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The Neglected Virome of Triatomine Insects

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The Triatominae subfamily (Reduviidae) harbors some hematophagous insect species that have been firmly connected to the transmission of *Trypanosoma cruzi*, the causative agent of Chagas disease. Triatomines not only host and transmit trypanosomatids, but also coexist with a variety of symbiotic microorganisms that generally reside in the insect's intestinal flora. The microbiome has profound effects on the physiology, immunity, fitness and survival of animals and plants. The interaction between triatomines and bacteria has been investigated to some extent and has revealed important bacteria symbionts. In contrast, the range of viral species that can infect triatomine insects is almost completely unknown. In some cases, genomic and metatranscriptomic approaches have uncovered sequences related to possible viral genomes, but, to date, only eight positive single-strand RNA viruses, namely Triatoma virus and *Rhodnius prolixus* viruses 1 - 7 have been investigated in more detail. Here, we review the literature available on triatomine viruses and the viruses-insect host relationship. The lack of broader metagenomic and metatranscriptomic studies in these medically relevant insects underscores the importance of expanding our knowledge of the triatomine virome both for surveillance purposes as well as to possibly harness their potential for insect vector population control strategies.

Keywords: Triatomine, virus, microbiome, metatranscriptome, Chagas disease

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INTRODUCTION

Hematophagous insect species belonging to the Triatominae (Reduviidae) sub-family are primary vectors of *Trypanosoma cruzi*, the etiologic agent of Chagas disease. This life-threatening parasitosis affects 6-7 million people in South and Central America (1) and is mainly transmitted by insect species belonging to three Triatomine genera: *Rhodnius*, *Triatoma* and *Panstrongylus.* However, more than 150 Triatomine species can support *T. cruzi* infections in laboratory settings or in the wild and therefore are potential vectors of the disease (2, 3). Triatomines are widely distributed in the Americas, from South Argentina to the South of USA causing concern in areas where Chagas disease is not endemic, but it is introduced by the increasing population mobility (2, 4, 5). Among triatomine species, *Rhodnius prolixus* has been extensively studied since the early 1900s proving an excellent model system to investigate several physiological and biochemical processes in insects (6–9). For instance, seminal studies by Sir V. B. Wigglesworth shed light on the hormonal control of oogenesis (10, 11) as well as the role of the juvenile hormone in metamorphosis (11). In recent years, the sequencing and partial assembly of *Rhodnius* genome has spun novel genetic and genomic studies, which have started to unveil the molecular mechanisms underlying triatomine development, reproduction, immunity and evolution (6, 12–18). Substantial progress has also been achieved in the characterization of the triatomine microbiome, where a special focus was placed on symbiotic bacteria. Triatomines are a reservoir of bacteria like *Serratia marcescens*, a Gram-negative bacteria that causes severe infections in humans and *Rhodococcus rhodnii*, the first symbiotic bacteria found in the triatomine gut (3, 19, 20). Because of space constraints, we refer the reader to excellent reviews on this topic as well as on the interaction between triatomines and *T. cruzi* (3, 13, 18, 21). Instead, here we focus on the virome of triatomine insects, a research area that is still in its infancy. Metagenomic approaches have helped uncover a wealth of viral species in arthropods especially in those of medical interest like the mosquito *Aedes aegypti* (22). Currently, 1,445 RNA viruses divided in more than 20 families clustered in 16 clades have been identified in insect tissues (23). Viral infections can prove harmless for the host or affect its survival and reproduction, while a few cases of beneficial virus-host interactions have been reported (24–27). Paradigmatic among the entomopathogenic class of viruses, the Slow Bee paralysis virus (SBPV) and related viral species (Deformed wing virus, Israeli acute paralysis virus, etc) are known to impair the development of bees (28), induce anterior leg paralysis (29) and malformations in wings and abdomen in the host (30, 31). SBPV is in fact responsible for the collapse of bee hives around the world causing large economic losses (32). Viruses however can represent beneficial symbionts in some cases (33). For instance, the plant virus Barley Yellow Dwarf Virus (BYDV) was shown to increase the fecundity and promote trophic facilitation of phytophagous vector species like the aphids *Rhopalosiphum maidis* and *Rhopalosiphum padi* (34). For the vast majority of the insect viruses however the characteristics of their interactions with the insect hosts are unknown. Despite the clinical relevance of some triatomine species, very little is known about their virome. Contigs related to viral genomes were independently identified in transcriptomic analyses of the salivary glands, fat bodies and testes of triatomine species like *Rhodnius prolixus*, *Panstrongylus megistus* and *Panstrongylus lignarius* (35–37). Viral sequences were also reported after sequencing the genome of *Rhodnius prolixus* (12). These observations however were not explored further and almost all that we know about triatomine viruses stems from the study of Triatoma virus (TrV) (38–40). TrV was discovered in a colony of field-collected *Triatoma infestans* (38, 40). Only recently, our group identified and characterized 7 novel viruses, namely *Rhodnius prolixus* virus 1-7 (RpV1-7), while investigating stage-specific *de novo* transcriptome assemblies in ovarian tissues of *Rhodnius prolixus* (16, 17). This review aims to describe the (limited) information available on triatomine viruses and underscore the importance of investing in this neglected area of scientific research.

