Local-scale virome depiction in Medellín, Colombia, supports significant differences between *Aedes aegypti* and *Aedes albopictus*

Arley Calle-Tobón1,2*, Juliana Pérez-Pérez1, Nicolás Forero-Pineda2, Omar Triana Chávez3, Winston Rojas-Montoya2, Guillermo Ruá-Uribe3, Andrés Gómez-Palacio2

1 Grupo Entomología Médica–GEM, Universidad de Antioquia, Medellín, Colombia, 2 Laboratorio de Investigación en Genética Evolutiva–LIGE, Universidad Pedagógica y Tecnológica de Colombia, Tunja, Boyacá, Colombia, 3 Grupo de Biología y Control de Enfermedades Infecciosas–BCEI, Universidad de Antioquia, Medellín, Colombia, 4 Grupo de Genética Molecular–GenMol, Universidad de Antioquia, Medellín, Colombia

* arley.calle@udea.edu.co

Abstract

*Aedes* spp. comprise the primary group of mosquitoes that transmit arboviruses such as dengue, Zika, and chikungunya viruses to humans, and thus these insects pose a significant burden on public health worldwide. Advancements in next-generation sequencing and metagenomics have expanded our knowledge on the richness of RNA viruses harbored by arthropods such as *Ae. aegypti* and *Ae. albopictus*. Increasing evidence suggests that vector competence can be modified by the microbiome (comprising both bacteriome and virome) of mosquitoes present in endemic zones. Using an RNA-seq-based metataxonomic approach, this study determined the virome structure, *Wolbachia* presence and mitochondrial diversity of field-caught *Ae. aegypti* and *Ae. albopictus* mosquitoes in Medellín, Colombia, a municipality with a high incidence of mosquito-transmitted arboviruses. The two species are sympatric, but their core viromes differed considerably in richness, diversity, and abundance; although the community of viral species identified was large and complex, the viromes were dominated by few virus species. BLAST searches of assembled contigs suggested that at least 17 virus species (16 of which are insect-specific viruses [ISVs]) infect the *Ae. aegypti* population. Dengue virus 3 was detected in one sample and it was the only pathogenic virus detected. In *Ae. albopictus*, up to 11 ISVs and one plant virus were detected. Therefore, the virome composition appears to be species-specific. The bacterial endosymbiont *Wolbachia* was identified in all *Ae. albopictus* samples and in some *Ae. aegypti* samples collected after 2017. The presence of *Wolbachia* spp. in *Ae. aegypti* was not related to significant changes in the richness, diversity, or abundance of this mosquito’s virome, although it was related to an increase in the abundance of *Aedes aegypti* To virus 2 (*Metavirus*). The mitochondrial diversity of these mosquitoes suggested that the *Ae. aegypti* population underwent a change that started in the second half of 2017, which coincides with the release of *Wolbachia*-infected mosquitoes in Medellín, indicating that the population of wMel-infected mosquitoes released has introduced new alleles into the wild *Ae. aegypti* population of Medellín. However, additional studies are required on the dispersal
speed and intergenerational stability of wMel in Medellin and nearby areas as well as on the introgression of genetic variants in the native mosquito population.

Introduction

Members of the mosquito genus *Aedes* (Diptera; Culicidae) are the primary vectors of arboviruses such as dengue virus (DENV), Zika virus (ZIKV), and chikungunya virus (CHIKV) to humans. Two species widely distributed globally are *Ae. (Stegomyia) aegypti* (Linnaeus, 1762)—the primary vector for various arboviruses that infect millions of people in tropical and subtropical countries every year [1]—and *Ae. (Stegomyia) albopictus* (Skuse, 1885)—a native to Asia but has expanded its distribution in the last 40 years, invading not only countries in the tropical and subtropical zones but also those in the temperate zones of North America and Europe, where it has been a primary vector of some arboviruses [2, 3].

Arboviruses affect several countries in the Americas, for example, Colombia; it is highly affected by DENV, ZIKV, and CHIKV [4–6]. The high incidence of arboviruses in Colombia is because of the wide distribution of *Ae. aegypti* throughout the country, where environmental and social conditions promote viral transmission [7, 8]. In 1998, *Ae. albopictus* was detected in the southern part of the country, and since then, this species has expanded its distribution to numerous cities such as Medellin, where both *Ae. aegypti* and *Ae. albopictus* are infected with DENV and ZIKV [9–11]. Since 2000, the number of dengue cases in Medellin has increased continuously, with periodic outbreaks, and nearly 20,000 dengue cases have been reported in each of the last major outbreaks occurring in 2010 and 2016 [12, 13]. Owing to the significant impact these mosquitoes have had on the health of this city’s residents, multiple vector surveillance and control strategies have been implemented. However, the success of these strategies has been questionable as dengue continues to occur in Medellin. The situation in Colombia reflects those in many other arbovirus-endemic countries, which require new approaches to control these vector-borne diseases.

Currently, there is growing scientific interest regarding the mechanism through which the mosquito microbiome, including insect-specific viruses (ISVs), are involved in arbovirus transmission. Among the microorganisms that can modify the vector competence of mosquitoes, the intracellular endosymbiotic bacterium *Wolbachia* has been demonstrated in vitro to reduce the replication of multiple arboviruses such as DENV, ZIKV, and CHIKV in *Ae. aegypti* [14–16]; moreover, it can also alter the native host microbiome in adult mosquitoes [17].

In addition to bacteria, the microbiome of mosquitoes also includes viruses. Recent analyses of virus sequences in metagenomics data have changed our understanding of viral diversity, abundance, evolution, and roles in host biology [18, 19]. In mosquitoes, pathogenic viruses represent only a fraction of the total set of viruses (virome) [20–25]. Furthermore, the identities of ISVs vary between populations and species; some ISVs are acquired from the environment, whereas others may circulate through vertical transmission, forming the virome core of mosquitoes populations [22, 26]. However, metagenomic studies for virome characterization may be conflated by the identification of endogenous viral elements (EVEs) that are not real viruses [27], entailing a challenge in these studies for the correct identification of viruses. Mosquitoes, like many organisms, possess EVEs that are remnants of viral integrations into their genomes [28]. And particularly, a large number of non-retroviral EVEs was detected in *Aedes* mosquito genomes [29, 30] (ref), increasing the interest in mosquito EVEs due to the hypothesis that they may serve as the source of immunological memory against exogenous viruses in insects [31].
The effect of many ISVs on the biology of mosquitoes remains unknown, some viruses may either suppress or enhance the replication of medically important arboviruses such as DENV, ZIKV, CHIKV, and West Nile viruses, suggesting that they play a role in modulating vector competence [32–34]. The insect-specific flavivirus Nhumirim virus is an example of an arbovirus suppressor as it can restrict the growth of ZIKV in mosquito cells in vitro and Ae. aegypti mosquitoes, resulting in significantly lower ZIKV infection rates in both orally infected and intrathoracically inoculated mosquitoes [35]. Although ISVs do not replicate in vertebrates, some are phylogenetically related to known pathogenic arboviruses from the families Flaviviridae, Bunyaviridae, Rhabdoviridae, Reoviridae, and Togaviridae. This finding has led to increasing efforts to discover and characterize more ISVs and explore ISVs as models for studying virus restriction or as potential biocontrol agents [33, 34]. A recent virome description of the global populations of Ae. aegypti and Ae. albopictus reveals significant differences in the composition and diversity of ISVs found in these mosquito species. Furthermore, the abundance of some ISVs such as Phasi Charoen-like virus (PCLV) and Humaita-Tubiacanga virus (HTV) may affect the arboviruses infection capacity as well as their transmission dynamics [36].

In Medellin, Colombia, the primary vector of arboviruses is Ae. aegypti, but Ae. albopictus may potentially be a secondary vector as it can be naturally infected with DENV2 [10, 37] and ZIKV [11, 38]. Both Medellin and its adjacent city, Bello, sustain arbovirus at consistent rates. Thus, these cities were selected for pioneering efforts to test an alternative control strategy based on the release of Wolbachia-transfected Ae. aegypti mosquitoes, which started in 2017 [39]. These releases have not yet provided any conclusive epidemiological evidence of arbovirus control in Medellin; however, in Yogyakarta, Indonesia, the introgression of wMel into Ae. aegypti population was related with a decrease in the incidence of symptomatic dengue [40]. Furthermore, other characteristics describing the native mosquitoes’ biology such as virome composition and mosquito population diversity before and after Wolbachia-transfected Ae. aegypti release remain unexplored. Our study used a metataxonomic approach employing RNA-seq to describe the virome composition, temporal stability, and wide mitochondrial diversity of wild sympatric Ae. aegypti and Ae. albopictus captured between 2015 and 2019 in Medellin, Colombia. To our knowledge, the present study is the first to provide the estimates of the diversity and abundance of viromes in wild-caught Ae. aegypti and Ae. albopictus in Colombia. It is also the first study to report on virome diversity in a city in the Americas where Wolbachia-transfected Ae. aegypti release has been explored. This study thus provides evidence showing the local differences in the richness, diversity, and abundance of ISVs between these two mosquito species and discusses the possible impacts of the Wolbachia-transfected mosquitoes on the core composition of the virome and diversity of the native mosquito population on a local scale.

Materials and methods

Mosquito sampling

The municipality of Medellín, Colombia, is located at 75° 34′05″ W and 6′13′55″ N and covers an area of approximately 376.2 km². Medellin is the second most populated city in Colombia, with a population of 2.5 million. Its location in the Andes’ central mountains at an altitude between 1500 and 1800 mts gives Medellin a humid subtropical climate with important rainfalls. The precipitation is characterized by a bi-modal distribution, while the temperatures range between 17 °C and 28 °C.

