Arbovirus de importancia en salud pública y Viroma en Mosquitos de Colombia: Un Enfoque Metagenómico

Alida Marcela Gómez Rodríguez

Documento de tesis presentado como requisito para optar al título de Doctora en Ciencias Biomédicas y Biológicas

Doctorado en Ciencias Biomédicas y Biológicas
Universidad Del Rosario
Bogotá, D.C.
2024

Arbovirus de importancia en salud pública y Viroma en Mosquitos de Colombia: Un Enfoque Metagenómico

Alida Marcela Gómez Rodríguez

Director:
Juan David Ramírez González Ph.D.
Profesor Titular
Facultad de Ciencias Naturales
Universidad del Rosario

Co-Directora:
Marina Muñoz Díaz
Profesora Asociada
Universidad Nacional de Colombia

Documento de tesis presentado como requisito para optar al título de Doctora en Ciencias Biomédicas y Biológicas

Doctorado en Ciencias Biomédicas y Biológicas
Universidad Del Rosario
Bogotá, D.C.
2024

AGRADECIMIENTOS

*Quiero expresar mi gratitud infinita a **Dios**, quien me ha mostrado que soy capaz de lograr todo lo que me proponga. Gracias por ayudarme a ser una mejor versión de mí misma, por enseñarme que el agradecimiento y el amor son la clave para superar cada momento de dificultad y disfrutar cada instante de alegría. Agradezco por llevarme siempre de la mano en este camino académico y en cada paso de mi vida, permitiéndome valorar cada experiencia. Ha sido un gran aprendizaje en todos los sentidos.*

*Agradezco profundamente a **mi hermosa familia** por ser mi fuente de motivación diaria, por su apoyo constante, por celebrar mis triunfos y por acompañarme en cada paso hacia la consecución de este objetivo. Todo lo que he logrado es por y para ustedes, quienes son mi mayor tesoro. Quiero expresar un agradecimiento especial a mi **hermosa Fanny Lu**, por regalarme tantas sonrisas, por alentarme siempre a ser mejor y a superar los obstáculos, y por representar el amor y la bondad incondicional en mi vida. Este logro es de nosotras!. A **mi padre, José David**, le agradezco su generosidad, su alegría, su fortaleza, sus sabios consejos y su ejemplo constante de resiliencia. A mis **maravillosas hermanas ("superpoderosas"), Raquel y Diana**, mis mejores amigas, les doy las gracias por estar siempre a mi lado, por sus abrazos reconfortantes y por ser un ejemplo de superación y mejora continua, son la mejor bendición!. También quiero agradecer a mis **adorables sobrinos, David y Victorya,** por ser la razón más hermosa de mi felicidad. Y a mis **cuñados, Felipe y Luis Gabriel**, les agradezco por su incondicional apoyo.*

*Agradezco de todo corazón **a mi novio, Luis Carlos**, por su amor incondicional. Gracias por demostrarme siempre, con ejemplo, que los sueños se hacen realidad con dedicación, disciplina, esfuerzo y mucha perseverancia. Aprecio profundamente su paciencia, su tranquilidad y su cariño, en cada momento, sé que hemos construido una visión del mundo más feliz y más fortalecida para los dos. También quiero agradecer por convertir en realidad nuestro pequeño Gran paraíso, **"Entre Lomas"**. Agradezco especialmente a mi querido cuñado **Nelson** por ser un líder visionario, y por demostrarme que la disciplina y el enfoque nos permiten alcanzar nuestras metas. Agradezco a su hermosa familia, **Anna y la princesa Juanita**, así como a sus padres y demás familiares, por su cariño, amistad y ánimo. Cada momento compartido con ustedes ha contribuido a mi crecimiento y avance en este camino.*

*Quiero expresar mi absoluto agradecimiento a mis Directores de Tesis Doctoral por su invaluable orientación en este camino de investigación. A **mi Maestro, Juan David Ramirez**, le agradezco de manera especial por ser un ejemplo maravilloso a seguir, tanto como profesional como persona. Gracias por enseñarme que el camino hacia la excelencia requiere constancia, disciplina y amor en todo lo que se hace. Gracias por Creer en mi, por su contribución para mejorar en mis debilidades, así como resaltar mis fortalezas. Aprecio enormemente su guía constante en este proceso de formación. A **mi querida Mari**, le doy las gracias por su acompañamiento durante estos años de doctorado. Especialmente valoro su ejemplo de optimismo y alegría, así como su capacidad para ver lo mejor en cada situación. Cada esfuerzo y cada dedicación ha valido la pena!*

*Agradezco sinceramente a todos los miembros del **Centro de Investigaciones en Microbiología y Biotecnología - CIMBIUR**, por su incondicional apoyo y por brindarme una invaluable experiencia de aprendizaje. Su compromiso con la excelencia ha sido fundamental para mi crecimiento tanto profesional como personal en estos años. Quiero expresar mi profunda gratitud a mis compañeros **Lissa, Guio, Caro, Adri, Nico, Angie, y David**, quienes han sido un ejemplo inspirador de investigadores apasionados y críticos. Agradezco sus valiosos aportes, los cuales han sido fundamentales para culminar este viaje académico con éxito.*

*Quiero expresar mi profundo agradecimiento a la **Universidad de Boyacá,** por el financiamiento de mis estudios doctorales en los mejores Centros de Investigación del país a través del Programa DIA (Doctorado para la Investigación y la Academia). Agradezco su apoyo constante en este proceso formativo. A mi grupo de investigación **NÚCLEO** y a mi querida **Andreita Wilches**, les estoy infinitamente agradecida por su liderazgo, consejo, amistad y apoyo incondicional para lograr este objetivo.*

*Agradezco sinceramente a **la Fundación Instituto de Inmunología de Colombia - FIDIC, al Dr. Manuel Elkin y al Dr. Manuel Alfonso Patarroyo** por brindarme la oportunidad de iniciar mi formación doctoral en su reconocido Instituto. Su exigencia, rigurosidad académica y disciplina han sido fundamentales en este camino de investigación y fortalecimiento personal.*

*A mis maravillosos amigos del doctorado, **David, Anny, Jessi y Lau,** les agradezco por ser mi segunda familia, por compartir el mismo sueño, por ser el mejor equipo!. La oportunidad de realizar este proyecto de doctorado me ha dado un gran regalo, que es su amistad y la de sus familias.*

*A las Amigas de mi corazón, **Linita, Vivi, Pili, Mayris, Rochi, Clau, Mafe y Nayda** gracias por su amistad incondicional, son mujeres sinónimo de alegría, fortaleza y tencidad, soy muy afortunada de tenerlas en mi vida.*

*Quiero expresar mi profundo agradecimiento al **Dr. Scott Weaver** por permitirme realizar mi pasantía internacional en la Universidad de Texas Medical Branch (UTMB). En especial, quiero expresar mi más sincero agradecimiento, respeto y admiración al **Dr. Kenneth Plante, a la Dra. Jessica Plante** y a su maravilloso equipo de investigación del **World Reference Center for Emerging Viruses and Arboviruses (WRCEVA),** por brindarme la oportunidad de afianzar y mejorar mis conocimientos en virología. Han sido una gran inspiración en mi carrera profesional y agradezco profundamente su acompañamiento durante mi estadía.*

*A mis estimados amigos de la **UTMB**, **Itizi, Miguel, Esteban, Tim, Mariia, Maria, Camila, Zonia, Anastasia y Michaella**, les doy las gracias por convertir mi estadía en Galveston, Texas, en una experiencia maravillosa.*

*Agradezco sinceramente a la Directora del Programa de Doctorado en Ciencias Biomédicas y Biológicas, **Dra. Carolina Ramírez**, por su constante colaboración y gentil orientación en cada paso del doctorado.*

*Quiero expresar mi sincero agradecimiento a los **jurados de mi tesis doctoral** por dedicar su valioso tiempo a la lectura y evaluación de mi documento de investigación. Es para mí un honor contar con la opinión de expertos en las áreas de mi estudio, agradezco sus importantes aportes a los resultados obtenidos y sus valuables consideraciones sobre los analisis realizados.*

*Agradezco a la **Dirección de Investigación e Innovación** de la Universidad del Rosario por su apoyo financiero durante el desarrollo de mis estudios doctorales. Igualmente, quiero expresar mi gratitud al **Comité de Becas de la Dirección Académica de la Universidad del Rosario** por otorgarme la beca que hizo posible mi pasantía internacional en la Universidad de Texas de la Rama de la Medicina - University of Texas Medical Branch (UTMB). Su generoso respaldo ha sido fundamental para mi formación académica y profesional.*

*Finalmente, agradezco al **Ministerio de Ciencia, Tecnología e Innovación de Colombia** por la financiación de mi proyecto doctoral bajo el contrato 143889685192-2021.*

Florecer implica pasar por todas las estaciones…

TABLA DE CONTENIDO

Contenido

1. LISTA DE PUBLICACIONES

Esta tesis se centra en cinco artículos científicos publicados durante la formación doctoral. Todos los artículos se encuentran anexos a este documento. La información suplementaria y tablas se anexan en archivos comprimidos siguiendo el número de artículos que se mencionan a continuación:

- **Artículo 1:** Gómez M, Martínez D, Hernández C, Luna N, Patiño LH, Bohórquez Melo R, Suarez LA, Palma-Cuero M, Murcia LM, González Páez L, Estrada Bustos L, Medina MA, Ariza Campo K, Padilla HD, Zamora Flórez A, De las Salas JL, Muñoz M and Ramírez JD. Arbovirus infection in *Aedes aegypti* from different departments of Colombia. Front. Ecol. Evol. 2022. 10:999169. doi: 10.3389/fevo.2022.999169.

- **Artículo 2:** Gómez M, Martínez D, Muñoz M, Ramírez JD. *Aedes aegypti* and *Ae. albopictus* microbiome/virome: new strategies for controlling arboviral transmission? Parasit Vectors. 2022 Aug 9;15(1):287. doi: 10.1186/s13071-022-05401-9.

- **Artículo 3:** Gómez M, Martínez D, Páez-Triana L, Luna N, Ramírez A, Medina J, Cruz-Saavedra L, Hernández C, Castañeda S, Bohórquez Melo R, Suarez LA, Palma-Cuero M, Murcia LM, González Páez L, Estrada Bustos L, Medina MA, Ariza Campo K, Padilla HD, Zamora Flórez A, De Las Salas JL, Muñoz M, Ramírez JD. Influence of dengue virus serotypes on the abundance of *Aedes aegypti* insect-specific viruses (ISVs). J Virol. 2023 Dec 14:e0150723. doi: 10.1128/jvi.01507-23.

- **Artículo 4:** Martínez D, Gómez M, De Las Salas JL, Hernández C, Flórez AZ, Muñoz M, Ramírez JD. Employing oxford nanopore technologies (ONT) for understanding the ecology and transmission dynamics of flaviviruses in mosquitoes (Diptera: Culicidae) from Eastern Colombia. Acta Trop. 2023 Sep; 245:106972. doi: 10.1016/j.actatropica.2023.106972.

- **Artículo 5:** Gómez M, Martínez D, Páez-Triana L, Luna N, De las Salas J, Hernández C, Zamora Flórez A, Muñoz M, Ramírez JD. Characterizing viral species in mosquitoes (Culicidae) in the Colombian Orinoco: insights from a preliminary metagenomic study. Sci Rep 2023.13, 22081. doi.org/10.1038/s41598-023-49232

2. LISTA DE ANEXOS

2.1 Anexos de artículos producto de la tesis

Artículo 1
Artículo 2
Artículo 3
Artículo 4
Artículo 5

2.2 Anexos de productos de la tesis

12.1 Anexos Artículos Científicos
12.2 Presentación En Eventos Científicos
12.3 Cursos
12.4 Pasantía Internacional
12.5 Becas

Anexos tesis doctoral Marcela G

3. LISTA DE FIGURAS

4. LISTA DE TABLAS

5. LISTA DE ABREVIATURAS

CHIKV: Virus Chikungunya

COI: gen de la subunidad I del citocromo C oxidasa mitocondrial

DENV*:* Virus del Dengue

DENV-1: Dengue virus serotipo 1

DENV-2: Dengue virus serotipo 2

DENV-3: Dengue virus serotipo 3

DENV-4: Dengue virus serotipo 4

HTV: *Humaita-Tubiacanga* (HTV)

ISV: Virus Específicos de Insecto

JEV: virus de la Encefalitis Japonesa

MAYV: virus Mayaro

NGS: secuenciación de nueva generación

ONT: Oxford Nanopore Technology

OROV: virus Oropouche

PCLV: *Phasi Charoen-like virus* (PCLV)

RVFV: virus de la fiebre del Valle del Rift

WNV: Virus del Nilo Occidental

YFV: virus de la fiebre amarilla

ZIKV: Virus del Zika

6. RESUMEN

Los arbovirus, virus transmitidos por artrópodos, son de gran importancia epidemiológica y socioeconómica a nivel mundial debido a su implicación en enfermedades febriles y neurológicas que afectan a una parte significativa de la población global. El virus del dengue (DENV) se destaca por causar la infección más prevalente, especialmente en países tropicales y subtropicales. En las últimas décadas, su incidencia ha experimentado un crecimiento exponencial a nivel mundial, con más de 4.000 millones de personas en riesgo de contagio en estas regiones. Por lo tanto, la vigilancia epidemiológica y el control vectorial juegan un papel fundamental en el control efectivo de estas enfermedades.

La propagación eficiente de arbovirus, como el DENV, se facilita principalmente por el mosquito *Aedes aegypti*, ampliamente reconocido como un vector altamente competente. Su marcado comportamiento antropofílico y su estrecha asociación con entornos urbanos y peridomésticos contribuyen significativamente a esta capacidad. Las características biológicas de los mosquitos *Aedes* promueven su rápida adaptación a nuevos hábitats y su extensa dispersión geográfica, muchas veces relacionada con intervenciones humanas como la urbanización no planificada, el comercio global y el cambio climático. Como resultado, esta especie vectorial se han convertido en una amenaza significativa, comprometiendo la eficacia de las medidas preventivas y la implementación operativa de los programas de control, así como la gestión de brotes de enfermedades asociadas. Asimismo, otros mosquitos de la familia Culicidae también han sido implicados en la transmisión de virus patógenos en ciclos tanto urbanos como rurales. Sin embargo, en las zonas rurales con riesgo de transmisión de enfermedades zoonóticas a los humanos, los vectores endémicos continúan siendo objeto de estudio.

Los avances en las tecnologías de secuenciación de nueva generación (NGS) han revolucionado las estrategias de vigilancia entomovirológica al permitir la detección de virus (re)emergentes y el descubrimiento de nuevas secuencias virales. Estos avances han llevado a la identificación de un grupo representativo de virus conocidos como virus específicos de insectos (ISVs), que infectan naturalmente a los artrópodos, pero no pueden replicarse en células de vertebrados ni infectar a humanos. Se ha observado que algunos de estos ISVs forman un "viroma central" estable y específico para cada especie de mosquito, lo que sugiere su capacidad para influir en la biología del hospedero. Específicamente, estos ISVs pueden modular la transmisión de agentes virales y la competencia vectorial, lo que los convierte en un componente crucial para el desarrollo de nuevas estrategias de control de arbovirus.

Colombia, un país tropical, ofrece condiciones óptimas para el desarrollo y adaptación de diversas especies de mosquitos, lo que crea un entorno propicio para la transmisión de arbovirus de importancia epidemiológica. Los casos de enfermedades transmitidas por arbovirus, especialmente el dengue, han sido prevalentes en el país debido a su alta incidencia estimada. Desde 2016, se ha observado la co-circulación de otros arbovirus como el chikungunya (CHIKV) y el Zika (ZIKV). Este fenómeno se atribuye a las características geográficas de Colombia, las condiciones favorables para la transmisión viral y la presencia generalizada del vector en la mayoría de los municipios del país. En este contexto, los estudios de epidemiología molecular de los arbovirus transmitidos por insectos son fundamentales para comprender la dinámica viral y anticipar posibles brotes epidémicos. Sin

embargo, la mayoría de estas investigaciones se han centrado en el análisis de muestras de pacientes infectados, y son escasos los estudios realizados en muestras de vectores. Esta limitación posiblemente ha obstaculizado un control integral y eficiente de este tipo de enfermedades.

Actualmente, uno de los desafíos principales en el estudio de las arbovirosis, es comprender la diversidad de éstos durante su ciclo de vida en el agente vectorial. Además, es crucial describir las comunidades virales asociadas a los mosquitos vectores, lo que podría proporcionar una comprensión más precisa de la competencia vectorial. Este enfoque permitiría obtener un conocimiento integral de la dinámica de transmisión de arbovirus, contribuiría a la vigilancia epidemiológica de alta resolución y facilitaría el diseño de estrategias eficientes de control y prevención. Por lo tanto, el objetivo general de esta tesis doctoral fue " Caracterizar arbovirus de importancia en salud pública y el viroma en mosquitos de Colombia mediante un enfoque metagenómico ", el cual se desglosó en tres objetivos específicos: 1. Analizar la frecuencia de infección de arbovirus e identificación molecular de las especies de mosquitos del género *Aedes* en distintos departamentos de Colombia. 2. Caracterizar el viroma de *Aedes aegypti* con infección natural por el virus del dengue (DENV) mediante análisis metagenómicos. 3. Describir la dinámica de transmisión y composición viral en poblaciones de mosquitos (Díptera: Culicidae) en ecosistemas rurales estratégicos. Cada uno de estos objetivos se encuentra asociado con un capítulo dentro de este trabajo de investigación.

En el primer capítulo se evidencia que la utilización de técnicas moleculares en la vigilancia entomovirológica constituye una herramienta poderosa para la detección temprana de arbovirus, facilitando así la toma de decisiones en las estrategias de control y prevención. Durante el período 2020-2021, se realizó un análisis en mosquitos *Ae. aegypti* recolectados en diversas regiones de Colombia, donde se observó una alta frecuencia de infección y co-infección natural por serotipos del virus del dengue (DENV-1, DENV-2 y DENV-3). A su vez, la presencia del virus Chikungunya (CHIKV) fue menos frecuente y no se detectó el virus Zika (ZIKV). Estos resultados reafirman el papel predominante de *Ae. aegypti* en la transmisión del dengue en Colombia y señalan una disminución en la circulación de otros arbovirus tras los brotes significativos de 2015 y 2016. Adicionalmente, el análisis de la diversidad genética de *Ae. aegypti*, realizado mediante la secuenciación del marcador molecular COI, reveló la predominancia del linaje I en el país. Este linaje está ampliamente distribuido en las Américas y está asociado con áreas de alta incidencia de dengue. Los hallazgos de este estudio subrayan la importancia de detectar infecciones y co-infecciones por arbovirus en mosquitos *Aedes*, así como la identificación de especies vectoriales y el análisis de su dinámica poblacional mediante técnicas moleculares. Esta información es crucial para establecer sistemas de alerta temprana que respalden la implementación oportuna de medidas de intervención frente a la emergencia o reemergencia de arbovirus.

En el segundo capítulo se llevó a cabo la caracterización del viroma de mosquitos *Aedes aegypti*, utilizando muestras que fueron previamente identificadas en el Capítulo 1 con infección natural por DENV (serotipos DENV-1 y DENV-2), así como muestras de mosquitos que resultaron negativas en las pruebas de infección viral (DENV-negativo). Para esto, se empleó un método de enriquecimiento viral seguido de secuenciación metagenómica utilizando la tecnología de Oxford Nanopore Technologies (ONT). Este enfoque permitió identificar los ISVs predominantes en estas poblaciones. Se observó una variación significativa en la abundancia de ciertas especies y familias de ISVs en respuesta a la infección natural por los diferentes serotipos de DENV. Estas variaciones pueden ser atribuidas a los complejos mecanismos de interacción entre los ISVs y los arbovirus dentro del

mosquito vector. Además, se constató una presencia diferencial del *Phasi-Charoen-like phasivirus* (PCLV) en las muestras infectadas por DENV. Este ISV ha sido descrito previamente como parte del viroma central en los mosquitos *Aedes*. Los análisis filogenéticos de PCLV revelaron asociaciones con secuencias de varias regiones a nivel mundial, destacando especialmente las similitudes con secuencias provenientes de Brasil y Guadeloupe, lo que sugiere una relación evolutiva compartida con estos hospederos. Los resultados de este estudio no solo mejoran nuestra comprensión de la diversidad viral en los mosquitos vectores, sino que también proporcionan información crucial sobre las posibles interacciones entre los ISVs y los arbovirus. Esta información es fundamental para el diseño de estrategias eficaces de control y prevención de las enfermedades transmitidas por *Ae. aegypti.*

En el tercer capítulo se investigó inicialmente la frecuencia de infección por Flavivirus y las preferencias alimentarias en las especies de mosquitos que habitan en un ecosistema rural de sabana en la Orinoquia de Colombia. A continuación, se efectuó un análisis metagenómico viral enfocado en las especies de mosquitos de mayor abundancia y relevancia para la salud pública, incluyendo géneros como *Ochlerotatus, Culex, Limatus, Mansonia, Psorophora* y *Sabethes.* La región estudiada se caracteriza por una mínima interferencia humana y una perturbación antropogénica limitada. Los resultados mostraron la presencia del virus del Nilo Occidental (WNV), un patógeno de importancia médica, en mosquitos de la especie *Culex browni*. Este hallazgo resalta el potencial impacto de las actividades humanas en ecosistemas sensibles a las intervenciones antropogénicas. Además, los patrones de alimentación demostraron que la mayoría de las especies de mosquitos exhiben un comportamiento generalista.

De igual manera, se identificó la presencia de diversos virus específicos de insectos (ISVs) y un grupo común de estos virus en todas las especies de mosquitos analizadas. Esto sugiere que los factores ambientales y del hábitat podrían influir en la composición de las comunidades virales en estos insectos, especialmente entre especies que cohabitan en ecosistemas locales con características ecológicas similares. Los resultados obtenidos destacan la importancia de mantener y ampliar los estudios de vigilancia entomovirológica, especialmente en áreas con mínima intervención humana. Estos hallazgos soportan la alta probabilidad de que virus potencialmente patógenos se propaguen entre las poblaciones humanas y animales en contextos de deforestación y cambios ambientales. Además, estos hallazgos resaltan la necesidad de comprender mejor la composición viral de los mosquitos en diversos ecosistemas. Estudiar la influencia de las condiciones del hábitat rural local en la estructura y composición del viroma de los mosquitos vectores, podría proporcionar una base más sólida para el desarrollo de estrategias efectivas en el control vectorial y la prevención de futuros brotes de enfermedades transmitidas por vectores.

En resumen, la presente tesis doctoral amplía el conocimiento sobre la vigilancia y detección de arbovirus en mosquitos *Aedes* de zonas urbanas y en otros mosquitos vectores de rurales de Colombia, siendo el primer estudio en usar la tecnología ONT en insectos y virus en el país. Además, esta investigación profundiza en la importancia de los virus específicos de insectos (ISV) en la biología y dinámica de los vectores. Utilizando herramientas moleculares, secuenciación de NGS y análisis metagenómicos, se describió la composición del viroma de *Ae. aegypti*, destacando cómo la presencia de los serotipos DENV-1 y DENV-2 influye en la estructura de los ISVs en este mosquito. Asimismo, se caracterizó los hábitos alimenticios y las comunidades virales en mosquitos de la familia Culicidae

en regiones rurales estratégicas, revelando la dinámica de los flavivirus y las especies virales compartidas -ISV- en mosquitos del oriente colombiano. Este estudio subraya la importancia de integrar la vigilancia entomovirológica en los sistemas de salud pública para comprender mejor la transmisión de arbovirus y su potencial impacto en la salud humana y animal. Igualmente, esta investigación establece una base sólida para futuras exploraciones sobre las interacciones entre mosquitos, hospederos y virus en sus hábitats naturales, así como sus implicaciones en la competencia vectorial.

7. MARCO TEORICO

7.1 **Arbovirus** (**Ar**thropod-**Bo**rne **Virus**es o Virus Transmitidos por artrópodos)

El término "arbovirus" deriva de las iniciales de "Arthropod Borne Viruses", que se refiere a los virus transmitidos por artrópodos (1). Este grupo de patógenos incluye una variedad de virus que se replican en insectos hematófagos, especialmente los arbovirus de mayor importancia médica se transmiten a través de mosquitos vectores (2). Los arbovirus representan más del 17% de las enfermedades infecciosas, afectan cada año a millones de personas en todo el mundo y constituyen una proporción significativa de los patógenos humanos emergentes (3-5). Además, la propagación de arbovirus endémicos en una región geográfica más amplia, que da lugar a brotes más grandes y frecuentes (6), lo que representa una considerable amenaza para la salud global.

Los arbovirus, dengue (DENV), chukungunya (CHIKV) y Zika (ZIKV) se consideran los agentes infecciosos de mayor importancia epidemiológica a nivel mundial (7) (**Figura 1**). Se estima que 3.900 millones de personas, en más de 120 países diferentes, corren el riesgo de infectarse con estos tres arbovirus principales (8). (9). Otros arbovirus, como los virus de la fiebre del valle del Rift (RVFV), Mayaro (MAYV), Oropuche (OROPV), West Nile (WNV), han captado la atención de la comunidad científica como amenazas emergentes para la salud pública (10). La expansión de las enfermedades arbovirales causada por estos agentes virales se ve influenciada por diversos factores, como las intervenciones humanas en áreas selváticas o forestales, la urbanización no planificada que favorece la proliferación de insectos en áreas cercanas a poblaciones humanas (1, 11, 12), el cambio climático (13), el crecimiento de la población (14, 15), el transporte globalizado y el comercio de productos (16, 17), así como las poblaciones de mosquitos vectores no controladas (10). Lo anterior, subraya la importancia de la gestión, el control y la vigilancia eficientes para reducir el riesgo de transmisión.

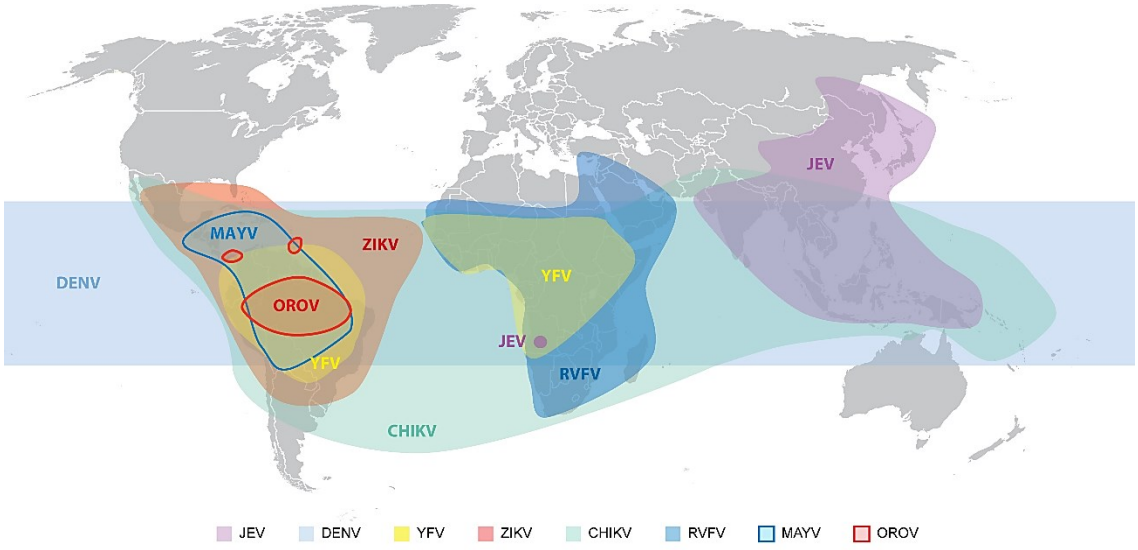

Figura 1. Mapa con la distribución global de los principales arbovirus emergentes. Abreviaturas: CHIKV, virus chikungunya; DENV, virus del dengue; JEV, virus de la encefalitis japonesa; MAYV, virus Mayaro; OROV, virus Oropouche; RVFV, virus de la fiebre del Valle del Rift; YFV, virus de la fiebre amarilla; ZIKV, virus Zika. Tomado de Weaver *et al.,* 2018. Ref.(10).

7.2 Ciclo de transmisión de arbovirus

Los arbovirus se mantienen en el ambiente mediante un ciclo de vida complejo que incluye un hospedero invertebrado (artrópodo) y un hospedero vertebrado (18). La mayoría de estos patógenos proliferan a través de un ciclo enzoótico (ciclo selvático), donde aves, roedores o primates no humanos sirven como reservorios y la transmisión del virus se produce por insectos vectores primarios (7). Los virus también pueden transmitirse entre insectos y animales domésticos, como cerdos y equinos (ciclo epizoótico/rural), ejemplos de este mecanismo incluyen la encefalitis japonesa (JEV), un arbovirus aviar que se amplifica en los cerdos, que a menudo viven muy cerca de las personas, y el virus de la fiebre del Valle del Rift (RVFV), que se amplifica en ovejas, vacas y otros animales domésticos (10). Así mismo, entre insectos y humanos (ciclo epidémico/urbano) (10). Este último, se desarrolla tras la entrada en contacto del ser humano con alguno de los ciclos previos (el selvático o el rural), donde se puede desencadenar la aparición de brotes de enfermedades en humanos (10) (**Figura 2**). Los arbovirus con potencial de propagación urbana se encuentran entre los más importantes para la salud pública. La transmisión arboviral tiende a ser estacional y podría ser variable en función de las condiciones climáticas locales, la transmisión viral y la actividad del vector (7, 19).

