandler et al. 2015).

The next metatranscriptomic analysis was published in 2021. In this study, unbiased metatranscriptomic sequencing of 148 individual field-collected adult *Aedes*, *Culex*, and *Culiseta* mosquitoes enabled the detection of sequences from eukaryotes, prokaryotes, and 24 known and 46 novel viral species (Batson et al. 2021). The fact that individual mosquitoes were sequenced added great value to the biological information that was obtained. Among others, it was possible to compute the prevalence of each microbe and the high frequency of viral co-infections, to establish an association between animal pathogens and specific blood meals, and to speciate the host mosquito (Batson et al. 2021).

Table 1: Comparative summary of metatranscriptomic studies (if available) in mosquitoes, sandflies, tsetse flies, triatomines and ticks. The list includes the host and species that were analyzed, country of origin of the specimens and if they were field-collected or lab-reared, developmental stage and sex that were analyzed, what part of the specimen/s was/were analyzed (whole body or certain tissues), the type of RNA that was sequenced (and if there was rRNA depletion), the NGS platform that was used, taxonomic and functional profiling (yes or no), and the corresponding reference. No information was available for tsetse flies, triatomines.

Host	Species	Country of origin (field-collected/ lab-reared)	Developmental stage (sex)	Body/ Tissue	RNA type	Sequencing platform	Taxonomic profiling	Functional profiling	Reference
Mosquitoes	*Culex pipiens*, *Culiseta incidens* and *Ochlerotatus sierrensis*	USA (field-collected)	Adults (females)	Whole body	Total RNA; rRNA subtraction	Illumina HiSeq2000	Yes	No	Chandler et al. 2015
	Aedes, *Culex* and *Culiseta*	USA (field-collected)	Adults (females)	Whole body	Total RNA; rRNA subtraction	Illumina NovaSeq or NextSeq sequencing system	Yes	No	Batson et al. 2021
	Aedes albopictus	USA (F1 lab-reared)	Adults (females)	Whole abdomen and midgut	Total RNA, rRNA subtraction	Illumina HiSeq	Yes	No	Calle-Tobón et al. 2021
	Ae. Albopictus	Germany (field-collected as larvae)	Recently emrged adults (female and male)	Whole body	Total RNA	Ion Torrent	Yes	No	Rau et al. 2022
Sandflies	*Lutzomyia longipalpis*	Argentina and Brazil (field-collected)	Adults (female and male)	Whole body	Total RNA	Pyrosequencing (454 GS FLX Titanium)	Yes	No	McCarthy et al. 2011
	Phlebotomus chinensis	China (field-collected)	Adults (don't specify sex)	Whole body	Total RNA; rRNA subtraction	Illumina NovaSeq	Yes	No	Wang et al. 2022
Ticks	*Ixodes holocyclus*, *Haemaphysalis bancrofti* and *Ixodes trichosuri*	Australia (field-collected)	Nymphs and adults (females and males)	Whole body	Total RNA	Illumina NovaSeq	Yes	No	Gofton et al. 2022

That same year another metatranscriptomic study analyzed total RNA extracted from dissected abdomens of *Ae. albopictus* females fed with sugar and human blood containing either normal or heat-inactivated serum, to evaluate the effect of heat inactivation on gene expression in the mosquitoes, and on the bacterial and viral components of their microbiome (Calle-Tobón et al. 2021). The authors found that at least 600 host genes showed a modified expression profile when mosquitoes were fed with normal vs. heat-inactivated-containing blood, and that the bacterial community changed at 6 hours post-feeding. Nevertheless, they did not observe differences in the core viral component of the mosquito microbiome. These results suggest that serum heat inactivation may have a profound effect on mosquito and microbiome metabolism. This study only described the bacterial and viral components of the microbiome.

The most recent metatranscriptomic analysis evaluated the microbiome of *Ae. albopictus* populations in Germany (Rau et al. 2022) where the mosquito specimens collected as larvae in the field from seven German locations were processed immediately after adult emergence, and adults were pooled according to sex before total RNA extraction. Sequence analysis revealed the presence of viruses, bacteria, and fungi. Some of the identified taxa had already been described in *Ae. albopictus*, such as *Wolbachia pipientis*, *Acinetobacter baumannii* or *Usinis virus*. Others had been detected previously in other mosquito species and invertebrates but not in *Ae. albopictus*, including High Island virus, Guapiaçu virus and *Elizabethkingia anophelis*. Lastly, some of the bacteria had not been identified previously in mosquitoes, including *Limnobacter humi*, *Zooglea resiniphila*, and *Chryseobacterium aureum*. The authors also found differences between males

and females: in females more contigs were assigned to bacteria, whereas in males most contigs were assigned to viruses (Rau et al. 2022).

Sandflies (*Diptera*: *Phlebotominae*)

Phlebotomine sandflies can transmit various diseases. Even though the most important are the leishmaniases (Maroli et al. 2013), they also transmit viruses (Alkan et al. 2013; Depaquit et al. 2010) and bacteria (Maroli et al. 2013), although little is known about the molecular interactions of sandflies with viruses and bacteria (Telleria et al. 2018). Phleboviruses are the most significant of the sandfly-borne viruses, causing symptoms that span from short term fever to haemorrhagic fever (Alkan et al. 2013). In South America, sandflies are the most important vectors of *Bartonella bacilliformis*, the etiological agent of bartonellosis (Battisti et al. 2015; Schultz 1968).

Sandflies lay their eggs in moist environments (leaves, soil, animal burrows, and/or tree trunk niches) and immature stages feed on organic materials (Volf et al. 2002). During adulthood they feed on sugars and females also ingest blood (Beaty and Marquardt 1996). Consequently, they are exposed to a wide range of microorganisms and viruses which can become part of their microbiome (Sant'Anna et al. 2012), mainly colonizing the sandfly midgut (Telleria et al. 2018). Sandflies become infected with *Leishmania* when they engorge on host blood to develop eggs and reproduce, and as the parasite develops exclusively in the mid- and hindgut of the sandfly, it coexists and interacts with the gut microbiome (Kelly et al. 2017). Moreover, the gut microbiome has a significant impact on *Leishmania* development (Louradour et al. 2017), and on sandfly fecundity and development (Telleria et al. 2018), which is why it has gained relevance over the last decade (Tabbabi et al. 2022).

Initial approaches to study the sandfly microbiota were culture-dependent (Akhoundi et al. 2012; Dillon et al. 1996; Oliveira et al. 2000; Perira de Oliveira et al. 2001; Volf et al. 2002) but, with the advent of molecular methods, standard bacteriological methods were combined with Sanger-sequencing of clones and culture-independent methods (Campolina et al. 2020; Fraihi et al. 2017; Gouveia et al. 2008; Guernaoui et al. 2011; Gunathilaka et al. 2020; Hillesland et al. 2008; Karimian et al. 2019; Li et al. 2016; Machado et al. 2014; Maleki-Ravasan et al. 2015; Mukhopadhyay et al. 2012; Sant'Anna et al. 2012; Vivero et al. 2016) (reviewed in Tabbabi et al. 2022). Notably, since the development of high throughput platforms, fewer studies have used an NGS approach to analyze the sandfly microbiome compared to other hematophagous arthropods such as mosquitoes and ticks. A few studies have used DNA metabarcoding to describe the bacterial community (*e.g.*, Kelly et al. 2017; Papadopoulos et al. 2020; Pires et al. 2017; Vivero et al. 2021, 2019), and the bacterial and fungal communities (Tabbabi et al. 2021), and one RNA metabarcoding study analyzed 16S rRNA transcripts (Monteiro et al. 2016).

Interestingly, even though sandfly-borne viruses have been extensively studied using traditional methods (reviewed in Ayhan and Charrel 2017; Depaquit et al. 2010; Jancarova et al. 2023), no metataxonomic approach has yet been used to analyze the viral component of the microbiome.

Metatranscriptomic studies in sandflies

To date, only two metatranscriptomic studies have been published for sandflies (Table 1). Neither study analyzed the metatranscriptomic data to determine the functional profile of the microbiome.

The first study analyzed the microbiome associated with field-caught adult male and female *Lutzomyia longipalpis* from an Argentine endemic (Posadas, Misiones) and a Brazilian non-endemic (Lapinha Cave, Minas Gerais) visceral leishmaniasis location (McCarthy et al. 2011). Total RNA was extracted from whole sandflies and submitted to high-throughput pyrosequencing. The diversity of bacterial, fungal, and protist transcripts that were identified mostly confirmed the sandflies' feeding habits and behavioral patterns. Nevertheless, it also suggested that these vectors could possibly be a chance source of dispersal of various animal and plant diseases, such as coccidiosis and malaria. Gregarines (protozoan invertebrate parasites) were also identified, which suggested they could be used as an efficient control method under natural conditions (McCarthy et al. 2011).

The other study analyzed the metatranscriptomes of several adult *Phlebotomus chinensis* populations in China (Wang et al. 2022). This analysis revealed actively replicating/transcribing bacteria, RNA and DNA viruses, and eukaryotic microbes. The authors found that the microbiome represented up to 1.8% of the total non-ribosomal RNA and comprised more than 87 species, 70 of which were novel, including divergent *Flavivirus* and Trypanosomatidae. Importantly, they identified four types of human and/or mammalian pathogens, including two phleboviruses (hedi and wuxiang viruses), one novel spotted fever group *Rickettsia*, and a member of the *Leishmania donovani* complex. This study also showed the ubiquitous presence of *Wolbachia*.

Tsetse flies (*Diptera*: *Glossinidae*)

Tsetse flies (*Glossina* sp.) are the primary vector of *Trypanosoma brucei*, the causal agent of human and domesticated animal African trypanosomiases in sub-Saharan Africa (Wang et al. 2013).

Adult tsetse (males and females) feed exclusively on vertebrate blood and, unlike other oviparous insects, females produce only one egg per gonotrophic cycle (Tobe 1978). Offspring develop in their mother's uterus, immediately pupate after being deposited as 3rd instar larvae (adenotrophic viviparity), and adults emerge after 30 days. A highly modified maternal accessory gland (or milk gland) provides nourishment during larvagenesis (Attardo et al. 2008; Benoit et al. 2012), and maternal milk is the route used by vertically-transmitted symbiotic bacteria to colonize the developing larvae (Wang et al. 2013).

Tsetse harbors various bacterial species. The bacterial community includes 3 maternally-transmitted endosymbionts, and a taxonomically diverse but reduced assemblage acquired from the environment (Wang et al. 2013), particularly from the host skin surface during blood meals (Farikou et al. 2010; Simo et al. 2008). The simplicity of the bacterial microbiota is most probably due to the unique aspects of tsetse fly biology, which significantly limit environmental microbial exposure. Namely, the obligate vertebrate blood feeding lifestyle of adults and the live birth of progeny following intrauterine larval development (Benoit et al. 2015).

Wigglesworthia, *Sodalis* and *Wolbachia* are the three endogenous symbionts. All field-collected tsetse flies examined to date harbor the obligate *Wigglesworthia*, whereas infection prevalence of *Sodalis* in field populations varies from 0 to 85% (Farikou et al. 2011; Maudlin et al. 1990), and *Wolbachia* infection prevalence in field-captured tsetse differs significantly between different host species, and between populations of the same species (Alam et al. 2012; Doudoumis et al. 2012).

Tsetse's association with *Wigglesworthia* is ancient (50-80 million years ago), and the significance of this mutualism has crystalized in the bacteriome structure (Aksoy et al. 1995). This specialized organ is an immunotolerant niche that only harbors *Wigglesworthia* within specialized epithelial cells (bacteriocytes) (Aksoy 2000, 1995). This bacterium is also found extracellularly in milk gland secretions (Attardo et al. 2008). *Wigglesworthia* provides its host with nutritional and immunological benefits, supplying the necessary nutrients that are lacking in the blood diet (Wang et al. 2009). Moreover, in the absence of *Wigglesworthia*, 1) intrauterine larval development is stunted and progeny aborted (Pais et al. 2008; Schlein 1977)), and 2) larval intrauterine development produces adults with a severely compromised immune system (Weiss et al. 2012, 2011).