Indoor resting adult mosquitoes were captured randomly from households using mouth aspirators and entomological nets between 2016 and 2019. The captured mosquitoes were transferred alive to the Medical Entomology Laboratory in the University of Antioquia.
(Medellin), where they were killed and identified using morphological keys [41]. The blood feeding status of female mosquitoes was determined through an external examination of the abdomen, females that had presence of blood were excluded from the study. Subsequently, the mosquitoes were stored at −80°C and sorted according to the sampling periods and species. In total, 430 mosquitoes were thus divided into 14 pools.

**RNA isolation and sequencing**

Total RNA was extracted from the mosquitoes using the RNeasy Mini Kit (Qiagen) according to the manufacturer’s instructions. The quality of the RNA extracts was evaluated using an Agilent 2100 Bioanalyzer (Agilent Technologies) operated by Macrogen (Seoul, Korea; www.macrogen.org). After confirming the RNA integrity number (> 7), sequencing libraries were constructed using a TruSeq total RNA library preparation kit (Illumina) after first removing the host rRNA with a Ribo-Zero-Gold (Human–Mouse–Rat) kit (Illumina). Each library was sequenced as 100-bp paired-ends on the Novaseq 6000 S4 platform (Illumina).

**Bioinformatic analysis**

The paired-end reads were processed using the fastp software v.0.20.0 to remove adaptor sequences and perform quality-based filtering [42]. The resulting high-quality reads (Phred quality score > 20) were mapped to their respective reference genomes (Ae. aegypti assembly AaegL5.2 [43] and Ae. albopictus assembly AaloF1.2 [44]) using the BWA-MEM option in VectorBase (https://vectorbase.org/vectorbase/app/) [45]. The unmapped reads were subsequently analyzed as described below.

Viral sequences were evaluated by first creating a custom database of ribosomal RNA sequences using SILVA v.132 LSU, SSU, and 5S rRNA (RF00001), 5.8S rRNA (RF00002), tRNA (RF00005), and ribonuclease P (RF000010, RF00011, and RF00373). Our custom database was then used via the software SortMeRNA v.2.1 [46] to identify, using an e-value cutoff of 10\(^{-5}\), ribosomal sequences in the unmapped reads [47]. Reads with 60% of the read length identical to ribosomal RNA sequences by >60% were excluded from further analysis. The remaining sequences were assembled using SPAdes v.3.12.0 [48] (S1 Table), and contigs > 450 nt were classified using DIAMOND v.2.0.8 [49] and the nr viral database (updated March 2021). The analysis employed the sensitive mode for taxonomic annotation with an e-value cutoff of 10\(^{-5}\) and a bit score cutoff of 100 [47, 50]. The output file of DIAMOND was parsed using KronaTools v.2.7.1 [51], which found the least common ancestor of the best 25 DIAMOND hits. Only contigs with identity scores of >60% were stored. Contigs identified as viral operational taxonomic units (vOTUs) were confirmed by performing an online BLASTn and BLASTx (https://blast.ncbi.nlm.nih.gov/Blast.cgi) searches, thus eliminating possible false positives. All OTUs were assigned to viral species based on their BLASTx identity. Although we used an RNA-seq strategy and perform a manual review of the viral contigs, allowing to identify and exclude some EVEs, we cannot completely exclude the possibility that some contigs are derived from EVEs. This must be assessed in future studies that evaluate both the identification of EVEs in these mosquito genome populations and their expression.

**Virome diversity analysis**

The number of viral reads per library was calculated by mapping the libraries against the viral contigs identified, then they were normalized by simply dividing viral reads number by the mosquito mapped reads number, and then multiplying the result by the size of the smaller sample. The alpha diversity index (i.e., diversity within each sample) was evaluated based on the observed virome richness, Shannon index, Simpson index, and evenness of each library at
the virus species level using the Rhea script set [52]. Finally, the indices of the host mosquito species were compared using Kruskal–Wallis rank sum and Mann–Whitney tests employing the R software [53].

Beta diversity (i.e., viral diversity between samples) was estimated by first constructing a Bray–Curtis dissimilarity matrix [54], which considers both the shared taxonomic composition and virus abundance in the viromes. The results were plotted using nonmetric multidimensional scaling (NMDS) ordination, and the significance of the resulting clusters was tested using permutational multivariate analysis of variance (PERMANOVA; Adonis tests) employing the R software.

Wide mitochondrial diversity between *Ae. aegypti* and *Ae. albopictus*

Paired-end reads were mapped using BWA-MEM v.0.7.17 [45, 55] against the mitochondrial genomes of *Ae. aegypti* and *Ae. albopictus* (NC_035159.1 and NC_006817.1, respectively). Only reads with a mapQ score of >20 were retained using SAMtools v.1.9 [56, 57]. Optical polymerase chain reaction (PCR) duplicates were removed, and read grouping was performed in Picard v.2.9.0 (http://broadinstitute.github.io/picard/). Variant calls in each pool were estimated using the “—pooled-discrete” option in the haplotype-based variant detection method implemented in freebayes v.1.2.0 [58]. Multiallelic positions at total depths of >10x were included and indels were excluded from variant calling. To analyze sequences, we retrieved a single sequence from each sample using FastaAlternateReferenceMaker in GATK v.4.1.2.0 [59]. Then, the overall nucleotide polymorphism per site (Theta-W) was estimated using DnaSP v.6 [60], and a Kimura-2p based distance tree was constructed using MEGA X [61].

**DENV PCR assay**

Because we detected DENV-3 in one of the RNA-seq samples, we performed a semi-nested reverse transcriptase (RT)-PCR to confirm this finding. The Luna Universal One-Step RT-qPCR kit was used according to manufacturer’s instructions with the primers D3_Fwd1 (5’ – GACCCAGAAGGCGGTTATTT-3’) and D3-Rev1 (5’ – GCCTCGAACATCTTCCCAATA–3’). These primers amplify a 1,260-bp region of the envelope gene [62]. The second PCR used the Thermo Scientific Taq DNA polymerase according to manufacturer instructions and employed the primers D3-Rev1 (5’ – GCCTCGAACATCTTCCCAATA–3’) and D3-MA (5’ – ACAAGCCCACGTTGGATAG-3’). These primers amplify a 1,057-bp fragment of the envelope gene. Amplicons were purified and sequenced (Macrogen). The forward and reverse sequences were joined using BioEdit v7.2 [63], generating the consensus sequences.

**Phylogenetic analysis**

The obtained consensus sequences were first aligned with reference sequences before phylogenetic analysis. The alignments were performed with MAFFT v.7.475 using the L-INS-i algorithm [64] and 1,000 cycles of iterative refinement. The phylogenetic tree was reconstructed from 1,000 ultrafast bootstrap maximum likelihood-based tree replicates using IQ-TREE v1.6.12 [65]. The best-fitting model was selected using ModelFinder [66]. The phylogenetic tree was drawn using FigTree v.1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/).

**Results**

In this study, a total of 10 and 4 pooled samples of *Ae. aegypti* and *Ae. albopictus*, respectively, were analyzed using mosquitoes captured between 2015 and 2019. Each pooled sample contained 15–36 mosquitoes (Table 1), and these were subjected to RNA extraction followed by
RNA sequencing. After processing the raw data, a total of 1,190,309,014 (range 93,199,032–131,645,868) 100-bp paired-end reads were generated from the 10 ribosomal RNA-depleted sequence libraries from *Ae. aegypti*; a total of 493,223,542 (range 105,740,360–131,645,868) 100-bp paired-end reads were generated from the four *Ae. albopictus* libraries. Approximately 65.3% of the reads corresponded to the mosquito sequences; therefore, a total of 405,798,910 and 191,058,189 reads were used to characterize the viromes of *Ae. aegypti* and *Ae. albopictus*, respectively, collected from Medellín, Colombia.

Virome characterization

After annotating and analyzing the contigs with DIAMOND BLASTx annotation and Krona-Tools, respectively, the contigs were verified; 125 of them were identified as viral OTUs (86 from *Ae. aegypti* and 39 from *Ae. albopictus*) comprising 21 virus species (Fig 1). These virus species were related to ISVs, arboviruses, and plant viruses belonging to nine different viral families: *Flaviviridae*, *Totiviridae*, *Reoviridae*, *Phenuiviridae*, *Iflaviridae*, *Bromoviridae*, *Metaviridae*, *Xinmoviridae*, and *Orthomyxoviridae*; however, six viruses remained unclassified.

Sequences from each library were mapped to the identified viral contigs to determine the abundance of each identified virus species. The rarefaction curves shown in Fig 2A are based on the number of viral sequences; these show that the curves describing samples are approaching the saturation plateau, suggesting that most viruses were detected in this experiment. In addition, *Ae. albopictus* curves showed a tendency to plateau faster, suggesting that the virome of this species is less diverse than that of *Ae. aegypti*. Furthermore, significantly lower numbers of viral sequences were detected in *Ae. albopictus* (Fig 2B).