TRIATOMA VIRUS

Triatoma virus (TrV) is a non-enveloped virus that displays a positive single strand RNA (+) ssRNA) genome and was first identified in the abdominal contents of infected *T. infestans* as small spherical particles about 30 nm in diameter (38). Due to its unique capsid structure, TrV originated a new genus within the *Dicistroviridae* family, namely the *Triatovirus* genus (PDB ID: 3NAP and 1B35, respectively) (41–43) (**Figure 1**). The TrV genome is 9010 nt and harbors two open reading frames (ORFs) (44). ORF1 (549-5936 nt) encodes motifs characteristic of viral RNA-dependent RNA polymerases (RdRp), cysteine proteases and RNA helicases, while ORF2 encodes capsid proteins (**Figure 1**). The two ORFs are separated by 172 nt of non-coding RNA (44). Two putative internal ribosomal entry sites (IRESs) in the TrV genome were identified: the 5'UTR IRES of 548 nt and the intergenic region (IGR) IRES of 172 nt (45). The TrV capsid displays icosahedral symmetry with pseudo-triangulation number P = 3 and is formed by 60 protomers comprising the three major VP1, VP2 and VP3 and one minor (VP0) structural polypeptides (40, 41, 46, 47). The capsid shows spikes forming a crown commonly present in members of the *Dicistroviridae* family (48). The exposed amino acid residues in those projections were proposed to exert a role in the interaction with the host (47). Infections by TrV were shown to induce 97.6% mortality rates in blood-fed fifth instar nymphs, delay the molting process (39, 49) and impair oogenesis (49). In accordance, the oviposition rate in adult *T. infestans* females infected with TrV drops by ~80% compared to uninfected females (49). Fifteen triatomine species have been shown to support TrV infections in laboratory settings, where the virus was inoculated *via* intrahemocelic injection or artificial ingestion of purified viral particles (50). In an artificial TrV infection experiment, fecal suspensions parenterally inoculated into uninfected triatomines killed all insects (n= 515) within 36 h (51), whereas viral particles could be detected at fecal samples from 5th instar *Triatoma infestans* fed in an artificial feeder with TrV-contaminated human blood (52). *T. infestans* also becomes infected with TrV when feeding on contaminated chickens (51). Some triatomines infected with TrV were collected in the field. For instance, *Triatoma sordida* from the Chaco province (53), *Psammolestes coreodes* in nests in a region endemic for Chagas disease (54) and *Triatoma breyeri* in sylvatic habitats mainly in the arid Chaco and Monte ecoregions in Argentina (50). Field-collected *Triatoma patagonica* (55), *Rhodnius neglectus*, an autochthonous Brazilian species, and *Meccus longipennis* could also be artificially infected by TrV (56). However, only 10% of *Triatoma infestans* from sylvatic populations from Argentine were found to harbor TrV infections (39). The discrepancy between the infection rates in wild-caught and colony-raised insects mostly likely lies on the variety of viral transmission routes like cannibalism, coprophagy and transovarial transmissions and their specific contribution to the spread of the virus in the population (39). Rearing conditions in the laboratory are particularly favorable for fecal-oral transmission, because insects are artificially overcrowded during feeding (51). The presence of a pheromone in feces freshly deposited by nymphs (57) attracts unfed nymphs while arresting the locomotion of fed ones. Hence, this phenomenon might also favor the spread of the TrV in the insectaries (58).