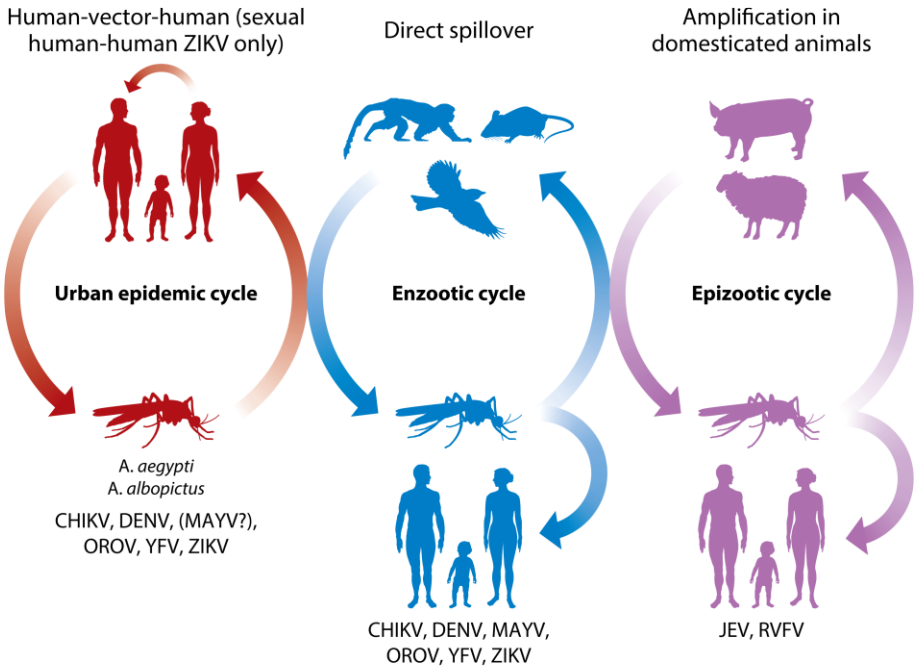

Figura 2. Ciclos de transmisión de arbovirus. Ciclos de transmisión de arbovirus, representando los orígenes selváticos (ciclo enzootico), la zona de emergencia donde los ciclos selváticos entran en contacto con las poblaciones humanas en las zonas rurales (Ciclo epizoótico) y en las zonas urbanas (ciclo epidémico). Abreviaturas: CHIKV, virus chikungunya; DENV, virus del dengue; JEV, virus de la encefalitis japonesa; MAYV, virus Mayaro; OROV, virus Oropouche; RVFV, virus de la fiebre del Valle del Rift; YFV, virus de la fiebre amarilla; ZIKV, virus Zika. Tomado de Weaver *et al.,* 2018. Ref. (10).

7.2.1. Ciclo de transmisión urbano en humanos y mosquitos *Aedes*

Los arbovirus de mayor importancia médica como DENV, ZIKV, CHIKV y YFV son transmitidos en zonas urbanas o periurbanas por mosquitos *Aedes* spp del subgénero Stegomyia (2, 20). Aunque los tres primeros son flavivirus pertenecientes a la familia Flaviviridae, el virus del chikungunya es un alfavirus de la familia Togaviridae. Todos estos virus se transmiten en ciclos zoonóticos que involucran a primates no humanos y mosquitos arborícolas, y han establecido ciclos urbanos entre humanos, principalmente a través de la transmisión por *Aedes aegypti* y, en algunos casos, por *Ae. albopictus*. Estos mosquitos urbanos son esenciales para mantener el ciclo de vida de los arbovirus, ya que la transmisión entre humanos suele ser suficiente para la persistencia de la enfermedad.

Durante el ciclo urbano, el adecuado funcionamiento del sistema humano-virus-vector permite la transmisión biológica de arbovirus, lo cual implica tres etapas distintas (**Figura 3**). Primero, la infección del vector (mosquitos hembra) después de la alimentación con sangre de un hospedero durante la fase aguda febril y virémica de la enfermedad (20). Segundo, la replicación del virus en el intestino medio del mosquito y su diseminación hacia los tejidos distales, hasta llegar a las glándulas salivales en un periodo denominado periodo de incubación extrínseco, que generalmente dura entre 8 a10 días (20). Este proceso está influenciado por la temperatura ambiente, la cepa del virus y la competencia del mosquito (13, 21). Tercero, una vez que se infectan las glándulas salivales, el mosquito es infeccioso y puede transmitir el virus a otra persona durante la alimentación con sangre (19). El mosquito sigue siendo infeccioso de por vida y puede transmitir el virus a todas las personas de las que se alimenta posteriormente. El tiempo desde la infección hasta el inicio de la enfermedad (el período de incubación intrínseco) en humanos varía de 3 a 14 días, con un promedio de 4 a 7 días, tiempo en el cual una persona sintomática o asintomática es capaz de transmitir el virus a un nuevo mosquito (18, 20). En el ciclo urbano, las personas actúan como huéspedes de amplificación y los mosquitos antropofílicos como *Aedes* transmiten el virus a menudo en entornos urbanos. La transmisión vertical puede ocurrir cuando el mosquito hembra infectado transmite el virus a través de los huevos a su descendencia, aunque la importancia epidemiológica de este modo de transmisión es incierta (22).

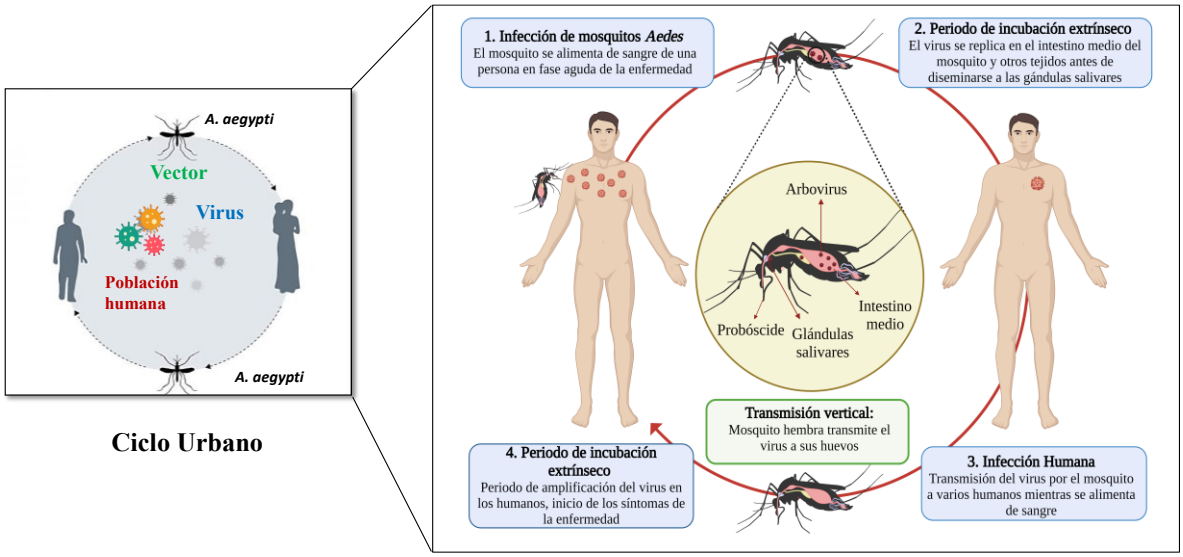

Figura 3. Ciclo de transmisión urbano del DENV. Tomado de Gómez *et al.,* 2022. Ref. (23)

7.3 Arbovirus de importancia para la salud pública

El dengue es actualmente una de las enfermedades tropicales desatendidas de mayor importancia mundial (24). Se estima que afecta aproximadamente el 50% de la población mundial, con proyecciones que señalan un aumento considerable en los riesgos futuros (8, 25). Cada año, se registran aproximadamente 400 millones de nuevas infecciones en los 157 países donde la enfermedad es endémica (1, 2, 8), alrededor de 500,000 desarrollan dengue grave (DS) y más de 40,000 casos resultan mortales Su expansión geográfica se ha visto impulsada por el aumento exponencial de casos y epidemias, facilitada por la co-circulación de los cuatro serotipos del virus del dengue (DENV 1, DENV-2, DENV-3 y DENV-4) (19, 26). Esta co-circulación ha dado lugar a diferentes manifestaciones clínicas, desde fiebre leve hasta casos graves como la fiebre hemorrágica y el síndrome de choque por dengue (19). Una vez recuperada la infección, la persona adquiere inmunidad contra ese serotipo específico (20), sin embargo, las infecciones posteriores causadas por otros serotipos aumentan el riesgo de desarrollar dengue grave (25).

Según la Organización Mundial de la Salud (OMS), históricamente entre los años 2000 y 2019 se evidenció un drástico aumento en el número de casos reportados, pasando de 500,000 a 5.2 millones (27). Después de una leve disminución entre 2020 y 2022, asociada a una menor notificación de casos, durante la pandemia de COVID-19, en el 2023 se observó un incremento global significativo (28). Este aumento en el número y la escala de los casos se caracterizó por múltiples brotes simultáneos, con más de cinco millones de infecciones y más de 5000 muertes reportadas en más de 80 países de África, las Américas, Asia Sudoriental, Pacífico Occidental y Mediterráneo Oriental (28). Cerca del 80% de estos casos (4.1 millones) se registró en la Región de las Américas (**Figura 4)**. El aumento del riesgo de propagación de la epidemia de dengue se ha atribuido a varios factores, incluyendo cambios en la distribución de los vectores (*Ae. aegypti* y *Ae. albopictus*), fenómenos climáticos severos, y el debilitamiento de los sistemas de salud durante la pandemia de COVID-19 (13, 24). Además, es importante destacar que la respuesta a esta situación se ha visto obstaculizada por la fragilidad de los sistemas de salud y la deficiencia en los sistemas de vigilancia en muchos países afectados.

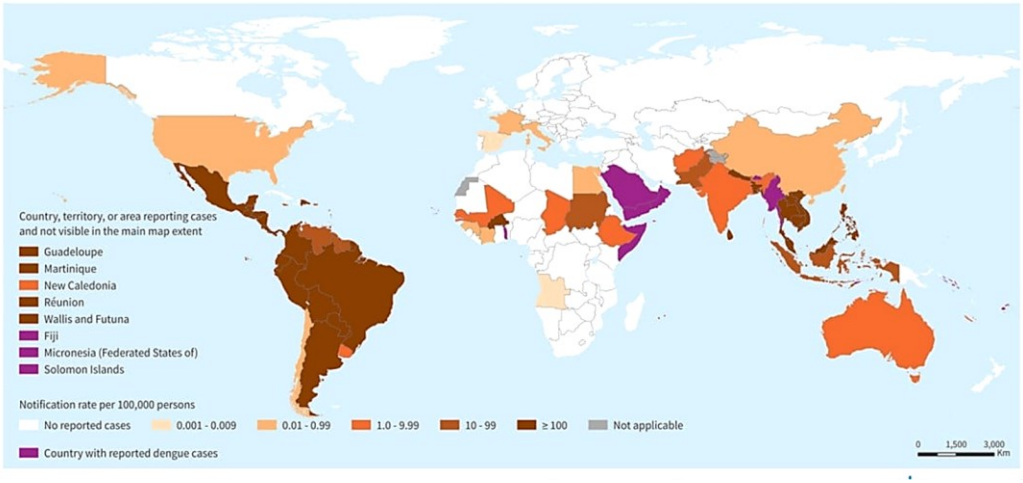

Figura 4. Países/territorios/áreas que reportan casos autóctonos de dengue (noviembre 2022 – noviembre 2023) Tomado de: World Health Organization, 2023. Ref (27).

El chikungunya es la segunda enfermedad arboviral más extendida después del dengue, designada por la OMS como una enfermedad emergente grave que afecta a más de 106 países/territorios, principalmente de Asia, Pacífico Sur, Europa Meridional, islas del Océano Indico, Caribe y América (7, 8, 10), con más de 2,5 millones de registros de casos sospechosos y confirmados. El CHIKV se considera un problema de salud pública debido a su rápida propagación y alta morbilidad (27). De igual forma, el virus del Zika (ZIKV) desde 2007, ha producido brotes importantes en África, el sudeste de Asia, las islas del Pacífico, el Caribe, Oceanía y las Américas, siendo endémico en 79 países (8, 10). Este virus, aunque produce una infección asintomática o una enfermedad clínicamente leve en la mayoría de los casos, puede causar defectos congénitos del neurodesarrollo como microcefalia y enfermedades neurológicas graves en adultos como el síndrome de Guillain-Barré (6, 7), por tal razón, la OMS lo declaró una amenaza para la seguridad de la salud pública mundial.

7.3.2 Arbovirus en la región de las Américas

En las Américas, el dengue se ha convertido en el arbovirus más prevalente, evidenciado por un incremento en los casos notificados, superando las cifras históricas de años anteriores. En las últimas cuatro décadas, los casos de dengue en esta región han experimentado un aumento significativo, pasando de 1,5 millones entre 1980 y 1989 a 17,5 millones entre 2010 y 2019 (27). En 2019, se alcanzó un máximo histórico con más de 3,18 millones de casos, de los cuales 28,208 fueron graves y se registraron 1823 fallecimientos, lo que representa una(27) tasa de letalidad del 0,06% (27). Para el año 2023, se notificaron 4,1 millones de presuntos casos de dengue, incluyendo 6710 casos graves y 2049 muertes, distribuidos en 42 países y territorios de la región.

El patrón de circulación de estos arbovirus en las Américas se representa en la **Figura 5**, que muestra que la introducción de chikungunya en diciembre de 2013 se observa claramente en 2014. Del mismo modo, tras la introducción del Zika en 2015, se produjo una circulación generalizada del virus en 2015. No obstante, la circulación del dengue ha seguido predominando (27).

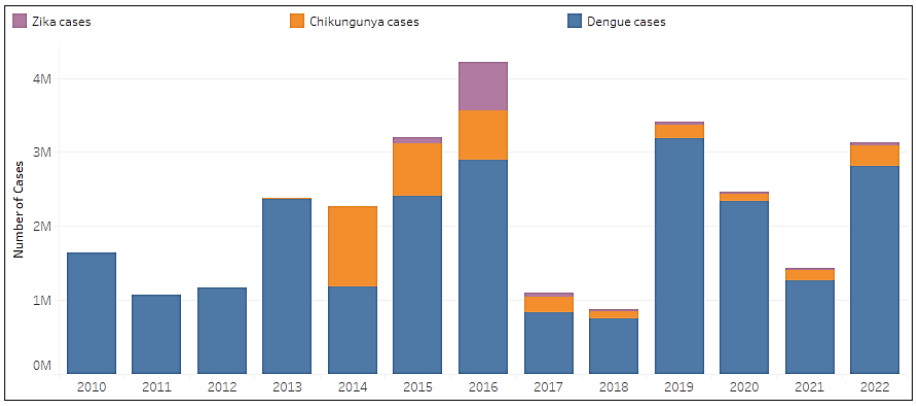

Figura 5. Casos notificados de dengue, chikungunya y Zika en la región de las Américas 2010-2022. Tomado de Organización Panamericana de la Salud, 2023. Ref. (27).

7.3.2 Arbovirus en Colombia

En Colombia, los arbovirus con mayor incidencia son el dengue, zika y chikungunya (23). Las características geográficas y eco-epidemiológicas del país favorecen la presencia de vectores y la transmisión de estos arbovirus en gran parte del territorio nacional (29-31). Actualmente, el dengue representa la enfermedad viral más prevalente, con los cuatro serotipos del virus circulando simultáneamente desde 2001 (29, 31), siendo DENV-1 y DENV-2 los más predominantes. Desde 2008, se ha observado un patrón fluctuante en la incidencia del dengue, con ciclos epidémicos que se repiten cada tres años y un cambio en la dominancia de serotipos de DENV-1 a DENV-2 (29). Investigaciones previas han asociado estos ciclos epidémicos con fenómenos meteorológicos como El Niño y La Niña, que influyen en el ciclo de vida de los vectores al proporcionar microambientes favorables para su desarrollo (32).

Durante los últimos ciclos epidémicos, particularmente en 2019, se registró un preocupante aumento en los casos de dengue, superando las 127,553 infecciones reportadas, con una incidencia de 600 casos por cada 100 mil habitantes y tasas de letalidad elevadas en comparación con los valores promedio en las Américas (33). Los años 2020 y 2021 representaron periodos inter-epidémicos, caracterizados por una disminución significativa en los casos notificados, con menos de 1,000 casos por semana epidemiológica y una incidencia que oscilaba entre 172.3 y 295.2 casos por cada 100,000 habitantes en riesgo (23) (**Figura 6**). Sin embargo, en 2022 se observó un resurgimiento de casos que persistió en 2023, con tasas de incidencia más elevadas, superando los 131,784 casos reportados (23), y vinculados a casos graves de la enfermedad. Estas cifras se asemejan a los registros de dengue en diversas regiones del mundo (23, 28).

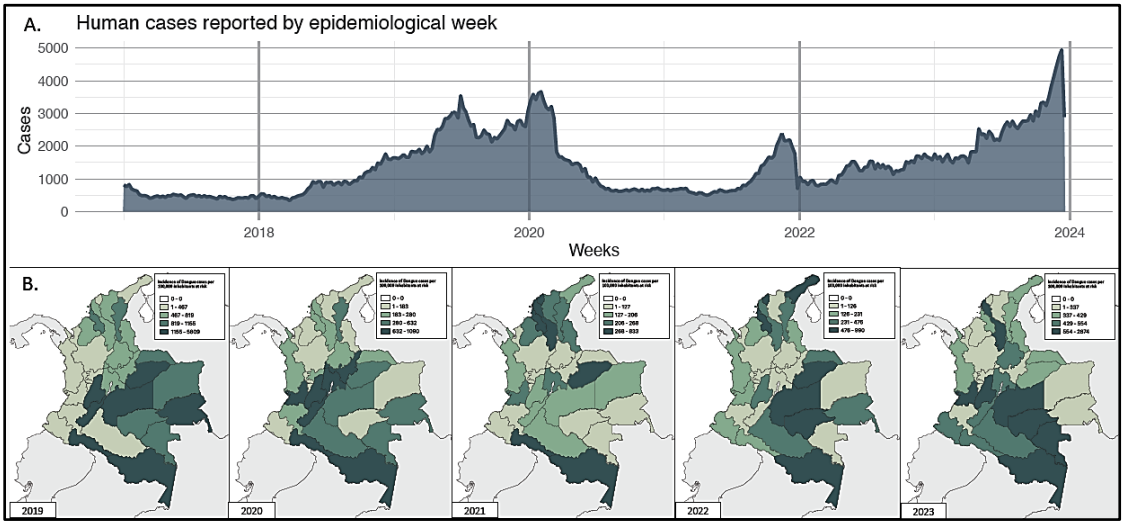

Figura 6. Incidencia de dengue en Colombia de 2017 a 2023. **A.** Casos de dengue notificados semanalmente en Colombia desde la semana 1 de 2017 hasta la semana 52 de 2023. **B.** Mapa de Colombia que representa la incidencia de dengue por 100.000 habitantes en riesgo por departamento de 2019 a 2023. Tomado de Martínez *et al*., 2023. Ref. (34).

Así mismo, desde la introducción del virus de chikungunya en el país en 2014, se han reportado más de 774.831 casos, lo que demuestra la endemicidad del CHIKV en Colombia. Según la Organización Panamericana de la Salud, Colombia fue el país con el tercer mayor número de casos después de Brasil y República Dominicana; entre 2014 y 2015 se registraron 460.484 casos diagnosticados por clínica y 4.658 confirmados por laboratorio Finalmente, se han notificado 21.149 casos entre 2016 y agosto de 2018 (35). Con respecto al ZIKV, desde su aparición en 2015, reporta 117.674 casos, lo que ubica a Colombia como el segundo país más afectado en las Américas después de Brasil (36, 37). Desde el 2015 y finales del 2016, 48 países y territorios de las Américas confirmaron casos autóctonos por transmisión vectorial del ZIKV y cinco países notificaron casos transmitidos sexualmente.

7.4 Mosquitos transmisores de enfermedades arbovirales

Los mosquitos, pertenecientes al orden Diptera y la familia Culicidae, son reconocidos a nivel mundial como vectores de agentes virales que afectan tanto a humanos como a animales. Estos insectos son responsables de millones de casos de enfermedades cada año, representando una significativa amenaza para la salud pública y veterinaria (38). Las especies de mosquitos hematófagos desempeñan un papel crucial en la transmisión de arbovirus epidémicos, en particular los pertenecientes a las familias Flaviviridae y Togaviridae (10, 39). En las regiones tropicales y subtropicales, los mosquitos capaces de transmitir virus patógenos tanto en ciclos urbanos como rurales se encuentran principalmente dentro de los géneros *Aedes y Culex* (40, 41). Estos vectores son los principales portadores de virus como el virus del dengue (DENV), el virus del Zika (ZIKV), el virus del Nilo Occidental (VNO) (42), la encefalitis de San Luis (SLEV) y la encefalitis japonesa (JEV) de la familia Flaviviridae, así como el virus chikungunya (CHIKV) de la familia Togaviridae. Las características biológicas de los mosquitos *Aedes y Culex* facilitan su rápida adaptación a nuevos hábitats y su amplia expansión geográfica, a menudo asociada a intervenciones antropogénicas en zonas boscosas, (38) urbanización no planificada (43), comercio global y cambio climático (44). Además, otros miembros de la familia Culicidae, pertenecientes a los géneros *Haemagogus, Sabethes, Mansonia y Psorophora*, han sido implicados en la transmisión de virus como el virus de la fiebre amarilla (YFV), el virus Mayaro (MAV) (45, 46) y el virus de la encefalitis equina venezolana (VEEV) en ciclos selváticos (43). Sin embargo, en las zonas rurales con riesgo de transmisión de enfermedades zoonóticas al ser humano (spillover), los vectores endémicos de estas zonas siguen estando poco estudiados.

7.4.1 Mosquitos vectores del género *Aedes*

Los arbovirus epidémicos DENV, CHIKV y ZIKV son transmitidos por mosquitos hembra, principalmente del género *A. aegypti* y, en menor medida, de la especie *A. albopictus* (10, 38). Estos mosquitos-vectores muestran una amplia plasticidad ecológica bajo diferentes condiciones antrópicas, climáticas y ambientales (39, 40), facilitando su propagación en zonas tropicales, subtropicales y algunas templadas (38, 44) **(Figura 7).**

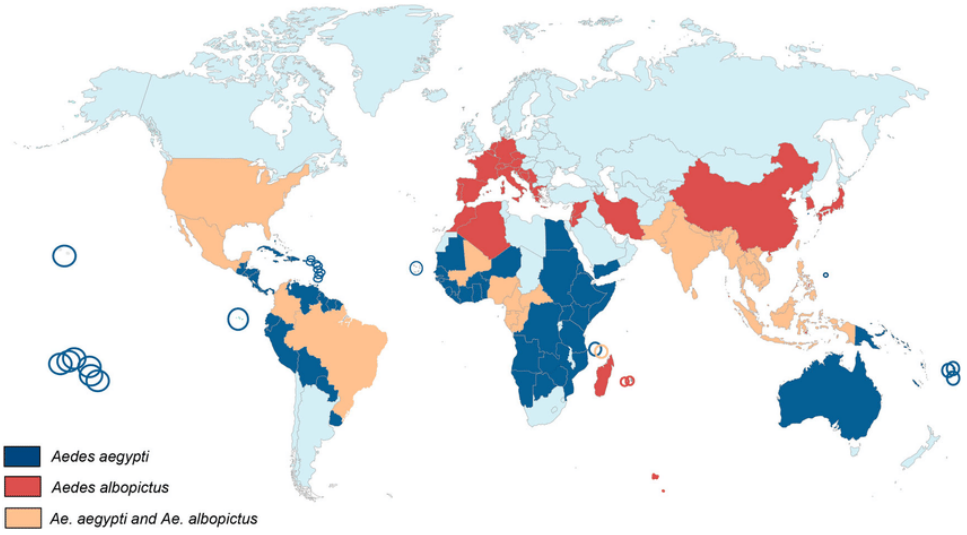

Figura 7. Distribución mundial de *Aedes aegypti y Aedes albopictus*.

Tomado de Houé *et al.*, 2019. Ref (47)

En los últimos años, la vigilancia virológica mediante técnicas moleculares se ha propuesto como una herramienta altamente sensible y eficiente para detectar la infección de arbovirus en vectores (48, 49). Los estudios realizados con este enfoque muestran que las tasas de infección en mosquitos *Aedes* spp. en zonas endémicas de América Latina pueden variar en función de la especie viral, lo que proporciona un enfoque prometedor para desencadenar estrategias de control. En los mosquitos *A. aegypti*, el DENV es más prevalente, con rangos entre cero y 16,2% (50), y el CHIKV está entre cero y 12,5% (51), mientras que el ZIKV representa la tasa más baja entre cero y 9,0% (52). Aunque los datos sobre coinfecciones arbovirales en mosquitos son limitados, algunos estudios demuestran diferencias en la susceptibilidad a infecciones mixtas por especie y serotipo en mosquitos, con frecuencias que oscilan entre el 0,9 y el 13,3% (34, 53).

También se ha descrito la identificación de especies de *Aedes* y su variabilidad genética mediante marcadores moleculares para conocer la dinámica poblacional y sus posibles implicaciones en la transmisión de enfermedades (54, 55). Esto indica que las tasas de infección y la identificación de la diversidad genética del vector son esenciales desde el punto de vista de la epidemiología molecular para determinar las posibles vías de transmisión arboviral y la progresiva dispersión y/o invasión del mosquito *Aedes* en zonas endémicas (55, 56).

7.4.2 Especies de mosquitos transmisores de arbovirus en Colombia

Colombia, reconocida por su megadiversidad, alberga una amplia variedad de especies de mosquitos (Diptera, Culicidae) y presenta una alta prevalencia de enfermedades transmitidas por estos vectores (42, 57). La enorme variedad de ecosistemas proporciona condiciones ambientales que favorecen la inmigración, adaptación, desarrollo y persistencia de una gran diversidad de especies de mosquitos (58). En Colombia y otras regiones de las Américas, existe una notable escasez de estudios enfocados en la caracterización entomológica de los mosquitos *Aedes* y otros vectores de arbovirus (34). Esta

carencia de información tiene consecuencias directas en la formulación de estrategias efectivas para el control de estos virus. Aunque existen investigaciones publicadas sobre vectores de arbovirus, la mayoría se centra en el principal transmisor del virus del dengue (DENV) en Colombia, el mosquito *Aedes* spp. Estos estudios suelen abordar aspectos como la distribución geográfica, la tasa de infección y los patrones de transmisión de arbovirus, elementos cruciales para entender y gestionar la propagación de estas enfermedades (30, 34, 53, 59, 60).

Los registros del Instituto Nacional de Salud indican que *Ae. aegypti* se encuentra ampliamente distribuido en el territorio nacional, con registros en 823 municipios que abarcan áreas rurales, periurbanas y urbanas de los 32 departamentos (36, 61, 62). En comparación, *A. albopictus*, de reciente introducción en Colombia (1998), (63), presenta una rápida expansión principalmente en zonas suburbanas y rurales con abundante vegetación en 52 localidades de 15 departamentos del país (64). En estas regiones los cambios en el uso del suelo, las condiciones de saneamiento básico deficiente, la alta población humana y la planificación urbana inapropiada crean escenarios aptos para la reproducción, dispersión y coexistencia de las especies vectoriales *Ae. aegypti y Ae. albopictus* (32, 35, 42, 65, 66). Se consideraba que estas especies se distribuían en altitudes que no sobrepasan los 2.200 m.s.n.m.; sin embargo, nuevos reportes muestran adaptaciones a 2.300 m.s.n.m., lo cual puede estar relacionado con múltiples factores socioeconómicos regionales y locales, así como factores climáticos **(Figura 8)** (32, 35).

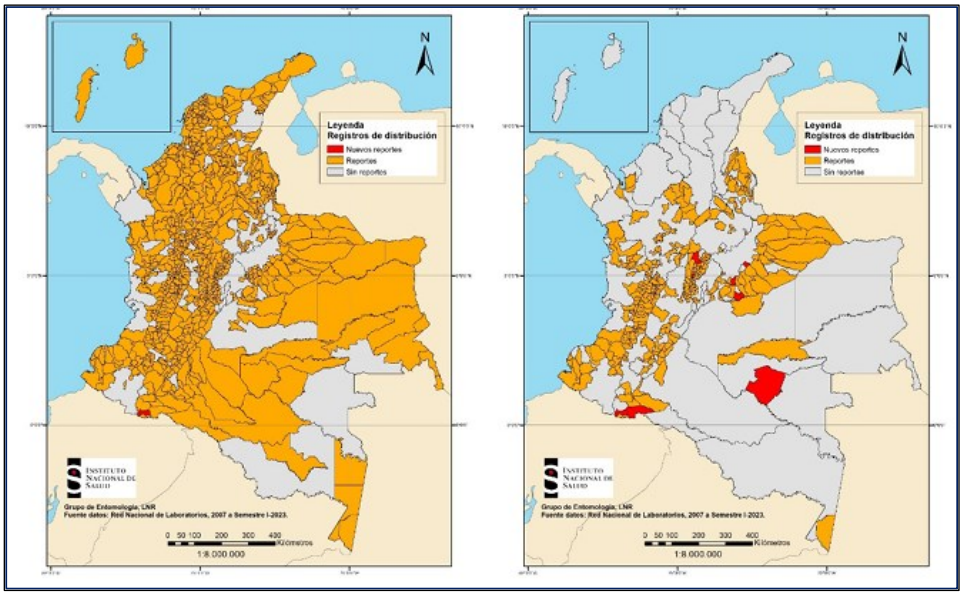

Figura 8. Distribución de *Ae. aegypti y Ae. albopictus en* Colombia desde el 2007-2023.