A total of 75,219,419 viral sequences (6.3% of libraries) were identified in *Ae. aegypti*, which were assigned to 17 viral species. The virome of this species was dominated by four viruses, which accounted for approximately 90% of the viral sequences. Of the total viral sequences, 52% corresponded to PCLV (*Phenuiviridae*); PCLV was present in all libraries and was the most abundant virus in almost all samples, except for *Ae. aegypti* 2015B, where *Aedes anphevirus* (*Xinmoviridae*) was the most abundant virus detected. The second and third most abundant viruses were Kwale mosquito virus (21.5%) and Guadeloupe mosquito virus (GMV; Table 1. Summary of the sequencing results showing data on pooled samples and the numbers of mosquitoes, mosquito species, read sequences, and mapped mosquito reads.

| Sample name   | Mosquito species | No. of mosquitoes | Sampling time         | No. of clean reads | Mosquito reads mapped |
|---------------|------------------|-------------------|-----------------------|--------------------|-----------------------|
| *Ae. aeg 2015A* | *Ae. aegypti*    | 15                | January–May, 2015     | 93,199,032         | 88,936,067            |
| *Ae. aeg 2015B* | *Ae. aegypti*    | 25                | September–November, 2015 | 113,389,586       | 104,933,849           |
| *Ae. aeg 2016A* | *Ae. aegypti*    | 35                | February–June, 2016   | 129,886,418        | 104,933,849           |
| *Ae. aeg 2016B* | *Ae. aegypti*    | 33                | July–November, 2016   | 122,388,802        | 74,256,344            |
| *Ae. aeg 2017A* | *Ae. aegypti*    | 31                | March–June, 2017      | 105,740,360        | 56,373,655            |
| *Ae. aeg 2017B* | *Ae. aegypti*    | 34                | July–December, 2017   | 128,342,842        | 75,574,534            |
| *Ae. aeg 2018A* | *Ae. aegypti*    | 35                | March–June, 2018      | 119,858,346        | 72,780,110            |
| *Ae. aeg 2018B* | *Ae. aegypti*    | 33                | July–November, 2018   | 131,645,868        | 85,013,726            |
| *Ae. aeg 2019A* | *Ae. aegypti*    | 31                | January–June, 2019    | 123,578,244        | 74,904,614            |
| *Ae. aeg 2019B* | *Ae. aegypti*    | 28                | July–November, 2019   | 122,279,516        | 76,372,131            |
| *Ae. alb 2017*  | *Ae. albopictus* | 25                | August–December, 2017 | 124,650,502        | 81,419,768            |
| *Ae. alb 2018*  | *Ae. albopictus* | 36                | March–August, 2018    | 114,442,616        | 63,806,581            |
| *Ae. alb 2019A* | *Ae. albopictus* | 34                | January–June, 2019    | 130,821,410        | 71,501,186            |
| *Ae. alb 2019B* | *Ae. albopictus* | 35                | July–November, 2019   | 123,309,014        | 85,437,818            |

https://doi.org/10.1371/journal.pone.0263143.t001
12.2%), respectively; both viruses are unclassified but are probably mosquito-specific viruses. Finally, cell fusing agent virus (CFAV; Flaviviridae) accounted for 3.7% of the total viral sequences. Interestingly, DENV3 (Flaviviridae) was detected in one of the samples from 2016. We confirmed the presence of this virus through RT-PCR targeting a fragment of the envelope gene. Subsequent phylogenetic analysis identified this sequence as DENV3 genotype III (S1 Fig). No other pathogenic arboviruses were identified in this study. The Ae. aegypti virome composition was fairly conserved among the samples throughout the 5 years of sampling, suggesting that the virome of Ae. aegypti in Medellín is stable over time.

The virome of Ae. albopictus had less viral sequences and species than that of Ae. aegypti (Fig 2). In Ae. Albopictus, a total of 12 viral species were identified, which were pooled into five families (84,339 reads, 0.02% of the total). Aedes flavivirus (AeFV; Flaviviridae) was the most abundant virus, accounting for 44.4% of the viral sequences in all samples (Fig 3). Australian
Anopheles totivirus (Totiviridae) accounted for 16% of viral sequences, and Katimi river virus (Flaviviridae) accounted for 15.3%. PCLV was predominant in the virome of *Ae. aegypti*, whereas it accounted for only 1% of the total viral sequence in *Ae. albopictus*. Of all viruses detected in *Ae. albopictus*, only eight were present in multiple samples (Fig 3). Tobacco streak virus (Bromoviridae)—a plant virus—and Kwale mosquito virus were detected in sample *Ae. alb 2017*, whereas GMV (unclassified viruses) and HTV were detected in sample *Ae. alb 2019A*.

**Virome comparison between the *Aedes* spp.**

The two *Aedes* spp. differed in the composition and abundance of virus species in their respective viromes. Fig 3 clearly shows the hierarchical clustering of virus species in each mosquito species based on the Euclidean distance matrix. In total, 21 virus species were identified, of which only eight were shared between the mosquito species studied (Australian Anopheles totivirus, CFAV, PCLV, GMV, HTV, Kwale mosquito virus, Kaiowa virus, and *Aedes aegypti* To virus 2). Similarly, viruses in both mosquito species had significantly different levels of alpha diversity, as evident from the indexes of diversity and richness (Fig 4A). Although the *Ae. aegypti* virome is richer in virus species, the *Ae. albopictus* virome is more diverse, based on Shannon and Simpson indices. This higher diversity may be because of the higher evenness of the *Ae. albopictus* viral community.

Beta diversity is based on Bray–Curtis dissimilarities that were calculated from the normalized abundance of viral species and then analyzed via unconstrained ordination using the PERMANOVA test on mosquito species (*p* = 0.004 and *R*² = 0.59) and NMDS. Fig 4B shows a clear separation of viral communities according to mosquito species, suggesting that the viromes of these two mosquito species differ significantly in their compositions.

**Effects of Wolbachia on the *Ae. aegypti* and *Ae. albopictus* viromes**

The presence of the intracellular symbiotic bacterium, *Wolbachia*, was detected in all *Ae. albopictus* samples, which might have been because this species is naturally infected by the
bacterium. In contrast, *Ae. aegypti* is not a natural host for *Wolbachia*; however, *Ae. aegypti* infected with *Wolbachia* have been released in Medellín since 2017. *Wolbachia* sequences were detected in five *Ae. aegypti* samples that were collected from mid-2017 to 2019. The consensus contigs of the gene encoding the surface protein (*wsp*) were used for phylogenetic analysis (Fig 5), which shows that *Ae. albopictus* was infected by *Wolbachia* spp. from clades A and B. In *Ae. aegypti*, *Wolbachia* sp. belonging to clade A, which is related to *Drosophila melanogaster* isolates, was detected. This result was expected because the released mosquitoes were transfected with the *wMel* strain, which originated from *D. melanogaster*.

Whether the *Wolbachia* infection in *Ae. aegypti* could influence the richness or diversity of the *Ae. aegypti* virome was investigated. For this purpose, *Ae. aegypti* samples with and without *Wolbachia* sp. were compared. No significant differences in richness or diversity were found between the samples; however, *Aedes aegypti* To virus 2, an *Errantivirus*, was significantly more abundant in samples with *Wolbachia* (Fig 4C).

**Mitochondrial diversity of *Aedes mosquitoes***

On average, 459,117 (± 222,695) reads were mapped in *Ae. aegypti*, whereas 443,887 (± 195,269) reads were mapped in *Ae. albopictus* (Table 2). A total of 285 and 150 single-
nucleotide polymorphisms (SNPs) were detected in *Ae. aegypti* and *Ae. albopictus*, respectively (Table 2).

In general, the number of SNPs per sample was higher in *Ae. aegypti* than in *Ae. albopictus*, except for sample *Ae. aeg 2015A*. However, variable sites were observed throughout the entire mitochondrial genome in both species. SNP density peaked at around position 7,000 of the mitochondrial genome, a region involved with coding for the ND5 gene in both species (Fig 4).
In addition, a second high-density SNP region located between positions 12,400 and 14,600 of the mitochondrial genome was observed in *Ae. aegypti* samples. This location harbors coding regions of the ND1, tRNA-Leu, tRNA-Val, and 16s, and 12s rRNA genes (Fig 6). The overall nucleotide polymorphism (Theta-W) was 0.004 for *Ae. aegypti*, whereas it was 0.002 for *Ae. albopictus*. Thus, the neighbor-joining tree showed two well-supported clades.

### Table 2. Results of mitochondrial diversity analysis showing the numbers of mitochondrial mapped reads and SNPs.

| Sample name | No. of mitochondrial mapped reads | No. of SNPs |
|-------------|----------------------------------|-------------|
| Ae. aeg 2015A | 859,461                          | 163         |
| Ae. aeg 2015B | 207,324                          | 143         |
| Ae. aeg 2016A | 352,912                          | 155         |
| Ae. aeg 2016B | 328,784                          | 150         |
| Ae. aeg 2017A | 109,532                          | 122         |
| Ae. aeg 2017B | 435,763                          | 197         |
| Ae. aeg 2018A | 565,002                          | 175         |
| Ae. aeg 2018B | 682,894                          | 177         |
| Ae. aeg 2019A | 495,284                          | 187         |
| Ae. aeg 2019B | 554,218                          | 195         |
| Average     | 459,117                          | 285         |
| Ae. alb 2017 | 308,580                          | 112         |
| Ae. alb 2018 | 591,312                          | 128         |
| Ae. alb 2019A | 630,538                          | 142         |
| Ae. alb 2019B | 245,118                          | 106         |
| Average     | 443,887                          | 150         |

https://doi.org/10.1371/journal.pone.0263143.t002
harboring both species. A secondary clade including samples collected after June 2017 (i.e., sample Ae. aeg 2017A) was also revealed in Ae. aegypti (Fig 7).