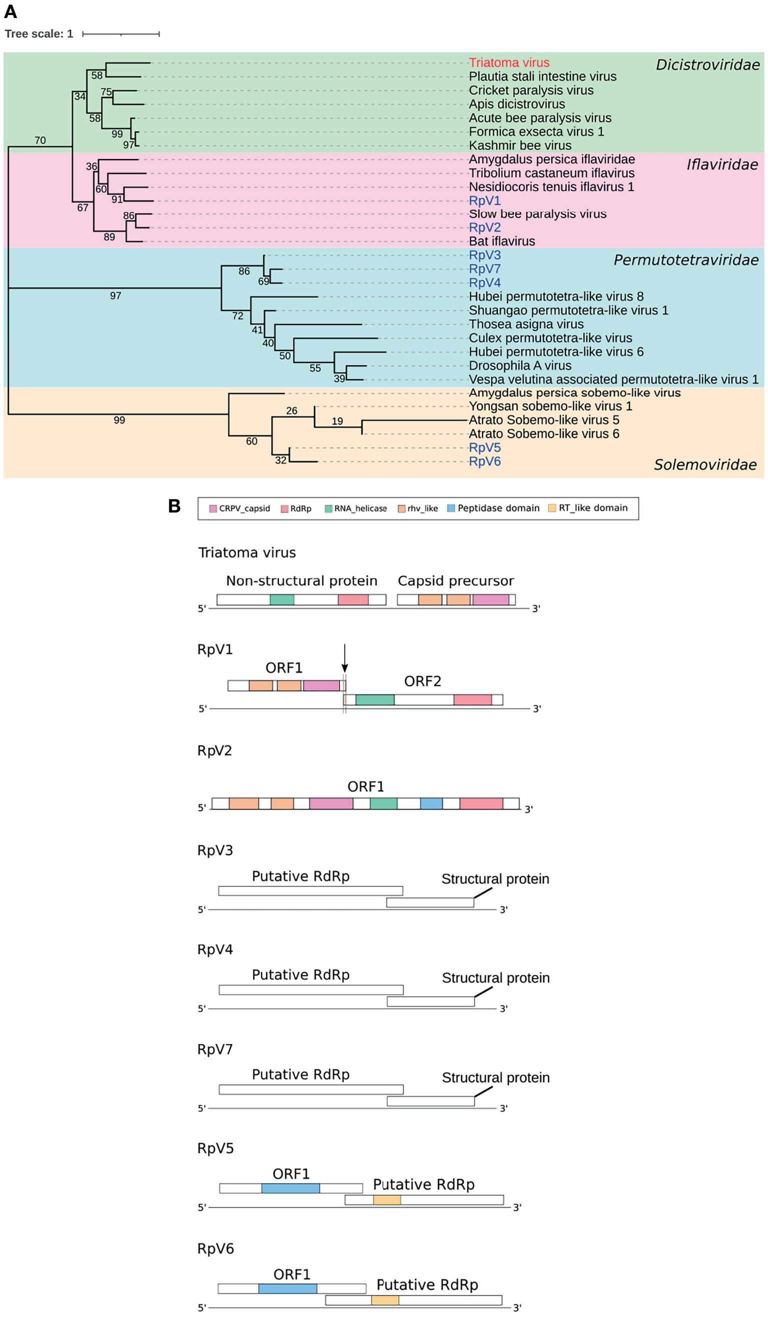

FIGURE 1 | Phylogenetic analysis and genome structure of the triatomine viruses. **(A)** Phylogenetic tree constructed from RdRp sequences of the (+) ssRNA viruses, TrV and RpV 1-7. Neighbor-Joining method with 1000 bootstrap replicates was used in the phylogenetic analysis. Bootstrap values are displayed in tree branches. Triatomine viruses belong to four different families: *Dicistroviridae*, *Iflaviridae*, *Permutotetraviridae* and *Solemoviridae.* **(B)** Genome organization of the TrV and RpVs displaying their structural and non-structural domains. CRPV_capsid, Cricket Paralysis Virus capsid protein, RdRp, RNA-dependent RNA Polymerase, RNA helicase, rhv-like, Picornavirus/Calicivirus coat protein, Peptidase domain, RT_like, Reverse Transcriptase_like. Adapted from Brito, et al. (16).