Sodalis is a gram-negative endosymbiont closely related to free-living Enterobacteriaceae, that is also found in other insects such as stink bugs (Kaiwa et al. 2010) and weevils (Toju et al. 2010). In contrast to *Wigglesworthia*, it exhibits a wide tissue tropism and can be found both intra and extracellularly in various tissues including midgut, fat body, milk gland, salivary glands and hemocoel (Balmand et al. 2013; Cheng and Aksoy 1999). Even though *Sodalis* lacks a clearly defined functional role within its host and is absent in several natural tsetse populations, various studies indicate that it may play a role in tsetse's ability to vector pathogenic trypanosomes. In contrast to *Wigglesworthia*, which increases tsetse refractoriness to trypanosomes, *Sodalis* appears to favor the establishment of trypanosome infections (Wang et al. 2013; Welburn et al. 1993).

Wolbachia is a widespread alpha-proteobacteria endosymbiont that infects approximately 70% of insects, including some tsetse populations (Hilgenboecker et al. 2008). *Wolbachia* is only found intracellularly in tsetse germ line tissues, and can be detected in early oocyte, embryo and larvae (Balmand et al. 2013; Cheng et al. 2000). It is thus transmitted transovarially via germ line cells, in contrast to *Sodalis* and *Wigglesworthia* which are transmitted via milk gland secretions.

Other environmentally acquired bacteria are found in tsetse flies and include members of the phyla Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria. They have been found consistently in different tsetse species captured in geographically distinct localities (Aksoy et al. 2014; Geiger et al. 2009; Lindh and Lehane 2011). Nevertheless, they account for less than 1% of tsetse's bacterial gut microbiota (Aksoy et al. 2014), and their effect on the biology of tsetse flies is still unclear (Gaithuma et al. 2020).

The bacterial component of the microbiome has been studied to a certain degree using culture dependent and independent approaches (Geiger et al. 2011, 2009; Lindh and Lehane 2011), DNA metabarcoding (Aksoy et al. 2014; Doudoumis et al. 2017; Griffith et al. 2018; Tsakeng et al. 2022), and one DNA metabarcoding analysis that simultaneously gauged the bacterial component and the blood meal source (Gaithuma et al. 2020). Compared to other VBDs, the number of NGS studies is very limited.

Many tsetse flies from colonies and natural populations, also harbor a salivary gland-associated rod-shaped, enveloped DNA virus called Salivary Gland Hypertrophy Virus (SGHV) (Jaenson 1978), that can

cause hypertrophy of the salivary glands and gonadal lesions (Jaenson 1978). It is vertically transmitted via maternal milk gland secretions, or horizontally during the feeding process (Abd-Alla et al. 2011). Most SGHV-infected tsetse are asymptomatic and have no apparent loss of host fitness, but flies infected with high virus titers show reduced fecundity and lifespan, and display hypertrophied salivary glands (Abd-Alla et al. 2011; Sang et al. 1999). Infection prevalence of SGHV in field populations varies according to location and species (Malele et al. 2013). Recently, two single-stranded RNA viruses of unknown impact were isolated from a *Glossina morsitans morsitans* colony (*Glossina morsitans morsitans iflavirus* (GmmIV) and *Glossina morsitans morsitans negevirus* (GmmNegeV)) (Meki et al. 2021). Results revealed potential horizontal viral transmission during feeding and/or vertical viral transmission from parent to offspring (Meki et al. 2021). Another study that analyzed public tsetse RNA-seq libraries (mainly from laboratory colonies), identified the genomes of four iflaviruses (Manni and Zdobnov 2021). The iflavirus identified in *G. morsitans* (GliflaV1) was found in all 136 available *G. morsitans* RNA-seq libraries, and displayed a broad tissue tropism and high abundance, reaching up to 15% of library content. Its ubiquitous distribution and presence in the reproductive tissues, intrauterine larvae, and teneral flies suggest it could be part of the initial core microbiota maternally transmitted to the progeny (Manni and Zdobnov 2021).

Nevertheless, no meta-RNA sequencing approach has been undertaken to characterize the viral component of the tsetse microbiome.

Metatranscriptomic studies in tsetse flies

No metatranscriptomic approach has yet been undertaken to identify the prokaryotic, eukaryotic, and viral composition of the tsetse microbiome (Table 1).

Two studies addressed tsetse-*Wigglesworthia* mutualism through dual RNA sequencing (Bing et al. 2017; Munoz et al. 2017). Briefly, one of these studies characterized the expression profile of the tsetse-*Wigglesworthia* association within the bacteriomes of field captured adult tsetse (*Glossina pallidipes*) from Kenya, with the objective of understanding these interactions within the host's natural setting (Munoz et al. 2017). The other study used colony-reared individuals to perform a dual RNA-seq analysis of the bacteriome, coupled with a metabolomic analysis of the bacteriome and haemolymph collected from normal and symbiont-cured (sterile) females (Bing et al. 2017).

Recently, a comparative transcriptomic analysis was performed between *Glossina morsitans* and *G. brevipalpis* tenerals (Medina Munoz et al. 2021). Because these newly emerged adults have not yet fed, their digestive tract microbiota only consists of the core bacteria seeded through maternal milk gland secretions, namely *Wigglesworthia* and *Sodalis* (Medina Munoz et al. 2021). Although a more diverse bacterial community has been reported in the digestive tracts of adults, these environmentally acquired bacteria are lacking within tenerals. Consequently, the mentioned study only compared the *Wigglesworthia*, *Sodalis* and tsetse transcriptomes (Medina Munoz et al. 2021).

As none of these studies included the eukaryotic and/or viral components of the microbiome, in this review they were not considered metatranscriptomic analyses.

Kissing bugs (*Hemiptera*: *Reduviidae*: *Triatominae*)

Triatomines, also known as kissing bugs, vector *Trypanosoma cruzi*, the etiological agent of Chagas' disease (WHO 2023c). An estimated 6-7 million people worldwide are infected with *T. cruzi*, leading to around 12,000 deaths each year and some 75 million people at risk of infection, mainly in Latin America (WHO 2023d).

Triatomines typically live in home walls or roof cracks and peridomiciliary structures of rural or suburban areas. They usually feed at night, and the parasites enter the body when the person inadvertently smudges the faeces or urine into the bite, other skin breaks, the eyes or the mouth (WHO 2023c).

Triatomines feed exclusively on vertebrate blood throughout their developmental cycle and, as other hematophagous vectors, they harbor beneficial symbionts whose primary role is to supply them with nutrients that are lacking in the diet (Salcedo-Porras et al. 2020). Symbionts are extracellular, reside in the midgut and hindgut lumens (Brecher and Wigglesworth 1944; Duncan 1926; Wigglesworth 1936), and are required for the insect's development and survival (Brecher and Wigglesworth 1944; Durvasula et al. 2008; Vallejo et al. 2009; Yassin 2005). Symbionts include *Rhodococcus rhodnii*, *Corynebacterium* sp. and *Nocardia* sp. (Salcedo-Porras et al. 2020), and are transmitted from parent to offspring by coprophagy (Salcedo-Porras et al. 2020).

The first study to use 16S rRNA gene amplification in triatomines identified only one bacterium in *Triatoma infestans* (Hypša and Dale 1997). Since then, culture-dependent (Lopez-Ordonez et al. 2018), culture-independent (da Mota et al. 2012; Gumiel et al. 2015), and high-throughput sequencing approaches have been used to gain a more comprehensive view of the bacterial component of the microbiome. The latter have mostly used bacterial metabarcoding (Brown et al. 2020; Díaz

et al. 2016; Kieran et al. 2019; Lima et al. 2018; Mann et al. 2020; McCall et al. 2018; Montoya-Porras et al. 2018; Oliveira et al. 2018; Orantes et al. 2018; Rodríguez-Ruano et al. 2018; Tarabai et al. 2023; Waltmann et al. 2019), a few have used DNA metabarcoding to identify various components simultaneously (bacteria, vertebrate hosts, parasite diversity and, in one case, triatomine bugs) (Dumonteil et al. 2020, 2018; Murillo-Solano et al. 2021), one metataxonomic study used shotgun pyrosequencing to describe cultivable bacteria (Carels et al. 2017), and another metataxonomic study used an interesting Restriction-site Associated DNA sequencing (RADSeq)-based analysis to simultaneously study the vector, the parasite, bacteria and feeding patterns (Orantes et al. 2018) (reviewed by Salcedo-Porras et al. 2020). Various of these studies have reported low bacterial diversity in the triatomine microbiome in comparison to other insect groups (da Mota et al. 2012; Gumiel et al. 2015; Lopez-Ordonez et al. 2018). Nevertheless, the triatomine microbiome harbors a broad spectrum of eukaryotic organisms and viruses, apart from bacteria (Song et al. 2022).

Very little is known about the viral component of the microbiome. To date, only 8 triatomine viruses have been identified and characterized: the Triatoma virus (TrV), that was discovered in a colony of field-collected *Triatoma infestans* (Muscio et al. 1988, 1987), and very recently seven *Rhodnius prolixus* viruses 1-7 (RpV1-7) (De Brito et al. 2021), which were initially discovered in transcriptome assemblies from ovarian tissues of *Rhodnius prolixus* (Coelho et al. 2021). Both RpVs and TrV are vertically transmitted to progeny (De Brito et al. 2021; Muscio et al. 1997). On the other hand, contigs related to viral genomes were incidentally identified in transcriptomic analyses of the salivary glands, fat bodies and testes of *Rhodnius prolixus*, *Panstrongylus megistus* and *P. lignarius* (Nevoa et al. 2018; Ribeiro et al. 2015; Schwarz et al. 2014), but these observations were not explored further. Finally, no metataxonomic analysis has yet studied the viral component of the microbiome and thus remains a pending assignment.

Metatranscriptomic studies in kissing bugs

Notably, no metatranscriptomic study has been performed in triatomines (Table 1).

Ticks (*Arachnida*: *Ixodida*)

Tick-borne pathogens cause most of the VBDs in temperate North America, Europe and Asia, and although these include viruses, bacteria, and parasites (Jongejan and Uilenberg 2004), Lyme disease is the most prevalent in the northern hemisphere (Rochlin and Toledo 2020). Some tick species may harbor numerous pathogens, whereas other species are typically associated with one major pathogen (Sanchez-Vicente et al. 2019).

There are two main tick families, *Argasidae* (soft ticks) and *Ixodidae* (hard ticks), that differ in their ecology and public health impact (Parola and Raoult 2001; Sonenshine and Roe 2014). Soft ticks have a more restricted habitat (Sonenshine 2014), feed quickly, can take several blood meals per stage (Vial 2009), and transmit fewer human pathogens than hard ticks (Parola and Raoult 2001). On the other hand, hard ticks are cosmopolitan (Sonenshine 2014), and have extended feeding periods (Sonenshine and Roe 2014) during the active feeding stages in their life cycle (larva, nymph and adult) (Parola and Raoult 2001), that facilitate the transmission of pathogens (Eisen 2018).

Ticks are obligate blood feeders and, because they are vulnerable to desiccation, they live in dark and humid conditions (*e.g.*, in leaf litter and animal burrows) (Goddard 2005). Consequently, exposure to these habitats, combined with the process of feeding on animals that host a diverse skin microbiome, provide opportunities for ticks to obtain part of their microbiome from the environment (Burtis et al. 2019). Like all blood-sucking arthropods, ticks lack key vitamins that are necessary for their development and rely on their bacterial symbionts to overcome this dietary limitation (Bonnet and Pollet 2021). The tick microbiome thus includes vertically transmitted symbionts and the environmentally acquired commensals.

Tick microbial diversity and composition has mostly been characterized by sequencing of the 16S rRNA gene (Bonnet and Pollet 2021). Some of these studies have used culture-independent approaches and Sanger sequencing (Clay et al. 2008; Hartelt et al. 2004; Moreno et al. 2006; Schabereiter-Gurtner et al. 2003; Van Overbeek et al. 2008), but most have used bacterial DNA metabarcoding (*e.g.*, Andreotti et al. 2011; Barraza-Guerrero et al. 2020; Beard et al. 2021; Budachetri et al. 2014; Carpi et al. 2011; Clayton et al. 2015; Gall et al. 2017; Guizzo et al. 2020; Heise et al. 2010; Lalzar et al. 2012; Narasimhan et al. 2014; Ponnusamy et al. 2014; Qiu et al. 2014; Sakamoto et al. 2020; Sperling et al. 2020; Zhang et al. 2020). One study performed DNA metabarcoding of various lineages (Bacteria, Archaea, Fungi and protists) (Landesman et al. 2019), whereas a couple of studies used shotgun metagenomics to analyze Bacteria and Archaea (Nakao et al. 2013) or only Bacteria (Díaz-Sánchez et al. 2019), and two studies analyzed bacterial transcriptomics (Hernández-Jarguín et al. 2018; Vayssier-Taussat et al.