Discussion

Recent studies on mosquito microbiomes have begun to elucidate the complex interactions between microorganisms and their hosts as well as the effects the former has on the latter. Most studies have focused on the bacteriome, thus the role and transmission of the virome, which comprises a highly diverse community of viruses, are less understood. Metagenomic studies rely on database analysis, which is a problem in virome studies because sequence information for many viral families and genera is still limited. Therefore, this study used stringent restrictions such as OTUs identity and cover greater than 60% to reduce the possibility of reporting false positives. Although false positive errors are minimized through this approach, it can also result in the underestimation of the true diversity of viruses in these mosquito populations.

This study, to the best of our knowledge, is the first to characterize the viromes of Ae. aegypti and Ae. albopictus populations in Colombia, where mosquito-borne diseases such as dengue, Zika, and chikungunya are major public health concerns [5]. The findings of the present study add significant information to the existing knowledge regarding the mosquito viromes in the Americas and the world. The study results reveal that the compositions of the viromes of sympatric, field-caught Ae. aegypti and Ae. albopictus differ significantly, suggesting that virome composition is species-specific and reflects differences in the host evolutionary history, host immunological response, virus–mosquito interactions, and perhaps vector competence. The two mosquito species also differ largely in the richness of virus species associated with them and the abundances of these viruses. This result was consistent across all comparisons (Fig 3).

A larger proportion of viral sequences were associated with Ae. aegypti; these sequences represented a greater richness of viruses than those associated with Ae. albopictus. These results support the hypothesis that Ae. aegypti has a higher capacity to carry viruses and,
therefore, to disperse these viruses more widely [36]. This also suggests that this mosquito species is more susceptible to infection by arboviruses such as DENV, ZIKV, and CHIKV [36]. Globally, the Ae. aegypti virome is highly variable in terms of the richness of viral species [22, 36]. In the present study, the Ae. aegypti virome comprised least 17 virus species predominated by PCLV, which is consistent with the results of previous studies showing that this virus...
superinfects *Ae. aegypti*, for example, PCLV has been reported to be a predominant ISV in *Ae. aegypti* populations in Australia [24], Guadeloupe [67], South China [68], Thailand [69], Brazil [36], and Grenada [70]. Interestingly, PCLV is less abundant in African populations such as those in Senegal, and it is absent in Gabon, where the dominant subspecies is related to *Ae. aegypti formosus*, which is considered less susceptible to arbovirus infection [71].

GMV and Kwale mosquito virus were also highly abundant in *Ae. aegypti* samples. GMV has been identified recently in *Ae. aegypti* from Guadeloupe, where it shows high abundance in the mosquito virome [67, 72]. Kwale mosquito virus was detected in a metagenomic analysis of *Ae. aegypti* mosquitoes from Kenya; it is phylogenetically closely related to Hubei mosquito virus 2 and GMV related viruses [22]. GMV and Kwale mosquito virus are currently considered unclassified viruses, and their effects on the biology of *Aedes* remain unknown. Another abundant member of the *Ae. aegypti* virome is CFAV, a flavivirus that was the first insect-specific flavivirus found in *Aedes* [73]. Since then, it has been detected in multiple regions [22, 24, 74–76]. Furthermore, CFAV infection can significantly enhance DENV replication in *Ae. aegypti* cells [77].

In this study, *Flaviviridae* was the most diverse family, and three ISVs as well as one arbovirus (CFAV, Menghai flavivirus, Xishuangbanna AeFV, and DENV3) were detected in most samples. Menghai flavivirus has been isolated from *Ae. albopictus* mosquitoes in China, and it is phylogenetically related to Xishuangbanna AeFV, which belongs to the *Aedes*-associated flaviviruses cluster [78]. Although ISVs are abundant and highly prevalent, arbovirus infections in wild mosquitoes are rare. Nonetheless, DENV3 was detected in sample *Ae. aeg. 2016A*. Results of the phylogenetic analysis revealed that this virus belongs to genotype III, the same genotype that has been circulating in Colombia and other South American countries [61]. A dengue epidemic occurred in Medellín in 2016 [13]; however, the *Ae. aegypti* virome from that year did not differ from those from other years.

ISVs from the *Totiviridae* family (*Aedes aegypti* toti-like virus and *Australian Anopheles* totivirus) and one from the *Iflaviridae* family (iflavirus) were detected in sample *Ae. aeg* 2016B. The latter is related to slow bee paralysis virus (72.2% BLASTx identity), although it may also be a new noncharacterized *iflavirus*. Similarly, several viruses from *Aedes* mosquitoes have been tentatively identified as *Iflaviruses*-like viruses [72, 79, 80]. These results indicate that this virus family needs to be expanded and revised. Furthermore, AaAV (*Xinmoviridae*) was detected in all samples. This *Ae. aegypti*-associated virus has been detected in field-caught and colony mosquitoes as well as in cells lines. AaAV can interact with *Wolbachia* and, in the process, influence DENV replication in cell lines [81].

Virus species richness was lower in *Ae. albopictus* samples, where 12 virus species were detected. The core virome of *Ae. albopictus* mainly comprised eight viruses that were found in multiple samples. The most diverse set of viruses belonged to the *Flaviviridae* family and included three ISVs, namely, AeFV, CFAV, and Kamiti river virus. AeFV is a flavivirus associated with both *Ae. albopictus* and *Ae. flavopictus* [82]. A temporal study of AeFV in *Ae. albopictus* in China suggests that it is a seasonal virus [83]. Because Colombia is a tropical country, AeFV may be perennially present in *Ae. albopictus*.

*Australian Anopheles* totivirus was the second most abundant species in *Ae. albopictus*, and it was present in all samples in addition to Kaiwa virus, *Aedes aegypti* To virus 2, Lamyprys noctiluca errantivirus 1 (*Metaviridae*), and PCLV. In contrast to the findings obtained from *Ae. aegypti*, PCLV was rare in *Ae. albopictus* samples. Notably, both PCLV and CFAV have been reported to inhibit ZIKV, DENV, and La Crosse virus (*Peribunyaviridae*) in the *Ae. albopictus* cell line Aa23 [84].

More unique viruses per sample were detected in *Ae. albopictus* than in *Ae. aegypti*; furthermore, Tobacco streak virus (*Bromoviridae*), HTV, Kwale mosquito virus, and GMV were each
detected in only one sample, suggesting that these viruses have been acquired from the environment. The higher level of variability in the virome composition of Ae. albopictus may be because of this mosquito’s tendencies to breed in natural habitats and feed on different types of vertebrates [85, 86], thus exposing itself to more viruses in the environment.

Mosquitoes are not known to be vectors of plant viruses; however, they can acquire these viruses from nectar during feeding; thus, some plant viruses have been detected in mosquito viromes [24, 25]. For example, in China, a plant-specific Tymoviridae-like virus that was isolated from Culex spp. was able to replicate in mosquito cells, suggesting that it had the potential to be transmitted by mosquitoes [87].

Despite the lower level of virus richness in Ae. albopictus than that in Ae. aegypti, the Alpha diversity of the Ae. albopictus virome was higher. This is because the abundance levels of viruses in Ae. albopictus were more evenly distributed. Multiple virus sequences identified in this study are consistent with other recently described sequences that currently lack a formal taxonomic classification. Therefore, the mosquito virome needs to be better characterized and considered in relationship with vector competence. However, studies relating the mosquito viroma profile and vector competence must take into consideration that the virome composition of mosquitoes is easily modified when they are used for establishing laboratory colonies from field-caught mosquitoes [22].

An important difference between Ae. albopictus and Ae. aegypti is the high prevalence of the intracellular bacterium Wolbachia in the former [88, 89] and its absence in the latter [90]. The effect of Wolbachia on mosquitoes viromes is still unclear, however, it has been hypothesized that Wolbachia colonization can reduce the abundance and richness of its associated viruses, which may explain the differences reported between the Ae. aegypti and Cx. quinquefasciatus viromes [67], and also the differences observed into this work between the Ae. albopictus and Ae. aegypti viromes [17]. The present study investigated whether differences between the microbiomes of Ae. aegypti and Ae. albopictus influence the compositions of their respective viromes. Samples collected between 2015 and the first half of 2017 that were negative for Wolbachia were compared with those that were positive for this bacterium. The results suggest that the presence of wMel Wolbachia did not influence the diversity or richness of the Ae. aegypti virome. However, the number of Wolbachia-infected mosquitoes in each of the five pools of mosquitoes was not quantified. In the wild populations of D. melanogaster, Wolbachia infection does not influence the diversity of native viruses in the insect [91]. Nevertheless, the results of the present study suggest that the presence of Wolbachia may enhance the level of infection of mosquitoes with Aedes aegypti To virus 2. A similar result was observed in other populations of field-caught Ae. aegypti mosquitoes, where Wolbachia enhanced ISF infection rates and loads, demonstrating that Wolbachia does not act as an antiviral against all flaviviruses [24, 92]. Therefore, we consider that Wolbachia presence in Ae. aegypti can alter the infection levels of low frequency viruses, but does not affect the mosquito core virome. However, this needs to be better addressed in future studies.

Although Wolbachia did not influence the Ae. aegypti virome diversity, a higher number of mitochondrial SNPs clustered apart from those of previous periods were observed in Ae. aegypti samples collected after the second half of 2017 (when the first Wolbachia-transfected mosquitoes were released; Table 2). Therefore, the release of these Wolbachia-transfected mosquitoes appeared to affect the native Ae. aegypti population (Figs 6 and 7). The transfected mosquitoes were the product of a cross between the native population and Wolbachia-bearing mosquitoes from Australia [93]. Therefore, a temporal interpopulation substructure might have formed after transfected mosquitoes were released in Bello and Medellín. In particular, some genetic variants of the Australian strain may have remained after the crosses, and these were introduced into the Medellín mosquito population,
increasing its diversity. Evidence for this can be obtained by analyzing biparental genetic markers and determining the levels of introgression between the native and introduced populations. Future Wolbachia-infected mosquitoes release can achieve faster rates of invasion at a lower cost by determining whether these variants become fixed in the native population and by assessing their effects on maintaining Wolbachia infections and its local spread capacities [94, 95].