Unlike some arboviruses that are capable of infecting both invertebrates and vertebrates like Dengue Virus and Zika Virus among others, *Insect-Specific Viruses* (ISVs) display a host range restricted to insects (59). TrV inoculation in different insect cell lines such as Sf9, Sf21 (from *Spodoptera frugiperda*), High Five (H5) and C6/36 (*Aedes albopictus*) resulted in signs of infection or cytopathic effect (CPE) after 24-48 h (60). However, this phenotype disappeared after successive passages. These results were confirmed with three independent approaches: transfection, direct inoculation of the purified viral particles or inoculation of the purified viral particles with trypsin (61). Thus, TrV appears to be a triatomine-specific entomopathogenic virus. This characteristic led to propose TrV as a possible biological tool for the control of Chagas disease Triatomine vector populations (39, 40).

RHODNIUS PROLIXUS VIRUSES

Recently, our group expanded the variety of viral species in triatomines by describing 7 unique viral genomes in *Rhodnius prolixus* (16). The RpVs are (+) single strand RNA viruses classified in three different families: *Iflaviridae* (RpV1 and RpV2), *Permutotetraviridae* (RpV3, RpV4 and RpV7) and *Solemoviridae* (RpV5 and RpV6) (16) and do not share any similarity with TrV (**Figure 1A** and **Table 1**).

RpV1 has the longest contig found (9.6 Kilobases) and it exhibits two main ORFs with amino acid sequence identity 36.99% and 40.88% with the proteins encoded by ORF1 and ORF2 of the Nesidiocoris tenuis virus 1 (NtV-1), respectively (16). NtV-1 has been classified in the *Iflavirus* genus, even though its genome revealed two different ORFs (62). ORF1 of RpV1 encodes putative structural capsid proteins related to Cricket Paralysis virus (CrPV) and *Picornavirales* order specific proteins such as RhV-like (**Figure 1B**). ORF2 instead encodes putative non-structural proteins such as the RdRp enzyme and a RNA helicase (16). ORF1 and ORF2 of RpV1 display a 1-nt overlap typical of the *Iflavirus* genus, which results in a -1nt frame-shift (**Figure 1B**). The RpV2 genome (contig ~8.9Kb) encodes a single polyprotein (2,890 amino acids) and it also seems to belong to the *Iflavirus* genus like RpV1 (16). The putative polyprotein of this virus shares ~70% amino acid sequence identity with the polyprotein of the Slow Bee Paralysis Virus (SBPV). For both viruses, the RhV-like and the CrPV structural capsid proteins are located at the N-terminal region, while the RdRp enzyme is at the C-terminal (16, 63). SBPV can be transmitted to *Apis mellifera* adult bees and pupae by the mite *Varroa destructor* (64). SBPV infections induce paralysis of the anterior legs and high mortality rates (29). Additionally, the bees that survived from one season to the next and were infected by Deformed Wing Virus (DWV) or SBPV showed a decrease in the infection rate by mite, suggesting equilibrium between entomopathogenic viruses and the mite (65). The large mite population can promote a starvation condition for the honeybee favoring condition-dependent SBPV virulence which was demonstrated in artificial infection assays in the *Bombus terrestris* pollinator (26). Since we first detected it in our ovarian RNA-seq datasets produced in 2014, RpV2 seems to have been lost to our colony (16). It is tempting to speculate that the pathogenicity of this virus might have eliminated the infected animals, eventually causing its disappearance from the colony. We cannot rule out however

TABLE 1 | GenBank database accession numbers from all sequences used in the phylogenetic tree.