Tomado de Instituto Nacional de Salud Colombia, 2023. Ref. (23)

Por otra parte, aunque algunos estudios se han centrado en la detección de arbovirus en poblaciones de mosquitos silvestres en Colombia, estos informes son relativamente escasos. Estudios realizados en mosquitos adultos colectados en zonas rurales del área central (región Andina) y norte (región caribe), identificados mediante códigos de barras de ADN y morfología, revelaron que los géneros *Culex* (42, 57) y *Mansonia* (67) son los más diversos y ricos en especies de los mosquitos muestreados

en estas zonas. Esto, posiblemente asociado a las características geográficas, climáticas y medioambientales de las áreas seleccionadas. Así mismo, investigaciones en áreas rurales el norte del país, (zonas con humedales y bosque seco tropical), identificaron arbovirus de importancia epidemiológica (Alphavirus y Flavivirus) (42). Los alfavirus detectados en los pools de *Culex spp., Deinocerites atlanticus, Mansonia titillans, Psorophora confinnis* y *Aedes scapularis* fueron identificados como virus de la encefalitis equina venezolana (VEEV). Los flavivirus se identificaron como DENV en *Ae. aegypti*, virus de la fiebre amarilla (YFV) en *Haemagogus splendens*, virus de la encefalitis de San Luis (SLEV) en *Cx erraticus,* y *Mansonia titillans* y Virus del Nilo Occidental (WNV) *en Cx. quinquefasciatus y Cx. erraticus* (40, 66). Los principales arbovirus encontrados en reportes en Colombia se presentan en la siguiente tabla:

Tabla 1. Arbovirus detectados en estudios de mosquitos vectores en Colombia

Especies de Mosquito	Ecosistema	Arbovirus identificado	Referencia
Aedes scapularis	Rural	VEEV	(42)
Aedes* (*Stegomyia*) *aegypti	Urbano Periurbano/Rural	DENV, CHIKV, ZIKV, YFV	(30, 31, 34, 59, 60, 68)
Aedes* (*Stegomyia*) *albopictus	Periurbano/ Rural	DENV, CHIKV	(31, 34)
Aedes serratus		YFV	(30)
Culex* (*Culex*) *quinquefasciatus	Rural	CxFv	(42)
***Culex* spp.**	Urbano/Rural	VEEV, SLEV, WNV, CxFv	(42, 69-72)
***Deinocerites atlanticus* aff.**	Rural	VEEV	(42)
***Haemagogus* sp.**	Rural	YFV	(30)
Mansonia titillans	Rural	VEEV, SLEV	(42, 69)
Psorophora* (*Grabhamia*) *confinnis	Rural	VEEV	(42)

SLEV y WNV son flavivirus emergentes que han sido detectados previamente en humanos, equinos y aves en la región del Caribe colombiano (71, 73). De igual forma, en Colombia, los vectores que participan en la transmisión selvática del YFV incluyen *Haemagogus equinus* y *Haemagogus janthinomys* (42). En el país, se han documentado epidemias de VEEV en varias regiones (72), con las siguientes especies de mosquitos implicadas en la transmisión: *Ma. venezuelensis, Ae. serratus, Aedes (Ochlerotatus) spp., Ae. fulvus y Culex (Melanoconion)* spp (42). En el caso de *Ma. titillans* se ha descrito que presenta hábitos alimentarios eclécticos donde animales domésticos (cerdos, caballos y vacas) y fauna selvática (principalmente aves y roedores), por lo que se consideró un candidato a vector puente, que puede transmitir SLEV del ambiente selvático/rural al peridoméstico donde podría infectar a los humanos (69). En comparación, *Cx. erraticus* tiene preferencia por una alta diversidad de aves, seguido de mamíferos con alta frecuencia de contacto con humanos (69). La presencia de distintos arbovirus en mosquitos vectores, indica el riesgo de emergencia o reemergencia de estos patógenos en esta zona de la región caribe del país y la necesidad de seguir realizando investigaciones en este campo en distintos ecosistemas de Colombia, lo cual puede contribuir al desarrollo de

estrategias más efectivas de prevención y control. Esto, teniendo en cuenta que las condiciones ecológicas del país son favorables a una gran biodiversidad viral en mosquitos. Además, los cambios abióticos en el ecosistema pueden contribuir a un aumento en el tamaño de las poblaciones de mosquitos y en los casos de enfermedades transmitidas por vectores (30, 74).

7.5 Microbiota y competencia vectorial

Durante los últimos años, estudios sobre la biología de insectos transmisores de arbovirus, señalan que además de los patógenos que causan enfermedades, los mosquitos también alojan una amplia variedad de microorganismos como bacterias, virus y hongos (75-77). La microbiota de los mosquitos juega un papel fundamental en muchos procesos biológicos, incluida la nutrición, la digestión, el apareamiento y la reproducción sexual, el desarrollo, las funciones de respuesta inmunitaria y la resistencia a los patógenos (78, 79). Recientes estudios demuestran que la microbiota de los mosquitos adultos puede afectar la capacidad vectorial (76, 79, 80), al influenciar la densidad del vector, la tasa de picaduras, la supervivencia, la competencia del vector y el período de incubación extrínseco del patógeno (81). Por lo tanto, se ha sugerido que los microorganismos pueden alterar la transmisión de patógenos en los mosquitos, ya sea positiva o negativamente (82-84), lo cual puede influir en el riesgo humano de exposición a enfermedades arbovirales febriles producidas por el DENV, el ZIKV y el CHIKV.

7.6.1 Estudios de metagenómica viral en insectos-vectores

La secuenciación metagenómica de nueva generación (mNGS) definida como “la aplicación de la técnica moderna de la genómica sin la necesidad de aislamiento y cultivo en laboratorio de especies individuales” (85), permite el estudio del material genético de una comunidad mixta de organismos en una muestra, para comprender la composición y diversidad de diferentes grupos de organismos. De esta manera, la metagenómica secuencia de manera imparcial todo el material genético (ADN o ARN) que existe en una muestra ambiental o biológica (86), lo que permite una amplia identificación de patógenos conocidos o incluso el descubrimiento de nuevos organismos (87). Esta metodología se usa comúnmente para investigar la diversidad taxonómica de una comunidad microbiana y/o para realizar una caracterización funcional a partir de los genes que están presentes (88-90). Esta técnica tiene la ventaja de reducir el tiempo durante análisis, al eliminar la necesidad de diseñar y sintetizar primers y sondas para PCR (91), lo cual es benéfico ante la necesidad de estudiar potenciales patógenos de muestras humanas y ambientales durante epidemias virales, como el caso de Zika (92) y Ébola (93).

Los estudios de metagenómica viral en mosquitos vectores son relativamente recientes, debido a que la mayoría de las investigaciones se centraban en técnicas de cultivo celular, microscopía electrónica, ensayos moleculares convencionales o técnicas de serología o aislamiento viral dirigido, para la detección casi exclusiva de arbovirus (94, 95). En la última década, gracias a las tecnologías de secuenciación de alto rendimiento denominadas “secuenciación de nueva generación” o “secuenciación masiva” como Illumina, Pacific Biosciences (PacBio), Oxford Nanopore technologies (ONT), la intensificación de la vigilancia de insectos vectoriales y necesidad de detectar patógenos emergentes y virus nuevos, se ha dilucidado que los mosquitos albergan un viroma rico y diverso, compuesto por arbovirus y en mayor proporción por ISV (virus específicos de insectos) (96-100). Estos últimos descritos como virus que infectan naturalmente a los artrópodos, pero no tienen

la capacidad de replicarse en células de vertebrados, ni infectar a seres humanos (101, 102). Así, los métodos de metagenómica, en especial durante la última década **(Figura 9),** han proporcionado nuevos conocimientos sobre la complejidad de los virus transportados por los insectos (96, 101, 103, 104), lo cual tiene una gran relevancia para la vigilancia dinámica de patógenos y la respuesta ante enfermedades infecciosas emergentes y re-emergentes (86, 95, 105, 106).

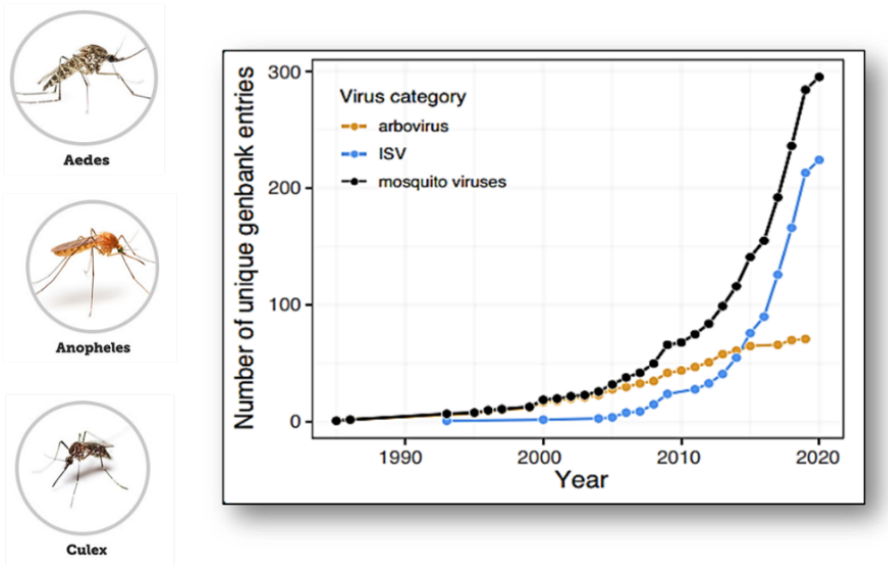

Figura 9. Descripción general de los virus que circulan en los mosquitos. Cantidad acumulada de entradas únicas en Genbank para virus asociados con mosquitos. Los números de arbovirus e ISV (virus específicos de insectos) también son indicado por separado.

Modificado de Almeida *et al.,* 2021. Ref. (70).

Análisis de los virus asociados a mosquitos recientemente descubiertos en la última década (2007-2017) identificaron 14 virus asociados a mosquitos que pueden infectar líneas celulares de mamíferos *in vitro* y que se ha demostrado serológicamente que infecta a los animales domésticos; sin embargo, no existe evidencia de enfermedad para todos estos otros virus (95). Por otra parte, la mayoría de las investigaciones sobre ISV se han enfocado en los mosquitos *Culex spp., Aedes spp.* y *Anopheles sp*, (99, 107-109), revelando que aunque existen variaciones de diversidad y abundancia de estos virus entre especies vectoriales, también se comparten familias virales como *Flaviviridae, Mesoniviridae, Rhabdovirida , Reoviridae* y *Peribunyaviridae* (95)**,** lo cual indica que los ISV podrían estar altamente relacionados con la competencia de estas especies vectoriales para transmitir arbovirus. Por esta razón algunos autores han propuesto que los ISV asociados a mosquitos podrían tener varias aplicaciones potenciales como (a) agentes de control biológico contra enfermedades transmitidas por vectores, (b) terapias de diagnóstico y (c) nuevas plataformas de vacunas (82, 94, 110).

Los análisis filogenéticos y los estudios experimentales han demostrado que muchos de estos virus específicos de insectos aislados de mosquitos están estrechamente relacionados con los arbovirus patógenos humanos (100, 111). Esto ha suscitado hipótesis sobre su posible papel en la modulación de la transmisión de arbovirus (84, 112). Además, se cree que ISV son ancestrales de los arbovirus, y probablemente evolucionaron y se diversificaron junto con sus hospedadores insectiles, lo que podría ayudar a entender la evolución del cambio de un hospedero único a un hospedero dual (84,

113). Los avances en la exploración del viroma en mosquitos tiene una importancia importante al reflejar el origen, la evolución, la diversidad y la distribución viral, así como contribuir al desarrollo de estrategias para controlar y prevenir enfermedades arbovirales (**Figura 10**).

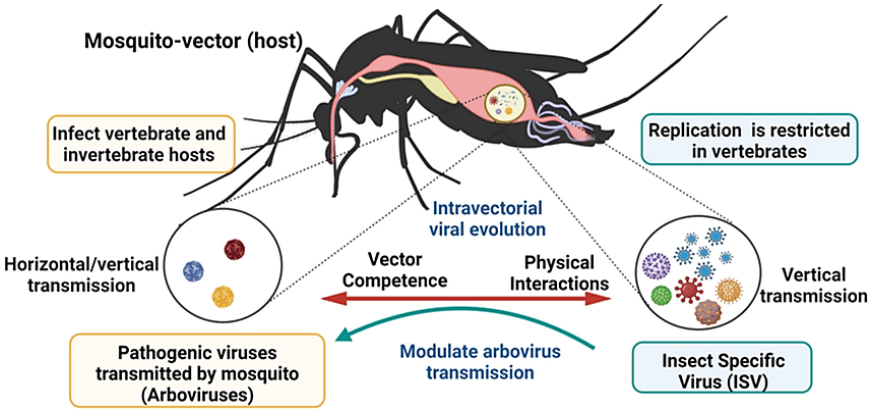

Figura 10. Resumen esquemático de algunas características del viroma del mosquito *Aedes* a partir de estudios metagenómicos. El viroma del *Aedes* está formado por arbovirus y en mayor proporción por virus específicos de insectos (ISV). Tomado de Gómez *et al.*, 2022. Ref.(22)**.**

7.7 Viroma de mosquitos *Aedes*

Análisis del viroma en los mosquitos *Aedes* spp. aún son muy limitados, y los estudios disponibles se enfocan especialmente en *Ae. aegypti*, posiblemente por ser considerado el vector principal de arbovirus (75, 96, 98). Recientemente, Shi *et al*. 2020 (114) realizaron una comparación de la composición viral de *Ae. aegypti* a partir de estudios metagenómicos realizados en Estados Unidos, Puerto Rico, Australia, Tailandia, Guadalupe, China y Kenia, en la que encontraron que existe un conjunto de virus que se encuentran en la mayoría de los individuos de las poblaciones de estudio, denominado “viroma central”, representado principalmente por el virus tipo *Phasi Charoen* (PCLV) y el virus *Humaita-Tubiacanga*, (96, 97) **(Figura 11)**. Por otra parte, el "viroma central" relativamente estable, evidenciado en *Ae. aegypti* y en otro tipo de vectores como *Culex quinquefasciatus* posiblemente tenga implicaciones importantes para la transmisión de arbovirus importantes desde el punto de vista médico (96); sin embargo, se requieren más estudios que permitan comprender mejor las interacciones entre ISV-arbovirus-vector.

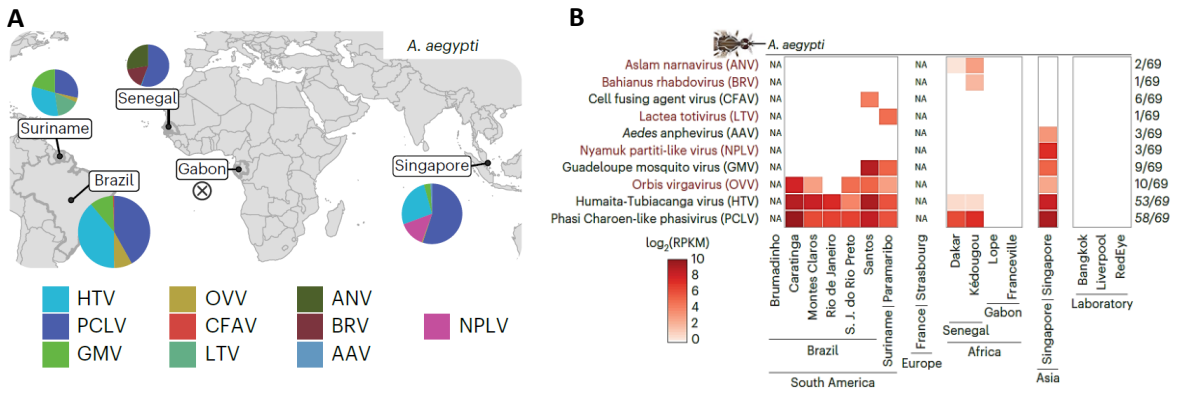

Figura 11. Análisis de metagenómica viral muestra la conservación de virus específicos de insectos (ISV) en el viroma de *A. aegypti* en países de diferentes zonas geográficas del mundo. **A.** Gráficos circulares de la carga global de virus y la diversidad viral de ISV para *A. aegypti* en distintas zonas del mundo. **B.** Carga viral mostrada como un mapa de calor para cada de los ISV prevalentes en poblaciones de mosquitos, el color blanco indica ausencia de un virus y NA indica ausencia de muestras en un lugar determinado. Los ISV en letra color rojo son virus nuevos, mientras los de color negro son virus previamente identificados. Tomado de Olmo *et al*, (2023) Ref. (115).

Por otra parte, se ha identificado que el perfil del viroma en *Aedes spp.* es muy estable en diferentes etapas de vida (larva, pupa y adulto), tanto en mosquitos cultivados en laboratorio como colectados en campo (114, 116)**,** lo que indica que el viroma de mosquitos puede adquirirse tanto en machos como hembras en todas las etapas de la vida y transmitirse principalmente de forma vertical (de parentales a descendencia) (84, 100, 110, 113). Adicionalmente, en mosquitos colectados en campo se ha evidenciado una mayor diversidad viral, que podría estar influenciada por factores relacionados con el hospedero y el medio ambiente (95, 117). De esta manera, el viroma en insectos podría estar influenciada tanto por factores abióticos y bióticos, incluida la genética viral y del hospedero, así como las condiciones climáticas y geográficas del sitio de reproducción (100, 104, 110, 114, 116, 118).

7.8 Viroma de diversas especies de mosquitos

Los estudios metagenómicos virales han impulsado el descubrimiento de virus asociados a mosquitos, que no sólo contribuye a la detección de arbovirus, sino más bien a una descripción general de los virus que circulan en los mosquitos (94). Estos estudios han demostrado la diversidad y abundancia del viroma en especies como *Culex* spp., *Aedes* spp., *Mansonia* spp., *Armigere* spp., *Psorophora* spp. en regiones específicas y en distintas zonas geográficas (96, 114, 115, 119, 120). Un reciente estudio demostró que existe un "pan viroma" o "viroma compartido" entre especies de importancia epidemiológica de los géneros de mosquitos, *Culex* spp., *Aedes* spp. y *Anopheles* spp., lo que ilustra el gran potencial de estos virus para la circulación viral entre diferentes géneros de mosquitos (121).

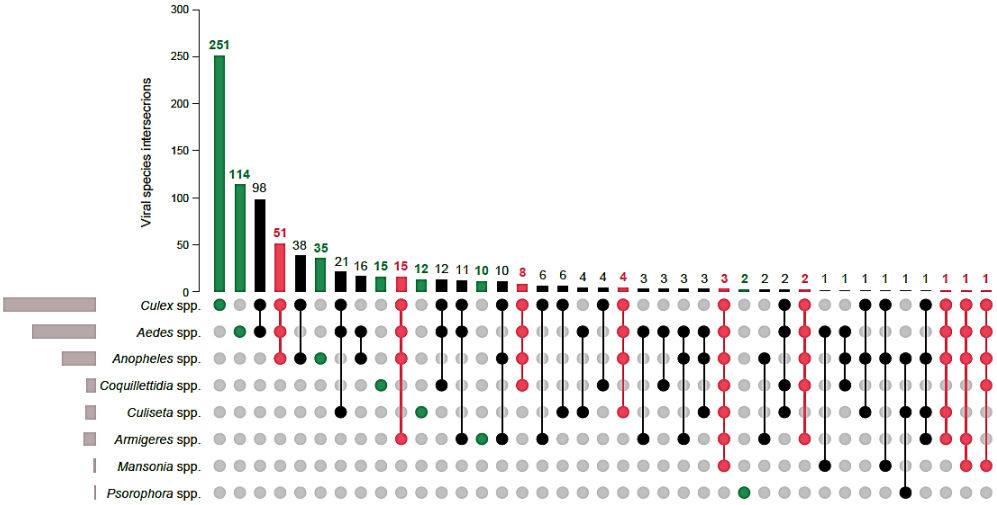

Figura 12. Especies virales compartidas y específicas en mosquitos vectores. El diagrama muestra la relación de viromas entre diferentes géneros de mosquitos, el número de especies virales compartidas por mosquitos entre distintas especies marcadas en rojo. El número de especies virales específicas de mosquitos se marcó en verde. Tomado de Zhao *et al.*, 2022. Ref (121).

Otras investigaciones han mostrado un viroma específico de cada género de mosquito, que podría estar relacionado con los atributos genéticos, el comportamiento, como las diferencias en los hábitos hematófagos o la influencia de factores ambientales, especialmente la preferencia del medio acuático en el que viven las larvas (40). Del mismo modo, otros estudios muestran que la dinámica vírica depende principalmente de las interacciones entre los mosquitos y su entorno, donde la alimentación se considera un factor crítico que afecta a la composición vírica (114, 120). Dado que un aumento en la tasa de alimentación de los mosquitos promueve la posibilidad de adquirir y propagar el virus ARN, la preferencia de fuente de alimento es un factor crítico que afecta la composición del viroma (114). Sin embargo, los factores que influyen en las complejas asociaciones de la composición vírica siguen siendo en gran medida desconocidos, especialmente en áreas naturales ambientalmente conservadas.

7.9 Interacciones entre virus específicos de mosquitos (ISV) y arbovirus

Algunos estudios han demostrado que los ISV Flavivirus como *Nhumirim virus* (NHUV) *Culex Flavivirus Cell-fusing agent virus* (CFAV) y Phasivirus como *Phasi Charoen-like* (PCLV), pueden interactuar con arbovirus en mosquitos, promover o inhibir la replicación y diseminación de arbovirus, y finalmente afectar la transmisibilidad de arbovirus de mosquito a humano o animal (84, 106). Uno de estos estudios evaluó la capacidad de NHUV, para suprimir la replicación del virus Zika (ZIKV) y el virus del dengue-2 (DENV-2) en células de *Ae. albopictus*, observando reducciones significativas en ZIKV (100.000 veces) y DENV-2 (10.000 veces) en células pre-inoculadas o inoculadas simultáneamente con NHUV (122). Adicionalmente, con individuos de *Ae. aegypti* se demostró una reducción en las tasas de infección por ZIKV en mosquitos inoculados con NHUV en comparación con los no expuestos (122). Estos resultados son similares a los reportados en *Cx. quinquefasciatus,* donde también se encontró disminución en las tasas de transmisión del virus del Nilo Occidental (WNV) cuando se expone previamente el vector al virus NHUV (123), lo que indica

que algunas especies de ISV podrían modular potencialmente la competencia vectorial en *Aedes* spp y *Culex*.

De forma similar, Schultz *et al* (2018) examinaron en líneas celulares de *Aedes* spp. la supresión de arbovirus en presencia de CFAV y PCLV, demostrando que la infección dual por ISV logró reducir hasta en un 90%, el crecimiento del ZIKV, el DENV y el virus La Crosse en células inmunocompetentes de *Ae. albopictus* y de *Ae. aegypti* (124). Estos datos sugieren que existen mecanismos de competencia viral (125), como el de exclusión por superinfección que se considera impulsa la exclusión de virus dentro de una misma familia (84, 124, 126) o mecanismos de regulación positiva de la respuesta inmune antiviral del vector (100). En contraste, un reciente análisis del viroma de *Ae. aegypti* en zonas urbanas endémicas de todo el mundo reveló la gran abundancia de ciertos ISV, como el virus PCLV y *el virus Humaita-Tubiacanga* (HTV), que podrían afectar a la capacidad de los mosquitos para transmitir el DENV y el ZIKV a huéspedes vertebrados (37).Sin embargo, los estudios entre ISV-arbovirus son hasta ahora incipientes y por lo tanto se requieren nuevas herramientas que ayuden a comprender mejor este tipo de las interacciones, lo cual redundaría en el desarrollo de nuevos enfoques para el control y prevención de enfermedades por arbovirus.

8. OBJETIVOS

8.1. Objetivo General

Caracterizar arbovirus de importancia en salud pública y el viroma en mosquitos de Colombia mediante un enfoque metagenómico.

8.2. Objetivos Específicos

1. Analizar la frecuencia de infección de arbovirus e identificación molecular de las especies de mosquitos del género *Aedes* en distintos departamentos de Colombia.

2. Caracterizar el viroma de *Aedes aegypti* con infección natural por el virus del dengue (DENV) mediante análisis metagenómicos

3. Describir la dinámica de transmisión y composición viral en poblaciones de mosquitos (Díptera: Culicidae) en ecosistemas rurales estratégicos.

9. INTRODUCCIÓN A LOS CAPÍTULOS

En la actualidad, el aumento de la incidencia y la amplia distribución geográfica de los vectores, y por consiguiente, de las enfermedades virales que transmiten, constituyen uno de los principales desafíos en materia de salud pública en zonas tropicales y subtropicales (41, 127). Los mosquitos (Diptera, Culicidae) son reconocidos a nivel mundial como vectores de arbovirus de importancia médica y veterinaria, contribuyendo a millones de casos de enfermedades cada año (2, 10) Específicamente, los mosquitos del género *Aedes* se destacan por su papel en la transmisión de arbovirus epidémicos como el virus del dengue (DENV), el virus del Zika (ZIKV), y así como el virus chikungunya (CHIKV). Recientemente, CHIKV y ZIKV han ampliado considerablemente su área de distribución geográfica y se han propagado a nuevos territorios, causando importantes brotes en la región de las Américas entre 2014 y 2016 (2, 40, 41), especialmente en países donde el dengue es endémico o hiperendémico, lo que ha generado altos índices de morbilidad y mortalidad (8), y ha requerido un aumento en los servicios de salud (15, 128). Asimismo, mosquitos del género *Culex* también se ha descrito en la transmisión de arbovirus de importancia medica como el virus del Nilo Occidental (WNV) (70, 129). La introducción de WNV en las Américas, donde los hospedadores vertebrados y artrópodos son susceptibles y capaces de mantener la infección, también ha contribuido a la aparición de grandes brotes (74). Estos eventos han puesto de manifiesto las limitaciones de las medidas de control disponibles, las cuales parecen no ser lo suficientemente efectivas para prevenir o reducir la circulación de arbovirus emergentes o reemergentes, especialmente en países en desarrollo.

En este contexto, se ha evidenciado la necesidad de mejorar la preparación ante la emergencia de arbovirus, tanto nuevos como existentes, que puedan impactar significativamente en los sistemas de salud. Esto requiere una comprensión profunda del potencial de propagación de enfermedades infecciosas emergentes, la implementación de estrategias de control y el fortalecimiento de sistemas integrales de vigilancia para proteger a las poblaciones en riesgo (74). En los últimos años, los avances en herramientas moleculares y tecnologías de secuenciación de nueva generación (NGS) han revolucionado la vigilancia viral, permitiendo la detección temprana de virus circulantes (82, 94, 95, 130). Estos progresos han facilitado los estudios de metagenómica viral en mosquitos, mejorando significativamente la capacidad de identificar rápidamente una amplia variedad de virus (40, 111, 131). Recientemente, las técnicas de secuenciación de tercera generación, como las ofrecidas por Oxford Nanopore Technologies (ONT), que ganaron prominencia durante la pandemia de COVID-19, han demostrado ser de bajo coste, fácil acceso y alta eficiencia, permitiendo una secuenciación profunda y detallada (132-134). Como resultado, se ha ampliado nuestro entendimiento de la diversidad viral, su taxonomía y las condiciones en las que estos agentes infecciosos existen en los mosquitos vectores. Este conocimiento es crucial para el desarrollo de nuevas estrategias de control de arbovirus, así como para el diseño de vacunas y plataformas de diagnóstico avanzadas (22).

Las investigaciones actuales sobre el viroma en mosquitos han revelado que estos albergan un viroma rico y diverso, compuesto principalmente por virus específicos de insectos (ISV). A diferencia de los arbovirus, que presentan un doble tropismo de hospedador (vertebrados y artrópodos vectores), los ISV se replican exclusivamente en insectos y no pueden infectar células de vertebrados ni afectar directamente a humanos (40, 94). Estudios recientes sobre el viroma de especies de *Aedes*, realizados

en diferentes partes del mundo, han evidenciado similitudes en la composición del viroma, lo que ha llevado a proponer el concepto del "viroma central"(96, 114). Este concepto engloba un conjunto de ISV compartidos entre poblaciones de mosquitos vectores de la misma especie (94, 96, 135). Sin embargo, también se han identificado ISV que pueden estar presentes en diferentes especies de mosquitos, probablemente debido a factores ambientales, asociados principalmente a los recursos alimentarios y los lugares de cría (114, 120). En ambos casos, los ISV podrían tener un impacto directo en la biología y evolución del mosquito hospedador, así como en la transmisión de arbovirus (40, 100, 135, 136). Los análisis de la composición y distribución global del viroma de los mosquitos sugieren que factores como el hábitat, los modos de transmisión del virus y las características genéticas de especies o géneros específicos de mosquitos son cruciales en la definición del viroma (115, 137, 138). Sin embargo, los determinantes de estas complejas asociaciones en la composición del viroma aún son mayormente desconocidos, especialmente en mosquitos que participan en ciclos epizoóticos rurales en áreas conservadas naturalmente. Dado este contexto, conocer el viroma vectorial y el papel de los ISV es de potencial importancia para el diseño de estrategias efectivas de prevención y control.

