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ISID Grant Report

RNA Interference: a tool for identifying and controlling Dengue virus and other arboviruses in insect vectors

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Introduction

Arboviruses, such as dengue virus (DENV), are a group of viruses transmitted to humans by insects and other arthropods. They infect millions of people annually and have great impact on public health. Most arboviral diseases lack effective treatments or vaccines. Therefore surveillance and vector control are the major strategies to help fight transmission of arboviruses (Weaver and Barrett, 2004).

Insects are productively infected by arboviruses and also need to control the infection to avoid detrimental effects. The antiviral defense in insects is mediated by RNA interference (RNAi). The RNAi pathway recognizes viral double stranded RNA and generates virus-derived small interfering RNAs (siRNAs) capable of inhibiting viral replication(Marques and Carthew, 2007; P.P.Vilela, et al., 2012). Notably, the antiviral siRNAs result from processing of viral dsRNA thus containing sequence information about their origin. Indeed, sequencing of virus-derived small RNAs can help identify viruses infecting insects and plants (Kreuze, et al., 2009; Wu, et al., 2010). Here, we applied a strategy based on small RNA deep sequencing data to discover viruses in two important insects vectors. We generated small RNA libraries from wild colonies of the sand fly *Lutzomyia longipalpis* and the mosquito *Aedes aegypti*. Using our strategy, we were able to identify and characterize 5 new viruses circulating in populations of these two dipteran insects.

Materials and Methods

1- *Samples: Aedes aegypti* colonies were established from wild populations collected in Rio de Janeiro state in Brazil. *Lutzomya longipalpis* colonies were established from wild populations collected in Teresina, state of Piaui in Brazil.

2- *Sequencing*: Libraries were prepared and sequenced using Illumina platform.

3- *Contig assembly*: Reads were filtered by quality and trimmed using FatsX Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/index.html). Reads that match common bacteria and reference host genomes were removed. Remaining reads separated by size: 20-23 nt reads, 21 nt only and 24-29 nt were assembled into contigs using Velvet assembler (Zerbino and Birney, 2008). Different assemblies were merged using cap3 software (Huang and Madan, 1999).

4- *Characterization*: Assembled contigs were characterized by sequence similarity searches

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against the nt database using BLAST (Altschul, et al., 1990), and analyzed for proteins domains using InterProScan (Mulder and Apweiler, 2007) and HMMER (Eddy, 2009).

Results and Discussion

Small RNA libraries were prepared from mosquitoes and sand flies and analyzed using an integrated pipeline for detection of viral sequences. Using this strategy, we were able to detect viral sequences that seem to correspond to 5 potential new viruses (Table 1).

Table 1: Contigs that show significant similarity to viral sequences:

Origin of library				
Virus family	Contig ID	Contig size	e-value (blastx)	Best hit
Mosquitoes				
Bunyaviridae				
	Aae_18	6807	0E+00	RdRP [Phasi Charoen-like virus]
Luteoviridae				
	Aae_5	2793	8E-34	RdRP [Penaeus monodon RNA virus]
Sand flies				
Reoviridae				
	Ll_94	3762	3E-173	RdRP [Choristoneura occidentalis cypovirus 16]
	Ll_132	3680	0E+00	RdRP [Bombyx mori cypovirus 1]
Nodaviridae				
	Ll_116	2018	0E+00	RdRP [Nodamura virus]

Mosquito libraries contained several contigs that showed around 99% nucleotide similarity to *Phasi charoen virus,* a bunyavirus first isolated from mosquitoes in Thailand (Yamao, et al., 2009). This is the first time this virus is reported in another continent. Further analysis is being performed to determine the relationship between Asian and American isolates of *Phasi charoen virus.* Bunyaviruses can cause severe disease in a wide range of animals, including humans, (Mertens, et al., 2013; Meyer and Schmaljohn, 2000). In mosquito libraries, we also found contigs that showed similarity to viruses from the *Luteoviridae* family but are distinct enough from any known species. Several luteoviruses are plants pathogens transmitted by aphids (Ali, et al., 2014).

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Sand fly libraries contained contigs that showed sequence similarity to two separate viruses of the *Reoviridae* family. These contigs were analyzed in details and, based on phylogeny utilizing viral RdRP sequences, we could infer that they represent two separate reoviruses. Both of them show sequence similarity to viruses belonging to the genus *Cypovirus* from the *Reoviridae* family. However, their sequences do not present enough nucleotide similarity, which suggests they represent two new Reovirus species. Cypoviruses have been mostly described to infect insects (Shapiro, et al., 2005; Zhao, et al., 2003). We also detected another set of contigs in sand fly libraries that showed sequence similarity to viruses of the genus *Alphanodavirus* from the *Nodaviridae* family. Sequence analysis suggests this Nodavirus is likely a new species. Nodaviruses are known to infect insects and also vertebrates (Ball, et al., 1992).

We have confirmed the presence of these viral sequences by PCR and Sanger sequencing and are currently trying to isolate each of these viruses in order to perform detailed biological characterization.

Conclusions

Our non-biased viral metagenomics approach applied to small RNA sequencing data allowed for successful identification of viruses in mosquitoes and sand flies without any prior information about their presence. The majority of the viruses we identified potentially represent new species, indicating the power of our strategy. Some of these viruses could directly impact public health, such as Bunyaviruses and Reoviruses, that are known to infect mammals. In addition, these viruses could have an indirect impact by affecting the circulation of other human pathogens in infected insects. In summary, our work highlights the importance of surveillance strategies to monitor viruses in wild vector insects, which can help design measures to prevent and control outbreaks.

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