Name	Genome	RdRp localization
Apis dicistrovirus	KY354239.1	ARO50048.1
Kashmir bee virus	NC_004807.1	NP_851403.1
Acute bee paralysis virus	NC_002548.1	NP_066241.1
Thosea asigna virus	NC_043231.1	YP_009665207.1
Bat iflavirus	NC_033823.1	YP_009345906.1
Atrato Sobemo-like virus 6	MN661101.1	QHA33729.1
Yongsan sobemo-like virus 1	MH703049.1	AXV43879.1
Vespa velutina associated permutotetra-like virus 1	MN565051.1	QGL51734.1
Culex permutotetra-like virus	LC505019.1	BBO25553.1
Tribolium castaneum iflavirus	MG012488.1	AUE23905.1
Amygdalus persica iflaviridae	MN823678.1	QKQ15124.1
Amygdalus persica sobemo-like virus	MN831439.1	QKI29238.1
RpV1	MZ328304.1	QYC92628.1
RpV2	MZ328305.1	QYC92629.1
RpV3	MZ328306.1	QYC92630.1
RpV4	MZ328307.1	QYC92632.1
RpV5	MZ328308.1	QYC92635.1
RpV6	MZ328309.1	QYC92637.1
RpV7	MZ328310.1	QYC92638.1
Triatoma virus	AF178440.1	AAF00472.1
Drosophila A virus	NC_012958.1	YP_003038595.1
Cricket paralysis virus	NC_003924.1	NP_647481.1
Formica exsecta virus 1	NC_023021.1	YP_008888535.1
Plautia stali intestine virus	NC_003779.1	NP_620555.1
Nesidiocoris tenuis iflavirus 1	KY969634.1	AVI05074.1
Slow bee paralysis virus	NC_014137.1	YP_003622540.1
Atrato Sobemo-like virus 5	MN661107.1	QHA33865.1
Hubei permutotetra-like virus 6	KX883442.1	APG76931.1
Hubei permutotetra-like virus 8	KX883453.1	APG76960.1
Shuangao permutotetra-like virus 1	KX883439.1	APG76925.1

Column 1: Name of the viruses analyzed; Column 2: Accession numbers of the viral genome sequences; Column 3: Accession numbers of the RdRp sequences.

that RpV2 might still be circulating in the insectary, but at threshold viral levels below the sensitivity of our assays.

RpV3, RpV4 and RpV7 belong to *Alphapermutotetravirus* genus, *Permutotetraviridae* family and they exhibit a genome around 5Kb long. The putative RdRp enzymes of RpV3, RpV4 and RpV7 share ~42% to ~47% amino acid sequence identity with proteins from Shuangao and Hubei permutotetra-like viruses (16). Only two *Alphapermutotetravirus* are classified by Taxonomy in the International Committee on Taxonomy of Viruses (ICTV): *Euprosterna elaeasa virus* and *Thosea asigna virus*. In a large assay analyzing invertebrate RNA viruses from several metatranscriptome libraries, thirteen Hubei permutotetra-like viruses and one Shuangao permutotetra-like virus were found in different arthropods (23). The host range of Hubei permutotetra-like viruses seems to be broad, including different host species like *Coleoptera* sp., *Aedes* sp., *Culex quinquefasciatus*, *Abraxas tenuisuffusa*, *Anopheles sinensis*, *Aedes albopictus*, *Aedes aegypti*, *Paracercion melanotum*, *Gryllidae* sp., *Neoscona nautica*, *Scutigeridae* sp. and *Spirurida sp1* (23, 66).

RpV5 and RpV6 (contigs ~2.8Kb for both) belong to the *Sobemovirus* genus and their encoded proteins share amino acid sequence identity ranging from 41% to 66%, respectively, when they were compared with Atrato sobemo-like virus. RpV5 and RpV6 also harbor two ORFs: ORF1 encodes Peptidase and ORF2 the RdRp enzyme (16) (**Figure 1B**). The *Sobemovirus* genus comprises some plant viruses whose natural host range is relatively narrow. Several sobemoviruses like the Rice Yellow Mottle Virus (RYMV) are of special interest as they infect crop plantations causing large economic losses (67). The main transmission route of sobemoviruses is through host plant wounds that can be caused by insects such as the beetle *Ceratoma trifurcota* (67, 68). Although sobemoviruses can infect invertebrates as beetles, aphids, mirid bugs, moths, grasshoppers and sucking bugs, the interaction of viruses with invertebrates has been poorly explored. It was recently shown that *R. prolixus* can feed on artificial sugar meals or even cherry tomatoes in laboratory settings, even though triatomines have been considered strictly hematophagous for over a century (69, 70). It is therefore possible that RpV5 and RpV6 infected the insects that originated our colony while they were feeding on the phloem of some plant.