The analysis of mitochondrial diversity during the 3 years of *Ae. albopictus* sampling revealed lower numbers of mitochondrial variable sites and SNPs than those observed in *Ae. aegypti*; moreover, *Ae. albopictus* diversity was similar between the sampling periods. *Ae. albopictus* is an invasive species detected in Medellín in 2011 [96], and its relatively low diversity suggests that its population size is smaller than that of *Ae. aegypti* and reflects a more recent founder event. To the best of our knowledge, however, no population genetic studies of this species have been conducted in either Medellín or Colombia. Therefore, it is not possible to establish how the diversity of this species has changed locally.

**Conclusions**

In conclusion, the levels of abundance and diversity between the viromes of *Ae. aegypti* and *Ae. albopictus* differ strikingly, indicating that virome profiles are species-specific and can be determined based on their evolutionary history. Most of the viruses associated with both species were detected throughout different sampling periods, suggesting that the viromes are temporally stable. Wolbachia spp. from clades A and B were prevalent in *Ae. albopictus*, whereas one strain of Wolbachia was present in post-release samples of *Ae. aegypti*. The presence of Wolbachia in *Ae. aegypti* was not related to any changes in the composition of its virome, except for an increase in the abundance of one ISV, *Aedes aegypti* To virus 2, which could be related to the presence of Wolbachia. Furthermore, mitochondrial genetic diversity was lower in *Ae. albopictus* than in *Ae. aegypti*, which suggests that the population size of the former is smaller and reflects a more recent founder event. The genetic diversity of *Ae. aegypti* increased after the second half of 2017, likely related to the release of mosquitoes transfected with Wolbachia. This study thus provides a baseline for future virome studies on *Ae. aegypti* and *Ae. albopictus* in Colombia and other countries in the Americas.

**Supporting information**

S1 Fig. Phylogenetic reconstruction of dengue virus sequences from *Aedes aegypti* mosquitoes in Medellín (Colombia) using a 1,057-bp region of the envelope gene. The contig was assembled from RNA-seq data, and the fragment was amplified and sequenced using the Sanger method. The sequences generated in this study are indicated in red. The scale bar indicates the number of substitutions per site. (TIF)

S1 Table. Summary of assembly metrics. (DOCX)

**Acknowledgments**

The authors express their sincere gratitude toward the people who allowed entry to their homes for entomological sampling as well as the Secretariat of Health of Medellín and the Medical Entomology Group of the University of Antioquia for their support during sampling.
Author Contributions

Conceptualization: Arley Calle-Tobón, Andrés Gómez-Palacio.

Formal analysis: Arley Calle-Tobón, Nicolás Forero-Pineda, Andrés Gómez-Palacio.

Funding acquisition: Juliana Pérez-Pérez, Guillermo Rúa-UrIBE, Andrés Gómez-Palacio.

Investigation: Arley Calle-Tobón, Juliana Pérez-Pérez, Omar Triana Chávez, Guillermo Rúa-UrIBE, Andrés Gómez-Palacio.

Methodology: Arley Calle-Tobón, Juliana Pérez-Pérez.

Writing – original draft: Arley Calle-Tobón, Andrés Gómez-Palacio.

Writing – review & editing: Arley Calle-Tobón, Juliana Pérez-Pérez, Nicolás Forero-Pineda, Omar Triana Chávez, Winston Rojas-Montoya, Guillermo Rúa-UrIBE, Andrés Gómez-Palacio.

References

1. World Health Organization. A Global Brief on Vector-Borne Diseases; WHO, 2014.

2. Paupy C.; Delatte H.; Bagny L.; Corbel V.; Fontenille D. Aedes Albopictus, an Arbovirus Vector: From the Darkness to the Light. *Microbes and Infection* 2009, 11, 1177–1185, https://doi.org/10.1016/j.micinf.2009.05.005 PMID: 19450706

3. Ding F.; Fu J.; Jiang D.; Hao M.; Lin G. Mapping the Spatial Distribution of Aedes Aegypti and Aedes Albopictus. *Acta Tropica* 2018, 178, 155–162, https://doi.org/10.1016/j.actatropica.2017.11.020 PMID: 29191515

4. Villar L.A.; Rojas D.P.; Besada-Lombana S.; Sarti E. Epidemiological Trends of Dengue Disease in Colombia (2000–2011): A Systematic Review. *PLOS Neglected Tropical Diseases* 2015, 9, e0003499, https://doi.org/10.1371/journal.pntd.0003499 PMID: 25790245

5. Rico-Mendoza A.; Alexandra P.-R.; Chang A.; Encinales L.; Lynch R. Co-Circulation of Dengue, Chikungunya, and Zika Viruses in Colombia from 2008 to 2018. *Revista Panamericana de Salud Pública* 2019, 43, 1, https://doi.org/10.26633/RPSP.2019.49 PMID: 31171921

6. Rodríguez-Morales A.J.; Galindo-Marquez M.L.; Carlos, ; García-Loaiza J.; Juan, ; Sabogal-Román A.; et al. Mapping Zika Virus Disease Incidence in Valle Del Cauca. *Infection* 45, https://doi.org/10.1007/s15010-016-0948-1 PMID: 27743307

7. Portilla Cabrera C.V.; Selvaraj J.J. Geographic Shifts in the Bioclimatic Suitability for Aedes Aegypti under Climate Change Scenarios in Colombia. *Heliyon* 2020, 6, e03101, https://doi.org/10.1016/j.heliyon.2019.e03101 PMID: 31909269

8. Singer, M. The Spread of Zika and the Potential for Global Arbovirus Syndemics. http://dx.doi.org/10.1080/17441692.2016.1225112 2016, 12, 1–18, 10.1080/17441692.2016.1225112.

9. Echeverry-Cárdenas E.; López-Castañeda C.; Carvajal-Castro J.D.; Aguirre-Obando O.A. Potential Geographic Distribution of the Tiger Mosquito Aedes Albopictus (Skuse, 1894) (Diptera: Culicidae) in Current and Future Conditions for Colombia. *PLOS Neglected Tropical Diseases* 2021, 15, e0008212, https://doi.org/10.1371/journal.pntd.0008212 PMID: 33974620

10. Gómez-Palacio A.; Suaza-Vasco J.; Castaño S.; Triana O.; Uribe S. Aedes Albopictus (Skuse, 1894) Infected with the American-Asian Genotype of Dengue Type 2 Virus in Medellín Suggests Its Possible Role as Vector of Dengue Fever in Colombia. *Biomédica* 2017, 37, 135, https://doi.org/10.7705/biomedica.v37i0.3474 PMID: 29161485

11. Calle-Tobón A.; Pérez-Pérez J.; Rojo R.; Rojas-Montoya W.; Triana-Chávez O.; Rúa-UrIBE G.; et al. Surveillance of Zika Virus in Field-Caught Aedes Aegypti and Aedes Albopictus Suggests Important Role of Male Mosquitoes in Viral Populations Maintenance in Medellín, Colombia. *Infection, Genetics and Evolution* 2020, 85, 104434, https://doi.org/10.1016/j.meegid.2020.104434 PMID: 32580028

12. Secretaría de Salud de Medellín. Boletín Epidemiológico Semana 52 de 2010; 2010.

13. Secretaría de salud de Medellín. Informe de Periodo Epidemiológico Medellín 2016. 2016, 52, 91.

14. Walker T.; Johnson P.H.; Moreira L.A.; Iturbe-Ormaetxe I.; Frentiu F.D.; McMeniman C.J.; et al. The WMel Wolbachia Strain Blocks Dengue and Invades Caged Aedes Aegypti Populations. *Nature* 2011 476:73612011, 476, 450–453, https://doi.org/10.1038/nature10355 PMID: 21866159
15. Caragata E.P.; Dutra H.L.C.; Moreira L.A. Inhibition of Zika Virus by Wolbachia in Aedes Aegypti. *Microbial Cell* 2016, 3, 293, https://doi.org/10.15698/mic2016.07.013 PMID: 28357366

16. van den Hurk A.F.; Hall-Mendelin S.; Pyke A.T.; Frentiu F.D.; McElroy K.; Day A.; et al. Impact of Wolbachia on Infection with Chikungunya and Yellow Fever Viruses in the Mosquito Vector Aedes Aegypti. *PLOS Neglected Tropical Diseases* 2012, 6, e1892, https://doi.org/10.1371/journal.pntd.0001892 PMID: 23133693

17. Audsley M.D.; Selezniev A.; Joubert D.A.; Woolfit M.; O'Neill S.L.; McGraw E.A. Wolbachia Infection Alters the Relative Abundance of Resident Bacteria in Adult Aedes Aegypti Mosquitoes, but Not Larvae. *Molecular Ecology* 2018, 27, 297–309, https://doi.org/10.1111/mec.14436 PMID: 29165845

18. Feschotte C.; Gilbert C. Endogenous Viruses: Insights into Viral Evolution and Impact on Host Biology. *Nature Reviews Genetics* 2012 13:4 2012, 13, 283–296, https://doi.org/10.1038/nrg3199 PMID: 22421730