2013) (reviewed in Narasimhan and Fikrig 2015 and Wu-Chuang et al. 2021).

Tick-borne viruses are a diverse group that includes members of *Flaviviridae*, *Bunyavirales*, *Orthomyxoviridae* and *Reoviridae* (Johnson et al. 2023). Probably this is why the viral component of the tick microbiome has recently been studied quite extensively through meta-RNA sequencing (*e.g.*, Bratuleanu et al. 2023; Cai et al. 2023; Guo et al. 2022; Harvey et al. 2019; Kong et al. 2022; Liu et al. 2022; Ni et al. 2023; Pettersson et al. 2017; Tokarz et al. 2018; Xu et al. 2021; Z. Yang et al. 2023).

Metatranscriptomic studies in ticks

The only metatranscriptomic study in ticks to date (Table 1) did not examine the metatranscriptomic data to determine the functional profile of the microbiome. The authors used untargeted metatranscriptomics to analyze the prokaryotic, eukaryotic and viral components of the microbiome in ticks (mainly *Ixodes holocyclus* and *Haemaphysalis bancrofti*) and in wildlife blood samples (from *Rattus rattus*, *Rattus fuscipes*, *Perame lesnasuta* and *Trichosurus vulpecula*) from urban and rural sites in Australia (Gofton et al. 2022). This study identified 32 unique tick-borne taxa, including 10 novel putative species. These included haemoprotozoa (*Babesia*, *Theileria*, *Hepatozoon* and *Trypanosoma* spp.), bacteria (*Borrelia*, *Rickettsia*, *Ehrlichia*, *Neoehrlichia* and *Anaplasma* spp.), and numerous viruses (including *Reoviridae* and a novel *Flaviviridae*-like jingmenvirus). A phylogenetic analysis of all the tick-borne microorganisms indicated that they were unique compared to their relatives from outside Australia, and no foreign tick-borne human pathogens were found (Gofton et al. 2022).

CONCLUSION

This review has addressed the status of metatranscriptomic and related studies in VBDs, focusing on some of the main hematophagous disease-transmitting arthropods namely mosquitoes, sandflies, tsetse flies, triatomines and ticks. The analysis was based on an extensive literature review of available microbial and viral studies for these hematophagous arthropods, and mainly focused on analyses that used high throughput sequencing approaches. Moreover, due to the lack of consensus terminology for these "meta-sequencing analyses", as a first step, these terms were defined to establish the necessary baseline for interpreting those studies and drawing consistent conclusions, namely:

- The majority of studies that used NGS approaches to analyze the microbiome of these vectors, carried out bacterial metataxonomic analyses using DNA metabarcoding.

- Most metataxonomic studies have been carried out in mosquitoes, followed by ticks, whereas the number of analyses for triatomines, sandflies, and tsetse flies is quite limited, particularly for sandflies and tsetse.

- The number of metatranscriptomic studies is notoriously low for all these hematophagous vectors: only 4 studies in mosquitoes, 2 in sandflies, 1 in ticks, and none in triatomines and tsetse flies (Table 1). Moreover, even though metatranscriptomics has the potential to unravel the taxonomic and functional profile of a sample, these studies only focused on identifying the different components of the microbiome and did not analyze the data to determine their expression profile.

Despite the fact that it is a challenge to assign functions and correctly interpret results in metatranscriptomic studies (Moran 2009; Rozadilla et al. 2020), the benefits of identifying all the processes that are simultaneously mediated by an undisturbed microbiome are evident. Ultimately, this review has helped to single out these gaps in knowledge for the VBDs included here, and this is a major step towards addressing them in future studies.

REFERENCES CITED

Abd-Alla AMM, Parker AG, Vreysen MJB, Bergoin M. 2011. Tsetse salivary gland hypertrophy virus: Hope or hindrance for tsetse control? *PLoS Negl Trop Dis.* doi:10.1371/journal.pntd.0001220

Akhoundi M, Bakhtiari R, Guillard T, Baghaei A, Tolouei R, Sereno D, Toubas D, Depaquit J, Abyaneh MR. 2012. Diversity of the Bacterial and Fungal Microflora from the Midgut and Cuticle of Phlebotomine Sand Flies Collected in North-Western Iran. *PLoS One* 7. doi:10.1371/journal.pone.0050259

Aksoy E, Telleria EL, Echodu R, Wu Y, Okedi LM, Weiss BL, Aksoy S, Caccone A. 2014. Analysis of multiple tsetse fly populations in Uganda reveals limited diversity and species-specific gut microbiota. *Appl Environ Microbiol* 80. doi:10.1128/AEM.00079-14

Aksoy S. 2000. Tsetse - A haven for microorganisms. *Parasitol Today.* doi:10.1016/S0169-4758(99)01606-3

Aksoy S. 1995. Wigglesworthia gen. nov. and Wigglesworthia glossinidia sp. nov., taxa consisting of the mycetocyte-associated, primary endosymbionts of tsetse flies. *Int J Syst Bacteriol* 45. doi:10.1099/00207713-45-4-848

Aksoy S, Pourhosseini AA, Chow A. 1995. Mycetome endosymbionts of tsetse flies constitute a distinct lineage related to Enterobacteriaceae. *Insect Mol Biol.* doi:10.1111/j.1365-2583.1995.tb00003.x

Alam U, Hyseni C, Symula RE, Brelsfoard C, Wu Y, Kruglov O, Wang J, Echodu R, Alioni V, Okedi LM, Caccone A, Aksoy S. 2012. Implications of microfauna-host interactions for trypanosome transmission dynamics in Glossina fuscipes fuscipes in Uganda. *Appl Environ Microbiol* 78. doi:10.1128/AEM.00806-12

Alkan C, Bichaud L, De Lamballerie X, Alten B, Gould EA, Charrel RN. 2013. Sandfly-borne phleboviruses of Eurasia and Africa: Epidemiology, genetic diversity, geographic range, control measures. *Antiviral Res.* doi:10.1016/j.antiviral.2013.07.005

Andreotti R, De León AAP, Dowd SE, Guerrero FD, Bendele KG, Scoles GA. 2011. Assessment of bacterial diversity in the cattle tick Rhipicephalus (Boophilus) microplus through tag-encoded pyrosequencing. *BMC Microbiol* 11. doi:10.1186/1471-2180-11-6

Aragão CF, da Silva SP, do Nascimento BLS, da Silva FS, Nunes Neto JP, Pinheiro VCS, Cruz ACR. 2023. Shotgun Metagenomic Sequencing Reveals Virome Composition of Mosquitoes from a Transition Ecosystem of North-Northeast Brazil. *Genes (Basel)* 14. doi:10.3390/genes14071443

Attardo GM, Lohs C, Heddi A, Alam UH, Yildirim S, Aksoy S. 2008. Analysis of milk gland structure and function in Glossina morsitans: Milk protein production, symbiont populations and fecundity. *J Insect Physiol* 54. doi:10.1016/j.jinsphys.2008.06.008

Ayhan N, Charrel RN. 2017. Of phlebotomines (sandflies) and viruses: a comprehensive perspective on a complex situation. *Curr Opin Insect Sci.* doi:10.1016/j.cois.2017.05.019

Balmand S, Lohs C, Aksoy S, Heddi A. 2013. Tissue distribution and transmission routes for the tsetse fly endosymbionts. *J Invertebr Pathol* 112. doi:10.1016/j.jip.2012.04.002

Barraza-Guerrero SI, Meza-Herrera CA, la Peña CG De, González-álvarez VH, Vaca-Paniagua F, Díaz-Velásquez CE, Sánchez-Tortosa F, Ávila-Rodríguez V, Valenzuela-Núñez LM, Herrera-Salazar JC. 2020. General microbiota of the soft tick ornithodoros turicata parasitizing the bolson tortoise (Gopherus flavomarginatus) in the mapimi biosphere reserve, Mexico. *Biology (Basel)* 9. doi:10.3390/biology9090275

Bashiardes S, Zilberman-Schapira G, Elinav E. 2016. Use of metatranscriptomics in microbiome research. *Bioinform Biol Insights* 10. doi:10.4137/BBI.S34610

Batson J, Dudas G, Haas-Stapleton E, Kistler AL, Li LM, Logan P, Ratnasiri K, Retallack H. 2021. Single mosquito metatranscriptomics identifies vectors, emerging pathogens and reservoirs in one assay. *Elife* 10. doi:10.7554/ELIFE.68353

Battisti JM, Lawyer PG, Minnick MF. 2015. Colonization of Lutzomyia verrucarum and Lutzomyia longipalpis Sand Flies (Diptera: Psychodidae) by Bartonella bacilliformis, the Etiologic Agent of Carrión's Disease. *PLoS Negl Trop Dis* 9. doi:10.1371/journal.pntd.0004128

Beard D, Stannard HJ, Old JM. 2021. Morphological identification of ticks and molecular detection of tick-borne pathogens from bare-nosed wombats (Vombatus ursinus). *Parasites and Vectors* 14. doi:10.1186/s13071-020-04565-6

Beaty BJ, Marquardt WC. 1996. The Biology of Disease Vectors. Colorado: University Press of Colorado.

Belda E, Coulibaly B, Fofana A, Beavogui AH, Traore SF, Gohl DM, Vernick KD, Riehle MM. 2017. Preferential suppression of Anopheles gambiae host sequences allows detection of the mosquito eukaryotic microbiome. *Sci Rep* 7. doi:10.1038/s41598-017-03487-1

Benoit JB, Attardo GM, Baumann AA, Michalkova V, Aksoy S. 2015. Adenotrophic viviparity in tsetse flies: Potential for population control and as an insect model for lactation. *Annu Rev Entomol.* doi:10.1146/annurev-ento-010814-020834

Benoit JB, Attardo GM, Michalkova V, Takáč P, Bohova J, Aksoy S. 2012. Sphingomyelinase activity in mother's milk is essential for juvenile development: A case from lactating tsetse flies. *Biol Reprod* 87. doi:10.1095/biolreprod.112.100008

Berg G, Rybakova D, Fischer D, Cernava T, Vergès MCC, Charles T, Chen X, Cocolin L, Eversole K, Corral GH, Kazou M, Kinkel L, Lange L, Lima N, Loy A, Macklin JA, Maguin E, Mauchline T, McClure R, Mitter B, Ryan M, Sarand I, Smidt H, Schelkle B, Roume H, Kiran GS, Selvin J, Souza RSC de, Van Overbeek L, Singh BK, Wagner M, Walsh A, Sessitsch A, Schloter M. 2020. Microbiome definition re-visited: old concepts and new challenges. *Microbiome.* doi:10.1186/s40168-020-00875-0

Bing X, Attardo GM, Vigneron A, Aksoy E, Scolari F, Malacrida A, Weiss BL, Aksoy S. 2017. Unravelling the relationship between the tsetse fly and its obligate symbiont Wigglesworthia: Transcriptomic and metabolomic landscapes reveal highly integrated physiological networks. *Proc R Soc B Biol Sci* 284. doi:10.1098/rspb.2017.0360

Boissière A, Tchioffo MT, Bachar D, Abate L, Marie A, Nsango SE, Shahbazkia HR, Awono-Ambene PH, Levashina EA, Christen R, Morlais I. 2012. Midgut microbiota of the malaria mosquito vector Anopheles gambiae and interactions with Plasmodium falciparum infection. *PLoS Pathog* 8. doi:10.1371/journal.ppat.1002742

Bonnet SI, Pollet T. 2021. Update on the intricate tango between tick microbiomes and tick-borne pathogens. *Parasite Immunol.* doi:10.1111/pim.12813

Bratuleanu BE, Raileanu C, Chrétien D, Guardado-Calvo P, Bigot T, Savuta G, Temmam S, Eloit M. 2023. A Search for Tick-Associated, Bronnoya-like Virus Spillover into Sheep. *Microorganisms* 11. doi:10.3390/microorganisms11010209