Colombia

Colombia, está ubicada en el noroeste de América del Sur, comprende una variedad de regiones biogeográficas que tienen características biofísicas contrastantes y una alta variabilidad ambiental (57). Esta diversidad proporciona condiciones epidemiológicas, geográficas y climáticas óptimas para la transmisión de arbovirus, los cuales son de significativa importancia para la salud pública (29). Actualmente, más de 28 millones de personas, correspondientes a más de la mitad de la población nacional, están expuestas a la infección por estos virus (31). La alta diversidad de mosquitos vectores en diversas regiones del país se asocia probablemente con modificaciones del paisaje, deforestación y cambios de temperatura, lo cual favorece la proximidad entre vectores silvestres y humanos (30, 42, 57), aumentando el riesgo de transmisión de arbovirus zoonóticos, tanto conocidos como emergentes. En particular, se ha documentado que el *Ae. aegypti* es el principal vector responsable de la transmisión de arbovirus como el dengue, el Zika y el chikungunya en el territorio nacional (29, 31). Este vector se encuentra ampliamente distribuido en áreas periurbanas y urbanas (23), lo que incrementa el riesgo de transmisión del dengue, especialmente en zonas situadas por debajo de los 2,300 metros sobre el nivel del mar (29, 32). Por otra parte, la región del Orinoco colombiano, comúnmente conocida como los Llanos Orientales, posee una importancia estratégica significativa debido a su excepcional biodiversidad y sus extensos recursos hídricos(139). Las áreas rurales en esta región han experimentado mínima interferencia humana, con perturbaciones antropogénicas limitada. Esta circunstancia plantea la posibilidad de que virus potencialmente patógenos generen eventos de desbordamiento bajo escenarios de deforestación (30).

En el país, los esfuerzos de vigilancia entomovirológica se han enfocado principalmente en la identificación de especies vectoriales clave para la salud pública, especialmente en zonas urbanas. Sin embargo, la exploración de otras especies en áreas rurales ha sido limitada. La composición viral en vectores significativos como *Ae. aegypti* y otros insectos de la familia Culicidae sigue siendo mayormente desconocida, tanto en Colombia como en la región de las Américas. Esto resalta la necesidad urgente de realizar investigaciones y estudios más completos en este campo. Ampliar nuestro conocimiento sobre la epidemiología molecular de los arbovirus, así como sobre las dinámicas de transmisión y las comunidades virales asociadas a los vectores, es crucial. Este enfoque

no solo contribuirá a la generación de nuevos conocimientos, sino que también fortalecerá las capacidades institucionales para desarrollar estrategias de prevención efectivas e innovadoras ante brotes epidémicos.

Dada la situación actual de la epidemiología de los arbovirus en Colombia y la necesidad imperante de comprender mejor su dinámica de transmisión en este contexto, así como de dilucidar la composición de las comunidades virales (viroma) de los mosquitos vectores en el país, esta tesis doctoral se plantea abordar el objetivo general "Caracterizar arbovirus de importancia en salud pública y el viroma en mosquitos de Colombia mediante un enfoque metagenómico" a través de tres capítulos distintos:

CAPÍTULO 1: Frecuencia de infección de arbovirus e identificación molecular de las especies de mosquitos del género *Aedes* en distintos departamentos de Colombia.

CAPÍTULO 2: Viroma de *Aedes aegypti* con infección natural por el virus del dengue (DENV) mediante análisis metagenómicos.

CAPÍTULO 3: Dinámica de transmisión y composición viral en poblaciones de mosquitos (Díptera: Culicidae) en ecosistemas rurales estratégicos

Capítulo 1.

Para cumplir con el objetivo específico 1, el primer capítulo examinó la prevalencia de infecciones naturales por los virus del dengue (DENV), Zika (ZIKV) y chikungunya (CHIKV) en mosquitos *Aedes* de varios departamentos de Colombia, además de identificar las especies mediante técnicas de barcoding. Todas las muestras positivas para arbovirus correspondieron a *Aedes aegypti*. Se observó que aproximadamente el 30% de los mosquitos *Ae. aegypti* capturados estaban infectados principalmente por DENV, con una predominancia de los serotipos DENV-1 y DENV-2 en todas las regiones estudiadas. La infección por CHIKV fue menos frecuente y no se detectaron casos de ZIKV. A través del análisis del gen COI del vector, se detectó una alta diversidad de haplotipos y la filogenia asoció estas secuencias al linaje I de *Ae. aegypti*, que es globalmente prevalente y asociado con áreas de alta incidencia de dengue. Estos hallazgos indican que la infección natural de *Ae. aegypti* por arbovirus puede reflejar la dinámica epidemiológica nacional y subrayan su potencial como indicador en sistemas de alerta temprana para la implementación de estrategias de control y prevención.

Como producto de este capítulo se adjunta el siguiente artículo científico:

• **Artículo 1:** Gómez M, Martínez D, Hernández C, Luna N, Patiño LH, Bohórquez Melo R, Suarez LA, Palma-Cuero M, Murcia LM, González Páez L, Estrada Bustos L, Medina MA, Ariza Campo K, Padilla HD, Zamora Flórez A, De las Salas JL, Muñoz M and Ramírez JD. Arbovirus infection in *Aedes aegypti* from different departments of Colombia. Front. Ecol. Evol. 2022. 10:999169. Doi: 10.3389/fevo.2022.999169.

CAPÍTULO 1

Frecuencia de infección de arbovirus e identificación molecular de las especies de mosquitos del género *Aedes* en distintos departamentos de Colombia.

frontiers | Frontiers in Ecology and Evolution

TYPE Original Research
PUBLISHED 16 September 2022
DOI 10.3389/fevo.2022.999169

OPEN ACCESS

EDITED BY
Jader Oliveira,
University of São Paulo, Brazil

REVIEWED BY
Fabiana Feitosa-Suntheimer,
Boston University, United States
Pedro Augusto Alves,
René Rachou Institute, Brazil

*CORRESPONDENCE
Juan David Ramírez
juand.ramirez@urosario.edu.co;
juan.ramirezgonzalez@mountsinai.org

SPECIALTY SECTION
This article was submitted to Phylogenetics, Phylogenomics, and Systematics, a section of the journal Frontiers in Ecology and Evolution

RECEIVED 20 July 2022
ACCEPTED 24 August 2022
PUBLISHED 16 September 2022

CITATION
Gómez M, Martínez D, Hernández C, Luna N, Patiño LH, Bohórquez Melo R, Suarez LA, Palma-Cuero M, Murcia LM, González Páez L, Estrada Bustos L, Medina MA, Ariza Campo K, Padilla HD, Zamora Flórez A, De las Salas JL, Muñoz M and Ramírez JD (2022) Arbovirus infection in *Aedes aegypti* from different departments of Colombia.
Front. Ecol. Evol. 10:999169.
doi: 10.3389/fevo.2022.999169

Arbovirus infection in *Aedes aegypti* from different departments of Colombia

Marcela Gómez[1,2], David Martínez[1], Carolina Hernández[1,3], Nicolás Luna[1], Luz Helena Patiño[1,4], Ramiro Bohórquez Melo[5], Luis Alejandro Suarez[5], Mónica Palma-Cuero[5], Luz Mila Murcia[5], Leonel González Páez[6], Leonardo Estrada Bustos[6], Manuel Alfonso Medina[6], Katiuska Ariza Campo[7], Holmer David Padilla[8], Alexander Zamora Flórez[9], Jorge Luis De las Salas[9], Marina Muñoz[1] and Juan David Ramírez[1,10]*

[1]Centro de Investigaciones en Microbiología y Biotecnología-UR, Facultad de Ciencias Naturales, Universidad del Rosario, Bogotá, Colombia, [2]Grupo de Investigación en Ciencias Básicas (NÚCLEO), Facultad de Ciencias e Ingeniería, Universidad de Boyacá, Tunja, Colombia, [3]Centro de Tecnología en Salud, Innovaseq SAS, Bogotá, Colombia, [4]Upqua S.A.S., Bogotá, Colombia, [5]Grupo de Estudios en Salud Pública de la Amazonía, Laboratorio de Salud Pública Departamental del Amazonas, Leticia, Colombia, [6]Secretaría de Salud de Boyacá, Tunja, Colombia, [7]Secretaría Seccional de Salud de Magdalena, Santa Marta, Colombia, [8]Hospital Local de El Retén, El Retén, Colombia, [9]Secretaría Departamental de Salud del Vichada, Puerto Carreño, Colombia, [10]Department of Pathology, Molecular and Cell-Based Medicine, Icahn School of Medicine at Mount Sinai, Molecular Microbiology Laboratory, New York, NY, United States

The lack of precise and timely knowledge about the molecular epidemiology of arboviruses of public health importance, particularly in the vector, has limited the comprehensive control of arboviruses. In Colombia and the Americas, entomovirological studies are scarce. Therefore, this study aimed to describe the frequency of natural infection and/or co-infection by Dengue (DENV), Zika (ZIKV), and Chikungunya (CHIKV) in *Aedes* spp. circulating in different departments of Colombia (Amazonas, Boyacá, Magdalena, and Vichada) and identifying vector species by barcoding. *Aedes* mosquitoes were collected in departments with reported prevalence or incidence of arbovirus cases during 2020–2021, located in different biogeographic zones of the country: Amazonas, Boyacá, Magdalena, and Vichada. The insects were processed individually for RNA extraction, cDNA synthesis, and subsequent detection of DENV (serotypes DENV1-4 by multiplex PCR), CHIKV, and ZIKV (qRT-PCR). The positive mosquitoes for arboviruses were sequenced (Sanger method) using the subunit I of the cytochrome oxidase (COI) gene for species-level identification. In total, 558 *Aedes* mosquitoes were captured, 28.1% (n = 157) predominantly infected by DENV in all departments. The serotypes with the highest frequency of infection were DENV-1 and DENV-2 with 10.7% (n = 58) and 14.5% (n = 81), respectively. Coinfections between serotypes represented 3.9% (n = 22). CHIKV infection

was detected in one individual (0.2%), and ZIKV infections were not detected. All infected samples were identified as *A. aegypti* (100%). From the COI dataset (593 bp), high levels of haplotype diversity (H = 0.948 ± 0.012) and moderate nucleotide diversity (π = 0.0225 ± 0.003) were identified, suggesting recent population expansions. Constructed phylogenetic analyses showed our COI sequences' association with lineage I, which was reported widespread and related to a West African conspecific. We conclude that natural infection in *A. aegypti* by arbovirus might reflect the country's epidemiological behavior, with a higher incidence of serotypes DENV-1 and DENV-2, which may be associated with high seroprevalence and asymptomatic infections in humans. This study demonstrates the high susceptibility of this species to arbovirus infection and confirms that *A. aegypti* is the main vector in Colombia. The importance of including entomovirological surveillance strategy within public health systems to understand transmission dynamics and the potential risk to the population is highlighted herein.

KEYWORDS

arbovirus, Dengue, Zika, Chikungunya, infection, *Aedes*

Introduction

Dengue virus (DENV), Zika virus (ZIKV), and Chikungunya virus (CHIKV) are considered to be arboviruses of major medical importance, capable of causing febrile syndromes, hemorrhagic fevers, and neurodegenerative diseases (Gould et al., 2017; Wilder-Smith et al., 2017; Paixao et al., 2018; Young, 2018). Dengue has been described as the most prevalent arboviral infection, with 40% of the human population at risk of infection, especially in tropical and subtropical countries (Wilder-Smith et al., 2010; Bhatt et al., 2013; Murray et al., 2013; Patterson et al., 2016; Sukhralia et al., 2019). Its incidence has increased 30 times in the last decades (Guzman et al., 2016; Pollett et al., 2018), with reports of more than 390 million infections annually (Bhatt et al., 2013). This virus has four serotypes, DENV-1, DENV-2, DENV-3, and DENV-4; each serotype confers specific lifelong immunity (Martina et al., 2009). However, with second infections by heterologous serotype, there is a higher risk of developing severe and/or lethal forms of the disease (Klungthong et al., 2004; Ngono and Shresta, 2018). CHIKV has caused widespread epidemics in Asian countries and East Africa; since 2004, it has spread to more than 60 countries, including the Americas, in 2013 (Leparc-Goffart et al., 2014; Gordon et al., 2018). Similarly, ZIKV has produced major outbreaks in 86 countries and territories in the Pacific, Asia, and the Americas, generating devastating congenital anomalies and neurological disorders, including microcephaly and Guillain-Barré syndrome post-2015 (Kazmi et al., 2020).

Aedes aegypti and *Aedes albopictus* are the main vectors of epidemic arboviruses DENV, CHIKV, and ZIKV (Kraemer et al., 2015; Leta et al., 2018; Lwande et al., 2020). These insects show broad ecological plasticity under different anthropic, climatic, and environmental conditions (Bonizzoni et al., 2013; Brady et al., 2014; Liu-Helmersson et al., 2016; Ciota et al., 2018), facilitating its spread in tropical, subtropical and some temperate zones (Leta et al., 2018; Kraemer et al., 2019; Ryan et al., 2019). In recent years, virological surveillance using molecular techniques has been proposed as a highly sensitive and efficient tool for detecting arboviral infection in vectors (Faye et al., 2013; Santiago et al., 2013; Alvarez-Diaz et al., 2021). Studies with this approach show that infection rates in *A. aegypti* and *A. albopictus* mosquitoes in endemic areas of Latin America may vary depending on the arboviral species, thus providing a promising approach to trigger control strategies. In the *Aedes* mosquitoes, DENV is more prevalent, with ranges between zero and 16.2% (Dos Santos et al., 2017; Kirstein et al., 2021), and CHIKV is between zero and 12.5% (Cevallos et al., 2018; Monteiro et al., 2020), while ZIKV represents the lowest rate between zero and 9.0% (Monteiro et al., 2020). Although data on arboviral co-infections in mosquitoes are limited, some studies demonstrate differences in susceptibility to mixed infections by species and serotype in mosquitoes, with frequencies ranging from 0.9 to 13.3% (Caron et al., 2012; Pena-Garcia et al., 2016; Pérez-Castro et al., 2016; Martínez et al., 2020).

The identification of *Aedes* species and their genetic variability has also been described using molecular markers to understand population dynamics and possible implications for

disease transmission (Scarpassa et al., 2008; Paupy et al., 2012; Jaimes-Duenez et al., 2015; Pereira et al., 2017). This indicates that the infection rates and the identification of vector genetic diversity are essential from the point of view of molecular epidemiology to determine the possible routes of arboviral transmission and the progressive dispersion and/or invasion of the *Aedes* mosquito in endemic areas (Caron et al., 2012; Paupy et al., 2012; Jimenez-Silva et al., 2018; Joyce et al., 2018).

Colombia is a hyper-endemic country for Dengue, where the four serotypes co-circulate (Padilla et al., 2017). Moreover, it is one of the countries with the highest reported cases in the Americas and the Caribbean. Since 2016, there has been co-circulation of other arboviruses, such as CHIKV and ZIKV, a condition given by the geographical characteristics, the conditions of virus transmission, and the presence of the vector in about 90% of the national territory (Instituto Nacional De Salud, 2019b). Currently, more than 26 million people, corresponding to more than half of the national population, are exposed to arbovirus infection (Hoyos-Lopez et al., 2016; Gómez and Zapata-Úsuga, 2019; Instituto Nacional De Salud, 2019b, 2020; Ministerio De Salud Y Protección Social, 2020). Despite this epidemiological situation, there is insufficient notification in some regions and a narrow active search for the circulation of different arboviral species (Gutierrez-Barbosa et al., 2020). An alarming aspect is the co-circulation of arboviral species in the same region by the same vector species (Wilder-Smith et al., 2017; Ciota, 2019; Vogels et al., 2019).

The lack of accurate and timely knowledge about the molecular epidemiology of arboviruses of public health importance, particularly in the mosquito vector, has limited the prediction of the genuine risk of emergence and spread of new arboviral strains in human populations. In Colombia and the Americas, studies on entomological characterization of *Aedes* mosquitoes and detection of arboviruses are scarce (Jaimes-Duenez et al., 2015; Pena-Garcia et al., 2016; Pérez-Castro et al., 2016; Peña-Garcia et al., 2017; Martínez et al., 2020; Carrasquilla et al., 2021), which has implications for effective arboviral control strategies. Therefore, this study aimed to describe the frequency of infection and/or natural co-infection by DENV, CHIKV, and ZIKV in *Aedes* spp. mosquitoes circulating in different Colombian departments and identify using molecular barcoding the vector species. We highlight the potential of molecular tests for the surveillance of arboviruses in mosquitoes and the identification of the vector species (entomovirologial surveillance).

Materials and methods

Location and capture of adult *Aedes*

Adult mosquitoes of the genus *Aedes* were captured between December 2020 and August 2021 in different municipalities of four departments of Colombia: the municipality of Leticia (4°12′29″S, 69°56′36″W) located in the department of Amazonas, in the south of the country in the Amazon region, shares borders with Brazil; municipalities of Puerto Boyacá (5°58′33.6″N 74°35.11″W) and Otanche (5°39′24.2″N 74°10.949′W), located in the department of Boyacá, in the central Andean region; municipality of Retén (10°36′40.9.9″ N 74°16.094′W), in the department of Magdalena, located on the Colombian Caribbean coast; and the municipalities of Santa Rosalia (5°07′1″N 70°52′1″W) and La Primavera (5°28′59″ N 70°24′ 0″W) in the department of Vichada, located on the east in the vast plains of the Orinoco region, bordering Venezuela to the east (**Figure 1**). The selection of the study sites was made considering the location of the departments in different biogeographic areas of Colombia and the records of incidence of cases of arbovirus infection (Dengue, Chikungunya, and Zika), as reported by the Colombian Public Health Surveillance System (*Sivigila*) (Instituto Nacional De Salud, 2020, 2022; **Figure 1**). With the support of the local Public Health Secretaries, a non-probabilistic convenience sampling was conducted to obtain a range of sampling efforts from 100 to 150 individuals in urban and/or peri-urban areas. Adult mosquitoes were collected in a single sampling moment per department. The department of Boyacá was sampled in November 2020 (end of rainy season), while the departments of Magdalena, Amazonas and Vichada were sampled in the months of April and June 2021 (rainy season). Black light traps placed in the selected areas for 24 h and/or manually captured with mechanical vacuum cleaners during the day were used. At each site, only mosquitoes of the *Aedes* genus were selected, and subsequently pooled to optimize both the transport and storage of insects from the sampling sites. The entomological material was preserved in RNA later (DNA/RNA shield, Zymo. R1100-50) at 4°C in flasks marked with the coordinates and/or collection site. The samples were then sent to the Universidad del Rosario microbiology laboratory in Bogotá, Colombia, for processing and molecular analysis.

RNA extraction and cDNA synthesis

In the laboratory, *Aedes* mosquitoes collected from each department were processed individually as a more accurate strategy for arbovirus detection, as previously reported (Martínez et al., 2020). Each mosquito (each specimen) was disrupted using ZR BashingBead^{TM} lysis tubes (Lysis Tubes-ref. S6003-50) with 200 μL of DNA/RNA Shield buffer in TissueLyser II$^{®}$ tissue homogenizer (Qiagen, Hilden, Germany), followed by centrifugation at 10,000 rpm for 2 min. RNA extraction was performed with the Quick-RNA Viral–ZYMO kit (Zymo, ref 1035), following the manufacturer's recommended instructions. Once RNA was obtained, its concentration and quality were measured using a

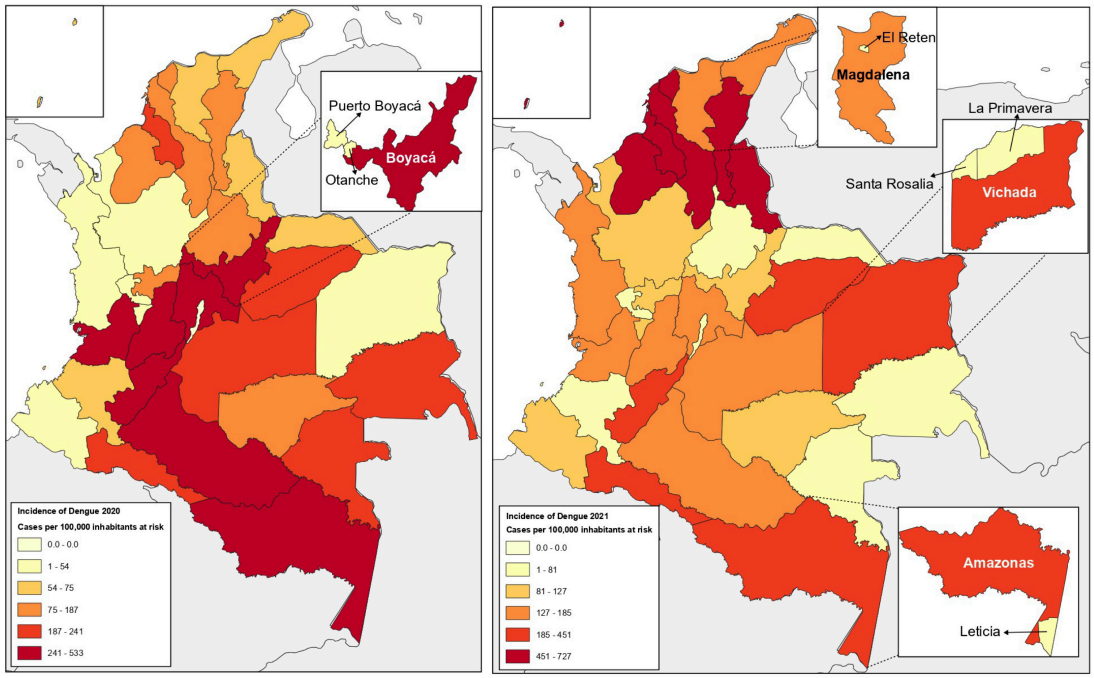

FIGURE 1
Dengue incidence in Colombia during 2020–2021 and study sites. Map of Colombia, showing Dengue incidence data (reported cases per 100,000 population at risk) for the years 2020 **(left)** and 2021 **(right)**. The sampled departments and municipalities are highlighted. Entomological sampling of *Aedes* mosquitoes in 2020 in the department of Boyacá (Dengue incidence data 241-533). Entomological sampling of *Aedes* mosquitoes in 2021 in the departments of Magdalena (Dengue incidence data 127-185), Amazonas and Vichada (Dengue incidence data 185-451). The data used for these maps were obtained from Colombia's Public Health Surveillance System (*Sivigila*) (Instituto Nacional De Salud, 2020, 2022).

Nanodrop-2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, United States) and stored at −80°C in a Revco freezer (Thermo Fisher Scientific, Waltham, MA, United States). From the RNA obtained, RT-PCR was performed to generate cDNA using LunaScript RT SuperMix Reverse Transcriptase Kit (NEB #E3010). Finally, the cDNA was stored at −30°C.

Conventional polymerase chain reaction for the identification of Dengue virus serotypes

Detection of DENV serotypes (DENV 1-4) was performed by multiplex PCR, using primers for the region of the C-prM gene previously reported (**Table 1**; Chien et al., 2006). GoTaq® Green PCR Master Mix (2×) enzyme (Promega, # M7123) was used with 10 μmol of each primer (MD1, rTS1, rTS2, rTS3, and rTS4) and 0.8 μl of cDNA. The thermal profile consisted of the following: an initial denaturation cycle at 95°C for 5 min, 35 cycles of 95°C for 30 s, 57°C for 45 s and 72°C for 33 s, and finally, a final extension cycle of 72°C for 10 min. Visualization of the gene fragments was carried out using 2% agarose gel electrophoresis in 1× TBE buffer and as an intercalating agent 1 μL of SYBR® Safe (Invitrogen®, Carlsbad, CA, United States), then the gel was placed under UV light to observe the amplification band of each serotype, in the case of DENV-1 a band of 208 base pairs (bp) was observed, DENV-2 a band of 119 bp, DENV-3 a band of 288 bp and DENV-4 a band of 260 bp. The DENV positive controls (supernatant of culture cells individually infected with serotypes DENV1, DENV2, DENV3, DENV4) were provided by the Universities of Antioquia and Antonio Nariño in Colombia. Finally, confirmation of positive samples was performed in duplicate.

Real-time polymerase chain reaction for the detection of Chikungunya and Zika

The identification of CHIKV and ZIKV was performed by real-time reverse transcription-polymerase chain reaction (qRT-PCR) using the corresponding primers and Taqman probes previously proposed (Faye et al., 2013; Alvarez-Diaz et al., 2021; **Table 1**). The qRT-PCR reaction for CHIKV and ZIKV (per sample) contained qScriptTM One-Step (2×), Low ROXTM (Quantabio, Carlsbad, CA,

TABLE 1 List of primers and Taqman probes used in PCR for the detection of DENV, serotypes DENV1-4 (Chien et al., 2006), CHIKV (Alvarez-Diaz et al., 2021), and ZIKV (Faye et al., 2013).

Arbovirus detection (CHIKV- ZIKV- DENV)					
Virus	**Primer/probe**	**Sequence (5′—3′)**	**Genomic position**	**Amplicon size**	**Genomic region amplified**
DENV-1	DENV1–F	mD1-TCAATATGCTGAAACGCGHGAGAAACCG	134–322	208 pb	C-prM
	DENV1–R	rTS1-CCCGTAACACTTTGATCGCT			
DENV-2	DENV2–F	mD1-TCAATATGCTGAAACGCGHGAGAAACCG	134–232	119 pb	
	DENV2–R	rTS2-CGCCACAAGGGCCATGAACAGTTT			
DENV-3	DENV3–F	mD1-TCAATATGCTGAAACGCGHGAGAAACCG	134–400	288 pb	
	DENV3–R	rTS3-TAACATCATCATGAGACAGAGC			
DENV-4	DENV4–F	mD1-TCAATATGCTGAAACGCGHGAGAAACCG	134–374	260 pb	
	DENV4–R	rTS4-TTCTCCCGTTCAGGATGTTC			
CHIKV	CHIKV –F	RAAGGAGTGCCGGAARGACAT	1,261–1,439	178 pb	nsP1
	CHIKV –R	GACAACCCGGACGACCACAG			
	CHIKV –P	FAM-GARAAGCTYYTGGGGGTCAGAGA-BHQ	1,289–1,311		
ZIKV	ZIKV–F	AARTACACATACCARAACAAAGTG GT	9,271–9,373	102 pb	NS5
	ZIKV–R	TCCRCTCCCYCTYTGGTCTTG			
	ZIKV–P	FAM-CTYAGACCAGCTGAAR-BBQ	9,304–9,320		

FAM, 6-carboxyfluorescein; BBQ, black berry quencher; Y = T or C, R = A or G; NS5, nsP1, non-structural protein 1, NS5 non-structural protein 5; C-prM, capsid-premembrane.

United States). For CHIKV, 10 μM of each primer (F-5′RAAGGAGTGAGTGCCGGAARGACAT) and (R-5′ GACAACCCCCGGACGACCACAG), 5 μM of the probe (5′FAM-GARAAGCTYYYTGGGGGGGTCAGAGA) and 5 μL of RNA were used. The thermal profile used consisted of one cycle at 50°C for reverse transcriptase activation for 10 min, followed by initial denaturation at 95°C for 2 min, 40 cycles of 95°C for 15 s, 55°C for 45 s. For ZIKV, 10 μM of each primer (F-5′ AARTACACATACCARAACACCARAACAAAGTG GT) and (R-5 TCCRCTCCCYCTYCTYTGGTCTTG), 25 μM of the probe (5′-FAM-CTYAGACCAGCTGAAR) and 5 μL of RNA were used. Amplification conditions were: one cycle at 50°C for 10 min, 95°C for 1 min, 40 cycles of 95°C for 15 s, and 56°C for 1 min. To perform the real-time PCR, the cut-off Ct value was $\leq$ 39 based on different criteria, among which are CDC (Centers for Disease Control and Prevention) interpretation criteria, considering a specimen positive if primer sets showed amplification with cycle threshold (Ct) values $\leq$ 38.5. Likewise, positive tests were confirmed in duplicate. The positive controls for CHIKV and ZIKV were donated by the Universidad de Antioquia and the Universidad Antonio Nariño. The frequency of infection for CHIKV and ZIKV was expressed as the infection rate.

Molecular identification of *Aedes* species

A fragment of subunit I of the mitochondrial Cytochrome Oxidase gene (mt-COI) (species-specific barcode) was amplified from the resulting positive mosquitoes for DENV, CHIKV, or ZIKV. Reactions contained 2× GoTaq®Green Master Mix, (Promega, # M7123), 10 μM of each primer LCO1490 (5′-GGTCAAATCATAATAAAGATATTGG-3′) and HCO2198 (5′-TAAACT TCAGGGTGACCAAAAAATCA-3′) (Joyce et al., 2018), and 5 μL of cDNA. Thermal profiling started with a DNA polymerase activation step at 95°C for 1 min, 45 cycles of 94°C for 10 s for denaturation, 60°C for 1 min for primer annealing, and a final extension at 72°C for 10 min. This was followed by 2% agarose gel electrophoresis and SYBR-Safe as an intercalating agent (Invitrogen®, Carlsbad, CA, United States) to observe a band of ∼650 base pairs (bp). The amplified products were sequenced using the Sanger sequencing platform, following cleanup using ExoSAP-IT™ PCR Product Cleanup Reagent (Applied Biosystems™ #78205). In addition to rule out degradation of the samples, the COI amplification was used as internal control. Therefore, a random selection of 10 negative mosquitoes per collection site were analyzed observing the amplification of the marker COI in all of them.

Cytochrome oxidase gene sequence analysis (barcoding)

Consensus sequences were obtained using Geneious Prime program.[1] Each sequence was then compared with the GenBank database using the BLAST (Basic Local Alignment Search Tool) sequence alignment tool (Mount, 2007), and

1 https://www.geneious.com/prime/

the *Aedes* species were assigned considering the percentage of identity (higher than 95%) and the best e-value result. Multiple sequence alignments were performed with the MAFFT algorithm (Katoh and Standley, 2013) in Unipro UGENE software (Okonechnikov et al., 2012) using sequences retrieved from Genbank (**Supplementary Table 1**).