19. Guégan M.; Zouache K.; Démiichel C.; Minard G.; Tran Van V.; Potier P.; et al. The Mosquito Holobiont: Fresh Insight into Mosquito-Microbiota Interactions. *Microbiome* 2018 6:1 2018, 6, 1–17, https://doi.org/10.1186/s40168-018-0435-2 PMID: 29554951

20. Du J.; Li F.; Han Y.; Fu S.; Liu B.; Shao N.; et al. Characterization of Viromes within Mosquito Species in China. *Science China Life Sciences* 2019, 63, 1089–1092, https://doi.org/10.1007/s11427-019-1583-9 PMID: 31834603

21. Nanfack-Minkeu F.; Mitri C.; Bischoff E.; Belda E.; Casademont I.; Vernick K.D. Interaction of RNA Viruses of the Natural Virome with the African Malaria Vector, Anopheles Coluzzii. *Scientific Reports* 2019, 9, 1–10, https://doi.org/10.1038/s41598-019-42825-3 PMID: 31040999

22. Shi C.; Zhao L.; Atoni E.; Zeng W.; Hu X.; Matthijnssens J.; et al. Stability of the Virome in Lab- and Field-Collected Aedes Albopictus Mosquitoes across Different Developmental Stages and Possible Core Viruses in the Publicly Available Virome Data of Aedes Mosquitoes. *mSystems* 2020, 5, 1–16, https://doi.org/10.1128/mSystems.00640-20 PMID: 32994288

23. Xiao P.; Han J.; Zhang Y.; Li C.; Guo X.; Wen S.; et al. Metagenomic Analysis of Flaviviridae in Mosquito Viromes Isolated From Yunnan Province in China Reveals Genes From Dengue and Zika Viruses. *Front Cell Infect Microbiol* 2018, 8, 359, https://doi.org/10.3389/fcimb.2018.00359 PMID: 30406308

24. Zakrzewski M.; Rašić G.; Darbro J.; Krause L.; Poo Y.S.; Filipović I.; et al. Mapping the Virome in Wild-Caught Aedes Aegypti from Cairns and Bangkok. *Scientific Reports* 2018, 8, 1–12, https://doi.org/10.1038/s41598-018-22945-y PMID: 29549363

25. Sadeghi M.; Altan E.; Deng X.; Barker C.M.; Fang Y.; Coffey L.L.; et al. Virome of > 12 Thousand Culex Mosquitoes from throughout California. *Virology* 2018, 523, 74–88 https://doi.org/10.1016/j.virol.2018.07.029 PMID: 30098450

26. Shi C.; Zhao L.; Atoni E.; Zeng W.; Hu X.; Matthijnssens J.; et al. The Conservation of a Core Virome in Aedes Mosquitoes across Different Developmental Stages and Continents. *bioRxiv* 2020, 2020.04.23.058701, https://doi.org/10.1101/2020.04.23.058701

27. de Almeida J.P.; Aguiar E.R.; Armache J.N.; Olmo R.P.; Marconcini M.; et al. The Conservation of a Core Virome in Aedes Mosquitoes across Different Developmental Stages and Continents. *bioRxiv* 2020, 2020.04.23.058701, https://doi.org/10.1101/2020.04.23.058701

28. Wallau G.L. RNA Virus EVEs in Insect Genomes. *Current Opinion in Insect Science* 2022, 49, 42–47, https://doi.org/10.1016/j.cois.2021.11.005 PMID: 34839033

29. Palatini U.; Miesen P.; Carballar-Lejarazu R.; Ometto L.; Rizzo E.; Tu Z.; et al. Comparative Genomics Shows That Viral Integrations Are Abundant and Express PiRNAs in the Arboviral Vectors Aedes Aegypti and Aedes Albopictus. *BMC Genomics* 2017, 18, 1–15, https://doi.org/10.1186/s12864-017-3903-3 PMID: 28676109

30. Crava C.M.; Varghesse F.S.; Pischedda E.; Halbach R.; Palatini U.; Marconcini M.; et al. Population Genomics in the Arboviral Vector Aedes Aegypti Reveals the Genomic Architecture and Evolution of Endogenous Viral Elements. *Molecular Ecology* 2021, 30, 1594–1611, https://doi.org/10.1111/mec.15796 PMID: 33432714

31. Blair C.D.; Olson K.E.; Bonizzoni M. The Widespread Occurrence and Potential Biological Roles of Endogenous Viral Elements in Insect Genomes. *Current Issues in Molecular Biology* 2020, 34, 13–29, https://doi.org/10.21775/cimb.034.013 PMID: 31167954

32. Nouri S.; Matsuruma E.E.; Kuo Y.W.; Falk B.W. Insect-Specific Viruses: From Discovery to Potential Translational Applications. *Current Opinion in Virology* 2018, 33, 33–41, https://doi.org/10.1016/j.coiv.2018.07.006 PMID: 30048906

33. Patterson E.I.; Villinger J.; Muthoni J.N.; Dobel-Ober L.; Hughes G.L. Exploiting Insect-Specific Viruses as a Novel Strategy to Control Vector-Borne Disease. *Current Opinion in Insect Science* 2020, 39, 50–56, https://doi.org/10.1016/j.cois.2020.02.006 PMID: 32278312
34. Öhlund P.; Lundén H.; Blomström A.-L. Insect-Specific Virus Evolution and Potential Effects on Vector Competence. *Virus Genes* 2019 55:2. 2019, 55, 127–137, https://doi.org/10.1007/s11262-018-01629-9 PMID: 30632016

35. Romo H.; Kenney J.L.; Bitvich B.J.; Brault A.C. Restriction of Zika Virus Infection and Transmission in Aedes Aegypti Mediated by an Insect-Specific Flavivirus. *Emerging Microbes and Infections* 2018, 7, https://doi.org/10.1038/s41424-018-0194-4 PMID: 30429457

36. Velez I.D.; Santacruz E.; Kutcher S.C.; Duque S.L.; Uribe A.; Barajas J.; et al. The Impact of City-Wide Deployment of Wolbachia-Carrying Mosquitoes on Arboviral Disease Incidence in Medellin and Bello, Colombia: Study Protocol for an Interrupted Time-Series Analysis and a Test-Negative Design Study. *F1000Res* 2019, 8, 1327, https://doi.org/10.12688/f1000research.19858.2 PMID: 34900237

37. Pérez-Pérez J.; Sanabria W.H.; Restrepo C.; Rojo R.; Henao E.; Triana O.; et al. Viral Metagenomic Analysis of Aedes Albopictus Mosquitoes. *Microbial Profiling Based on 16S RRNA Gene Amplicons*. *BMC Bioinformatics* 2012, 19, 455–477, https://doi.org/10.1089/cmb.2012.0021 PMID: 22506599

38. Kopylova E.; Nóe L.; Touzet H. SortMeRnA: Fast and Accurate Filtering of Ribosomal RNAs in Metatranscriptomic Data. *Bioinformatics* 2012, 28, 3211–3217, https://doi.org/10.1093/bioinformatics/bts611 PMID: 23071270

39. Zhao L.; Atoni E.; Shi C.; Yuan Z.; Xia H. Mapping the Virome in Lab-Reared and Wild-Caught Aedes Albopictus Mosquitoes. *Virology Surveillance of Aedes (Stegomyia) Aegypti and Aedes (Stegomyia) Albopictus as Support for Decision Making for Dengue Control in Medellin*. *Biomédica* 2017, 37, 155, https://doi.org/10.7705/biomedicav37i0.3467 PMID: 29161487

40. Pérez-Pérez J.; Rojo R.; Henao E.; García-Huerta P.; Triana-Chavez O.; Rúa-Urbi G. Natural Infection of Aedes Aegypti, Ae. Albopictus and Culex Spp. with Zika Virus in Medellin, Colombia. *CES Medicina* 2019, 33, 175–181, https://doi.org/10.21615/cesmedicina.33.3.2

41. Matthews B.J.; Dudchenko O.; Kingan S.B.; Koren S.; Antoshechkin I.; Crawford J.E.; et al. Improved Reference Genome of Aedes Aegypti Informs Arbovirus Vector Control. *New England Journal of Medicine* 2018, 384, 2177–2186, https://doi.org/10.1056/NEJMoa2030243 PMID: 34107180

42. Umarini A.; Indriani C.; Ahmad R.A.; Tantowijoyo W.; Arguni E.; Ansari M.R.; et al. Efficacy of Wolbachia-Infected Mosquito Deployments for the Control of Dengue. *Bioinformatics* 2014, 25, 1754–1760, https://doi.org/10.1093/bioinformatics/btp324 PMID: 19451168

43. Li H.; Durbin R. Fast and Accurate Short Read Alignment with Burrows-Wheeler Transform. *Bioinformatics* 2009, 25, 1754–1760, https://doi.org/10.1093/bioinformatics/btp560 PMID: 30430866

44. Chen X.-G.; Jiang X.; Gu J.; Xu M.; Wu Y.; Deng Y.; et al. Genome Sequence of the Asian Tiger Mosquito, Aedes Albopictus, Reveals Insights into Its Biology, Genetics, and Evolution. *Proceedings of the National Academy of Sciences* 2015, 112, E5907–E5915, https://doi.org/10.1073/PNAS.1516410112 PMID: 26483478

45. Li H.; Durbin R. Fast and Accurate Short Read Alignment with Burrows-Wheeler Transform. *Bioinformatics* 2009, 25, 1754–1760, https://doi.org/10.1093/bioinformatics/btp560 PMID: 19451168