Brecher G, Wigglesworth VB. 1944. The transmission of actinomyces rhodnii Erikson in rhodnius prolixus stål (hemiptera) and its influence on the growth of the host. *Parasitology* 35. doi:10.1017/S0031182000021648

Brown JJ, Rodríguez-Ruano SM, Poosakkannu A, Batani G, Schmidt JO, Roachell W, Zima J, Hypša V, Nováková E. 2020. Ontogeny, species identity, and environment dominate microbiome dynamics in wild populations of kissing bugs (Triatominae). *Microbiome.* doi:10.1186/s40168-020-00921-x

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

Budachetri K, Browning RE, Adamson SW, Dowd SE, Chao CC, Ching WM, Karim S. 2014. An insight into the Microbiome of the Amblyomma maculatum (Acari: Ixodidae). *J Med Entomol* 51. doi:10.1603/ME12223

Burtis JC, Yavitt JB, Fahey TJ, Ostfeld RS. 2019. Ticks as Soil-Dwelling Arthropods: An Intersection between Disease and Soil Ecology. *J Med Entomol.* doi:10.1093/jme/tjz116

Cai Xianglong, Cai Xiaojing, Xu Y, Shao Y, Fu L, Men X, Zhu Y. 2023. Virome analysis of ticks and tick-borne viruses in Heilongjiang and Jilin Provinces, China. *Virus Res* 323. doi:10.1016/j.virusres.2022.199006

Calle-Tobón A, Holguin-Rocha AF, Moore C, Rippee-Brooks M, Rozo-Lopez P, Harrod J, Fatehi S, Rua-Uribe GL, Park Y, Londoño-Rentería B. 2021. Blood Meals With Active and Heat-Inactivated Serum Modifies the Gene Expression and Microbiome of Aedes albopictus. *Front Microbiol* 12. doi:10.3389/fmicb.2021.724345

Campolina TB, Villegas LEM, Monteiro CC, Pimenta PFP, Secundino NFC. 2020. Tripartite interactions: Leishmania, microbiota and lutzomyia longipalpis. *PLoS Negl Trop Dis* 14. doi:10.1371/journal.pntd.0008666

Caragata EP, Tikhe C V., Dimopoulos G. 2019. Curious entanglements: interactions between mosquitoes, their microbiota, and arboviruses. *Curr Opin Virol.* doi:10.1016/j.coviro.2019.05.005

Carels N, Gumiel M, da Mota FF, de Carvalho Moreira CJ, Azambuja P. 2017. A Metagenomic Analysis of Bacterial Microbiota in the Digestive Tract of Triatomines. *Bioinform Biol Insights* 11. doi:10.1177/1177932217733422

Carpi G, Cagnacci F, Wittekindt NE, Zhao F, Qi J, Tomsho LP, Drautz DI, Rizzoli A, Schuster SC. 2011. Metagenomic profile of the bacterial communities associated with Ixodes ricinus ticks. *PLoS One* 6. doi:10.1371/journal.pone.0025604

Chandler JA, Liu RM, Bennett SN. 2015. RNA Shotgun Metagenomic Sequencing of Northern California (USA) Mosquitoes Uncovers Viruses, Bacteria, and Fungi. *Front Microbiol* 6. doi:10.3389/fmicb.2015.00185

Cheng Q, Aksoy S. 1999. Tissue tropism, transmission and expression of foreign genes in vivo in midgut symbionts of tsetse flies. *Insect Mol Biol* 8. doi:10.1046/j.1365-2583.1999.810125.x

Cheng Q, Ruel TD, Zhou W, Moloo SK, Majiwa P, O'Neill SL, Aksoy S. 2000. Tissue distribution and prevalence of Wolbachia infections in tsetse flies, Glossina spp. *Med Vet Entomol* 14. doi:10.1046/j.1365-2915.2000.00202.x

Clay K, Klyachko O, Grindle N, Civitello D, Oleske D, Fuqua C. 2008. Microbial communities and interactions in the lone star tick, Amblyomma americanum. *Mol Ecol* 17. doi:10.1111/j.1365-294X.2008.03914.x

Clayton KA, Gall CA, Mason KL, Scoles GA, Brayton KA. 2015. The characterization and manipulation of the bacterial microbiome of the Rocky Mountain wood tick, Dermacentor andersoni. *Parasites and Vectors* 8. doi:10.1186/s13071-015-1245-z

Clements AN. 1992. The biology of mosquitoes: Development, nutrition and reproductionThe Biology of Mosquitoes. Chapman & Hall.

Coelho VL, de Brito TF, de Abreu Brito IA, Cardoso MA, Berni MA, Araujo HMM, Sammeth M, Pane A. 2021. Analysis of ovarian transcriptomes reveals thousands of novel genes in the insect vector Rhodnius prolixus. *Sci Rep* 11. doi:10.1038/s41598-021-81387-1

Coon KL, Brown MR, Strand MR. 2016. Mosquitoes host communities of bacteria that are essential for development but vary greatly between local habitats. *Mol Ecol* 25. doi:10.1111/mec.13877

Coon KL, Vogel KJ, Brown MR, Strand MR. 2014. Mosquitoes rely on their gut microbiota for development. *Mol Ecol* 23. doi:10.1111/mec.12771

Cox JW, Ballweg RA, Taft DH, Velayutham P, Haslam DB, Porollo A. 2017. A fast and robust protocol for metataxonomic analysis using RNAseq data. *Microbiome* 5. doi:10.1186/s40168-016-0219-5

Creer S, Fonseca VG, Porazinska DL, Giblin-Davis RM, Sung W, Power DM, Packer M, Carvalho GR, Blaxter ML, Lambshead PJD, Thomas WK. 2010. Ultrasequencing of the meiofaunal biosphere: Practice, pitfalls and promises. *Mol Ecol* 19. doi:10.1111/j.1365-294X.2009.04473.x

da Mota FF, Marinho LP, de Moreira CJC, Lima MM, Mello CB, Garcia ES, Carels N, Azambuja P. 2012. Cultivation-independent methods reveal differences among bacterial gut microbiota in triatomine vectors of Chagas disease. *PLoS Negl Trop Dis.* doi:10.1371/journal.pntd.0001631

Dada N, Benedict AC, López F, Lol JC, Sheth M, Dzuris N, Padilla N, Lenhart A. 2021a. Comprehensive characterization of internal and cuticle surface microbiota of laboratory-reared F1 Anopheles albimanus originating from different sites. *Malar J* 20. doi:10.1186/s12936-021-03934-5

Dada N, Jupatanakul N, Minard G, Short SM, Akorli J, Villegas LM. 2021b. Considerations for mosquito microbiome research from the Mosquito Microbiome Consortium. *Microbiome.* doi:10.1186/s40168-020-00987-7

Dada N, Lol JC, Benedict AC, López F, Sheth M, Dzuris N, Padilla N, Lenhart A. 2019. Pyrethroid exposure alters internal and cuticle surface bacterial communities in Anopheles albimanus. *ISME J* 13. doi:10.1038/s41396-019-0445-5

De Brito TF, Coelho VL, Cardoso MA, De Abreu Brito IA, Berni MA, Zenk FL, Iovino N, Pane A. 2021. Transovarial transmission of a core virome in the Chagas disease vector Rhodnius prolixus. *PLoS Pathog* 17. doi:10.1371/journal.ppat.1009780

Depaquit J, Grandadam M, Fouque F, Andry PE, Peyrefitte C. 2010. Arthropod-borne viruses transmitted by Phlebotomine sandflies in Europe: a review. *Euro Surveill* 15:19507.

Díaz-Sánchez S, Hernández-Jarguín A, Torina A, de Mera IGF, Blanda V, Caracappa S, Gortazar C, de la Fuente J. 2019. Characterization of the bacterial microbiota in wild-caught Ixodes ventalloi. *Ticks Tick Borne Dis* 10. doi:10.1016/j.ttbdis.2018.11.014

Díaz S, Camargo C, Avila FW. 2021. Characterization of the reproductive tract bacterial microbiota of virgin, mated, and blood-fed Aedes aegypti and Aedes albopictus females. *Parasites and Vectors* 14. doi:10.1186/s13071-021-05093-7

Díaz S, Villavicencio B, Correia N, Costa J, Haag KL. 2016. Triatomine bugs, their microbiota and Trypanosoma cruzi: Asymmetric responses of bacteria to an infected blood meal. *Parasites and Vectors.* doi:10.1186/s13071-016-1926-2

Dickson LB, Jiolle D, Minard G, Moltini-Conclois I, Volant S, Ghozlane A, Bouchier C, Ayala D, Paupy C, Moro CV, Lambrechts L. 2017. Carryover effects of larval exposure to different environmental bacteria drive adult trait variation in a mosquito vector. *Sci Adv* 3. doi:10.1126/sciadv.1700585

Dillon RJ, el Kordy E, Shehata M, Lane RP. 1996. The prevalence of a microbiota in the digestive tract of Phlebotomus papatasi. *Ann Trop Med Parasitol* 90:669–673.

Doudoumis V, Blow F, Saridaki A, Augustinos A, Dyer NA, Goodhead I, Solano P, Rayaisse JB, Takac P, Mekonnen S, Parker AG, Abd-Alla AMM, Darby A, Bourtzis K, Tsiamis G. 2017. Challenging the Wigglesworthia, Sodalis, Wolbachia symbiosis dogma in tsetse flies: Spiroplasma is present in both laboratory and natural populations. *Sci Rep* 7. doi:10.1038/s41598-017-04740-3

Doudoumis V, Tsiamis G, Wamwiri F, Brelsfoard C, Alam U, Aksoy E, Dalaperas S, Abd-Alla A, Ouma J, Takac P, Aksoy S, Bourtzis K. 2012. Detection and characterization of Wolbachia infections in laboratory and natural populations of different species of tsetse flies (genus Glossina). *BMC Microbiol* 12. doi:10.1186/1471-2180-12-S1-S3

Duguma D, Hall MW, Smartt CT, Debboun M, Neufeld JD. 2019. Microbiota variations in Culex nigripalpus disease vector mosquito of west nile virus and saint louis encephalitis from different geographic origins. *PeerJ* 2019. doi:10.7717/peerj.6168

Dumonteil E, Pronovost H, Bierman EF, Sanford A, Majeau A, Moore R, Herrera C. 2020. Interactions among Triatoma sanguisuga blood feeding sources, gut microbiota and Trypanosoma cruzi diversity in southern Louisiana. *Mol Ecol.* doi:10.1111/mec.15582

Dumonteil E, Ramirez-Sierra MJ, Pérez-Carrillo S, Teh-Poot C, Herrera C, Gourbière S, Waleckx E. 2018. Detailed ecological associations of triatomines revealed by metabarcoding and next-generation sequencing: Implications for triatomine behavior and Trypanosoma cruzi transmission cycles. *Sci Rep.* doi:10.1038/s41598-018-22455-x

Duncan JT. 1926. On a Bactericidal Principle Present in the Alimentary Canal of Insects and Arachnids. *Parasitology* 18. doi:10.1017/S0031182000005205

Dupré J, O'Malley MA. 2013. Varieties of Living Things: Life at the Intersection of Lineage and MetabolismHistory, Philosophy and Theory of the Life Sciences. doi:10.1007/978-94-007-2445-7_13

Durvasula R V., Sundaram RK, Kirsch P, Hurwitz I, Crawford C V., Dotson E, Beard CB. 2008. Genetic transformation of a Corynebacterial symbiont from the Chagas disease vector Triatoma infestans. *Exp Parasitol*. doi:10.1016/j.exppara.2007.12.020

Eisen L. 2018. Pathogen transmission in relation to duration of attachment by Ixodes scapularis ticks. *Ticks Tick Borne Dis*. doi:10.1016/j.ttbdis.2018.01.002

Farajollahi A, Fonseca DM, Kramer LD, Marm Kilpatrick A. 2011. "Bird biting" mosquitoes and human disease: A review of the role of Culex pipiens complex mosquitoes in epidemiology. *Infect Genet Evol*. doi:10.1016/j.meegid.2011.08.013

Farikou O, Njiokou F, Simo G, Asonganyi T, Cuny G, Geiger A. 2010. Tsetse fly blood meal modification and trypanosome identification in two sleeping sickness foci in the forest of southern Cameroon. *Acta Trop* 116. doi:10.1016/j.actatropica.2010.06.002