Subsequently, the genetic variability of the COI marker for *A. aegypti* in the samples from the study departments was evaluated through genetic diversity indices, including the number of haplotypes (h), the number of segregation sites (s), the average number of differences (k), haplotype diversity (hd), nucleotide diversity (π), using DnaSP software (Rozas et al., 2017). Using the same software, the divergence test was performed using Tajima's D (Rozas et al., 2017). In addition, haplotype networks were constructed using the median joint method (MJN) in PopART (Population Analysis with Reticulate Trees) software (Leigh and Bryant, 2015) in order to observe the relationships between the sequences of COI haplotypes obtained for Colombia. Additionally, to infer relationships between the COI haplotypes of *A. aegypti* generated in our study with COI haplotypes from outside Colombia, a Maximum Likelihood (ML) phylogenetic analysis was performed on the haplotypes from each department, together with haplotypes from Africa, Asia, America, and the Caribbean regions published in similar studies, and obtained from the GenBank database (**Supplementary Table 1**). Representative COI haplotype sequences between and within each department were selected for phylogenetic analysis. IQtree version 1.6.12 software was used (Nguyen et al., 2015), with the TIM3 + F + I + G4 substitution model (chosen as the best in software). Branch support was performed using 10,000 repeats by ultrafast bootstrap approximation (UFBoot), visualized iTOL.[2] The COI gene sequence of *Culex spinosus* (KM593059.1) was used as outgroup (Martínez et al., 2020).

Statistical analyses

Initially, the results of arbovirus infection were analyzed in terms of absolute and relative frequencies for the variables considered in the study (departments and arboviral species and/or serotypes). Subsequently, the Chi-square test was used to establish possible associations between these variables. In order to perform multiple comparisons between the different departments under study with the arbovirus species, *post hoc* tests were implemented through the vcd package in R software using the chisq.*post hoc*.test function that allows pairwise comparisons using the Bonferroni adjustment method. Statistical analyses were carried out using the R software (RStudio Team, 2019). All significance tests were two-tailed, and p-values < 0.01 were considered statistically significant.

2 https://itol.embl.de

Results

Natural arbovirus infection in *Aedes* mosquitoes

A total of 558 *Aedes* specimens were captured (female and male), with 150 individuals collected in Amazonas, Boyacá, and Magdalena, respectively, and 108 in the department of Vichada (**Table 2**). In general, in all study departments, the arbovirus with the highest frequency was DENV, found in 28.1% (n = 157) of *Aedes* specimens, with a predominance of serotypes DENV-1 and DENV-2, with a frequency of 9.9% (n = 55) and 14.2% (n = 79), respectively (**Supplementary Table 2**). Only one individual was infected by CHIKV (0.18%), and none by ZIKV (0.0%). The DENV-1 serotype was detected with a higher frequency in Boyacá at 18% (n = 27), followed by the department of Amazonas with a frequency of 11.3% (n = 17) and Magdalena with 6.0% (n = 9), while Vichada with 1.9% (n = 2) registered the lowest frequency of infection by this serotype (**Figure 2** and **Supplementary Table 2**). In contrast, DENV-2 was detected with a higher frequency in samples from Vichada 30.6% (n = 33), with the highest infection rate per serotype, followed by 14.7% in Amazonas (n = 22), 9.3% in Boyacá (n = 14) and 6.7% in Magdalena (n = 10). A significant relationship was found between the distribution of DENV among departments (chi-square tests, $p < 0.005$). Multiple comparisons showed that only DENV-2 was specifically associated with the department of Vichada (chisq.*post hoc*.test $p < 0.000036$). In addition, the total study areas found a frequency of 3.9% (n = 22) of mixed infections by DENV serotypes. Co-infections by DENV-1/DENV-2 were found in all departments, with a higher frequency in Amazonas at 5.3% (n = 8). Only in Magdalena were two individuals coinfected with DENV-1/DENV-3 (1.3%) and three individuals with DENV-1/DENV-2/DENV-3 (2.0%), while in Vichada, one (1) individual was coinfected with DENV-2/DENV-3 (0.9%) (**Figure 2**). No relationship was found between the distribution of DENV co-infections between departments (chi-square tests, $p < 0.1549$).

Cytochrome oxidase gene sequence analysis (haplotypes)

Based on the analysis of the COI sequences of the arbovirus-positive mosquito samples infected with DENV and CHIKV (157 sequences), they were all classified as *A. aegypti* using the BLASTn algorithm in the NCBI database. Subsequently, a total of 145 sequences were used to study genetic variations in the COI gene: 44 from the department of Amazonas, 42 from the department of Boyacá, 28 from the department of Magdalena, and 31 from the department of Vichada (**Table 3**).

TABLE 2 Frequency of infection by arbovirus in Colombian departments.

	N = 150	*N* = 150	*N* = 150	*N* = 108	*N* = 558	*P*-value (Chi-Squared)
	Amazonas *n* (%)	**Boyacá *n* (%)**	**Magdalena *n* (%)**	**Vichada *n* (%)**	**Total *n* (%)**	
DENV	47 (31.3%)	44 (29.3%)	29 (19.3%)	37 (34.3%)	157 (28.1%)	0.000023
CHIKV	0%	0 (0.0%)	1 (0.7%)	0 (0%)	1 (0.2%)	NA
ZIKV	0%	0 (0.0%)	0 (0.0%)	0 (0%)	0 (0%)	NA
Total infected	47 (31.3%)	44 (29.3%)	30 (20%)	37 (34.3%)	158 (28.3%)	
Total No infected	103 (68.7%)	106 (70.7%)	120 (80%)	71 (65.7%)	400 (71.7%)	

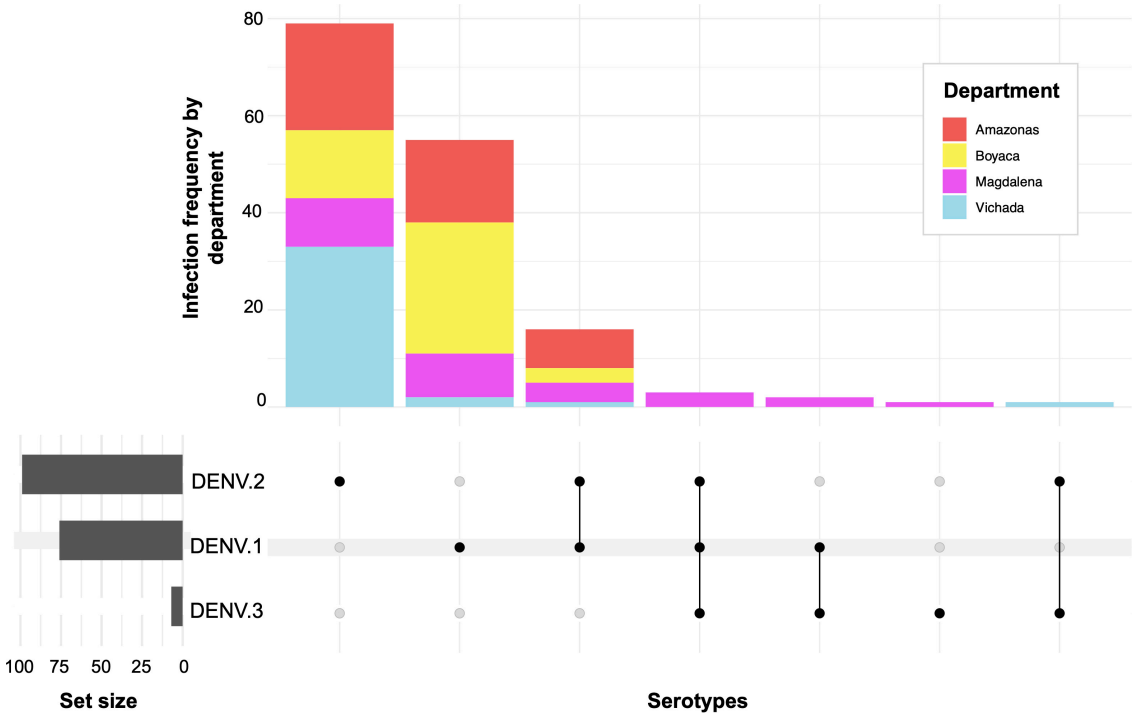

FIGURE 2
Frequency of infection and co-infection by Dengue serotypes in *Aedes aegypti* mosquitoes in Colombia.

Eighty-seven haplotypes were identified from the COI dataset, with 197 variable sites. High levels of haplotypic diversity (H = 0.948 ± 0.012) and moderate nucleotide diversity were observed (π = 0.0225 ± 0.003) (Table 3). The number of haplotypes per department was similar in Boyacá (h = 28), Magdalena, and Vichada (h = 24), while it varied in Amazonas (h = 19). Haplotypic diversity was comparatively higher in the departments of Magdalena (H = 0.9814 ± 0.018), Vichada (H = 0.9655 ± 0.022), and Boyacá (H = 0.8919 ± 0.033) compared to the departments of Amazonas (H = 0. 78647 ± 0.048), while nucleotide diversity was similar in all departments ($\sim \pi$ = 0.017) and marginally higher in the department of Vichada (π = 0.0330 ± 0.0036). The mean number of haplotypic differences (K) for the populations of the departments of Amazonas, Boyacá, Magdalena, and Vichada were, respectively, 9,016, 10,683, 11,010, and 19,217.

The mean value of Tajima's D in the analyzed sequences from the four study departments was negative (-2.283) (Table 3). In addition, the Tajima D values for Amazonas and Boyacá were negative and statistically significant ($p < 0.01$), suggesting recent population expansions. In Magdalena and Vichada, however, Tajima's D was negative but not significant ($p > 0.1$). A significant difference in π values was observed between the municipalities.

Figure 3A shows the COI haplotype network of *A. aegypti* from the study departments produced by the median junction network (MJN). Of the 145 mitochondrial sequences analyzed, we obtained 87 haplotypes distributed among the four

TABLE 3 Genetic diversity analysis in *Aedes aegypti* mosquitoes based on molecular analysis of the COI gene.

Department	Number of samples	h	S	K	Hd ± SD	π ± SD	D Tajima	*P*-value
Amazonas	44	19	83	9.016	0.78647 ± 0.048	0.0163 ± 0.00411	−1.98738	0.05
Boyacá	42	27	109	10.683	0.8919 ± 0.033	0.0183 ± 0.00490	−2.2732	0.01
Magdalena	28	24	69	11.010	0.9814 ± 0.018	0.0189 ± 0.00328	−1.6399	0.1
Vichada	31	24	129	19.217	0.9655 ± 0.022	0.0330 ± 0.0036	−1.7883	0.1
Total data Estimated	145	87	197	12.481	0.948 ± 0.012	0.0225 ± 0.003	−2.283	0.010

Department, study department name; Number of samples, number of samples analyzed by department; h, number of haplotypes; S, number of segregation sites; K, average number of differences; Hd, haplotype diversity; π (Pi), nucleotide diversity; SD, standard deviation. *p*-value = 0.05.

departments, with many low-frequency haplotypes. A dispersed haplotype network was observed; 51.72% of the haplotypes were shared among the departments. The network shows two main haplogroups of higher frequency and geographic distribution. The first (haplotype color network) predominantly consists of Amazon haplotypes, composed of ancestral haplotypes (H1). The second haplogroup (haplotype color red, pink, yellow, and blue) consists of haplotypes from all locations (H6).

A multiple sequence alignment of *A. aegypti* haplotype sequences was constructed with the COI sequences generated in this study and with the 22 COI haplotype sequences retrieved from NCBI genebank worldwide (**Supplementary Table 1**). Phylogenetic relationships between COI gene haplotype sequences from a representative group of our samples (30%) were examined along with sequences from around the world using maximum likelihood methods. The phylogenetic tree showed two main clusters, with Bootstrap support > 80% (**Figure 3B**). One group was associated with COI sequences of haplotypes from East Africa (Tanzania), the Caribbean region (H2 Martinique), and the Americas (Mexico, H1 Brazil, Colombia, and H1, H2, H3, and H4 Bolivia) reported as *Lineage II* (Bracco et al., 2007; Paupy et al., 2012). The second cluster was associated with haplotypes from West Africa (H1 Cameroon, H1 Guinea, and H1 Cambodia) and the Americas (H1 Venezuela, H1 United States, H1 Colombia, H4, H58.) reported as *Lineage I*. (Bracco et al., 2007; Paupy et al., 2012). All *A. aegypti* sequences in the present study were associated with *Lineage I* (**Figure 3B**).

Discussion

Currently, vector control efforts and active surveillance for the early detection of epidemic events caused by arboviruses are fundamental for the integrated management of arboviral diseases (Kilpatrick and Randolph, 2012; Golding et al., 2015; Kauffman and Kramer, 2017). In the present study, we identified natural infection by DENV and CHIKV in specimens of *Aedes* spp., collected from different departments of Colombia. However, ZIKV was not detected in this study. DENV was the arbovirus with the highest frequency of infected mosquitoes (28.1%), while CHIKV and ZIKV had low (0.2%) or none (0.0%) infection rates, respectively (**Table 2**). These results are consistent with the behavior of arboviruses in Colombia, where in recent years, Dengue notifications have represented 99.5% of the total number of arbovirus notifications in the country (Instituto Nacional De Salud, 2020, 2022). They are also related to the high seroprevalence of asymptomatic cases of DENV infections, more than 85% in children and 90% in adults identified in the national territory (Velandia-Romero et al., 2020), which may play an important role in the silent maintenance and progression of the transmission cycle, between humans and mosquitoes (Pollett et al., 2018). In addition, the detection of CHIKV in only one individual and the non-detection of ZIKV in *Aedes* mosquitoes may reflect the decrease in transmission of these viruses following the outbreaks that occurred in 2015 and 2016. In recent years, a gradual decrease in the notification of Chikungunya and Zika cases has been observed (Instituto Nacional De Salud, 2020, 2022), with a low incidence in 2020 and 2021 (0.3–0.5 cases per 100.000 inhab) for both arboviruses. The reduced circulation of these arboviruses may be associated with low rates of susceptible population turnover and reintroduction (Carrasquilla et al., 2021), which limits the circulation of these viruses in humans and *Aedes*.

Our results on the frequency of infection by arboviruses are within the ranges found in other studies of infection in *A. aegypti* in Latin America. The most prevalent viral infection in *A. aegypti* is DENV, with frequencies in Brazil of 0.81% from 2012 to 2013 (Dos Santos et al., 2017) and 0.2% in 2017 (Monteiro et al., 2020), while in Mexico, 7.7% frequency was found for 2016 and 2017 (Kirstein et al., 2021). In Colombia, studies on DENV infection frequencies in this mosquito-vector show infection rates between 12% in the north and center of the country in 2012–2013 (Pena-Garcia et al., 2016) of 62% in the central region by 2015 (Pérez-Castro et al., 2016) and 29.6% for eastern Colombia in 2018–2019 (Martínez et al., 2020). These results may be closely related to the epidemic and inter-epidemic periods for Dengue in Colombia and the Americas (Ramos-Castaneda et al., 2017), as well as sampling effort, local abundance of *A. aegypti* and intensity of arbovirus circulation (Kirstein et al., 2021). Several studies reveal the vast and permanent circulation of DENV in the continent, which is related to the extensive distribution of *A. aegypti* in the region, the co-circulation of the four serotypes (DENV

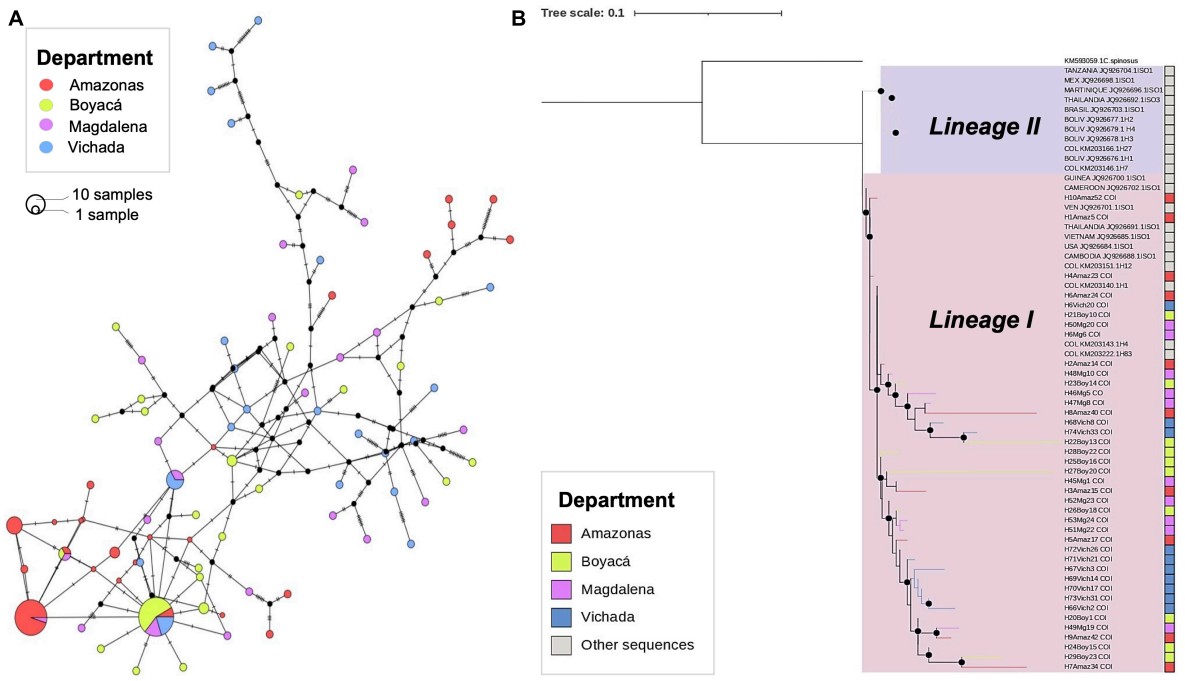

FIGURE 3
Median-joining network (MJN) and phylogenetic analysis of mitochondrial COI haplotypes between *Aedes aegypti* populations in different departments of Colombia. **(A)** The haplotype network from *A. aegypti* sequences was collected in the departments of Amazonas, Boyacá, Magdalena, and Vichada. The lines on each branch indicate the number of mutational steps between haplotypes, and the size of the circle is proportional to the number of individuals. **(B)** A maximum likelihood (ML) tree based on the TIM3 + F + I + G4 nucleotide substitution model of *A. aegypti* individuals from the study departments. Black dots indicate bootstrap support > 80. The red, yellow, purple, and turquoise indicate haplotypes found in Colombia's Amazonas, Boyacá, Magdalena, and Vichada departments. Shown in gray are haplotype sequences from Africa, Asia, the Americas, and the Caribbean, obtained from the GenBank database.

1-4) (Ramos-Castaneda et al., 2017; Gutierrez-Barbosa et al., 2020; Instituto Nacional De Salud, 2020) and the susceptibility profiles of the local populations (Jimenez-Silva et al., 2018; Velandia-Romero et al., 2020). In addition, some authors indicate that the successful transmission of arboviruses such as DENV by *A. aegypti* may also be due to transovarial or vertical transmission (Pena-Garcia et al., 2016; Pérez-Castro et al., 2016; Ferreira-De-Lima and Lima-Camara, 2018), which can become an efficient strategy to maintain the constant circulation of arboviruses in a territory.

We reported the circulation of DENV-1-3 serotypes in *A. aegypti* mosquitoes. The highest frequency of infection by serotypes DENV-1 and DENV-2 was identified in all the departments studied, while DENV-3 was detected only in the department of Magdalena in one individual, and no infection by DENV-4 was detected (**Figure 2**). This finding is consistent with epidemiological trends for Colombia, which indicate that DENV-1 and DENV-2 are the predominant serotypes in the national territory (Gutierrez-Barbosa et al., 2020; Instituto Nacional De Salud, 2022), while DENV-3, followed by DENV-4 are the serotypes with the lowest incidence (Gutierrez-Barbosa et al., 2020). According to the historical dynamics of Dengue transmission in Colombia, DENV-1 and DENV-2 are responsible for the last epidemic outbreaks from 2010 to 2019. National registries report changes in the dominant serotype over time, evidencing the shift from the most prevalent serotype of DENV-1 toward DENV-2 between 2014 and 2016; and subsequently reported DENV-1 as the predominant serotype, followed by DENV-2 in 2019 (Instituto Nacional De Salud, 2019a,b, 2020; Gutierrez-Barbosa et al., 2020). Also, studies of natural infection in *Aedes* mosquitoes in central departments of the country in 2016 reported high rates of DENV-2 infection in *A. aegypti*, possibly associated with the epidemic that occurred during that year (Pérez-Castro et al., 2016; Ruiz-López et al., 2016; Gómez-Palacio et al., 2017; Pérez-Pérez et al., 2017). Similarly, reports of infection in *Aedes* during 2018 and 2019 showed DENV-1 infections related to the latest outbreak in Colombia (Martínez et al., 2020; Carrasquilla et al., 2021). Given this scenario, our findings on the frequency of infection in *A. aegypti* could be associated with this national trend and reveal the predominant serotype transition from serotype DENV-1 to DENV-2 in all departments, suggesting that the latter serotype could be the cause of the next outbreak in the country. In addition, some authors suggest that changing serotype prevalence has been associated with lineage extinction and replacement (Allicock et al., 2012; Salvo et al., 2019).

However, epidemiological and entomovirological surveillance should be continued to verify these assertions coupled with genomic surveillance.

Interestingly, we demonstrate that in departments located in different biogeographic regions, there is an active circulation of DENV in *A. aegypti* mosquitoes. The department of Vichada presented the lowest frequency of DENV-1 infection (1.9%) and the highest frequency of DENV-2 infection (30.6%) in *A. aegypti* mosquitoes with significant differences compared to the other departments studied. These findings may be explained by the proportion of dependent serotypes per department and the local viral population dynamics, as previously reported for other Colombian departments (Jimenez-Silva et al., 2018; Gutierrez-Barbosa et al., 2020; Velandia-Romero et al., 2020). In addition, it may imply a regional evolutionary process of the virus probably associated with vector populations, immunological characteristics, and geographical barriers that prevent the homogenization of circulating serotypes (Gutierrez-Barbosa et al., 2020). Furthermore, the geographic proximity between Vichada and Venezuela probably reflects one of the most significant viral diffusion routes between geographic locations and shows a migratory pathway of Dengue viral lineages (Jimenez-Silva et al., 2018). Therefore, this information highlights the importance of comprehensive monitoring DENV transmission in transboundary mosquito and human regions, such as Vichada and even Amazonas (an area bordering Brazil), which can contribute to controlling the diversification, transmission, and persistence of strains or gene variants of this or other arboviruses.

The data on co-infections between species, serotypes, or viral genotypes in *Aedes* mosquitos are limited. However, laboratory evidence confirms that *A. aegypti* mosquitoes can be simultaneously or sequentially infected with different arbovirus species in all combinations and that they are capable of co-transmitting simultaneously without affecting vectorial competence (Nuckols et al., 2015; Goertz et al., 2017; Ruckert et al., 2017; Magalhaes et al., 2018; Mourya et al., 2018; Vogels et al., 2019). Here, we found natural co-infection in *A. aegypti* by different Dengue serotypes, with the highest frequency between DENV-1 and DENV-2 (2.9%, $n = 16$). Recent investigations in Colombia also found mixed infections in *Aedes* with serotypes DENV-1 and DENV-2, DENV-2 and DENV-4 (Pérez-Castro et al., 2016), DENV-4 and DENV-1 (Pena-Garcia et al., 2016), and by arboviral species DENV-CHIKV (Martínez et al., 2020), which could be related to the differences in the endemic and entomological patterns. Some reports indicate that *Aedes* mosquitoes have a high probability of infection by two or more DENV serotypes (Guzman et al., 2016; Vazeille et al., 2016), and may be related to increased susceptibility to infection by one of these serotypes. Currently, the implications of co-infection and co-transmission on the epidemiology, pathogenesis, and evolution of these viral agents remain unclear, mainly due to limited clinical information, underdiagnosis, and poor detection in both humans and mosquitoes (Carrillo-Hernandez et al., 2018; Mercado-Reyes et al., 2019; Vogels et al., 2019). Nevertheless, in regions where there is active co-circulation of arboviral agents, especially in areas with high endemicity for Dengue, it is essential to clarify the dynamics of infection in mosquitoes and possible implications for arboviral transmission and evolution. The implementation of accurate diagnostic tests for single or multiple arboviral detections deserves greater attention as part of epidemiological surveillance.

The present study shows that *A. aegypti* populations sampled in different departments of Colombia exhibited high haplotype diversity 0.948 ± 0.012, but medium nucleotide diversity (0.0225 ± 0.003) (**Table 3**). Similar results were found in studies on the genetic structure of *A. aegypti* in Latin American countries such as Brazil (Bracco et al., 2007), Mexico (Gorrochotegui-Escalante et al., 2002), Venezuela (Herrera et al., 2006), and Colombia (Jaimes-Duenez et al., 2015). The high diversity of haplotypes in different populations of *A. aegypti* in the continent indicates a rapid population demographic expansion in a relatively recent period (Joyce et al., 2018) and a gene flow between countries in the region (Jaimes-Duenez et al., 2015). In contrast, other countries such as Bolivia (Paupy et al., 2012) show low genetic diversity, probably related to geographical isolation and limited terrestrial access, which prevents reinvasion processes by *A. aegypti* in this country. Analysis of Tajima's D test showed negative values in all study departments and significant values for *A. aegypti* samples in Amazonas and Boyacá ($P < 0.05$), suggesting an excess of rare alleles, which may reflect demographic instability and confirm the population expansion of this vector species (Paupy et al., 2012). These analyses are related to the haplotype network (**Figure 3A**), which shows the genetic diversity of *A. aegypti* in the study populations, with greater nucleotide diversity for samples from the department of Vichada. It also reveals a haplogroup among most Amazon sequences, which presented the lowest haplotype diversity, while departments with the highest haplotype diversity (Boyacá, Magdalena, and Vichada) are in the second-highest haplogroup. This may suggest that the Amazonas populations present some degree of isolation from the other *A. aegypti* populations in the study areas, while the populations of the other departments may present a greater genetic flow between them. As the Colombian *Aedes* populations from Amazonas seem to be different from the rest of the country, future studies should study the complex ecological, evolutionary, and epidemiological context of Amazonas, as this department limits with the Brazilian, Venezuelan, and Peruvian amazon comprising a fascinating hotspot for understanding *Aedes* population structure.

In addition, phylogenetic analyses of haplotype sequences from our study and haplotype sequences from other countries suggest the predominance of a *Lineage I* of *A. aegypti* in Colombia, where all sequences from Amazonas, Boyacá, Magdalena, and Vichada were related (**Figure 3B**). This

lineage has been described as the most widespread in countries of the Americas (Herrera et al., 2006; Bracco et al., 2007; Paupy et al., 2012) and is related to West African ancestors (Moore et al., 2013). Recent studies on the genetic characteristics and population structure of *A. aegypti* in Colombia, based on COI and NAD4 (dehydrogenase subunit 4) molecular markers, show the existence of a second lineage (*Lineage II*), recently introduced to the country and related to East African analogs (Jaimes-Duenez et al., 2015). According to the authors, *Lineage I* is found in areas with a high incidence of Dengue and chemical insecticides, while *Lineage II* is present in cities with a low incidence of Dengue where chemical insecticides are not constant (Jaimes-Duenez et al., 2015). This suggests that the epidemiology of Dengue may be influenced by the population dynamics of *A. aegypti* in the territory. However, it is necessary to continue molecular epidemiology studies in this type of vector populations, including more molecular markers or even comparative genomics, which would help to clarify the associations between vector competence and the origin of the populations of *Aedes* species.

Finally, our study shows that entomovirological surveillance using molecular tools is a powerful tool that can support decision-making for preventing and controlling vector-borne diseases such as Dengue. Thus, individual detection of infection and/or co-infection by arboviruses (DENV, CHIKV, ZIKV) in field-collected *Aedes* mosquitoes, and molecular discrimination of vector species using molecular markers, can become a strategy that generates highly accurate entomological information. It could be used as an early warning system to support the adoption of vector control and prevention measures in emergency or re-emergence of arboviral outbreaks, especially in predicting infections up to six weeks before the onset of cases (Pena-Garcia et al., 2016). On the other hand, considering the installed capacity due to the Coronavirus disease (COVID-19) sanitary contingency (Alvarez-Diaz et al., 2021), health entities could take advantage of the Next Generation Sequencing (NGS) technology initiative of viral strains to detect new DENV lineages, in both humans and mosquitoes, and learn about regional dissemination patterns. These sequencing strategies, routinely implemented for pathogen detection and monitoring by public health agencies in Europe (Public Health England) and North America (Centers for Disease Control and Prevention, Public Health Agency of Canada), have enabled the successful surveillance of arboviruses such as West Nile virus (WNV) (Wollants et al., 2018) and Zika (Quick et al., 2017), which provided crucial information for early decision making to prevent the spread of these diseases, as well as assisting in the development of new schemes for the comprehensive management of these outbreaks.

It is essential to recognize some limitations in our study, such as the sample size of *Aedes* mosquitoes in the study areas, as well as the use of a single molecular marker to perform the population structure analysis, that would allow us to carry out a more robust analysis. We consider that our findings on entomovirological surveillance in different areas of Colombia provide valuable information that supports comprehensive surveillance and control systems for *A. aegypti* (*Lineage I*) as the primary vector of arboviruses in Colombia. Likewise, our findings highlight the importance of protecting the population at risk of serotype transmission, especially DENV-1 and DENV-2, and focusing on the potential serotype change in the next epidemic outbreak.