46. Matthews B.J.; Dudchenko O.; Kingan S.B.; Koren S.; Antoshechkin I.; Crawford J.E.; et al. Improved Reference Genome of Aedes Aegypti Informs Arbovirus Vector Control. *Nature* 2018, 563, 7732, 2018, 563, 501–507, https://doi.org/10.1038/s41586-018-0692-z PMID: 30293615

47. Chen X.-G.; Jiang X.; Gu J.; Xu M.; Wu Y.; Deng Y.; et al. Genome Sequence of the Asian Tiger Mosquito, Aedes Albopictus, Reveals Insights into Its Biology, Genetics, and Evolution. *Proceedings of the National Academy of Sciences* 2015, 112, E5907–E5915, https://doi.org/10.1073/PNAS.1516410112 PMID: 26483478

48. Li H.; Durbin R. Fast and Accurate Short Read Alignment with Burrows-Wheeler Transform. *Bioinformatics* 2009, 25, 1754–1760, https://doi.org/10.1093/bioinformatics/btp560 PMID: 19451168

49. Matthews B.J.; Dudchenko O.; Kingan S.B.; Koren S.; Antoshechkin I.; Crawford J.E.; et al. Improved Reference Genome of Aedes Aegypti Informs Arbovirus Vector Control. *Nature* 2018, 563, 7732, 2018, 563, 501–507, https://doi.org/10.1038/s41586-018-0692-z PMID: 30293615

50. Kopylova E.; Nóe L.; Touzet H. SortMeRnA: Fast and Accurate Filtering of Ribosomal RNAs in Metatranscriptomic Data. *Bioinformatics* 2012, 28, 3211–3217, https://doi.org/10.1093/bioinformatics/bts611 PMID: 23071270

51. Zhao L.; Atoni E.; Shi C.; Yuan Z.; Xia H. Mapping the Virome in Lab-Reared and Wild-Caught Aedes Albopictus Mosquitoes. *Access Microbiology* 2019, 1, 4, https://doi.org/10.1099/acmi.imav2019.00009 PMID: 32974496

52. Bankevich A.; Nurk S.; Antipov D.; Gurevich A.A.; Dvorkin M.; Kulikov A.S.; et al. SPAdes: A New Genome Assembly Algorithm and Its Applications to Single-Cell Sequencing. *Journal of Computational Biology* 2012, 19, 455–477, https://doi.org/10.1089/cmb.2012.0021 PMID: 22506599

53. Buchfink B.; Xie C.; Huson D.H. Fast and Sensitive Protein Alignment Using DIAMOND. *Nature Methods* 2014, 12, 59–60, https://doi.org/10.1038/nmeth.3176 PMID: 25402007

54. Kubacki J.; Flacio E.; Qi W.; Guidi V.; Tonolla M.; Fraefel C. Viral Metagenomic Analysis of Aedes Albopictus Mosquitoes from Southern Switzerland. *Viruses* 2020, 12, 929, https://doi.org/10.3390/v12090929 PMID: 32846990

55. Ondov B.D.; Bergman N.H.; Phillippy A.M. Interactive Metagenomic Visualization in a Web Browser. *BMC Bioinformatics* 2011, 12, https://doi.org/10.1186/1471-2105-12-385 PMID: 21961884

56. Lagkouvardos I.; Fischer S.; Kumar N.; Clavel T. Rhea: A Transparent and Modular R Pipeline for Microbial Profiling Based on 16S rRNA Gene Amplicons. *PeerJ* 2017, 5, e2836, https://doi.org/10.7717/peerj.2836 PMID: 28097056

57. R Core Team R. A Language and Environment for Statistical Computing 2020.

58. Anderson M.J.; Crist T.O.; Chase J.M.; Velland M.; Inouye B.D.; Freestone A.L.; et al. Navigating the Multiple Meanings of B Diversity: A Roadmap for the Practicing Ecologist. *Ecology Letters* 2011, 14, 19–28, https://doi.org/10.1111/j.1461-0248.2010.01552.x PMID: 21070562
55. Li, H. Aligning Sequence Reads, Clone Sequences and Assembly Contigs with BWA-MEM. 2013.
56. Li H.; Handsaker B.; Wysoker A.; Fennell T.; Ruan J.L; Homer N.; et al. The Sequence Alignment/Map Format and SAMtools. Bioinformatics 2009, 25, 2078–2079, https://doi.org/10.1093/bioinformatics/ btp352 PMID: 19505943
57. H L. A Statistical Framework for SNP Calling, Mutation Discovery, Association Mapping and Population Genetical Parameter Estimation from Sequencing Data. Bioinformatics 2011, 27, 2987–2993, https://doi.org/10.1093/bioinformatics/btr327
58. Garrison, E.; Marth, G. Haplotype-Based Variant Detection from Short-Read Sequencing. arXiv preprint arXiv:1207.3907, 2012.
59. DePristo M.A.; Banks E.; Poplin R.; Garimella K. V; Maguire J.R.; Hartl C.; et al. A Framework for Variation Discovery and Genotyping Using Next-Generation DNA Sequencing Data. Nature Genetics 2011 43:5 2011, 43, 491–498, https://doi.org/10.1038/ng.806 PMID: 21478889
60. Rozas J.; Ferrer-Mata A.; Sánchez-DelBarrio J.C.; Guirao-Rico S.; Librado P.; Ramos-Onsins S.E.; et al. DnaSP 6: DNA Sequence Polymorphism Analysis of Large Data Sets. Molecular Biology and Evolution 2017, 34, 3299–3302, https://doi.org/10.1093/molbev/msx248 PMID: 29029172
61. Kumar S.; Stecher G.; Knyaz C.; Tamura K. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Molecular Biology and Evolution 2018, 35, 1547, https://doi.org/10.1093/molbev/msy096 PMID: 29722887
62. Jiménez-Silva C.L.; Carreño M.F.; Ortiz-Baez A.S.; Rey L.A.; Villabona-Arenas C.J.; Ocazionez R.E. Evolutionary History and Spatio-Temporal Dynamics of Dengue Virus Serotypes in an Endemic Region of Colombia. PLOS ONE 2018, 13, e0203090, https://doi.org/10.1371/journal.pone.0203090 PMID: 30157270
63. Hall, T. BioEdit v.7.0.5. Biological Sequences Alignment Editor for Windows. Ibis Therapeutics, a Division of Isis Pharmaceuticals. 2005.
64. Katoh K.; Standley D.M. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. Molecular Biology and Evolution 2013, 30, 772–780, https://doi.org/10.1093/molbev/mst010 PMID: 23326960
65. Nguyen L.-T.; Schmidt H.A.; von Haeseler A.; Minh B.Q. IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. Molecular Biology and Evolution 2015, 32, 268–274, https://doi.org/10.1093/molbev/msu300 PMID: 25371430
66. Kalyaanamoorthy S.; Minh B.Q.; Wong T.K.F.; Von Haeseler A.; Jermiin L.S. ModelFinder: Fast Model Selection for Accurate Phylogenetic Estimates. Nature Methods 2017, 14, 587–589, https://doi.org/10.1038/nmeth.4285 PMID: 28481363
67. Shi C.; Beller L.; Deboutte W.; Yinda K.C.; Delang L.; Vega-Rú a A.; et al Stable Distinct Core Eukaryotic Viromes in Different Mosquito Species from Guadeloupe, Using Single Mosquito Viral Metagenomics. Microbiome 2019, 7, 1–20, https://doi.org/10.1186/s40168-019-0734-2 PMID: 31462331
68. Zhang X.; Huang S.; Jin T.; Lin P.; Huang Y.; Wu C.; et al. Discovery and High Prevalence of Phasi Charoen-like Virus in Field-Captured Aedes Aegypti in South China. Virology 2018, 523, 35–40, https://doi.org/10.1016/j.virol.2018.07.021 PMID: 30077072
69. Chandler J.A.; Thongsrirong P.; Green A.; Kittayapong P.; Wilcox B.A.; Schrot G.P.; et al. Metagenomic Shotgun Sequencing of a Bunyavirus in Wild-Caught Aedes Aegypti from Thailand Informs the Evolutionary and Genomic History of the Phleboviruses. Virology 2014, 464–465, 312–319, https://doi.org/10.1016/j.virol.2014.06.036 PMID: 25108381
70. Ramos-Nino M.E.; Fitzpatrick D.M.; Tighe S.; Eckstrom L.M.; Hseuh A.N.; et al. High Prevalence of Phasi Charoen-like Virus from Wild-Caught Aedes Aegypti in Grenada, W.I. as Revealed by Metagenomic Analysis. PLOS ONE 2020, 15, e0227998, https://doi.org/10.1371/journal.pone.0227998 PMID: 32004323
71. Aubry F.; Dabo S.; Manez C.; Filipović I.; Rose N.H.; Miot E.F.; et al. Enhanced Zika Virus Susceptibility of Globally Invasive Aedes Aegypti Populations. Science (1979) 2020, 370, 991–996, https://doi.org/10.1126/science.abc3663 PMID: 33214283
72. de Oliveira Ribeiro G.; Morais V.S.; Monteiro F.J.C.; Ribeiro E.S.D.A.; da Rego M.O.S.; Souto R.N.P.; et al. Aedes Aegypti from Amazon Basin Harbor High Diversity of Novel Viral Species. Viruses 2020, 12, https://doi.org/10.3390/v12080868 PMID: 32784421
73. Stollar V.; Thomas V.L. An Agent in the Aedes Aegypti Cell Line (Peleg) Which Causes Fusion of Aedes Albopictus Cells. Virology 1975, 64, 367–377, https://doi.org/10.1016/0042-6822(75)90113-0 PMID: 806166
74. Espinoza-Gómez, F.; López-Lemus, A.U.; Rodríguez-Sanchez, I.P.; Martínez-Fierro, M.L.; Newton-Sánchez, O.A.; Chávez-Flores, E.; et al. Detection of Sequences from a Potentially Novel Strain of Cell Fusing Agent Virus in Mexican Stenomgyia (Aedes) Aegypti Mosquitoes., 10.1007/s00705-011-0967-2.
75. Cook S.; Bennett S.N.; Holmes E.C.; De Chesse R.; Moureau G.; de Lamballerie X. Isolation of a New Strain of the Flavivirus Cell Fusing Agent Virus in a Natural Mosquito Population from Puerto Rico. *Journal of General Virology* 2006, 87, 735–748. [https://doi.org/10.1099/vir.0.81475-0](https://doi.org/10.1099/vir.0.81475-0) PMID: 16528021