Farikou O, Thevenon S, Njiokou F, Allal F, Cuny G, Geiger A. 2011. Genetic diversity and population structure of the secondary symbiont of tsetse flies, Sodalis glossinidius, in sleeping sickness foci in Cameroon. *PLoS Negl Trop Dis* 5. doi:10.1371/journal.pntd.0001281

Fauver JR, Grubaugh ND, Krajacich BJ, Weger-Lucarelli J, Lakin SM, Fakoli LS, Bolay FK, Diclaro JW, Dabiré KR, Foy BD, Brackney DE, Ebel GD, Stenglein MD. 2016. West African Anopheles gambiae mosquitoes harbor a taxonomically diverse virome including new insect-specific flaviviruses, mononegaviruses, and totiviruses. *Virology* 498. doi:10.1016/j.virol.2016.07.031

Feng Y, Gou QY, Yang WH, Wu WC, Wang J, Holmes EC, Liang G, Shi M. 2022. A time-series meta-transcriptomic analysis reveals the seasonal, host, and gender structure of mosquito viromes. *Virus Evol* 8. doi:10.1093/ve/veac006

Fraihi W, Fares W, Perrin P, Dorkeld F, Sereno D, Barhoumi W, Sbissi I, Cherni S, Chelbi I, Durvasula R, Ramalho-Ortigao M, Gtari M, Zhioua E. 2017. An integrated overview of the midgut bacterial flora composition of Phlebotomus perniciosus, a vector of zoonotic visceral leishmaniasis in the Western Mediterranean Basin. *PLoS Negl Trop Dis* 11. doi:10.1371/journal.pntd.0005484

Gabrieli P, Caccia S, Varotto-Boccazzi I, Arnoldi I, Barbieri G, Comandatore F, Epis S. 2021. Mosquito Trilogy: Microbiota, Immunity and Pathogens, and Their Implications for the Control of Disease Transmission. *Front Microbiol*. doi:10.3389/fmicb.2021.630438

Gaithuma A, Yamagishi J, Hayashida K, Kawai N, Namangala B, Sugimoto C. 2020. Blood meal sources and bacterial microbiome diversity in wild-caught tsetse flies. *Sci Rep* 10. doi:10.1038/s41598-020-61817-2

Galen SC, Borner J, Williamson JL, Witt CC, Perkins SL. 2020. Metatranscriptomics yields new genomic resources and sensitive detection of infections for diverse blood parasites. *Mol Ecol Resour* 20. doi:10.1111/1755-0998.13091

Gall CA, Scoles GA, Magori K, Mason KL, Brayton KA. 2017. Laboratory colonization stabilizes the naturally dynamic microbiome composition of field collected Dermacentor andersoni ticks. *Microbiome* 5. doi:10.1186/s40168-017-0352-9

Gao H, Cui C, Wang L, Jacobs-Lorena M, Wang S. 2020. Mosquito Microbiota and Implications for Disease Control. *Trends Parasitol* 36:98–111. doi:10.1016/j.pt.2019.12.001

Geiger A, Fardeau ML, Grebaut P, Vatunga G, Josénando T, Herder S, Cuny G, Truc P, Ollivier B. 2009. First isolation of Enterobacter, Enterococcus, and Acinetobacter spp. as inhabitants of the tsetse fly (Glossina palpalis palpalis) midgut. *Infect Genet Evol* 9. doi:10.1016/j.meegid.2009.09.013

Geiger A, Fardeau ML, Njiokou F, Joseph M, Asonganyi T, Ollivier B, Cuny G. 2011. Bacterial Diversity Associated with Populations of Glossina spp. from Cameroon and Distribution within the Campo Sleeping Sickness Focus. *Microb Ecol* 62. doi:10.1007/s00248-011-9830-y

Gimonneau G, Tchioffo MT, Abate L, Boissière A, Awono-Ambéné PH, Nsango SE, Christen R, Morlais I. 2014. Composition of Anopheles coluzzii and Anopheles gambiae microbiota from larval to adult stages. *Infect Genet Evol* 28:715–724. doi:10.1016/j.meegid.2014.09.029

Goddard J. 2005. Biology of Disease Vectors, 2nd ed. *Emerg Infect Dis* 11. doi:10.3201/eid1108.050610

Gofton AW, Blasdell KR, Taylor C, Banks PB, Michie M, Roy-Dufresne E, Poldy J, Wang J, Dunn M, Tachedjian M, Smith I. 2022. Metatranscriptomic profiling reveals diverse tick-borne bacteria, protozoans and viruses in ticks and wildlife from Australia. *Transbound Emerg Dis* 69. doi:10.1111/tbed.14581

Gouveia C, Asensi MD, Zahner V, Rangel EF, Oliveira SM. 2008. Study on the bacterial midgut microbiota associated to different Brazilian populations of Lutzomyia longipalpis (Lutz & Neiva) (Diptera: Psychodidae). *Neotrop Entomol* 37:597–601. doi:S1519-566X2008000500016 [pii]

Griffith BC, Weiss BL, Aksoy E, Mireji PO, Auma JE, Wamwiri FN, Echodu R, Murilla G, Aksoy S. 2018. Analysis of the gut-specific microbiome from field-captured tsetse flies, and its potential relevance to host trypanosome vector competence. *BMC Microbiol* 18. doi:10.1186/s12866-018-1284-7

Guégan M, Zouache K, Démichel C, Minard G, Tran Van V, Potier P, Mavingui P, Valiente Moro C. 2018. The mosquito holobiont: fresh insight into mosquito-microbiota interactions. *Microbiome*. doi:10.1186/s40168-018-0435-2

Guernaoui S, Garcia D, Gazanion E, Ouhdouch Y, Boumezzough A, Pesson B, Fontenille D, Sereno D. 2011. Bacterial flora as indicated by PCR-temperature gradient gel electrophoresis (TGGE) of 16S rDNA gene fragments from isolated guts of phlebotomine sand flies (Diptera: Psychodidae). *J Vector Ecol* 36. doi:10.1111/j.1948-7134.2011.00124.x

Guizzo MG, Neupane S, Kucera M, Perner J, Frantová H, da Silva Vaz I, Oliveira PL d., Kopacek P, Zurek L. 2020. Poor Unstable Midgut Microbiome of Hard Ticks Contrasts With Abundant and Stable Monospecific Microbiome in Ovaries. *Front Cell Infect Microbiol* 10. doi:10.3389/fcimb.2020.00211

Gumiel M, Da Mota FF, Rizzo VDS, Sarquis O, Castro DP De, Lima MM, Garcia EDS, Carels N, Azambuja P. 2015. Characterization of the microbiota in the guts of Triatoma brasiliensis and Triatoma pseudomaculata infected by Trypanosoma cruzi in natural conditions using culture independent methods. *Parasites and Vectors*. doi:10.1186/s13071-015-0836-z

Gunathilaka N, Perera H, Wijerathna T, Rodrigo W, Wijegunawardana NDAD. 2020. The Diversity of Midgut Bacteria among Wild-Caught Phlebotomus argentipes (Psychodidae: Phlebotominae), the Vector of Leishmaniasis in Sri Lanka. *Biomed Res Int* 2020. doi:10.1155/2020/5458063

Guo L, Ma J, Lin J, Chen M, Liu W, Zha J, Jin Q, Hong H, Huang W, Zhang L, Zhang K, Wei Z, Liu Q. 2022. Virome of Rhipicephalus ticks by metagenomic analysis in Guangdong, southern China. *Front Microbiol* 13. doi:10.3389/fmicb.2022.966735

Hameed M, Wahaab A, Shan T, Wang X, Khan S, Di D, Xiqian L, Zhang JJ, Anwar MN, Nawaz M, Li B, Liu K, Shao D, Qiu Y, Wei J, Ma Z. 2021. A Metagenomic Analysis of Mosquito Virome Collected From Different Animal Farms at Yunnan–Myanmar Border of China. *Front Microbiol* 11. doi:10.3389/fmicb.2020.591478

Hartelt K, Oehme R, Frank H, Brockmann SO, Hassler D, Kimmig P. 2004. Pathogens and symbionts in ticks: prevalence of Anaplasma phagocytophilum (Ehrlichia sp.), Wolbachia sp., Rickettsia sp., and Babesia sp. in Southern Germany. *Int J Med Microbiol* 293 Suppl:86–92. doi:10.1016/s1433-1128(04)80013-5

Harvey E, Rose K, Eden J-S, Lo N, Abeyasuriya T, Shi M, Doggett SL, Holmes EC. 2019. Extensive Diversity of RNA Viruses in Australian Ticks. *J Virol* 93. doi:10.1128/jvi.01358-18

Hebert PDN, Cywinska A, Ball SL, DeWaard JR. 2003. Biological identifications through DNA barcodes. *Proc R Soc B Biol Sci* 270. doi:10.1098/rspb.2002.2218

Hegde S, Khanipov K, Albayrak L, Golovko G, Pimenova M, Saldaña MA, Rojas MM, Hornett EA, Motl GC, Fredregill CL, Dennett JA, Debboun M, Fofanov Y, Hughes GL. 2018. Microbiome interaction networks and community structure from laboratory-reared and field-collected Aedes aegypti, Aedes albopictus, and Culex quinquefasciatus mosquito vectors. *Front Microbiol* 9. doi:10.3389/fmicb.2018.02160

Heise SR, Elshahed MS, Little SE. 2010. Bacterial diversity in amblyomma americanum (Acari: Ixodidae) with a focus on members of the genus rickettsia. *J Med Entomol* 47. doi:10.1603/ME09197

Hernández-Jarguín A, Díaz-Sánchez S, Villar M, de la Fuente J. 2018. Integrated metatranscriptomics and metaproteomics for the characterization of bacterial microbiota in unfed Ixodes ricinus. *Ticks Tick Borne Dis* 9. doi:10.1016/j.ttbdis.2018.04.020

Hilgenboecker K, Hammerstein P, Schlattmann P, Telschow A, Werren JH. 2008. How many species are infected with Wolbachia? - A statistical analysis of current data. *FEMS Microbiol Lett* 281. doi:10.1111/j.1574-6968.2008.01110.x

Hillesland H, Read A, Subhadra B, Hurwitz I, McKelvey R, Ghosh K, Das P, Durvasula R. 2008. Identification of aerobic gut bacteria from the kala azar vector, Phlebotomus argentipes: A platform for potential paratransgenic manipulation of sand flies. *Am J Trop Med Hyg* 79. doi:10.4269/ajtmh.2008.79.881

Hypša V, Dale C. 1997. In vitro culture and phylogenetic analysis of "Candidatus arsenophonus triatominarum," an intracellular bacterium from the triatomine bug, Triatoma infestans. *Int J Syst Bacteriol* 47. doi:10.1099/00207713-47-4-1140

Jaenson TGT. 1978. Virus-like rods associated with salivary gland hyperplasia in tsetse, glossina pallidipes. *Trans R Soc Trop Med Hyg* 72. doi:10.1016/0035-9203(78)90200-6

Jancarova M, Polanska N, Volf P, Dvorak V. 2023. The role of sand flies as vectors of viruses other than phleboviruses. *J Gen Virol*. doi:10.1099/jgv.0.001837

Johnson N, Migné C V., Gonzalez G. 2023. Tick-borne encephalitis. *Curr Opin Infect Dis*. doi:10.1097/QCO.0000000000000924

Jones S. 2013. Trends in microbiome research. *Nat Biotechnol*. doi:10.1038/nbt.2546

Jongejan F, Uilenberg G. 2004. The global importance of ticks. *Parasitology*. doi:10.1017/S0031182004005967

Kaiwa N, Hosokawa T, Kikuchi Y, Nikoh N, Meng XY, Kimura N, Ito M, Fukatsu T. 2010. Primary gut symbiont and secondary, sodalis-allied symbiont of the scutellerid stinkbug cantao ocellatus. *Appl Environ Microbiol* 76. doi:10.1128/AEM.00421-10

Karimian F, Vatandoost H, Rassi Y, Maleki-Ravasan N, Mohebali M, Shirazi MH, Koosha M, Choubdar N, Oshaghi MA. 2019. Aerobic midgut microbiota of sand fly vectors of zoonotic visceral leishmaniasis from northern Iran, a step toward finding potential paratransgenic candidates 06 Biological Sciences 0605 Microbiology. *Parasites and Vectors* 12. doi:10.1186/s13071-018-3273-y