Conclusion

This study reports on the natural infection by DENV-1, DENV-2, DENV-3, and CHIKV of *A. aegypti* from departments in different biogeographical regions of Colombia. Furthermore, it confirms that co-infection with DENV serotypes in different combinations is possible. This demonstrates the high susceptibility of this species to arbovirus infection and confirms that *A. aegypti* is the primary vector in Colombia. The high rates of DENV infection may be associated with the high seroprevalence and asymptomatic infections in the human population, which may influence the constant maintenance of the arboviral infection cycle. In contrast, the low and null detection of CHIKV and ZIKV, respectively, may reflect the decrease in transmission after the outbreaks in 2015 and 2016. Finally, from the analysis of genetic diversity, we demonstrate the predominance of *lineage I* of *A. aegypti* in Colombia, widely spread in the Americas, and previously related to areas with a high incidence of Dengue. Our findings highlight the importance of including entomovirological surveillance to better understand its epidemiological dynamics and take preventive measures against future outbreaks. We suggest, especially to health and government entities, to take advantage of the installed capacity in the face of sanitary containment by COVID-19 to monitor the circulation of DENV serotypes and genotypes as a strategy for comprehensive disease management of arboviruses. We also recommend that future studies include and identify other disease-carrying vectors circulating in the area (e.g., *Culex* spp.). This would be useful to inform the public system of new viruses and vectors. Finally, we believe that our results on the molecular epidemiology of arboviruses in different areas of Colombia provide valuable information that supports institutional capacities for the prevention and control of arboviral agents.

Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding author.

Author contributions

JDR and MG conceived the study. RBM, LAS, MP-C, LMM, LGP, LEB, MAM, KAC, HDP, AZF, and JLD collected the samples. DM, LHP, and MG performed the processing of the samples in the laboratory and analysis. MG drafted the manuscript. DM, CH, NL, MM, and JDR contributed to interpretation and critical review. All authors read and approved the final manuscript.

Funding

This work was supported by Dirección de Investigación e Innovación from Universidad del Rosario. We thank the Ministerio de Ciencia y Tecnologia e Innovacion from Colombia in the framework of the call 891 "Convocatoria fortalecimiento de vocaciones y formacion en CTeI para la reactivacion economica en el marco de la postpandemia 2020" for funding LHP.

Acknowledgments

We thank the local health authorities from Amazonas, Boyacá, Magdalena, and Vichada departments for their support and assistance with the fieldwork. Equally, thanks to the Universidad del Rosario for covering the publication fees. We thank the Universidad de Boyacá for their support in financing MG's doctoral studies. Also, we thank Sergio Castañeda for his expertise in statistical analysis.

Conflict of interest

CH was employed by Innovaseq S.A.S. LHP was employed by Upqua S.A.S.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The handling editor JO declared a past co-authorship with the author JDR.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.999169/full#supplementary-material

References

Allicock, O. M., Lemey, P., Tatem, A. J., Pybus, O. G., Bennett, S. N., Mueller, B. A., et al. (2012). Phylogeography and population dynamics of dengue viruses in the Americas. *Mol. Biol. Evol.* 29, 1533–1543. doi: 10.1093/molbev/msr320

Alvarez-Diaz, D. A., Valencia-Alvarez, E., Rivera, J. A., Rengifo, A. C., Usme-Ciro, J. A., Pelaez-Carvajal, D., et al. (2021). An updated RT-qPCR assay for the simultaneous detection and quantification of chikungunya, dengue and zika viruses. *Infect. Genet. Evol.* 93:104967. doi: 10.1016/j.meegid.2021.104967

Bhatt, S., Gething, P. W., Brady, O. J., Messina, J. P., Farlow, A. W., Moyes, C. L., et al. (2013). The global distribution and burden of Dengue. *Nature* 496, 504–507. doi: 10.1038/nature12060

Bonizzoni, M., Gasperi, G., Chen, X., and James, A. A. (2013). The invasive mosquito species *Aedes albopictus*: Current knowledge and future perspectives. *Trends Parasitol.* 29, 460–468. doi: 10.1016/j.pt.2013.07.003

Bracco, J. E., Capurro, M. L., Lourenco-De-Oliveira, R., and Sallum, M. A. (2007). Genetic variability of *Aedes aegypti* in the Americas using a mitochondrial gene: Evidence of multiple introductions. *Mem. Inst. Oswaldo Cruz* 102, 573–580. doi: 10.1590/S0074-02762007005000062

Brady, O. J., Golding, N., Pigott, D. M., Kraemer, M. U., Messina, J. P., Reiner, R. C. Jr., et al. (2014). Global temperature constraints on *Aedes aegypti* and *Ae. albopictus* persistence and competence for dengue virus transmission. *Parasit. Vectors* 7:338. doi: 10.1186/1756-3305-7-338

Caron, M., Paupy, C., Grard, G., Becquart, P., Mombo, I., Nso, B. B., et al. (2012). Recent introduction and rapid dissemination of Chikungunya virus and Dengue virus serotype 2 associated with human and mosquito co-infections in Gabon, central Africa. *Clin. Infect. Dis.* 55, e45–e53. doi: 10.1093/cid/cis530

Carrasquilla, M. C., Ortiz, M. I., Leon, C., Rondon, S., Kulkarni, M. A., Talbot, B., et al. (2021). Entomological characterization of *Aedes* mosquitoes and arbovirus detection in Ibague, a Colombian city with co-circulation of Zika, dengue and chikungunya viruses. *Parasit. Vectors* 14:446. doi: 10.1186/s13071-021-04908-x

Carrillo-Hernandez, M. Y., Ruiz-Saenz, J., Villamizar, L. J., Gomez-Rangel, S. Y., and Martinez-Gutierrez, M. (2018). Co-circulation and simultaneous co-infection of Dengue, chikungunya, and zika viruses in patients with febrile syndrome at the Colombian-Venezuelan border. *BMC Infect. Dis.* 18:61. doi: 10.1186/s12879-018-2976-1

Cevallos, V., Ponce, P., Waggoner, J. J., Pinsky, B. A., Coloma, J., Quiroga, C., et al. (2018). Zika and Chikungunya virus detection in naturally infected *Aedes aegypti* in Ecuador. *Acta Trop.* 177, 74–80. doi: 10.1016/j.actatropica.2017.09.029

Chien, L. J., Liao, T. L., Shu, P. Y., Huang, J. H., Gubler, D. J., and Chang, G. J. (2006). Development of real-time reverse transcriptase PCR assays to detect and serotype dengue viruses. *J. Clin. Microbiol.* 44, 1295–1304. doi: 10.1128/JCM.44.4.1295-1304.2006

Ciota, A. T. (2019). The role of co-infection and swarm dynamics in arbovirus transmission. *Virus Res.* 265, 88–93. doi: 10.1016/j.virusres.2019.03.010

Ciota, A. T., Chin, P. A., Ehrbar, D. J., Micieli, M. V., Fonseca, D. M., and Kramer, L. D. (2018). Differential effects of temperature and mosquito genetics determine transmissibility of arboviruses by *Aedes aegypti* in Argentina. *Am. J. Trop. Med. Hyg.* 99, 417–424. doi: 10.4269/ajtmh.18-0097

Dos Santos, T. P., Cruz, O. G., Da Silva, K. A. B., De Castro, M. G., De Brito, A. F., Maspero, R. C., et al. (2017). Dengue serotype circulation in natural populations of *Aedes aegypti*. *Acta Trop.* 176, 140–143. doi: 10.1016/j.actatropica.2017.07.014

Faye, O., Faye, O., Diallo, D., Diallo, M., Weidmann, M., and Sall, A. A. (2013). Quantitative real-time PCR detection of Zika virus and evaluation with field-caught mosquitoes. *Virol J.* 10:311. doi: 10.1186/1743-422X-10-311

Ferreira-De-Lima, V. H., and Lima-Camara, T. N. (2018). Natural vertical transmission of dengue virus in A*edes aegypti* and *Aedes albopictus*: A systematic review. *Parasit. Vectors* 11:77. doi: 10.1186/s13071-018-2643-9

Goertz, G. P., Vogels, C. B. F., Geertsema, C., Koenraadt, C. J. M., and Pijlman, G. P. (2017). Mosquito co-infection with Zika and chikungunya virus allows simultaneous transmission without affecting vector competence of *Aedes aegypti*. *PLoS Negl. Trop. Dis.* 11:e0005654. doi: 10.1371/journal.pntd.0005654

Golding, N., Wilson, A. L., Moyes, C. L., Cano, J., Pigott, D. M., Velayudhan, R., et al. (2015). Integrating vector control across diseases. *BMC Med.* 13:249. doi: 10.1186/s12916-015-0491-4

Gómez, W., and Zapata-Úsuga, G. (2019). Presencia de *Aedes aegypti* y *Aedes albopictus* (Diptera: Culicidae) en área rural del departamento de Santander, Colombia. *Biosalud* 18, 7–21.

Gómez-Palacio, A., Suaza-Vasco, J., Castaño, S., Triana, O., and Uribe, S. (2017). Infección de *Aedes albopictus* (Skuse, 1894) con el genotipo asiático-americano del virus del dengue serotipo 2 en Medellín y su posible papel como vector del dengue en Colombia. *Biomédica* 37, 135–142. doi: 10.7705/biomedica.v37i0.3474

Gordon, A., Gresh, L., Ojeda, S., Chowell, G., Gonzalez, K., Sanchez, N., et al. (2018). Differences in transmission and disease severity between 2 successive waves of Chikungunya. *Clin. Infect. Dis.* 67, 1760–1767. doi: 10.1093/cid/ciy356

Gorrochotegui-Escalante, N., Gomez-Machorro, C., Lozano-Fuentes, S., Fernandez-Salas, L., De Lourdes Munoz, M., Farfan-Ale, J. A., et al. (2002). Breeding structure of *Aedes aegypti* populations in Mexico varies by region. *Am. J. Trop. Med. Hyg.* 66, 213–222. doi: 10.4269/ajtmh.2002.66.213

Gould, E., Pettersson, J., Higgs, S., Charrel, R., and De Lamballerie, X. (2017). Emerging arboviruses: Why today? *One Health* 4, 1–13. doi: 10.1016/j.onehlt.2017.06.001

Gutierrez-Barbosa, H., Medina-Moreno, S., Zapata, J. C., and Chua, J. V. (2020). Dengue infections in Colombia: Epidemiological trends of a hyperendemic country. *Trop. Med. Infect. Dis.* 5:156. doi: 10.3390/tropicalmed5040156

Guzman, M. G., Gubler, D. J., Izquierdo, A., Martinez, E., and Halstead, S. B. (2016). Dengue infection. *Nat. Rev. Dis. Primers* 2:16055. doi: 10.1038/nrdp.2016.55

Herrera, F., Urdaneta, L., Rivero, J., Zoghbi, N., Ruiz, J., Carrasquel, G., et al. (2006). Population genetic structure of the dengue mosquito *Aedes aegypti* in Venezuela. *Mem. Inst. Oswaldo Cruz* 101, 625–633. doi: 10.1590/S0074-02762006000600008

Hoyos-Lopez, R., Suaza-Vasco, J., Rua-Uribe, G., Uribe, S., and Gallego-Gomez, J. C. (2016). Molecular detection of flaviviruses and alphaviruses in mosquitoes (Diptera: Culicidae) from coastal ecosystems in the Colombian Caribbean. *Mem. Inst. Oswaldo Cruz* 111, 625–634. doi: 10.1590/0074-02760160096

Instituto Nacional De Salud (2019b). *Informe técnico entomológico de arbovirus–Colombia, 2018*. Bogota, CO: Ministerio de Salud y Protección Social.

Instituto Nacional De Salud (2019a). *Boletín epidemiológico semana 30. Dirección de vigilancia y análisis del riesgo en salud pública publicación en línea*. Bogota, CO: Ministerio de Salud y Protección Social. doi: 10.33610/23576189.2019.30

Instituto Nacional De Salud (2020). *Instituto nacional de salud. Boletín epidemiológico semana 41. Dirección de vigilancia y análisis del riesgo en salud pública publicación en línea: ISSN 2357-6189 2018*. Bogota, CO: Ministerio de Salud y Protección Social. doi: 10.33610/23576189.2020.41

Instituto Nacional De Salud (2022). *Instituto nacional de salud. Boletín epidemiológico semana 33. Dirección de vigilancia y análisis del riesgo en salud pública publicación en línea: ISSN 2357-6189 2018*. Bogota, CO: Ministerio de Salud y Protección Social. doi: 10.33610/23576189.2019.20

Jaimes-Duenez, J., Arboleda, S., Triana-Chavez, O., and Gomez-Palacio, A. (2015). Spatio-temporal distribution of *Aedes aegypti* (Diptera: Culicidae) mitochondrial lineages in cities with distinct dengue incidence rates suggests complex population dynamics of the dengue vector in Colombia. *PLoS Negl. Trop. Dis.* 9:e0003553. doi: 10.1371/journal.pntd.0003553

Jimenez-Silva, C. L., Carreno, M. F., Ortiz-Baez, A. S., Rey, L. A., Villabona-Arenas, C. J., and Ocazionez, R. E. (2018). Evolutionary history and spatio-temporal dynamics of dengue virus serotypes in an endemic region of Colombia. *PLoS One* 13:e0203090. doi: 10.1371/journal.pone.0203090

Joyce, A. L., Torres, M. M., Torres, R., and Moreno, M. (2018). Genetic variability of the *Aedes aegypti* (Diptera: Culicidae) mosquito in El Salvador, vector of Dengue, yellow fever, chikungunya and Zika. *Parasit. Vectors* 11:637. doi: 10.1186/s13071-018-3226-5

Katoh, K., and Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Mol. Biol. Evol.* 30, 772–780. doi: 10.1093/molbev/mst010

Kauffman, E. B., and Kramer, L. D. (2017). Zika virus mosquito vectors: Competence, biology, and vector control. *J. Infect. Dis.* 216, S976–S990. doi: 10.1093/infdis/jix405

Kazmi, S. S., Ali, W., Bibi, N., and Nouroz, F. (2020). A review on Zika virus outbreak, epidemiology, transmission and infection dynamics. *J. Biol. Res. (Thessalon)* 27:5. doi: 10.1186/s40709-020-00115-4

Kilpatrick, A. M., and Randolph, S. E. (2012). Drivers, dynamics, and control of emerging vector-borne zoonotic diseases. *Lancet* 380, 1946–1955. doi: 10.1016/S0140-6736(12)61151-9

Kirstein, O. D., Ayora-Talavera, G., Koyoc-Cardena, E., Chan Espinoza, D., Che-Mendoza, A., Cohuo-Rodriguez, A., et al. (2021). Natural arbovirus infection rate and detectability of indoor female *Aedes aegypti* from Merida. Yucatan, Mexico. *PLoS Negl. Trop. Dis.* 15:e0008972. doi: 10.1371/journal.pntd.0008972

Klungthong, C., Zhang, C., Mammen, M. P. Jr., Ubol, S., and Holmes, E. C. (2004). The molecular epidemiology of dengue virus serotype 4 in Bangkok, Thailand. *Virology* 329, 168–179. doi: 10.1016/j.virol.2004.08.003

Kraemer, M. U. G., Reiner, R. C. Jr., Brady, O. J., Messina, J. P., Gilbert, M., Pigott, D. M., et al. (2019). Past and future spread of the arbovirus vectors *Aedes aegypti* and *Aedes albopictus*. *Nat. Microbiol.* 4, 854–863. doi: 10.1038/s41564-019-0376-y

Kraemer, M. U., Sinka, M. E., Duda, K. A., Mylne, A. Q., Shearer, F. M., Barker, C. M., et al. (2015). The global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. albopictus*. *Elife* 4:e08347. doi: 10.7554/eLife.08347

Leigh, J., and Bryant, D. (2015). POPART: Full-feature software for haplotype network construction. *Methods Ecol. Evol.* 6, 1110–1116. doi: 10.1111/2041-210X.12410

Leparc-Goffart, I., Nougairede, A., Cassadou, S., Prat, C., and De Lamballerie, X. (2014). Chikungunya in the Americas. *Lancet* 383:514. doi: 10.1016/S0140-6736(14)60185-9

Leta, S., Beyene, T. J., De Clercq, E. M., Amenu, K., Kraemer, M. U. G., and Revie, C. W. (2018). Global risk mapping for major diseases transmitted by *Aedes aegypti* and *Aedes albopictus*. *Int. J. Infect. Dis.* 67, 25–35. doi: 10.1016/j.ijid.2017.11.026

Liu-Helmersson, J., Quam, M., Wilder-Smith, A., Stenlund, H., Ebi, K., Massad, E., et al. (2016). Climate change and *Aedes* vectors: 21st century projections for dengue transmission in Europe. *EBioMedicine* 7, 267–277. doi: 10.1016/j.ebiom.2016.03.046

Lwande, O. W., Obanda, V., Lindstrom, A., Ahlm, C., Evander, M., Naslund, J., et al. (2020). Globe-trotting *Aedes aegypti* and *Aedes albopictus*: Risk factors for arbovirus pandemics. *Vector Borne Zoonotic Dis.* 20, 71–81. doi: 10.1089/vbz.2019.2486

Magalhaes, T., Robison, A., Young, M. C., Black, W. C. T., Foy, B. D., Ebel, G. D., et al. (2018). Sequential infection of *Aedes aegypti* Mosquitoes with Chikungunya virus and Zika virus enhances early Zika virus transmission. *Insects* 9:177. doi: 10.3390/insects9040177

Martina, B. E., Koraka, P., and Osterhaus, A. D. (2009). Dengue virus pathogenesis: An integrated view. *Clin. Microbiol. Rev.* 22, 564–581. doi: 10.1128/CMR.00035-09

Martínez, D., Hernández, C., Muñoz, M., Armesto, Y. A. C., and Jd, R. (2020). Identification of *Aedes* (Diptera: Culicidae) species and arboviruses circulating in Arauca,Eastern Colombia. *Front. Ecol. Evol.* 8:602190. doi: 10.3389/fevo.2020.602190

Mercado-Reyes, M., Acosta-Reyes, J., Navarro-Lechuga, E., Corchuelo, S., Rico, A., Parra, E., et al. (2019). Dengue, chikungunya and zika virus coinfection: Results of the national surveillance during the zika epidemic in Colombia. *Epidemiol. Infect.* 147:e77. doi: 10.1017/S095026881800359X

Ministerio De Salud Y Protección Social (2020). *Lineamiento para la gestion y operación del programa de enfermedades transmitidas por vectores y zoonosis y otras consideraciones para la ejecución de sus transferencias nacionales de funcionamiento. Dirección de Promoción y Prevención*, ed. S. D. E. T. G. Endemoepidémicas (Combia: Ministerio de Salud y Protección Social. República de Colmbia).

Monteiro, F. J. C., Mourao, F. R. P., Ribeiro, E. S. D., Rego, M., Frances, P., Souto, R. N. P., et al. (2020). Prevalence of dengue, Zika and chikungunya viruses in *Aedes* (Stegomyia) *aegypti* (Diptera: Culicidae) in a medium-sized city, Amazon, Brazil. *Rev. Inst. Med. Trop. Sao Paulo* 62:e10. doi: 10.1590/s1678-9946202062010

Moore, M., Sylla, M., Goss, L., Burugu, M. W., Sang, R., Kamau, L. W., et al. (2013). Dual African origins of global *Aedes aegypti* s.l. Populations revealed by mitochondrial DNA. *PLoS Negl. Trop. Dis.* 7:e2175. doi: 10.1371/journal.pntd.0002175

Mount, D. W. (2007). Using the basic local alignment search tool (BLAST). *CSH Protoc.* 2007:pdb top17. doi: 10.1101/pdb.top17

Mourya, D. T., Gokhale, M. D., Majumdar, T. D., Yadav, P. D., Kumar, V., and Mavale, M. S. (2018). Experimental Zika virus infection in *Aedes aegypti*: Susceptibility, transmission & co-infection with dengue & chikungunya viruses. *Indian J. Med. Res.* 147, 88–96. doi: 10.4103/ijmr.IJMR_1142_17

Murray, N. E., Quam, M. B., and Wilder-Smith, A. (2013). Epidemiology of Dengue: Past, present and future prospects. *Clin. Epidemiol.* 5, 299–309. doi: 10.2147/CLEP.S34440

Ngono, A. E., and Shresta, S. (2018). Immune response to dengue and zika. *Annu. Rev. Immunol.* 36, 279–308. doi: 10.1146/annurev-immunol-042617-053142

Nguyen, L. T., Schmidt, H. A., Von Haeseler, A., and Minh, B. Q. (2015). IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol. Biol. Evol.* 32, 268–274. doi: 10.1093/molbev/msu300

Nuckols, J. T., Huang, Y. J., Higgs, S., Miller, A. L., Pyles, R. B., Spratt, H. M., et al. (2015). Evaluation of simultaneous transmission of chikungunya virus and dengue virus type 2 in infected *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae). *J. Med. Entomol.* 52, 447–451. doi: 10.1093/jme/tjv017

Okonechnikov, K., Golosova, O., Fursov, M., and Team, U. (2012). Unipro UGENE: A unified bioinformatics toolkit. *Bioinformatics* 28, 1166–1167. doi: 10.1093/bioinformatics/bts091

Padilla, J., Lizarazo, F., Murillo, O., Mendigaña, F., Pachón, E., and Vera, M. (2017). Epidemiología de las principales enfermedades transmitidas por vectores en Colombia, 1990–2016. *Biomedica* 37, 27–40. doi: 10.7705/biomedica.v37i0.3769

Paixao, E. S., Teixeira, M. G., and Rodrigues, L. C. (2018). Zika, chikungunya and Dengue: The causes and threats of new and re-emerging arboviral diseases. *BMJ Glob. Health* 3:e000530. doi: 10.1136/bmjgh-2017-000530

Patterson, J., Sammon, M., and Garg, M. (2016). Dengue, zika and chikungunya: Emerging arboviruses in the new world. *West. J. Emerg. Med.* 17, 671–679. doi: 10.5811/westjem.2016.9.30904

Paupy, C., Le Goff, G., Brengues, C., Guerra, M., Revollo, J., Barja Simon, Z., et al. (2012). Genetic structure and phylogeography of *Aedes aegypti*, the Dengue and yellow-fever mosquito vector in Bolivia. *Infect. Genet. Evol.* 12, 1260–1269. doi: 10.1016/j.meegid.2012.04.012

Peña-Garcia, V. H., Triana-Chavez, O., and Arboleda-Sanchez, S. (2017). Estimating Effects of temperature on dengue transmission in Colombian Cities. *Ann. Glob. Health* 83, 509–518. doi: 10.1016/j.aogh.2017.10.011

Pena-Garcia, V. H., Triana-Chavez, O., Mejia-Jaramillo, A. M., Diaz, F. J., Gomez-Palacio, A., and Arboleda-Sanchez, S. (2016). Infection rates by dengue virus in mosquitoes and the influence of temperature may be related to different endemicity patterns in three Colombian Cities. *Int. J. Environ. Res. Public Health* 13:734.

Pereira, L., Brito, M. C. A., Araruna, F. B., De Andrade, M. S., Moraes, D. F. C., Borges, A. C. R., et al. (2017). Molecular studies with *Aedes* (Stegomyia) *aegypti* (Linnaeus, 1762), mosquito transmitting the dengue virus. *Parasitol. Res.* 116, 2057–2063. doi: 10.3390/ijerph13070734

Pérez-Castro, R., Castellanos, J. E., Olano, V. A., Matiz, M., Jaramillo, J., Vargas, S., et al. (2016). Detection of all four dengue serotypes in *Aedes aegypti* female mosquitoes collected in a rural area in Colombia. *Mem. Inst. Oswaldo Cruz* 111, 233–240. doi: 10.1007/s00436-017-5484-0

Pérez-Pérez, J., Sanabria, W., Restrepo, C., Rojo, R., Henao, E., Triana, O., et al. (2017). Vigilancia virológica de *Aedes* (Stegomyia) *aegypti yAedes* (Stegomyia) *albopictus* como apoyo para la adopción de decisiones en el control del dengue en Medellín. *Biomédica* 37, 155–166. doi: 10.1590/0074-02760150363

Pollett, S., Melendrez, M. C., Maljkovic Berry, I., Duchene, S., Salje, H., Cummings, D. A. T., et al. (2018). Understanding dengue virus evolution to support epidemic surveillance and counter-measure development. *Infect. Genet. Evol.* 62, 279–295. doi: 10.7705/biomedica.v37i0.3467

Quick, J., Grubaugh, N. D., Pullan, S. T., Claro, I. M., Smith, A. D., Gangavarapu, K., et al. (2017). Multiplex PCR method for MinION and Illumina sequencing of Zika and other virus genomes directly from clinical samples. *Nat. Protoc.* 12, 1261–1276. doi: 10.1016/j.meegid.2018.04.032

Ramos-Castaneda, J., Barreto Dos, Santos, F., Martinez-Vega, R., Galvao, De Araujo, J. M., et al. (2017). Dengue in latin America: Systematic review of molecular epidemiological trends. *PLoS Negl. Trop. Dis.* 11:e0005224. doi: 10.1038/nprot.2017.066

Rozas, J., Ferrer-Mata, A., Sanchez-Delbarrio, J. C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S. E., et al. (2017). DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Mol. Biol. Evol.* 34, 3299–3302. doi: 10.1371/journal.pntd.0005224

RStudio Team (2019). *RStudio: Integrated development for R.* RStudio, PBC: Boston, MA.

Ruckert, C., Weger-Lucarelli, J., Garcia-Luna, S. M., Young, M. C., Byas, A. D., Murrieta, R. A., et al. (2017). Impact of simultaneous exposure to arboviruses on infection and transmission by *Aedes aegypti* mosquitoes. *Nat. Commun.* 8:15412. doi: 10.1093/molbev/msx248

Ruiz-López, R., González-Mazo, A., Vélez-Mira, A., Gómez, G., Zuleta, L., and Vélez-Bernal, I. (2016). Presencia de *Aedes* (Stegomyia) *aegypti* (Linnaeus, 1762) y su infección natural con el virus del dengue en alturas no registradas para Colombia. *Biomédica* 36, 303–308. doi: 10.1038/ncomms15412

Ryan, S. J., Carlson, C. J., Mordecai, E. A., and Johnson, L. R. (2019). Global expansion and redistribution of *Aedes*-borne virus transmission risk with climate change. *PLoS Negl. Trop. Dis.* 13:e0007213. doi: 10.7705/biomedica.v36i2.3301

Salvo, M. A., Aliota, M. T., Moncla, L. H., Velez, I. D., Trujillo, A. I., Friedrich, T. C., et al. (2019). Tracking dengue virus type 1 genetic diversity during lineage replacement in an hyperendemic area in Colombia. *PLoS One* 14:e0212947. doi: 10.1371/journal.pntd.0007213

Santiago, G. A., Vergne, E., Quiles, Y., Cosme, J., Vazquez, J., Medina, J. F., et al. (2013). Analytical and clinical performance of the CDC real time RT-PCR assay for detection and typing of dengue virus. *PLoS Negl. Trop. Dis.* 7:e2311. doi: 10.1371/journal.pone.0212947

Scarpassa, V. M., Cardoza, T. B., and Cardoso Junior, R. P. (2008). Population genetics and phylogeography of *Aedes aegypti* (Diptera: Culicidae) from Brazil. *Am. J. Trop. Med. Hyg.* 78, 895–903. doi: 10.1371/journal.pntd.0002311

Sukhralia, S., Verma, M., Gopirajan, S., Dhanaraj, P. S., Lal, R., Mehla, N., et al. (2019). From dengue to zika: The wide spread of mosquito-borne arboviruses. *Eur. J. Clin. Microbiol. Infect. Dis.* 38, 3–14. doi: 10.4269/ajtmh.2008.78.895

Vazeille, M., Gaborit, P., Mousson, L., Girod, R., and Failloux, A. B. (2016). Competitive advantage of a dengue 4 virus when co-infecting the mosquito *Aedes aegypti* with a dengue 1 virus. *BMC Infect. Dis.* 16:318. doi: 10.1007/s10096-018-3375-7

Velandia-Romero, M. L., Coronel-Ruiz, C., Castro-Bonilla, L., Camacho-Ortega, S., Calderon-Pelaez, M. A., Castellanos, A., et al. (2020). Prevalence of dengue antibodies in healthy children and adults in different Colombian endemic areas. *Int. J. Infect. Dis.* 91, 9–16. doi: 10.1186/s12879-016-1666-0

Vogels, C. B. F., Ruckert, C., Cavany, S. M., Perkins, T. A., Ebel, G. D., and Grubaugh, N. D. (2019). Arbovirus co-infection and co-transmission: A neglected public health concern? *PLoS Biol.* 17:e3000130. doi: 10.1016/j.ijid.2019.10.045

Wilder-Smith, A., Gubler, D. J., Weaver, S. C., Monath, T. P., Heymann, D. L., and Scott, T. W. (2017). Epidemic arboviral diseases: Priorities for research and public health. *Lancet Infect. Dis.* 17, e101–e106. doi: 10.1371/journal.pbio.3000130

Wilder-Smith, A., Ooi, E. E., Vasudevan, S. G., and Gubler, D. J. (2010). Update on Dengue: Epidemiology, virus evolution, antiviral drugs, and vaccine development. *Curr. Infect. Dis. Rep.* 12, 157–164. doi: 10.1016/S1473-3099(16)30518-7

Wollants, E., Smolders, D., Naesens, R., Bruynseels, P., Lagrou, K., Matthijnssens, J., et al. (2018). Use of next-generation sequencing for diagnosis of west nile virus infection in patient returning to Belgium from Hungary. *Emerg. Infect. Dis.* 24, 2380–2382. doi: 10.1007/s11908-010-0102-7

Young, P. R. (2018). Arboviruses: A family on the move. *Adv. Exp. Med. Biol.* 1062, 1–10. doi: 10.3201/eid2412.180494

Capítulo 2

Para alcanzar el objetivo planteado en este capítulo, se utilizó una metodología metagenómica para examinar en profundidad el viroma de los mosquitos *Ae. aegypti* en diversas zonas urbanas y periurbanas de Colombia. Este análisis se basó en los hallazgos previos sobre la prevalencia de infección por DENV, detallados en el capítulo anterior. Los resultados revelaron que el viroma de estos mosquitos está compuesto principalmente por virus específicos de insectos (ISV), los cuales son agentes virales no patógenos exclusivos de los insectos. Se observó que la infección por distintos serotipos de DENV, especialmente DENV-1 y DENV-2, podría influir en la abundancia relativa de las distintas familias y especies virales que conforman el viroma central en *Aedes* spp. Además, este estudio permitió identificar y analizar las relaciones evolutivas del *Phasi Charoen-like virus* (PCLV, Family: Phenuviridae) en *Ae. aegypti* en Colombia, un ISV prevalente en regiones con alta incidencia de DENV. La caracterización detallada del viroma de *Ae. aegypti*, presentada en este capítulo, enriquece nuestra comprensión sobre la diversidad viral en los mosquitos vectores y proporciona datos cruciales para futuras investigaciones sobre las interacciones entre ISV – arbovirus – mosquito, así como su posible uso en estrategias de control vectorial.