76. Yamanaka A.; Thongrungkit S.; Ramasoota P.; Konishi E. Genetic and Evolutionary Analysis of Cell-Fusing Agent Virus Based on Thai Strains Isolated in 2008 and 2012. *Infection, Genetics and Evolution* 2013, 19, 188–194. [https://doi.org/10.1016/j.meegid.2013.07.012](https://doi.org/10.1016/j.meegid.2013.07.012) PMID: 23871775

77. Zhang G.; Asad S.; Khromykh A.A.; Asgari S. Cell Fusing Agent Virus and Dengue Virus Mutually Interact in Aedes Aegypti Cell Lines. *Scientific Reports* 2017, 7:12017, 7, 1–8. [https://doi.org/10.1038/s41598-017-07279-5](https://doi.org/10.1038/s41598-017-07279-5) PMID: 28761113

78. Zhang X.; Guo Xiaofang; Fan Hang; Zhao Q.; Zuo S.; Qiang Sun, et al. Complete Genome Sequence of Menghai Flavivirus, a Novel Insect-Specific Flavivirus from China. *Archives of Virology* 162, [https://doi.org/10.1007/s00705-017-3232-5](https://doi.org/10.1007/s00705-017-3232-5) PMID: 28175982

79. Parr R.; Naccache F.; Ndiaye E.H.; Fall G.; Castelli I.; Lühken R.; et al. Identification and RNAi Profile of a Novel Flavivirus Infecting Senegalese Aedes Vexans Arabiensis Mosquitoes. *Viruses* 2020, Vol. 12, *Page 440* 2020, 12, 440. [https://doi.org/10.3390/v12040440](https://doi.org/10.3390/v12040440) PMID: 32295109

80. Kobayashi D.; Isawa H.; Fujita R.; Murota K.; Itokawa K.; Higa Y.; et al. Isolation and Characterization of a New Flavivirus from Armigeres Spp. Mosquitoes in the Philippines. *Journal of General Virology* 2017, 98, 2876–2881, [https://doi.org/10.1099/jgv.0.009929](https://doi.org/10.1099/jgv.0.009929) PMID: 29048274

81. Parr R.; Asgari S. Aedes Anopheles: An Insect-Specific Virus Distributed Worldwide in Aedes Aegypti Mosquitoes That Has Complex Interplays with Wolbachia and Dengue Virus Infection in Cells. *Journal of Virology* 2018, 92, 1–19. [https://doi.org/10.1128/JVI.00224-18](https://doi.org/10.1128/JVI.00224-18) PMID: 29950416

82. Hoshino K.; Isawa H.; Tsuda Y.; Sawabe K.; Kobayashi M. Isolation and Characterization of a New Insect Flavivirus from Aedes Albopictus and Aedes Flavopictus Mosquitoes in Japan. *Virology* 2009, 391, 119–129, [https://doi.org/10.1016/j.virol.2009.06.025](https://doi.org/10.1016/j.virol.2009.06.025) PMID: 19580982

83. Fang Y.; Zhang Y.; Zhou Z.-B.; Shi W.-Q.; Xia S.; Li Y.-Y.; et al. Co-Circulation of Aedes Flavivirus, Culex Flavivirus, and Quang Binh Virus in Shanghai, China. *Infectious Diseases of Poverty* 2018, 7:1, 7, 1–10. [https://doi.org/10.1186/S40249-018-0457-9](https://doi.org/10.1186/S40249-018-0457-9) PMID: 30021614

84. Schultz M.J.; Frydman H.M.; Connor J.H. Dual Insect Specific Virus Infection Limits Arbovirus Replication in Aedes Mosquito Cells. *Virology* 2018, 518, 406–413, [https://doi.org/10.1016/j.virol.2018.03.022](https://doi.org/10.1016/j.virol.2018.03.022) PMID: 29625404

85. Gratz N.G. Critical Review of the Vector Status of Aedes Albopictus. *Medical and Veterinary Entomology* 2004, 18, 215–227, [https://doi.org/10.1111/j.0269-2585.2004.00513.x](https://doi.org/10.1111/j.0269-2585.2004.00513.x) PMID: 15347388

86. Richards S.L.; Ponnusamy L.; Unnasch T.R.; Hassan H.K.; Apperson C.S. Host-Feeding Patterns of *Aedes Albopictus* (Diptera: Culicidae) in Relation to Availability of Human and Domestic Animals in Urban Landscapes of Central North Carolina. *Journal of Medical Entomology* 2006, 43, 543–551, [https://doi.org/10.1603/0022-2585(2006)43[543:hpoaad]2.0.co;2](https://doi.org/10.1603/0022-2585(2006)43[543:hpoaad]2.0.co;2) PMID: 16739414

87. Wang L.; Lv X.; Zhai Y.; Fu S.; Wang D.; Rayner S.; et al. Genomic Characterization of a Novel Virus of the Family Tymoviridae Isolated from Mosquitoes. *PLOS ONE* 2012, 7, e39845, [https://doi.org/10.1371/journal.pone.0039845](https://doi.org/10.1371/journal.pone.0039845) PMID: 22848363

88. Ahmad N.A.; Vythilingam I.; Lim Y.A.L.; Zabari N.Z.A.M.; Lee H.L. Detection of Wolbachia in Aedes Albopictus and Their Effects on Chikungunya Virus. *American Journal of Tropical Medicine and Hygiene* 2017, 96, 148–156, [https://doi.org/10.4269/ajtmh.16-0516](https://doi.org/10.4269/ajtmh.16-0516) PMID: 27920393

89. Seabournid P.; Spafford H.; Yoneishi N.; Medeirosid M. The Aedes Albopictus (Diptera: Culicidae) Microbiome Varies Spatially and with Ascosporagin Formation. *PLOS Neglected Tropical Diseases* 2020, 14, 1–21, [https://doi.org/10.1371/journal.pntd.0008615](https://doi.org/10.1371/journal.pntd.0008615) PMID: 32813707

90. Ross P.A.; Callahan A.G.; Yang Q.; Jasper M.; Anf M.A.K.; Alizah A.N.; et al. An Elusive Endosymbiont: Does Wolbachia Occur Naturally in Aedes Aegypti? *Ecology and Evolution* 2020, 10, 1581–1591, [https://doi.org/10.1002/ece3.6012](https://doi.org/10.1002/ece3.6012) PMID: 32076535

91. Shi M.; White V.L.; Schluib T.; Eden J.S.; Hoffmann A.A.; Holmes E.C. No Detectable Effect of Wolbachia WMel on the Prevalence and Abundance of the RNA Virome of Drosophila Melanogaster. *Proceedings of the Royal Society B. Biological Sciences* 2018, 285, [https://doi.org/10.1098/rspb.2018.1165](https://doi.org/10.1098/rspb.2018.1165) PMID: 30051873

92. Amnuzo H.E.; Tsyganov K.; Koh C.; Herbert R.I.; Powell D.R.; McGraw E.A. Wolbachia Enhances Insect-Specific Flavivirus Infection in Aedes Aegypti Mosquitoes. *Ecology and Evolution* 2018, 8, 5441–5454, [https://doi.org/10.1002/ece3.4066](https://doi.org/10.1002/ece3.4066) PMID: 29938064

93. Aliota M.T.; Peinado S.A.; Velez I.D.; Orsorio J.E. The WMel Strain of Wolbachia Reduces Transmission of Zika Virus by Aedes Aegypti. *Scientific Reports* 2016 6:1 2016, 6, 1–7, [https://doi.org/10.1038/srep28792](https://doi.org/10.1038/srep28792) PMID: 27364935
94. Schmidt T.L.; Filipović I.; Hoffmann A.A.; Rašić G. Fine-Scale Landscape Genomics Helps Explain the Slow Spatial Spread of Wolbachia through the Aedes Aegypti Population in Cairns, Australia. *Heredity* 2018 120:52018, 120, 386–395, https://doi.org/10.1038/s41437-017-0039-9 PMID: 29356725

95. Jasper M.; Schmidt T.L.; Ahmad N.W.; Sinkins S.P.; Hoffmann A.A. A Genomic Approach to Inferring Kinship Reveals Limited Intergenerational Dispersal in the Yellow Fever Mosquito. *Molecular Ecology Resources* 2019, 19, 1254–1264, https://doi.org/10.1111/1755-0998.13043 PMID: 31125998

96. Rúa-Uribe Guillermo.; Suárez Acosta C.; Londoño Viviana.; Sanchez James.; Rojo R.; Novoa B. Primera Evidencia de Aedes Albopictus (Skuse) (Diptera: Culicidae) En La Ciudad de Medellín, Antioquia —Colombia. *Revista Salud Pública de Medellín* 2011, 5, 89–98.