Kelly PH, Bahr SM, Serafim TD, Ajami NJ, Petrosino JF, Meneses C, Kirby JR, Valenzuela JG, Kamhawi S, Wilson ME. 2017. The Gut Microbiome of the Vector *Lutzomyia longipalpis* Is Essential for Survival of *Leishmania infantum*. *MBio*. doi:10.1128/mBio.01121-16

Kieran TJ, Arnold KMH, Thomas JC, Varian CP, Saldaña A, Calzada JE, Glenn TC, Gottdenker NL. 2019. Regional biogeography of microbiota composition in the Chagas disease vector Rhodnius pallescens. *Parasites and Vectors* 12. doi:10.1186/s13071-019-3761-8

Kong Y, Zhang G, Jiang L, Wang P, Zhang S, Zheng X, Li Y. 2022. Metatranscriptomics Reveals the Diversity of the Tick Virome in Northwest China. *Microbiol Spectr* 10. doi:10.1128/spectrum.01115-22

Lalzar I, Harrus S, Mumcuoglu KY, Gottlieb Y. 2012. Composition and seasonal variation of Rhipicephalus turanicus and Rhipicephalus sanguineus bacterial communities. *Appl Environ Microbiol* 78. doi:10.1128/AEM.00323-12

Landesman WJ, Mulder K, Page Fredericks L, Allan BF. 2019. Cross-kingdom analysis of nymphal-stage Ixodes scapularis microbial communities in relation to Borrelia burgdorferi infection and load. *FEMS Microbiol Ecol* 95. doi:10.1093/femsec/fiz167

Ledesma N, Harrington L. 2011. Mosquito vectors of dog heartworm in the United States: Vector status and factors influencing transmission efficiency. *Top Companion Anim Med* 26. doi:10.1053/j.tcam.2011.09.005

Li C, Liu S, Zhou H, Zhu W, Cui M, Li J, Wang J, Liu J, Zhu J, Li W, Bi Y, Carr MJ, Holmes EC, Shi W. 2023. Metatranscriptomic Sequencing Reveals Host Species as an Important Factor Shaping the Mosquito Virome. *Microbiol Spectr* 11. doi:10.1128/spectrum.04655-22

Li K, Chen H, Jiang J, Li X, Xu J, Ma Y. 2016. Diversity of bacteriome associated with Phlebotomus chinensis (Diptera: Psychodidae) sand flies in two wild populations from China. *Sci Rep* 6. doi:10.1038/srep36406

Lima MS, Laport MS, Lorosa ES, Jurberg J, dos Santos KRN, da Silva Neto MAC, Rachid CTC da C, Atella GC. 2018. Bacterial community composition in the salivary glands of triatomines (Hemiptera: Reduviidae). *PLoS Negl Trop Dis* 12. doi:10.1371/journal.pntd.0006739

Lindh JM, Lehane MJ. 2011. The tsetse fly Glossina fuscipes fuscipes (Diptera: Glossina) harbours a surprising diversity of bacteria other than symbionts. *Antonie van Leeuwenhoek, Int J Gen Mol Microbiol* 99. doi:10.1007/s10482-010-9546-x

Liu Q, Cui F, Liu X, Fu Y, Fang W, Kang X, Lu H, Li S, Liu B, Guo W, Xia Q, Kang L, Jiang F. 2023. Association of virome dynamics with mosquito species and environmental factors. *Microbiome* 11. doi:10.1186/s40168-023-01556-4

Liu Z, Li L, Xu W, Yuan Y, Liang X, Zhang L, Wei Z, Sui L, Zhao Y, Cui Y, Yin Q, Li D, Li Q, Hou Z, Wei F, Liu Q, Wangid Z. 2022. Extensive diversity of RNA viruses in ticks revealed by metagenomics in northeastern China. *PLoS Negl Trop Dis* 16. doi:10.1371/journal.pntd.0011017

Lopez-Ordonez T, Flores-López CA, Montejo-Lopez R, Cruz-Hernandez A, Conners EE. 2018. Cultivable bacterial diversity in the gut of the chagas disease vector Triatoma dimidiata: Identification of possible bacterial candidates for a paratransgenesis approach. *Front Ecol Evol* 5. doi:10.3389/fevo.2017.00174

Louradour I, Monteiro CC, Inbar E, Ghosh K, Merkhofer R, Lawyer P, Paun A, Smelkinson M, Secundino N, Lewis M, Erram D, Zurek L, Sacks D. 2017. The midgut microbiota plays an essential role in sand fly vector competence for Leishmania major. *Cell Microbiol*. doi:10.1111/cmi.12755

Machado VE, Martins PMM, Ferreira H, Ferro M, Bacci M, Pinto MC. 2014. Bacterial groups associated with Nyssomyia neivai (Diptera: Psychodidae) sandflies. *J Vector Borne Dis*.

Maleki-Ravasan N, Oshaghi MA, Afshar D, Arandian MH, Hajikhani S, Akhavan AA, Yakhchali B, Shirazi MH, Rassi Y, Jafari R, Aminian K, Fazeli-Varzaneh RA, Durvasula R. 2015. Aerobic bacterial flora of biotic and abiotic compartments of a hyperendemic Zoonotic Cutaneous Leishmaniasis (ZCL) focus. *Parasites and Vectors* 8. doi:10.1186/s13071-014-0517-3

Malele II, Manangwa O, Nyingilili HH, Kitwika WA, Lyaruu EA, Msangi AR, Ouma JO, Nkwangulila G, Abd-Alla AMM. 2013. Prevalence of SGHV among tsetse species of economic importance in Tanzania and their implication for SIT application. *J Invertebr Pathol*. doi:10.1016/j.jip.2012.07.018

Mancini M V., Damiani C, Accoti A, Tallarita M, Nunzi E, Cappelli A, Bozic J, Catanzani R, Rossi P, Valzano M, Serrao A, Ricci I, Spaccapelo R, Favia G. 2018. Estimating bacteria diversity in different organs of nine species of mosquito by next generation sequencing. *BMC Microbiol* 18. doi:10.1186/s12866-018-1266-9

Mann AE, Mitchell EA, Zhang Y, Curtis-Robles R, Thapa S, Hamer SA, Allen MS. 2020. Comparison of the Bacterial Gut Microbiome of North American Triatoma spp. With and Without Trypanosoma cruzi. *Front Microbiol* 11. doi:10.3389/fmicb.2020.00364

Manni M, Zdobnov E. 2021. Tsetse RNA Virome: Novel Iflavirus Genomes in Glossina morsitans and Other Tsetse Species. *bioRxiv*.

Marcelino VR, Irinyi L, Eden JS, Meyer W, Holmes EC, Sorrell TC. 2019. Metatranscriptomics as a tool to identify fungal species and subspecies in mixed communities – A proof of concept under laboratory conditions. *IMA Fungus* 10. doi:10.1186/s43008-019-0012-8

Marchesi JR, Ravel J. 2015. The vocabulary of microbiome research: a proposal. *Microbiome* 3. doi:10.1186/s40168-015-0094-5

Maroli M, Feliciangeli MD, Bichaud L, Charrel RN, Gradoni L. 2013. Phlebotomine sandflies and the spreading of leishmaniases and other diseases of public health concern. *Med Vet Entomol* 27:123–147. doi:10.1111/j.1365-2915.2012.01034.x

Maudlin I, Welburn SC, Mehlitz D. 1990. The relationship between rickettsia-like-organisms and trypanosome infections in natural populations of tsetse in Liberia. *Trop Med Parasitol* 41.

McCall LI, Tripathi A, Vargas F, Knight R, Dorrestein PC, Siqueira-Neto JL. 2018. Experimental Chagas disease-induced perturbations of the fecal microbiome and metabolome. *PLoS Negl Trop Dis* 12. doi:10.1371/journal.pntd.0006344

McCarthy CB, Diambra LA, Pomar R V. 2011. Metagenomic analysis of taxa associated with Lutzomyia longipalpis, vector of visceral leishmaniasis, using an unbiased high-throughput approach. *PLoS Negl Trop Dis* 5:e1304. doi:10.1371/journal.pntd.0001304

Medina Munoz M, Brenner C, Richmond D, Spencer N, Rio RVM. 2021. The holobiont transcriptome of teneral tsetse fly species of varying vector competence. *BMC Genomics* 22. doi:10.1186/s12864-021-07729-5

Meki IK, Huditz HI, Strunov A, van der Vlugt RAA, Kariithi HM, Rezapanah M, Miller WJ, Vlak JM, van Oers MM, Abd-Alla AMM. 2021. Characterization and tissue tropism of newly identified iflavirus and negeviruses in Glossina morsitans morsitans tsetse flies. *Viruses* 13. doi:10.3390/v13122472

Mengoni A, Tatti E, Decorosi F, Viti C, Bazzicalupo M, Giovannetti L. 2005. Comparison of 16S rRNA and 16S rDNA T-RFLP approaches to study bacterial communities in soil microcosms treated with chromate as perturbing agent. *Microb Ecol* 50. doi:10.1007/s00248-004-0222-4

Monteiro CC, Villegas LEM, Campolina TB, Pires ACMHA, Miranda JC, Pimenta PFP, Secundino NFC. 2016. Bacterial diversity of the American sand fly Lutzomyia intermedia using high-throughput metagenomic sequencing. *Parasites and Vectors*. doi:10.1186/s13071-016-1767-z

Montoya-Porras LM, Omar TC, Alzate JF, Moreno-Herrera CX, Cadavid-Restrepo GE. 2018. 16S rRNA gene amplicon sequencing reveals dominance of Actinobacteria in Rhodnius pallescens compared to Triatoma maculata midgut microbiota in natural populations of vector insects from Colombia. *Acta Trop*. doi:10.1016/j.actatropica.2017.11.004

Moran MA. 2009. Metatranscriptomics: Eavesdropping on complex microbial communities. *Microbe* 4. doi:10.1128/microbe.4.329.1

Moreno CX, Moy F, Daniels TJ, Godfrey HP, Cabello FC. 2006. Molecular analysis of microbial communities identified in different developmental stages of Ixodes scapularis ticks from Westchester and Dutchess Counties, New York. *Environ Microbiol* 8. doi:10.1111/j.1462-2920.2005.00955.x

Mukhopadhyay J, Braig HR, Rowton ED, Ghosh K. 2012. Naturally occurring culturable aerobic gut flora of adult Phlebotomus papatasi, vector of Leishmania major in the old world. *PLoS One* 7. doi:10.1371/journal.pone.0035748

Munoz MM, Pollio AR, White HL, Rio RVM. 2017. Into the wild: Parallel transcriptomics of the Tsetse-Wigglesworthia Mutualism within Kenyan populations. *Genome Biol Evol* 9. doi:10.1093/gbe/evx175

Murillo-Solano C, López-Domínguez J, Gongora R, Rojas-Gulloso A, Usme-Ciro J, Perdomo-Balaguera E, Herrera C, Parra-Henao G, Dumonteil E. 2021. Diversity and interactions among triatomine bugs, their blood feeding sources, gut microbiota and Trypanosoma cruzi in the Sierra Nevada de Santa Marta in Colombia. *Sci Rep* 11. doi:10.1038/s41598-021-91783-2

Muscio OA, La Torre JL, Bonder MA, Scodeller EA. 1997. Triatoma Virus Pathogenicity in Laboratory Colonies of Triatoma infestans (Hemiptera: Reduviidae). *J Med Entomol* 34. doi:10.1093/jmedent/34.3.253

Muscio OA, La Torre JL, Scodeller EA. 1988. Characterization of Triatoma virus, a picorna-like virus isolated from the triatomine bug Triatoma infestans. *J Gen Virol* 69. doi:10.1099/0022-1317-69-11-2929

Muscio OA, LaTorre JL, Scodeller EA. 1987. Small nonoccluded viruses from triatomine bug Triatoma infestans (hemiptera: Reduviidae). *J Invertebr Pathol* 49. doi:10.1016/0022-2011(87)90163-7