Como producto de este capítulo se adjuntan los siguientes artículos científicos:

- **Artículo 3:** Gómez M, Martínez D, Muñoz M, Ramírez JD. *Aedes aegypti* and *Ae. Albopictus* microbiome/virome: new strategies for controlling arboviral transmission? Parasit Vectors. 2022 Aug 9;15(1):287. Doi: 10.1186/s13071-022-05401-9.

- **Artículo 4:** Gómez M, Martínez D, Páez-Triana L, Luna N, Ramírez A, Medina J, Cruz-Saavedra L, Hernández C, Castañeda S, Bohórquez Melo R, Suarez LA, Palma-Cuero M, Murcia LM, González Páez L, Estrada Bustos L, Medina MA, Ariza Campo K, Padilla HD, Zamora Flórez A, De Las Salas JL, Muñoz M, Ramírez JD. Influence of dengue virus serotypes on the abundance of Aedes aegypti insect-specific viruses (ISVs). J Virol. 2023 Dec 14:e0150723. Doi: 10.1128/jvi.01507-23.

CAPÍTULO 2

Viroma de *Aedes aegypti* con infección natural por el virus del dengue (DENV) mediante análisis metagenómicos.

Parasites & Vectors

REVIEW Open Access

Aedes aegypti and *Ae. albopictus* microbiome/virome: new strategies for controlling arboviral transmission?

Marcela Gómez[1,2], David Martinez[1], Marina Muñoz[1] and Juan David Ramírez[1,3*]

Abstract

Abstract: *Aedes aegypti* and *Aedes albopictus* are the main vectors of highly pathogenic viruses for humans, such as dengue (DENV), chikungunya (CHIKV), and Zika (ZIKV), which cause febrile, hemorrhagic, and neurological diseases and remain a major threat to global public health. The high ecological plasticity, opportunistic feeding patterns, and versatility in the use of urban and natural breeding sites of these vectors have favored their dispersal and adaptation in tropical, subtropical, and even temperate zones. Due to the lack of available treatments and vaccines, mosquito population control is the most effective way to prevent arboviral diseases. Resident microorganisms play a crucial role in host fitness by preventing or enhancing its vectorial ability to transmit viral pathogens. High-throughput sequencing and metagenomic analyses have advanced our understanding of the composition and functionality of the microbiota of *Aedes* spp. Interestingly, shotgun metagenomics studies have established that mosquito vectors harbor a highly conserved virome composed of insect-specific viruses (ISV). Although ISVs are not infectious to vertebrates, they can alter different phases of the arboviral cycle, interfering with transmission to the human host. Therefore, this review focuses on the description of *Ae. aegypti* and *Ae. albopictus* as vectors susceptible to infection by viral pathogens, highlighting the role of the microbiota-virome in vectorial competence and its potential in control strategies for new emerging and re-emerging arboviruses.

Keywords: Arbovirus, ISV, Metagenomics, Microbiota, Vectors, Virome

Background

Vector-borne diseases significantly impact public health, affecting approximately 30% of the world's population [1–3]. In particular, viral pathogens transmitted to humans by insects—arboviruses—are one of the main concerns due to the accelerated increase in their incidence and geographical distribution in recent years [4–6], with dengue (DENV), Zika (ZIKV), and chikungunya (CHIKV) the arboviruses of most significant medical importance in the world and requiring active epidemiological surveillance [7–9]. DENV, the most prevalent viral infection in tropical and subtropical countries [10–14], infects between 100 and 400 million people per year, and its incidence has increased 30-fold in recent decades [15, 16]. Since 2000, CHIKV and ZIKV have spread and caused significant outbreaks in Asia and the Americas [17–19].

DENV, CHIKV, and ZIKV are transmitted to humans predominantly through the highly competent mosquitoes *Aedes aegypti* and *Aedes albopictus* [20, 21]. *Aedes aegypti* is a mosquito vector with a wide distribution worldwide, especially in tropical and subtropical environments, and is closely associated with urban areas and areas with environmental disturbances [22, 23]. Contrastingly, *Ae. albopictus* presents a greater geographical

*Correspondence: juand.ramirez@urosario.edu.co; juan.ramirezgonzalez@mountsinai.org

[1] Centro de Investigaciones en Microbiología y Biotecnología-UR (CIMBIUR), Facultad de Ciencias Naturales, Universidad del Rosario, Bogotá, Colombia
Full list of author information is available at the end of the article

expansion [24], colonizing all five continents [20]. Despite vector control efforts, in recent years an increase in the geographical distribution of *Aedes* spp. has been detected due to factors associated with climate change [5, 25–27], globalization [28–30], urbanization [29–31], and resistance to different insecticides [32]. As a result, these vector species are considered a serious threat [33], compromising the effectiveness of preventive measures, control programs, and the management of outbreaks of the diseases they are involved in.

Furthermore, the search and discovery of viruses in insect vectors have accelerated in the last decade, thanks to advances in metagenomic sequencing technologies [34–37]. However, studies on vector virome are still scarce [37], and the role of insect-specific viruses—ISVs (viruses that only replicate in arthropod cells)—is virtually unknown, despite the importance in the prevention and control of mosquito-borne diseases. Different investigations indicate that ISVs are closely related to families of pathogenic viruses such as *Flaviviridae* and *Togaviridae* [38, 39], where DENV, CHIKV, and ZIKV are found. Therefore, ISVs can become potential pathogenic viruses in invertebrates [40, 41]; on the contrary, they could be highly related to the competition of vectors to transmit arboviruses [42, 43], or serve in the future as biological control agents against known arboviruses [44, 45]. Knowledge about the composition of viral communities in mosquitoes of the genus *Aedes* will contribute to the understanding of the mosquito–virus–pathogen interaction, as well as to elucidating new control strategies in the face of new arboviral epidemics. This review focuses on the description of the composition and diversity of the microbiota-virome of vectors involved in the transmission of arboviruses such as *Ae. aegypti* and *Ae. albopictus*. Also, the role of these microbes in the modulation of vectorial capacity and in the potential strategy of biological control is highlighted.

Aedes aegypti and *Aedes albopictus:* competent vectors in arboviral transmission

Aedes aegypti (*Stegomyia aegypti*) and *Ae. albopictus* (*Stegomyia albopicta*) are of interest primarily because of their association with emerging and re-emerging infectious diseases [17]. These two mosquito vectors have been described as highly competent in the transmission of arboviral pathogens such as DENV, ZIKV, and CHIKV [5, 22, 23, 29, 33, 46]. The species share several characteristics that give them adaptive advantages over others, making them successful invaders. The rapid spread and adaptation in tropical, subtropical, and temperate zones, and thus expansion of global coverage [5, 21, 26], may be related to large-scale epidemics and recent simultaneous outbreaks [20, 29]. *Aedes aegypti* is originally from Africa, considered a primary vector of some arboviruses. It has a high potential for pathogen transmission to humans due to its purely anthropophilic habits, reproduction in domestic (urban) and peridomestic environments, use of artificial containers as breeding places [5, 47], and greater availability of natural containers for oviposition [48, 49]. In comparison, *Ae. albopictus*, known as the tiger mosquito (Asian), is ecologically more flexible, with a more comprehensive geographical range than *Ae. aegypti* [24, 50]. It is found in suburban, rural, and sylvatic habitats, where it presents a wide range of hosts including humans, livestock, amphibians, reptiles, and birds [24, 29]. Both vectorial species present high ecological plasticity in heterogenous anthropic, climatic, and environmental conditions [25, 31, 50, 51]. Thus, *Ae. aegypti* and *Ae. albopictus* also reveal opportunistic feeding patterns in multiple human hosts during a gonotrophic cycle [52], diapause states (metabolism decreased at meager rates of energy expenditure and subsequent inactivity) during the development of eggs in drought conditions [53], resistance to insecticides such as DDT (dichlorodiphenyltrichloroethane) and pyrethroids [54], and versatility in the use of clean or stagnant water hatcheries in urban and natural environments [24].

Aedes aegypti and *Ae. albopictus* are considered two of the most invasive mosquito species [23, 50]. Competition between the two species in their ranges, whether native or invaded, frequently causes competitive displacement of one of the species [55], which can modify the epidemiology of arboviral diseases [56–58]. However, today, the two still coexist in large regions of the world [21, 59]. Several authors have revealed that the coexistence of *Ae. aegypti* and *Ae. albopictus* vector species in the same geographical areas can increase the risk of infection or co-infection for humans, especially during outbreaks or arboviral expansion [22, 26, 46, 60, 61]. Braks et al. [48] demonstrated that habitat is a determining factor in the abundance of the two species. Although *Ae. aegypti* predominates in urban areas and *Ae. albopictus* in rural areas, the two species can coexist in peri-urban areas, as demonstrated in several regions of Brazil and in the state of Florida in the United States. Thus, the segregation of different habitats may be a mechanism promoting coexistence between species, which avoids direct competition [55, 62].

Different factors influence the spatio-temporal relationships of virus–vector–human transmission, including vector capacity, vector competence, and host susceptibility [63]. Vector competence is defined as the intrinsic ability of the vector to successfully transmit a virus [63]. It normally comprises the capacity of a vector to acquire, maintain, and transmit a pathogen agent [64]. In contrast, vector capacity involves environmental factors such

as temperature, vertebrate host availability, vector feeding behavior, population density, longevity, and predation [63]. Scientific evidence indicates that factors such as climate, vegetation, and building density affect the distribution of both species [5, 51, 57, 65], which determines the probability of arbovirus transmission in each region. In addition, virus–vector dynamics associated with the genetic and immunology background of each population of *Aedes* and the associated microbiota, including ISVs and arboviral variants, also play essential roles in the spread of the virus by vector species [23, 66, 67].

In epidemic arboviruses such as DENV, ZIKV, and CHIKV, the urban (enzootic) cycle is essential for maintaining transmission without requiring other cycles to achieve disease persistence [8, 9, 15]. Transmission of arboviruses by vectors is carried out in various steps (Fig. 1): (1) infection of the vector (female mosquitoes) after feeding with blood from a host during the acute febrile or viremic phase of the disease [15]; (2) extrinsic incubation period, with the replication of the virus in the middle intestine of the mosquito and its spread to distal tissues, until it reaches the salivary glands (reservoir organs for the virus) [68, 69], a process influenced by the ambient temperature, the strain of the virus, immunological response of the mosquito, and the vector capacity [63]; (3) transmission of the virus from the infectious mosquito to a human during a new blood-feeding [9]; and (4) intrinsic incubation period and appearance of disease symptoms. Symptoms develop within an incubation period of 4 to 10 days after being bitten by an infected mosquito and usually last 2 to 7 days. The asymptomatic or symptomatic person can transmit the virus to a new mosquito and keep the epidemic cycle active [15, 33].

Interestingly, vertical or transovarial transmission, defined as the transfer of pathogens from the infected parent to part of the offspring [70], also occurs when the infected female mosquito transmits the virus through eggs [68] (Fig. 1). This phenomenon can occur especially during interepidemic periods and periods of drought [59, 71, 72]. In endemic areas, during unfavorable periods for horizontal transmission, the ability of some arboviruses to persist in the environment after long periods of few or no documented human cases is not clear [73, 74]. Although vertical transmission occurs at low rates, it can limit effective surveillance for infectious diseases and the achievement of comprehensive arboviral control. In particular, during the extrinsic incubation period, the genetic diversity of the virus population decreases stochastically as the virus crosses different anatomical barriers for transmission [69, 71], such as the midgut, where

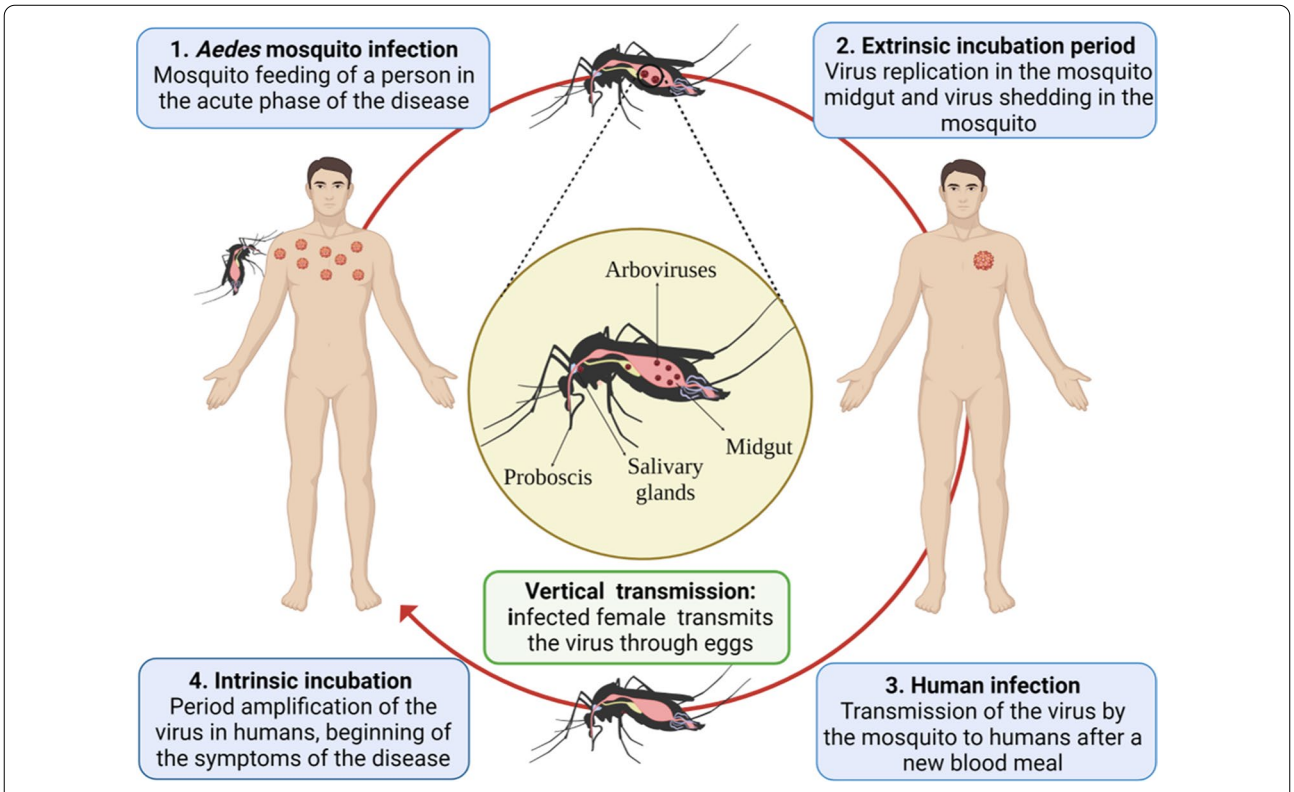

Fig. 1 The urban cycle of arboviruses in humans and mosquitoes. Figure created with BioRender.com

viral replication occurs in distal organs for dissemination, and salivary glands where transmission is finally ensured. Therefore, virus populations capable of overcoming these tissue barriers are considered to undergo positive or purifying selection where new genotypes may emerge [15, 40, 69], influencing vector competence in mosquitoes.

Microbiota and vector competence in *Ae. aegypti and Ae. albopictus*

In recent years, studies on the biology of arbovirus-transmitting insects have shown that in addition to disease-causing pathogens, mosquitoes harbor many microorganisms such as bacteria, viruses, fungi, and parasites [23, 75–77]. It has been shown that microbial composition and functionality in mosquitoes are influenced by genetic factors of the host and the environment [78]. Therefore, it is believed that mosquito microbiomes can vary substantially among individuals, life stages, species, and geographical area [36, 79]. In *Ae. aegypti* and *Ae. albopictus*, there is evidence that acquisition of the microbiota is attributed primarily to trans-stage transmission from larva to adult and consumption of water, nectar, or other environmental food sources [35, 78, 80]. The vectorial competence, blood consumption, and infection by different pathogens may persistently or transiently change the bacterial composition through alterations in the redox state of metabolism. However, despite these differences, there appears to be a "core microbiota," a collection of critical bacterial taxa that commonly colonize different mosquito species [35, 81], although the specific roles of these taxa and their relationship to vectorial competence remain unclear.

In the case of adult *Ae. aegypti* and *Ae. albopictus* mosquitoes, Proteobacteria, Bacteroides, Firmicutes, and Actinobacteria are the phyla that group more than 99% of the total components of the microbiota community [36]. Some of the bacteria associated with the different body organs of *Aedes* mosquito species are *Acetobacter*, *Burkholderia*, *Cupriavidus*, *Elizabethkingia*, *Escherichia-Shigella*, *Ochrobacterium*, *Pantoea*, *Serratia*, and *Sphingomonas* at the salivary gland level; *Asaia*, *Bacillus*, *Chryseobacterium*, *Chromobacterium*, *Cupriavidus*, *Enterobacter*, *Enterococcus*, *Klebsiella*, *Kluyvera*, *Leucobacter*, *Pantoea*, *Pichia*, *Pseudomonas*, *Serratia*, and *Sphingomonas* at the midgut level; and *Pseudomonas*, *Acinetobacter*, *Cupriavidus*, *Ochrobacterium*, *Stenotrophomonas*, and *Wolbachia* at the reproductive organ level [35, 36, 75, 82, 83]. Although the bacterial components of the mosquito microbiota are widely investigated [76, 84–86], some studies also include other entities such as fungi and yeasts identified from metagenomic shotgun sequencing [87] and targeted sequencing of the 28S marker (28S rRNA). In laboratory-reared and field-collected *Aedes*, mainly yeasts such as *Candida* and *Pichia* have been identified, as well as a variety of filamentous fungi such as *Penicillium* [35]. The protists of helminths comprising this microbiota and their influence on the insect physiology remain unknown.

Recent studies have shown that bacterial communities of adult *Ae. aegypti* and *Ae. albopictus* can play an essential role in regulating viral invasion by generating resistance or susceptibility to infection against arboviruses and other pathogens [64, 75, 84, 86, 88] (Fig. 2). Thus, some patterns of microbial regulation have been described that ultimately modulate the vectorial competence. Strategies of viral regulation include modulation of physical barriers in midgut epithelial cells (MEC), activation of immune response signaling pathways, and release of antipathogenic components. One of the symbionts that promote susceptibility to arbovirus infection is *Serratia marcescens*. This can degrade mucins bound to the intestinal membrane of *Ae. aegypti* by releasing the Sm enhancin protein, which decreases the natural protection of intestinal mucus [89] and thereby promotes the spread of pathogenic viruses such as DENV in the mosquito gut. Similarly, the fungus *Talaromyces* sp. in this same vector species can suppress the expression of digestive enzymes (trypsin) in the midgut of *Aedes* mosquitoes and, consequently, increase susceptibility to DENV infection [90] (Fig. 2).

Wolbachia, an intracellular symbiont prevalent in some insects, has been identified as capable of reducing vectorial competition by manipulating the reproduction of its host insects and generating cytoplasmic incompatibility [75]. Experimental trials show a significant reduction in the transmission of DENV, ZIKV, CHIKV, and yellow fever virus (YFV) in *Aedes* mosquitoes inoculated with this bacterium [67, 91]. Curiously, negative interactions between *Wolbachia* and arboviruses are mainly associated with enhanced insect immune response. This occurs by inducing antimicrobial peptides (AMP), melanization, and the production of reactive oxygen species (ROS) [84]. Likewise, it has been demonstrated that this microorganism, by inducing microRNA (miRNA) production, suppresses the expression of essential genes during viral genome methylation [92, 93]. In addition, *Wolbachia* can compete for resources by sequestering cholesterol and other lipids in insect cells [94], which ultimately limits arboviral infections (Fig. 2).

Interestingly, bacteria with broad-spectrum antipathogenic activity against arboviruses, such as *Chromobacterium* sp. Panama (*Csp_P*), can reduce DENV infection in *Ae. aegypti* by degrading the viral envelope protein through the production of a type of aminopeptidase [95, 96]. This bacterium can also restrict *Plasmodium falciparum* infection in *Ae. gambiae* through the antiparasitic

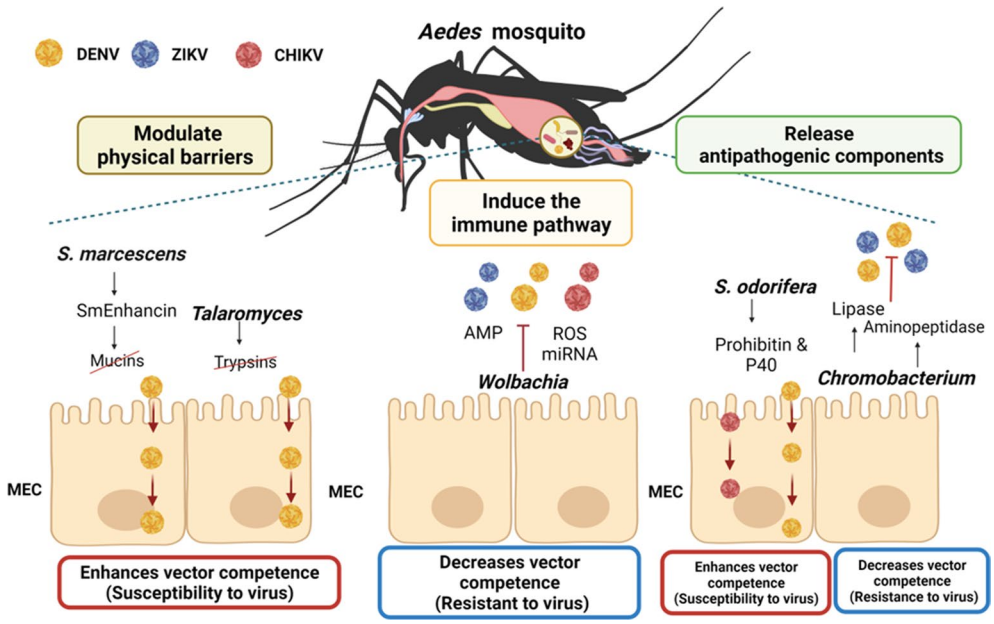

Fig. 2 *Aedes* mosquito–microbiota–arbovirus interactions may modulate vectorial competence. Some viral regulatory strategies include the following: Modulation of physical barriers in midgut epithelial cells (MEC). *Serratia marcescens* by releasing Sm enhancin protein and the fungus *Talaromyces* by suppressing the expression of digestive enzymes (trypsin) in the midgut of *Aedes* can promote susceptibility to DENV infection. Activation of immune response signaling pathways and release of antipathogenic components. *Wolbachia* in the presence of arboviruses can induce antimicrobial peptides (AMP), melanization, and the production of reactive oxygen species (ROS), among others, that restrict arboviral activity. Release of antipathogenic compounds. *Chromobacterium* sp. Panama (Csp_P), by degrading the arbovirus coat protein, limits the replication of DENV and ZIKV, while *Serratia odorifera* participates in the interaction between P40 polypeptide and prohibitin, proteins associated with DENV and CHIKV infection in *Aedes*. Red boxes indicate interactions that increase vector competence (susceptibility to virus infection). Blue boxes indicate interactions that decrease vector competence (resistance to virus infection). *CHIKV* chikungunya virus, *ZIKV* Zika virus, *DENV* dengue virus, *ROS* reactive oxygen species, *AMP* antimicrobial peptides, *miRNA* microRNA, *MEC* midgut epithelial cells, *P40* polyvinentide P40, *Pr* prohibitin. Figure created with BioRender.com

protein romidepsin [97]. In contrast, *Serratia odorifera* can increase DENV or CHIKV infection in *Ae. aegypti* due to the effect generated by the interaction between the P40 polypeptide, encoded by the bacterium, and prohibitin, a protein related to arboviral infection in mosquito cells [98, 99] (Fig. 2).

These results have opened a window for further research to understand mosquito–-microbiota interactions, their influence on arboviral infection in *Ae. aegypti* and *Ae. albopictus*, and the development of novel vector control strategies. Paratransgenesis stands out among the main strategies developed with the use of microbiota to control arboviral and vector-borne diseases [35]. This technique is based on the genetic manipulation of symbiotic bacteria to produce antipathogen effector molecules, followed by the reintroduction of the modified symbiont into the arthropod host to reduce vector competence [75]. Interestingly, genetic engineering research has evaluated molecules generated by "stable" species of the vector microbiota that are commonly present in different mosquito species ("core" microbiota).

Most of these studies have focused on persistent symbionts, capable of secreting antagonistic molecules that are horizontally and/or vertically transmitted, thus allowing self-sustainment of the modified symbionts in the field [36]. In addition, these symbionts must present the potential to survive long enough in the mosquito to ensure the effective and constant production of effectors that limit pathogen replication in the vector [35, 67, 84]. The primary investigations have studied genetic modifications of symbiont microbiota of insects such as *Rhodnius prolixus* to control the *Trypanosoma cruzi* parasite, the causative agent of Chagas disease, and *Anopheles* spp. for the control of the *Plasmodium* parasite, the causative agent of malaria [84]. In the case of *Aedes*, the bacterium *Asaia* is postulated as a promising option for arboviral control due to its ability to colonize both laboratory and field mosquitoes [35, 80, 82, 83, 100]. In addition, this bacterial species has been used in paratransgenesis for malaria control, demonstrating limitations in larval development in *Anopheles* spp. [35]. Despite the advances achieved, most studies are still in vitro trials due to the possible environmental risk that could be generated by

the release of genetically modified organisms and the potential implications of interactions between modified microorganisms and native insect vectors.

Based on advances in next-generation sequencing, especially shotgun metagenomics, much better understanding of the complexity of the *Ae. aegypti* and *Ae. albopictus* microbiota in the presence or absence of viral pathogens is expected. Currently, it is important to move towards a deeper understanding of the inherent molecular mechanisms of interaction between the microbiota, the pathogens of *Aedes* mosquitoes, and their impact on the modulation of vectorial capacity. This new metagenomic next-generation sequencing (mNGS) approach can favor the detection of microorganisms that can be exploited to develop different applications for the efficient management of *Aedes* vectors and the diseases they transmit. In addition, considering that the population structure of *Ae. aegypti* and *Ae. albopictus* is strongly influenced by geography and the type of breeding site, the anthropogenic, environmental, and geographical factors that affect acquisition should also be considered in future studies and abundance of microbial communities. This information could be used to better understand the potential of the microbiome to prevent or increase mosquitoes' ability to transmit medically important arboviral pathogens, depending on the conditions of the habitat where these vector species circulate or coexist.

Metagenomics and the rise of virome studies in mosquitoes

Metagenomic next-generation sequencing (mNGS), also known as shotgun deep-sequencing, is a high-throughput sequencing strategy with high efficiency and a short turnaround time [101]. mNGS is defined as "the application of the modern genomics without the need for isolation and laboratory culture of individual species" [102], and facilitates the understanding of the composition of all genetic material (DNA or RNA) in a clinical or environmental sample [103]. The deployment of mNGS has identified structural and functional diversity of vertebrates and invertebrates microbiomes [35, 78, 104]. In the case of viral communities in insects, even recently there was a "biased" conception of viruses as agents that cause disease; however, today, with the advances in metagenomics studies, the proper structural and functional diversity of insect viromes has begun to be elucidated [104–106]. Thus, metagenomics methods, especially during the last decade, have provided new insights into the complexity of insect-borne viruses [41, 107–109], relevant for active pathogen surveillance and response to emerging and re-emerging infectious diseases [37, 103, 110, 111].

Metagenomic studies in mosquito vectors are relatively recent. This technique is one of the most common sequencing approaches for the characterization of mosquito microbial communities and the description of phylogenetic relationships [35, 100, 112]. With this new approach, it has been possible to elucidate that *Ae. aegypti* and *Ae. albopictus* mosquitoes harbor a rich and diverse virome composed mainly of ISVs [39, 107, 113–115]. Unlike arboviruses, which have dual host tropism (vertebrates and arthropod vectors), ISVs replicate exclusively in insect populations and cannot replicate in vertebrate cells or infect humans [41, 116, 117]. Therefore, being naturally associated with arthropods, they can be considered members of viral communities of insects exclusively (viromes), and are not considered pathogens [104, 117]. In adult insects, the highest proportion of ISVs characterized so far correspond to RNA viruses [104] without causing apparent affectation, suggesting their high adaptation and relationship with insects [104].