Both RpVs and TrV are vertically transmitted to progeny, which likely contributes to establish persistent infections (16, 39). Vertical transmission is of special interest as it has been linked to the emergence of seasonal pandemics of some arboviroses like Dengue and Zika (71, 72). Furthermore, DWV and other viruses that infect honeybees were found in the queen's ovaries and transmitted to the offspring including eggs and young larvae (24). Additional transmission routes have been demonstrated for TrV, but they remain still unexplored for the RpVs. For these viruses, also the host range restriction needs to be addressed in the future.

Because only one virus was known until recently, the investigation of antiviral systems in triatomines is also lagging behind. It has been suggested that haemocytes, phenoloxidase and nitric oxide might provide defense mechanisms against viral infections in these insect species (18), but this hypothesis has not been tested yet. However, the discovery of viral small interfering RNAs against the RpVs and the evolutionary conservation of key RNAi players in *Rhodnius prolixus* ovaries demonstrates that a RNAi-based antiviral system is active in triatomines and might keep the viral load below lethal levels (15–17). It will be interesting to explore these mechanisms especially in embryos, where the viral titers appear to be several orders of magnitudes lower than in other stages of *Rhodnius prolixus* early development. Recently, another class of small non-coding RNAs, namely the Piwi-interacting RNAs or piRNAs, was implicated in antiviral activity in Aedes mosquitoes (73, 74). Viral piRNAs can originate from viral sequences, known as Endogenous Viral Elements (EVEs), harbored in the genome of the insect (73). However, we did not identify either piRNAs or EVEs related to the RpVs in R. prolixus ovary (16). In the absence of similar studies in other Triatomines, it is therefore likely that the piRNA pathway does not contribute to antiviral defenses in ovarian tissues of Triatomine insects.

DISCUSSION

Triatomine insect species are of public health importance since they can vector the protozoan *T. cruzi*, the etiologic agent of Chagas Disease. Surprisingly, the characterization of the virome in these insects is still in the embryonic phase mostly due to the lack of broad unbiased metagenomic and metatranscriptomic studies. The metatranscriptomic studies from different Triatomine species including field-captured bugs together with functional assays for viral pathogenicity are some of the strategies which might be used to better understand the Triatomine virome diversity. This goal is of paramount importance and might be achieved by including the analysis of viral small interfering RNAs (vsiRNAs), which not only provide valuable information on the antiviral systems acting in the insect, but can also be employed for the assembly of the genome of new viruses (16, 75). If some triatomines are capable of transmitting *T. cruzi* to vertebrates and humans, we cannot rule out that they might also be competent vectors capable of transmitting pathogenic viruses (3). This hypothesis receives some support by the observation that most of the human sera samples from patients with Chagas disease in the Argentine, Bolivia and Mexico were positive for anti-TrV antibodies (76). The vectorial competence of triatomines has been tested with HIV and HBV, which were not able to replicate in the insect tissues or be transmitted to mammalian hosts (77, 78). To our knowledge however the interaction between triatomines and arboviruses like Dengue, Zika and Chikungynya viruses has not been tested yet. Conversely, some triatomine ISVs might be harnessed as biological tools for the control of insects of economic and medical relevance. When applied to triatomines, these strategies might lead to the control of triatomine vector populations in the wild and therefore, the reduction of Chagas disease diffusion. Finally, TrV Viral-Like Particles (TrV-VLPs) have been proposed to act as a vaccine adjuvant by enhancing the humoral immune response against *T. cruzi* chimeric antigens (79). A thorough characterization of the triatomine virome, its interactions with the insect host and host range restriction are therefore a challenge of paramount importance for the next future.

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