Muturi EJ, Kim CH, Bara J, Bach EM, Siddappaji MH. 2016. Culex pipiens and Culex restuans mosquitoes harbor distinct microbiota dominated by few bacterial taxa. *Parasites and Vectors* 9. doi:10.1186/s13071-016-1299-6

Nakao R, Abe T, Nijhof AM, Yamamoto S, Jongejan F, Ikemura T, Sugimoto C. 2013. A novel approach, based on BLSOMs (Batch Learning Self-Organizing Maps), to the microbiome analysis of ticks. *ISME J* 7. doi:10.1038/ismej.2012.171

Narasimhan S, Fikrig E. 2015. Tick microbiome: The force within. *Trends Parasitol*. doi:10.1016/j.pt.2015.03.010

Narasimhan S, Rajeevan N, Liu L, Zhao YO, Heisig J, Pan J, Eppler-Epstein R, Deponte K, Fish D, Fikrig E. 2014. Gut microbiota of the tick vector Ixodes scapularis modulate colonization of the Lyme disease spirochete. *Cell Host Microbe* 15. doi:10.1016/j.chom.2013.12.001

Nevoa JC, Mendes MT, da Silva M V., Soares SC, Oliveira CJF, Ribeiro JMC. 2018. An insight into the salivary gland and fat body transcriptome of Panstrongylus lignarius (Hemiptera: Heteroptera), the main vector of Chagas disease in Peru. *PLoS Negl Trop Dis* 12. doi:10.1371/journal.pntd.0006243

Ni XB, Cui XM, Liu JY, Ye RZ, Wu YQ, Jiang JF, Sun Y, Wang Q, Shum MHH, Chang QC, Zhao L, Han XH, Ma K, Shen SJ, Zhang MZ, Guo W Bin, Zhu JG, Zhan L, Li LJ, Ding SJ, Zhu DY, Zhang J, Xia LY, Oong XY, Ruan XD, Shao HZ, Que TC, Liu GY, Du CH, Huang EJ, Wang X, Du LF, Wang CC, Shi WQ, Pan YS, Zhou YH, Qu JL, Ma J, Gong CW, Chen QQ, Qin Q, Lam TTY, Jia N, Cao WC. 2023. Metavirome of 31 tick species provides a compendium of 1,801 RNA virus genomes. *Nat Microbiol* 8. doi:10.1038/s41564-022-01275-w

Oliveira JL, Cury JC, Gurgel-Gonçalves R, Bahia AC, Monteiro FA. 2018. Field-collected Triatoma sordida from central Brazil display high microbiota diversity that varies with regard to developmental stage and intestinal segmentation. *PLoS Negl Trop Dis*. doi:10.1371/journal.pntd.0006709

Oliveira SM, Moraes BA, Goncalves CA, Giordano-Dias CM, D'Almeida JM, Asensi MD, Mello RP, Brazil RP. 2000. [Prevalence of microbiota in the digestive tract of wild females of Lutzomyia longipalpis Lutz & Neiva, 1912) (Diptera: Psychodidae)]. *Rev Soc Bras Med Trop* 33:319–322.

Orantes LC, Monroy C, Dorn PL, Stevens L, Rizzo DM, Morrissey L, Hanley JP, Rodas AG, Richards B, Wallin KF, Helms Cahan S. 2018. Uncovering vector, parasite, blood meal and microbiome patterns from mixed-DNA specimens of the Chagas disease vector Triatoma dimidiata. *PLoS Negl Trop Dis*. doi:10.1371/journal.pntd.0006730

Ortiz-Baez AS, Cousins K, Eden JS, Chang WS, Harvey E, Pettersson JHO, Carver S, Polkinghorne A, Šlapeta J, Rose K, Holmes EC. 2020. Meta-transcriptomic identification of Trypanosoma spp. In native wildlife species from Australia. *Parasites and Vectors* 13. doi:10.1186/s13071-020-04325-6

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

Pais R, Lohs C, Wu Y, Wang J, Aksoy S. 2008. The obligate mutualist Wigglesworthia glossinidia influences reproduction, digestion, and immunity processes of its host, the tsetse fly. *Appl Environ Microbiol* 74. doi:10.1128/AEM.00741-08

Papadopoulos C, Karas PA, Vasileiadis S, Ligda P, Saratsis A, Sotiraki S, Karpouzas DG. 2020. Host species determines the composition of the prokaryotic microbiota in phlebotomus sandflies. *Pathogens* 9. doi:10.3390/pathogens9060428

Parola P, Raoult D. 2001. Ticks and tickborne bacterial diseases in humans: An emerging infectious threat. *Clin Infect Dis*. doi:10.1086/319347

Perira de Oliveira SM, de Morais BA, Goncalves CA, Giordano-Dias CM, Vilela ML, Brazil RP, D'Almeida JM, Asensi MD, Mello RP. 2001. [Digestive tract microbiota in female Lutzomyia longipalpis (Lutz & Neiva, 1912) (Diptera: Psychodidae) from colonies feeding on blood meal and sucrose plus blood meal]. *Cad Saude Publica* 17:229–232.

Pettersson JHO, Shi M, Bohlin J, Eldholm V, Brynildsrud OB, Paulsen KM, Andreassen Å, Holmes EC. 2017. Characterizing the virome of Ixodes ricinus ticks from northern Europe. *Sci Rep* 7. doi:10.1038/s41598-017-11439-y

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

Ponnusamy L, Gonzalez A, Van Treuren W, Weiss S, Parobek CM, Juliano JJ, Knight R, Roe RM, Apperson CS, Meshnick SR. 2014. Diversity of rickettsiales in the microbiome of the lone star tick, amblyomma americanum. *Appl Environ Microbiol* 80. doi:10.1128/AEM.02987-13

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

Ramírez AL, Colmant AMG, Warrilow D, Huang B, Pyke AT, McMahon JL, Meyer DB, Graham RMA, Jennison A V., Ritchie SA, van den Hurk AF. 2020. Metagenomic Analysis of the Virome of Mosquito Excreta. *mSphere* 5. doi:10.1128/msphere.00587-20

Rau J, Werner D, Beer M, Höper D, Kampen H. 2022. The microbial RNA metagenome of Aedes albopictus (Diptera: Culicidae) from Germany. *Parasitol Res* 121. doi:10.1007/s00436-022-07576-7

Ribeiro JMC, Schwarz A, Francischetti IMB. 2015. A deep insight into the sialotranscriptome of the chagas disease vector, Panstrongylus megistus (hemiptera: Heteroptera). *J Med Entomol* 52. doi:10.1093/jme/tjv023

Rochlin I, Toledo A. 2020. Emerging tick-borne pathogens of public health importance: A mini-review. *J Med Microbiol*. doi:10.1099/jmm.0.001206

Rodríguez-Ruano SM, Škochová V, Rego ROM, Schmidt JO, Roachell W, Hypša V, Nováková E. 2018. Microbiomes of North American triatominae: The grounds for Chagas disease epidemiology. *Front Microbiol*. doi:10.3389/fmicb.2018.01167

Rozadilla G, Moreiras Clemente J, McCarthy C. 2020. HoSeIn: A Workflow for Integrating Various Homology Search Results from Metagenomic and Metatranscriptomic Sequence Datasets. *BIO-PROTOCOL*. doi:10.21769/bioprotoc.3679

Sadeghi M, Altan E, Deng X, Barker CM, Fang Y, Coffey LL, Delwart E. 2018. Virome of > 12 thousand Culex mosquitoes from throughout California. *Virology* 523:74–88. doi:10.1016/j.virol.2018.07.029

Sakamoto JM, Diaz GES, Wagner EA. 2020. Bacterial communities of ixodes scapularis from central Pennsylvania, USA. *Insects* 11. doi:10.3390/insects11100718

Salcedo-Porras N, Umaña-Diaz C, Bitencourt R de OB, Lowenberger C. 2020. The role of bacterial symbionts in triatomines: An evolutionary perspective. *Microorganisms*. doi:10.3390/microorganisms8091438

Sanchez-Vicente S, Tagliafierro T, Coleman JL, Benach JL, Tokarz R. 2019. Polymicrobial nature of tick-borne diseases. *MBio* 10. doi:10.1128/mBio.02055-19

Sang RC, Jura WGZO, Otieno LH, Mwangi RW, Ogaja P. 1999. The effects of a tsetse DNA virus infection on the functions of the male accessory reproductive gland in the host fly Glossina morsitans centralis (Diptera; Glossinidae). *Curr Microbiol* 38. doi:10.1007/PL00006815

Sant'Anna MRV, Darby AC, Brazil RP, Montoya-Lerma J, Dillon VM, Bates PA, Dillon RJ. 2012. Investigation of the bacterial communities associated with females of Lutzomyia sand fly species from South America. *PLoS One* 7. doi:10.1371/journal.pone.0042531

Schabereiter-Gurtner C, Lubitz W, Rölleke S. 2003. Application of broad-range 16S rRNA PCR amplification and DGGE fingerprinting for detection of tick-infecting bacteria. *J Microbiol Methods* 52. doi:10.1016/S0167-7012(02)00186-0

Schlein Y. 1977. Lethal effect of tetracycline on tsetse flies following damage to bacterioid symbionts. *Experientia* 33. doi:10.1007/BF01922204

Schultz MG. 1968. A history of bartonellosis (Carrión's disease). *Am J Trop Med Hyg* 17. doi:10.4269/ajtmh.1968.17.503

Schwarz A, Medrano-Mercado N, Schaub GA, Struchiner CJ, Bargues MD, Levy MZ, Ribeiro JMC. 2014. An Updated Insight into the Sialotranscriptome of Triatoma infestans: Developmental Stage and Geographic Variations. *PLoS Negl Trop Dis* 8. doi:10.1371/journal.pntd.0003372

Sharma P, Sharma S, Maurya RK, De T Das, Thomas T, Lata S, Singh N, Pandey KC, Valecha N, Dixit R. 2014. Salivary glands harbor more diverse microbial communities than gut in Anopheles culicifacies. *Parasites and Vectors* 7. doi:10.1186/1756-3305-7-235

Shi C, Liu Y, Hu X, Xiong J, Zhang B, Yuan Z. 2015. A metagenomic survey of viral abundance and diversity in mosquitoes from hubei province. *PLoS One*. doi:10.1371/journal.pone.0129845

Shi M, Neville P, Nicholson J, Eden J-S, Imrie A, Holmes EC. 2017. High-Resolution Metatranscriptomics Reveals the Ecological Dynamics of Mosquito-Associated RNA Viruses in Western Australia. *J Virol* 91. doi:10.1128/jvi.00680-17

Simo G, Njiokou F, Mbida Mbida JA, Njitchouang GR, Herder S, Asonganyi T, Cuny G. 2008. Tsetse fly host preference from sleeping sickness foci in Cameroon: Epidemiological implications. *Infect Genet Evol* 8. doi:10.1016/j.meegid.2007.09.005

Sonenshine DE. 2014. The Biology of Tick Vectors of Human DiseaseTick-Borne Diseases of Humans. doi:10.1128/9781555816490.ch2

Sonenshine DE, Roe RM. 2014. Biology of Ticks , Second Edition. *Oxford Univ Press*.

Song X, Zhong Z, Gao L, Weiss BL, Wang J. 2022. Metabolic interactions between disease-transmitting vectors and their microbiota. *Trends Parasitol*. doi:10.1016/j.pt.2022.05.002

Sperling JLH, Fitzgerald D, Sperling FAH, Magor KE. 2020. Microbiome Composition and Borrelia Detection in Ixodes scapularis Ticks at the Northwestern Edge of Their Range. *Trop Med Infect Dis* 5. doi:10.3390/tropicalmed5040173

Strand MR. 2018. Composition and functional roles of the gut microbiota in mosquitoes. *Curr Opin Insect Sci*. doi:10.1016/j.cois.2018.05.008

Tabbabi A, Mizushima D, Yamamoto DS, Kato H. 2022. Sand Flies and Their Microbiota. *Parasitologia* 2. doi:10.3390/parasitologia2020008

Tabbabi A, Watanabe S, Mizushima D, Caceres AG, Gomez EA, Yamamoto DS, Cui L, Hashiguchi Y, Kato H. 2021. Comparative analysis of bacterial communities in lutzomyia ayacuchensis populations with different vector competence to Leishmania parasites in Ecuador and Peru. *Microorganisms* 9. doi:10.3390/microorganisms9010068

Tarabai H, Floriano AM, Zima J, Filová N, Brown JJ, Roachell W, Smith RL, Beatty NL, Vogel KJ, Nováková E. 2023. Microbiomes of Blood-Feeding Triatomines in the Context of Their Predatory Relatives and the Environment. *Microbiol Spectr*. doi:10.1128/spectrum.01681-23

Tawidian P, Coon KL, Jumpponen A, Cohnstaedt LW, Michel K. 2021. Host-Environment Interplay Shapes Fungal Diversity in Mosquitoes. *mSphere* 6. doi:10.1128/msphere.00646-21

Telleria EL, Martins-Da-Silva A, Tempone AJ, Traub-Cseko YM. 2018. Leishmania, microbiota and sand fly immunity. *Parasitology*. doi:10.1017/S0031182018001014

Thongsripong P, Chandler JA, Green AB, Kittayapong P, Wilcox BA, Kapan DD, Bennett SN. 2018. Mosquito vector-associated microbiota: Metabarcoding bacteria and eukaryotic symbionts across habitat types in Thailand endemic for dengue and other arthropod-borne diseases. *Ecol Evol* 8. doi:10.1002/ece3.3676

Thongsripong P, Chandler JA, Kittayapong P, Wilcox BA, Kapan DD, Bennett SN. 2021. Metagenomic shotgun sequencing reveals host species as an important driver of virome composition in mosquitoes. *Sci Rep* 11. doi:10.1038/s41598-021-87122-0

Tobe SS. 1978. Reproductive physiology of Glossina. *Annu Rev Entomol*. doi:10.1146/annurev.en.23.010178.001435

Toju H, Hosokawa T, Koga R, Nikoh N, Meng XY, Kimura N, Fukatsu T. 2010. Candidatus Curculioniphilus buchneri,quot; a novel clade of bacterial endocellular symbionts from weevils of the genus Curculio. *Appl Environ Microbiol* 76. doi:10.1128/AEM.02154-09

Tokarz R, Sameroff S, Tagliafierro T, Jain K, Williams SH, Cucura DM, Rochlin I, Monzon J, Carpi G, Tufts D, Diuk-Wasser M, Brinkerhoff J, Lipkin WI. 2018. Identification of Novel Viruses in Amblyomma americanum , Dermacentor variabilis, and Ixodes scapularis Ticks . *mSphere* 3. doi:10.1128/msphere.00614-17

Tringe SG, Hugenholtz P. 2008. A renaissance for the pioneering 16S rRNA gene. *Curr Opin Microbiol* 11. doi:10.1016/j.mib.2008.09.011

Trzebny A, Slodkowicz-Kowalska A, Björkroth J, Dabert M. 2023. Microsporidian Infection in Mosquitoes (Culicidae) Is Associated with Gut Microbiome Composition and Predicted Gut Microbiome Functional Content. *Microb Ecol* 85. doi:10.1007/s00248-021-01944-z

Tsakeng CUB, Tanekou TTM, Soffack SF, Tirados I, Noutchih C, Njiokou F, Bigoga JD, Wondji CS. 2022. Assessing the Tsetse Fly Microbiome Composition and the Potential Association of Some Bacteria Taxa with Trypanosome Establishment. *Microorganisms* 10. doi:10.3390/microorganisms10061141

Vallejo GA, Guhl F, Schaub GA. 2009. Triatominae-Trypanosoma cruzi/T. rangeli: Vector-parasite interactions. *Acta Trop* 110. doi:10.1016/j.actatropica.2008.10.001

Van Overbeek L, Gassner F, Van Der Plas CL, Kastelein P, Nunes-Da Rocha U, Takken W. 2008. Diversity of Ixodes ricinus tick-associated bacterial communities from different forestsFEMS Microbiology Ecology. doi:10.1111/j.1574-6941.2008.00468.x

Vayssier-Taussat M, Moutailler S, Michelet L, Devillers E, Bonnet S, Cheval J, Hébert C, Eloit M. 2013. Next generation sequencing uncovers unexpected bacterial pathogens in ticks in western Europe. *PLoS One* 8. doi:10.1371/journal.pone.0081439

Vial L. 2009. Biological and ecological characteristics of soft ticks (Ixodida: Argasidae) and their impact for predicting tick and associated disease distriblftion. *Parasite* 16. doi:10.1051/parasite/2009163191

Villegas LEM, Campolina TB, Barnabe NR, Orfano AS, Chaves BA, Norris DE, Pimenta PFP, Secundino NFC. 2018. Zika virus infection modulates the bacterial diversity associated with Aedes aegypti as revealed by metagenomic analysis. *PLoS One* 13. doi:10.1371/journal.pone.0190352

Vivero RJ, Castañeda-Monsalve VA, Romero LR, Hurst GD, Cadavid-Restrepo G, Moreno-Herrera CX. 2021. Gut microbiota dynamics in natural populations of pintomyia evansi under experimental infection with leishmania infantum. *Microorganisms* 9. doi:10.3390/microorganisms9061214

Vivero RJ, Jaramillo NG, Cadavid-Restrepo G, Soto SIU, Herrera CXM. 2016. Structural differences in gut bacteria communities in developmental stages of natural populations of Lutzomyia evansi from Colombia's Caribbean coast. *Parasites and Vectors* 9. doi:10.1186/s13071-016-1766-0

Vivero RJ, Villegas-Plazas M, Cadavid-Restrepo GE, Herrera CXM, Uribe SI, Junca H. 2019. Wild specimens of sand fly phlebotomine Lutzomyia evansi, vector of leishmaniasis, show high abundance of Methylobacterium and natural carriage of Wolbachia and Cardinium types in the midgut microbiome. *Sci Rep* 9. doi:10.1038/s41598-019-53769-z

Volf P, Kiewegova A, Nemec A. 2002. Bacterial colonisation in the gut of Phlebotomus duboscqi (Diptera: Psychodidae): transtadial passage and the role of female diet. *Folia Parasitol (Praha)* 49:73–77.

Waltmann A, Willcox AC, Balasubramanian S, Borrini Mayori K, Mendoza Guerrero S, Salazar Sanchez RS, Roach J, Condori Pino C, Gilman RH, Bern C, Juliano JJ, Levy MZ, Meshnick SR, Bowman NM. 2019. Hindgut microbiota in laboratory-reared and wild Triatoma infestans. *PLoS Negl Trop Dis*. doi:10.1371/journal.pntd.0007383

Wang J, Gou Q yu, Luo G yan, Hou X, Liang G, Shi M. 2022. Total RNA sequencing of Phlebotomus chinensis sandflies in China revealed viral, bacterial, and eukaryotic microbes potentially pathogenic to humans. *Emerg Microbes Infect* 11. doi:10.1080/22221751.2022.2109516

Wang J, Weiss BL, Aksoy S. 2013. Tsetse fly microbiota: Form and function. *Front Cell Infect Microbiol*. doi:10.3389/fcimb.2013.00069

Wang J, Wu Y, Yang G, Aksoy S. 2009. Interactions between mutualist Wigglesworthia and tsetse peptidoglycan recognition protein (PGRP-LB) influence trypanosome transmission. *Proc Natl Acad Sci U S A* 106. doi:10.1073/pnas.0901226106

Wang Y, Gilbreath TM, Kukutla P, Yan G, Xu J. 2011. Dynamic gut microbiome across life history of the malaria mosquito anopheles gambiae in Kenya. *PLoS One* 6. doi:10.1371/journal.pone.0024767

Weaver SC, Charlier C, Vasilakis N, Lecuit M. 2018. Zika, Chikungunya, and Other Emerging Vector-Borne Viral Diseases. *Annu Rev Med* 69. doi:10.1146/annurev-med-050715-105122

Weiss BL, Maltz M, Aksoy S. 2012. Obligate Symbionts Activate Immune System Development in the Tsetse Fly. *J Immunol* 188. doi:10.4049/jimmunol.1103691

Weiss BL, Wang J, Aksoy S. 2011. Tsetse immune system maturation requires the presence of obligate symbionts in larvae. *PLoS Biol* 9. doi:10.1371/journal.pbio.1000619

Welburn SC, Arnold K, Gooday GW, Maudlin I. 1993. Rickettsia-like organisms and chitinase production in relation to transmission of trypanosomes by tsetse flies. *Parasitology* 107. doi:10.1017/S003118200006724X

Westreich ST, Ardeshir A, Alkan Z, Kable ME, Korf I, Lemay DG. 2019. Fecal metatranscriptomics of macaques with idiopathic chronic diarrhea reveals altered mucin degradation and fucose utilization. *Microbiome* 7. doi:10.1186/s40168-019-0664-z

Whipps JM, Karen L, Cooke RC. 1988. Mycoparasitism and plant disease controlFungi in Biological Control Systems.

WHO. 2023a. Vector-borne diseases. *WHO*. https://www.who.int/news-room/fact-sheets/detail/vector-borne-diseases

WHO. 2023b. Malaria - Fact sheets. https://www.who.int/en/news-room/fact-sheets/detail/malaria

WHO. 2023c. Chagas disease (American trypanosomiasis). https://www.who.int/health-topics/chagas-disease#tab=tab_1

WHO. 2023d. World Chagas Disease Day 2023 to focus on integrating universal care and surveillance at the primary care level. https://www.who.int/news/item/14-04-2023-world-chagas-disease-day-2023-to-focus-on-integrating-universal-care-and-surveillance-at-the-primary-care-level

WHO. 2017. Global Vector Control response 2017 -2030: An integrated approach for the control of vector borne diseases. *WHO*.

Wigglesworth VB. 1936. Symbiotic Bacteria in a Blood-sucking Insect, Rhodnius Prolixus Stål. (Hemiptera, Triatomidae). *Parasitology* 28. doi:10.1017/S0031182000022459

Wikipedia. 2023. Metabarcoding. https://en.wikipedia.org/wiki/Metabarcoding

Wu-Chuang A, Hodžić A, Mateos-Hernández L, Estrada-Peña A, Obregon D, Cabezas-Cruz A. 2021. Current debates and advances in tick microbiome research. *Curr Res Parasitol Vector-Borne Dis*. doi:10.1016/j.crpvbd.2021.100036

Wu Q, Guo C, Li X kang, Yi BY, Li QL, Guo ZM, Lu JH. 2023. A meta-transcriptomic study of mosquito virome and blood feeding patterns at the human-animal-environment interface in Guangdong Province, China. *One Heal* 16. doi:10.1016/j.onehlt.2023.100493

Xu L, Guo M, Hu B, Zhou H, Yang W, Hui L, Huang R, Zhan J, Shi W, Wu Y. 2021. Tick virome diversity in Hubei Province, China, and the influence of host ecology. *Virus Evol* 7. doi:10.1093/ve/veab089

Yadav KK, Bora A, Datta S, Chandel K, Gogoi HK, Prasad GBKS, Veer V. 2015. Molecular characterization of midgut microbiota of Aedes albopictus and Aedes aegypti from Arunachal Pradesh, India. *Parasites and Vectors* 8. doi:10.1186/s13071-015-1252-0

Yang X, Qin S, Liu X, Zhang N, Chen J, Jin M, Liu F, Wang Y, Guo J, Shi H, Wang C, Chen Y. 2023. Meta-Viromic Sequencing Reveals Virome Characteristics of Mosquitoes and Culicoides on Zhoushan Island, China . *Microbiol Spectr* 11. doi:10.1128/spectrum.02688-22

Yang Z, Wang H, Yang S, Wang X, Shen Q, Ji L, Zeng J, Zhang W, Gong H, Shan T. 2023. Virome diversity of ticks feeding on domestic mammals in China. *Virol Sin* 38. doi:10.1016/j.virs.2023.02.001

Yassin AF. 2005. Rhodococcus triatomae sp. nov., isolated from a blood-sucking bug. *Int J Syst Evol Microbiol* 55. doi:10.1099/ijs.0.63571-0

Zhang R, Yu G, Huang Z, Zhang Z. 2020. Microbiota assessment across different developmental stages of Dermacentor silvarum (Acari: Ixodidae) revealed stage-specific signatures*Ticks Tick Borne Dis* 11. doi:10.1016/j.ttbdis.2019.101321

Submitted date: November 1, 2023, Accepted Date: January 31, 2024, Published date: June 30, 2024.