Metaviromic analyses in mosquitoes carried out recently show a significant increase in the number of specific viral or ISV sequences, both in natural populations and in mosquito-derived cell lines [37, 116, 118]. These findings indicate a higher abundance and prevalence of ISVs in mosquito populations than in arboviruses in approximately 1–2% of individuals [34]. Research on ISVs in mosquito vectors has focused mainly on *Culex* spp. mosquitoes, *Aedes* spp., and *Anopheles* spp. [109, 115, 119–121]. Phylogenetic analyses on the reconstruction of ancestral traits in ISVs indicate that there are variations in the diversity and abundance of viruses between vector species; however, viral families such as *Flaviviridae* (positive-sense [+] single-stranded RNA [ssRNA]), *Bunyaviridae* (negative-sense ssRNA [−]), *Rhabdoviridae* (ssRNA), *Reoviridae* (double-stranded RNA [dsRNA]), and *Togaviridae* (+ssRNA) are shared [34, 37–39, 122]. These discoveries have opened a new view on the diversity and evolution of ISVs [38, 39, 116] and their influence on vectorial competence [123] for efficient transmission of human pathogens (arboviruses) [118], in addition to their potential use as biological control agents or new vaccine platforms [44, 45] (Fig. 3).

Phylogenetic analyses and experimental studies have shown that many ISVs isolated from mosquitoes contain ancient and diverse lineages, which possibly evolved and diversified with their host insects [122, 124]. Research on the reconstruction of ancestral traits in ISVs of *Bunyavirales* has evidenced a basal phylogenetic relationship between the dual-host bunyavirus and insect-specific ancestors [125]. The close relationship found between ISVs and human pathogenic arboviruses [38, 39, 122] has generated the hypothesis about the role they can play in modulating arboviral transmission [44, 45] (Fig. 3). ISVs

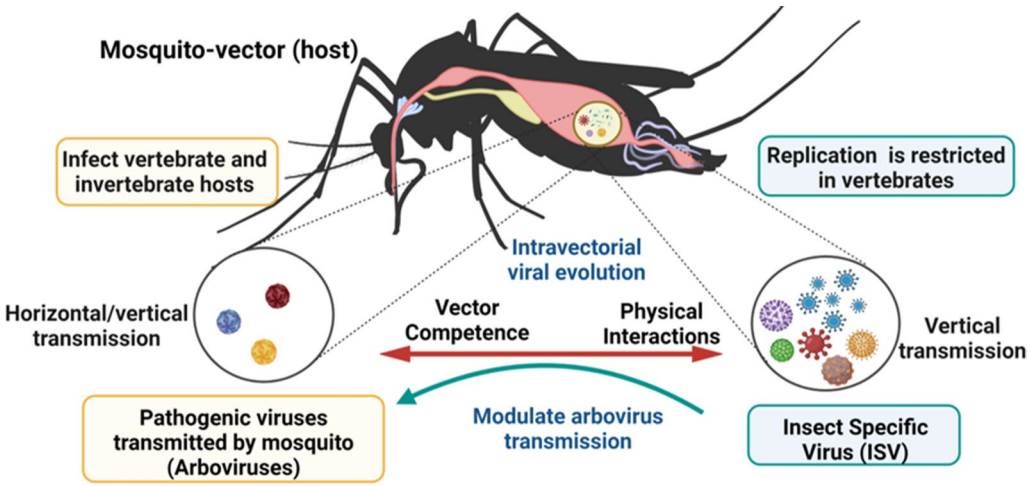

Fig. 3 Schematic summary of some characteristics of the *Aedes* mosquito virome from metagenomic studies. The *Aedes* virome is formed by arboviruses and in greater proportion by insect-specific viruses (ISV). Arboviruses are pathogenic viruses that are transmitted to humans by mosquito vectors (dual host), while ISVs are viruses that replicate exclusively in insects and are not capable of infecting humans (single host). ISVs possibly evolved and diversified with their insect hosts. Some ISV species could potentially modulate vector competence in *Aedes* spp. Advances in vector viromics may contribute to the development of strategies to control and prevent arboviral diseases. Figure created with BioRender.com

are found at all stages of life in both male and female mosquitoes. This is associated with their efficient transmission to offspring (transovarial transmission) [104, 118] and coexistence with their insect host over a long period [39, 124]. In addition, there is evidence of the presence of viral RNA in the insect transcriptome and a high incidence of endogenous copies in the insect genome, suggesting a crucial role of ISV in the evolution of RNA viruses [126]. In this way, it could be inferred that, if ISVs are ancestral to arboviruses, they could be studied to understand the evolution from a single host to a dual host, as well as to elucidate the factors that influence the "*switch*" of viruses from arthropods to viruses with emerging potential.

Virome in *Ae. aegypti* and *Ae. albopictus*

Metagenomics studies in *Ae. aegypti* and *Ae. albopictus* conducted in different geographical areas of the world (Americas, Asia, Africa, Europe, and Australia) [73, 107, 121, 127–134] have identified that *Aedes* host a viral community with high diversity. Currently, the available data focus mainly on *Ae. aegypti*, compared with the virome of *Ae. albopictus*, possibly because it is considered the primary vector of arboviruses and with a vast geographical distribution [23, 107, 114]. It should be noted that most of the viruses discovered lack a formal taxonomic classification (unclassified viruses), which limits the proper understanding of the diversity of the circulating virome in these vector species. This condition highlights the need to generate more robust databases allowing us to improve the viral characterization of mosquitoes that transmit infectious diseases.

Curiously, in these investigations, similarity in the composition of the virome has been evidenced in *Aedes* species, made up mainly of the families *Flaviviridae*, *Totiviridae*, *Phenuiviridae*, *Orthomyxoviridae*, *Virgaviridae*, and *Secoviridae*. In addition, this research highlights the conservation of viral species in the *Aedes* virome, such as Phasi Charoen-like virus (PCLV) (*Phenuiviridae*), Humaita-Tubiacanga (HTV) (unclassified viruses), Guadeloupe mosquito virus (GMV)/Wenzhou sobemo-like virus 4 (unclassified viruses), cell fusing agent virus (CFAV; *Flaviviridae*), Guadeloupe mosquito quaranja-like virus 1/Aedes alboannulatus orthomyxo-like virus (*Orthomyxoviridae*), and Australian Anopheles totivirus (*Totiviridae*) (Fig. 4), demonstrating the presence of a "core virome," perhaps associated with its ecology, similar food sources, selective host pressures, and microbial interactions [67, 112]. Therefore, it is considered that the "core virome" in *Ae. aegypti* and *Ae. albopictus* likely plays a role in mosquito homeostasis and may also have implications in vector competition for arbovirus transmission [39, 118, 132]. Future studies are needed to fully understand these complex interactions.

In most metavirome studies carried out so far, the viral families *Flaviviridae*, *Orthomyxoviridae*, *Totiviridae*, and *Phenuiviridae* have been the most prevalent (Fig. 4). This suggests a possible origin from the ancestral *Aedes* mosquito and evolution with the vector in different parts of the planet. This is consistent with several authors that propose processes of co-evolution and diversification

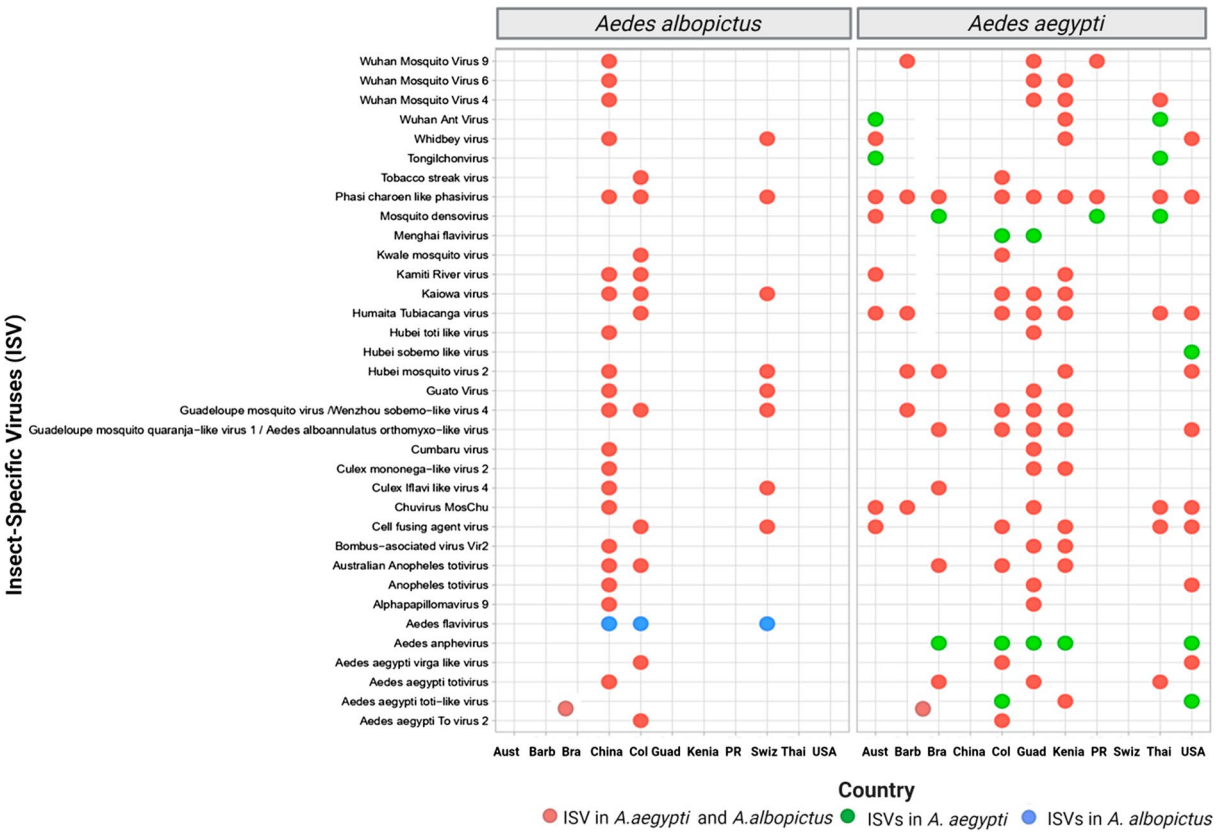

Fig. 4 Conservation of insect-specific viruses (ISV) (virome) in *Ae. aegypti* and *Ae. albopictus* in countries of different geographical areas of the world. Pink circles indicate the presence of shared ISVs in *Ae. aegypti* and *Ae. albopictus*, yellow circles indicate unique ISVs in *Ae. aegypti*, and blue circles indicate unique ISVs in *Ae. albopictus*. *Aust* Australia, *Barb* Barbados, *Bra* Brazil, *Nig* Nigeria, *Guad* Guadeloupe, *Ken* Kenya, *PR* Puerto Rico, *Suiz* Switzerland, *Tha* Thailand, *USA* United States of America

between ISVs and the insect [113, 116, 121, 135], as previously suggested for the families *Bunyaviridae*, *Flaviviridae*, and *Rhabdoviridae* [38, 123]. In particular, the family *Totiviridae* has been mainly associated with fungi and plants [136]. However, its prevalence has increased among arthropods, possibly due to horizontal virus transfer mechanisms [132]. According to Shi et al. [137], interspecies virus transmission is a joint event across the ISV landscape, and it has likely been a significant factor in the evolutionary history of such viruses. Considering this new vision on the virome of *Ae. aegypti* and *Ae. albopictus* mosquitoes, subsequent studies could focus their attention on investigating how these viruses could influence mosquito–virus–pathogen interactions in the dynamics of arbovirus–insect transmission.

Some investigations of viral communities in *Ae. aegypti* and *Ae. albopictus* also show a stable virome profile at different life stages (larva, pupa, and adult), grown in the laboratory and collected in the field [107, 127]. In this way, several studies suggest that the "core virome" can be acquired vertically (from parent to offspring) [127] or from the environment [127]. Accordingly, studies by Coatsworth et al. [132] confirmed the vertical transfer of ISVs in *Ae. aegypti* by metagenomic studies conducted over several generations. Similarly, Thannesberger et al. [128] showed that the local ecosystem could play a preponderant role in the composition of viral communities in mosquito vectors. Interestingly, most metaviromic analyses in *Aedes* mosquitoes collected from the field show the presence of a "core virome" with more extraordinary diversity than the virome of laboratory mosquitoes. This is probably associated with the variable geographical and environmental conditions found in nature versus the standardized conditions of water and resources available in the laboratory [37, 129, 130]. The evident contrast between the scenarios in which field and laboratory mosquitoes are exposed could reflect the viral diversity of their respective environments [132]. Thus, future investigations could identify the impact of different biological and environmental variables on ISVs.

Comparative analyses between the virome of *Ae. aegypti* and *Ae. albopictus* show differences in viral

composition and diversity. Metagenomic studies show a higher ISV richness in *Ae. aegypti* [73, 107, 128, 131, 132], related to its high susceptibility to arbovirus infection [23, 29, 63]. In contrast, the virome of *Ae. albopictus* presents a wide diversity of viruses associated with vertebrates, insects, plants, bacteria, and fungi [24, 127, 129, 130], which can probably be explained by the different cycle, ecotope, and environmental factors, including breeding and feeding sites [29, 80]. In addition, it must be highlighted that specific viral species have been identified for each vector and geographical region. However, unique ISVs have been detected in Aedes anphevirus (AeAV; *Xinmoviridae*) and Aedes aegypti totivirus (*Totiviridae*), while in *Ae. albopictus* only the ISV Aedes flavivirus (AeFV; *Flaviviridae*) has been found. These differences in the virome composition of these vectors could reflect essential differences in evolutionary history and host immune responses [78, 81] and differences in virus–mosquito interactions, potentially related to vector competence [35, 45, 112].

ISVs seems to modulate arbovirus infections in *Ae. aegypti and Ae. albopictus*

Considering the role of some symbiont members of the microbiota of insect vectors, it is considered that some ISVs may also have a similar effect on vector competition by suppressing or enhancing arbovirus replication in insect vectors [45, 104, 111, 117]. Some authors have proposed that mosquito-associated ISVs could have potential applications as (i) biological control agents against vector-borne diseases, (ii) diagnostic therapies, and (iii) new vaccine platforms [34, 35, 44, 45, 104, 117, 118].

The first characterized ISVs belong to the genus *Flavivirus*, as the CFAV, isolated from a culture in a cell line of *Ae. aegypti* [138]. This virus can also replicate in *Ae. albopictus* cell lines; however, it does not show a cytopathic effect on invertebrate cell lines. Recent studies by Zhang et al. [139] found that CFAV infection significantly improved DENV replication, possibly due to an increase in the expression of ribonuclease kappa (RNASEK), known to promote infection of endocytosis-dependent viruses and pH-dependent entry. The authors indicate that increased CFAV-induced RNASEK expression will likely contribute to improved DENV replication in CFAV-infected cells [139].

Research conducted by Schultz et al. [42] examined cell lines of *Aedes* species. The suppression of the arbovirus in the presence of ISV CFAV and PCLV demonstrates that dual ISV infection managed to decrease the growth of ZIKV, DENV, and La Crosse encephalitis virus (LACV) by up to 90% in immunocompetent cells of *Ae. albopictus* and *Ae. aegypti*. Another study characterized Aedes anphevirus (AeAV), a negative-sense RNA virus of the order *Mononegavirales*, capable of infecting laboratory colonies, wild mosquitoes, and cell lines of *Ae. aegypti* and *Ae. albopictus* worldwide [140]. It was identified that *Ae. aegypti* cells, co-infected with AeAV and *Wolbachia*, improved AeAV replication and slightly reduced DENV replication in vitro [140]. These data suggest that there are mechanisms of viral competition [141] as exclusion by superinfection. They may involve competition or modification of cellular resources that reduce receptor binding, viral entry, RNA replication, and translation of the secondary virus [42, 45, 142] or mechanisms of both positive and negative regulation of the antiviral immune response of the vector [39].

A similar study evaluated Nhumirim virus (NHUV) (*Flaviviridae*) pre-inoculated or inoculated simultaneously with ZIKV and dengue-2 virus (DENV-2) in *Ae. albopictus* cells, showing a significant reduction of these viruses [43]. Additionally, trials with individuals of *Ae. aegypti* also demonstrated decreased ZIKV infection rates in mosquitoes inoculated with NHUV compared with those not exposed [43]. Likewise, in *Culex quinquefasciatus,* a decrease in the transmission rates of West Nile virus (WNV) was observed when the vector was previously exposed to NHUV [143]. These results indicate that some ISV species could modulate vector competition in *Aedes* spp. and *Culex*.

On the other hand, Nazar et al. [144] investigated the ability of Eilat virus (EILV), an ISV of the *Togaviridae* family, to interfere with viruses of the same family, such as Sindbis virus (SINV), CHIKV, western equine encephalitis viruses (WEEV), eastern equine encephalitis virus (EEEV), and Venezuelan equine encephalitis virus (VEEV). In vitro results in *Ae. aegypti* C7/10 cells showed that EILV infection reduced replication of pathogenic viruses regardless of virus or multiplicity of infection. In addition, in vivo trials in *Aedes* mosquitoes pre-inoculated with EILV and fed blood containing CHIKV also showed a reduction in the rate of infection and the spread of CHIKV [144]. These results suggest different interactions between ISVs and arboviruses, such as competitive inhibition and superinfection exclusion [123]. However, it should be noted that studies on the relationship between ISV–arbovirus–microbiota of insects are only just emerging. Therefore, new investigations are needed to better understand this type of interaction, which may result in the development of new approaches for the control and prevention of arboviral transmission.

Conclusions

Aedes aegypti and *Ae. albopictus* mosquitoes that transmit viruses of medical and economic importance, such as DENV, CHIKV, and ZIKV, among others, continue to be a major threat to global public health. Currently, the geographical spread of these mosquito vectors, and thus of arboviruses, is increasingly extending to new regions under the influence of multiple social, environmental, and ecological factors. In recent years, shotgun metagenomic sequencing has improved our understanding of the composition and, in part, the functionality of the *Aedes* microbiota/virome. These advances have revealed the critical role that some of the resident microorganisms play in host fitness, especially in the presence and absence of viral pathogens. It is known that *Ae. aegypti* and *Ae. albopictus* share a core set of microorganisms, especially bacterial and viral. However, many aspects of this area of knowledge remain unclear. Therefore, understanding the true diversity of the mosquito microbiome and its interactions with the host, as well as its dynamics in the face of arbovirus infection events, is critical, given the evidence as potential biological control agents. Recent metavirological analyses indicate that the "core virome" of *Ae. aegypti* and *Ae. albopictus*, composed mainly of ISVs, has the potential to alter the susceptibility of mosquitoes to certain arboviruses, as well as to show evolutionary relationships with these pathogens. Further studies, especially of ISVs, including isolation, whole-genome sequencing, and even functional assays, are thus warranted to better understand their origins, pathogenic potential, molecular mechanisms affecting vector competence, and potential biotechnological applications.

It is therefore emphasized that microbiota–ISV–arbovirus interactions in the mosquito (host) form an ecologically complex system, influenced by the geographical and environmental conditions to which the vector is exposed. For this reason, future analyses should also consider the anthropogenic, environmental, and geographical factors that influence the acquisition and abundance of the microbial communities of *Ae. aegypti* and *Ae. albopictus*, and identify the change in the composition and diversity of the microbiota/virome in the different life stages of *Aedes* (pupa, larva and adult), at both the field and laboratory levels. This information could be used to better understand the potential of the microbiota to prevent or enhance the mosquito's ability to transmit arboviral pathogens of medical importance, depending on the habitat conditions where these vector species circulate or coexist. Finally, it is concluded that the path is open for further strengthening of omics and bioinformatics sciences, towards a better understanding of *Aedes* insect biology and arbovirus epidemiology, and the development of potential "novel" arboviral intervention strategies.

Abbreviations

ISV: Insect-specific viruses; AMP: Antimicrobial peptides; CHIKV: Chikungunya virus; DENV: Dengue virus; DDT: Dichlorodiphenyltrichloroethane; miRNA: MicroRNA; MEC: Midgut epithelial cells; PCR: Polymerase chain reaction; P40: Polyvinentide P40; Pr: Prohibitin.; ROS: Reactive oxygen species; ZIKV: Zika virus.

Acknowledgements

We thank the Dirección de Investigación e Innovación from Universidad del Rosario for covering the publication fees of this manuscript.

Author contributions

JDR conceived the article. MG drafted the manuscript, with contributions from DM, MM and JDR. All authors read and approved the final manuscript.

Funding

Open Access funding provided by Colombia Consortium. This work was supported by Dirección de Investigación e Innovación from Universidad del Rosario.

Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

All authors declare no conflict of interest.

Author details

[1]Centro de Investigaciones en Microbiología y Biotecnología-UR (CIMBIUR), Facultad de Ciencias Naturales, Universidad del Rosario, Bogotá, Colombia. [2]Grupo de Investigación en Ciencias Básicas (NÚCLEO) Facultad de Ciencias e Ingeniería, Universidad de Boyacá, Tunja, Colombia. [3]Molecular Microbiology Laboratory, Department of Pathology, Molecular and Cell-Based Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, USA.

Received: 12 May 2022 Accepted: 14 July 2022
Published online: 09 August 2022

References

1. Golding N, Wilson AL, Moyes CL, Cano J, Pigott DM, Velayudhan R, et al. Integrating vector control across diseases. BMC Med. 2015;13:249.
2. World Health Organization. Global vector control response 2017–2030. Geneva: World Health Organization; 2017.
3. Mboera LE, Mweya CN, Rumisha SF, Tungu PK, Stanley G, Makange MR, et al. The risk of dengue virus transmission in Dar es Salaam, Tanzania during an epidemic period of 2014. PLoS Negl Trop Dis. 2016;10:e0004313.
4. Guzman MG, Harris E. Dengue. Lancet. 2015;385:453–65.
5. Kraemer MUG, Reiner RC Jr, Brady OJ, Messina JP, Gilbert M, Pigott DM, et al. Past and future spread of the arbovirus vectors *Aedes aegypti* and *Aedes albopictus*. Nat Microbiol. 2019;4:854–63.
6. Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, et al. Global trends in emerging infectious diseases. Nature. 2008;451:990–3.
7. Paixao ES, Teixeira MG, Rodrigues LC. Zika, chikungunya and dengue: the causes and threats of new and re-emerging arboviral diseases. BMJ Glob Health. 2018;3:e000530.
8. Young PR. Arboviruses: a family on the move. Adv Exp Med Biol. 2018;1062:1–10.

9. Gould E, Pettersson J, Higgs S, Charrel R, de Lamballerie X. Emerging arboviruses: Why today? One Health. 2017;4:1–13.
10. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global distribution and burden of dengue. Nature. 2013;496:504–7.
11. Murray NE, Quam MB, Wilder-Smith A. Epidemiology of dengue: past, present and future prospects. Clin Epidemiol. 2013;5:299–309.
12. Wilder-Smith A, Ooi EE, Vasudevan SG, Gubler DJ. Update on dengue: epidemiology, virus evolution, antiviral drugs, and vaccine development. Curr Infect Dis Rep. 2010;12:157–64.
13. Patterson J, Sammon M, Garg M. Dengue, Zika and Chikungunya: emerging arboviruses in the new world. West J Emerg Med. 2016;17:671–9.
14. Sukhralia S, Verma M, Gopirajan S, Dhanaraj PS, Lal R, Mehla N, et al. From dengue to Zika: the wide spread of mosquito-borne arboviruses. Eur J Clin Microbiol Infect Dis. 2019;38:3–14.
15. Guzman MG, Gubler DJ, Izquierdo A, Martinez E, Halstead SB. Dengue infection. Nat Rev Dis Primers. 2016;2:16055.
16. Pollett S, Melendrez MC, Maljkovic Berry I, Duchene S, Salje H, Cummings DAT, et al. Understanding dengue virus evolution to support epidemic surveillance and counter-measure development. Infect Genet Evol. 2018;62:279–95.
17. Weaver SC, Charlier C, Vasilakis N, Lecuit M. Zika, Chikungunya, and other emerging vector-borne viral diseases. Annu Rev Med. 2018;69:395–408.
18. Ketkar H, Herman D, Wang P. Genetic determinants of the re-emergence of arboviral diseases. Viruses. 2019;11:150.
19. Jones R, Kulkarni MA, Davidson TMV. Arbovirus vectors of epidemiological concern in the Americas: a scoping review of entomological studies on Zika, dengue and chikungunya virus vectors. PLoS ONE. 2020;15:e0220753.
20. Kraemer MU, Sinka ME, Duda KA, Mylne AQ, Shearer FM, Barker CM, et al. The global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. albopictus*. Elife. 2015;4:e08347.
21. Leta S, Beyene TJ, De Clercq EM, Amenu K, Kraemer MUG, Revie CW. Global risk mapping for major diseases transmitted by *Aedes aegypti* and *Aedes albopictus*. Int J Infect Dis. 2018;67:25–35.
22. Espinal MA, Andrus JK, Jauregui B, Waterman SH, Morens DM, Santos JI, et al. Emerging and reemerging *Aedes*-transmitted arbovirus infections in the region of the Americas: implications for health policy. Am J Public Health. 2019;109:387–92.
23. Souza-Neto JA, Powell JR, Bonizzoni M. *Aedes aegypti* vector competence studies: a review. Infect Genet Evol. 2019;67:191–209.
24. Paupy C, Delatte H, Bagny L, Corbel V, Fontenille D. *Aedes albopictus*, an arbovirus vector: from the darkness to the light. Microbes Infect. 2009;11:1177–85.
25. Brady OJ, Golding N, Pigott DM, Kraemer MU, Messina JP, Reiner RC Jr, et al. Global temperature constraints on *Aedes aegypti* and *Ae. albopictus* persistence and competence for dengue virus transmission. Parasit Vectors. 2014;7:338.
26. Ryan SJ, Carlson CJ, Mordecai EA, Johnson LR. Global expansion and redistribution of Aedes-borne virus transmission risk with climate change. PLoS Negl Trop Dis. 2019;13:e0007213.
27. Goindin D, Delannay C, Ramdini C, Gustave J, Fouque F. Parity and longevity of *Aedes aegypti* according to temperatures in controlled conditions and consequences on dengue transmission risks. PLoS ONE. 2015;10:e0135489.
28. Morens DM, Folkers GK, Fauci AS. The challenge of emerging and re-emerging infectious diseases. Nature. 2004;430:242–9.
29. Kraemer MU, Sinka ME, Duda KA, Mylne A, Shearer FM, Brady OJ, et al. The global compendium of *Aedes aegypti* and *Ae. albopictus* occurrence. Sci Data. 2015;2:150035.
30. Mayer SV, Tesh RB, Vasilakis N. The emergence of arthropod-borne viral diseases: a global prospective on dengue, chikungunya and zika fevers. Acta Trop. 2017;166:155–63.
31. Liu-Helmersson J, Quam M, Wilder-Smith A, Stenlund H, Ebi K, Massad E, et al. Climate change and *Aedes* vectors: 21st century projections for dengue transmission in Europe. EBioMedicine. 2016;7:267–77.
32. Morales D, Ponce P, Cevallos V, Espinosa P, Vaca D, Quezada W. Resistance status of *Aedes aegypti* to deltamethrin, malathion, and temephos in Ecuador. J Am Mosq Control Assoc. 2019;35:113–22.
33. Conway MJ, Colpitts TM, Fikrig E. Role of the vector in arbovirus transmission. Annu Rev Virol. 2014;1:71–88.
34. de Almeida JP, Aguiar ER, Armache JN, Olmo RP, Marques JT. The virome of vector mosquitoes. Curr Opin Virol. 2021;49:7–12.
35. Scolari F, Casiraghi M, Bonizzoni M. *Aedes* spp. and their microbiota: a review. Front Microbiol. 2019;10:2036.
36. Mancini MV, Damiani C, Accoti A, Tallarita M, Nunzi E, Cappelli A, et al. Estimating bacteria diversity in different organs of nine species of mosquito by next generation sequencing. BMC Microbiol. 2018;18:126.
37. Atoni E, Zhao L, Karungu S, Obanda V, Agwanda B, Xia H, et al. The discovery and global distribution of novel mosquito-associated viruses in the last decade (2007–2017). Rev Med Virol. 2019;29:e2079.
38. Roundy CM, Azar SR, Rossi SL, Weaver SC, Vasilakis N. Insect-specific viruses: a historical overview and recent developments. Adv Virus Res. 2017;98:119–46.
39. Ohlund P, Lunden H, Blomstrom AL. Insect-specific virus evolution and potential effects on vector competence. Virus Genes. 2019;55:127–37.
40. Halbach R, Junglen S, van Rij RP. Mosquito-specific and mosquito-borne viruses: evolution, infection, and host defense. Curr Opin Insect Sci. 2017;22:16–27.
41. Bolling BG, Vasilakis N, Guzman H, Widen SG, Wood TG, Popov VL, et al. Insect-specific viruses detected in laboratory mosquito colonies and their potential implications for experiments evaluating arbovirus vector competence. Am J Trop Med Hyg. 2015;92:422–8.
42. Schultz MJ, Frydman HM, Connor JH. Dual Insect specific virus infection limits Arbovirus replication in Aedes mosquito cells. Virology. 2018;518:406–13.
43. Romo H, Kenney JL, Blitvich BJ, Brault AC. Restriction of Zika virus infection and transmission in *Aedes aegypti* mediated by an insect-specific flavivirus. Emerg Microbes Infect. 2018;7:181.
44. Nouri S, Matsumura EE, Kuo YW, Falk BW. Insect-specific viruses: from discovery to potential translational applications. Curr Opin Virol. 2018;33:33–41.
45. Patterson EI, Villinger J, Muthoni JN, Dobel-Ober L, Hughes GL. Exploiting insect-specific viruses as a novel strategy to control vector-borne disease. Curr Opin Insect Sci. 2020;39:50–6.
46. Lozano-Fuentes S, Kenney JL, Varnado W, Byrd BD, Burkhalter KL, Savage HM. Susceptibility and vectorial capacity of American *Aedes albopictus* and *Aedes aegypti* (Diptera: Culicidae) to American Zika virus strains. J Med Entomol. 2019;56:233–40.
47. Shragai T, Tesla B, Murdock C, Harrington LC. Zika and chikungunya: mosquito-borne viruses in a changing world. Ann N Y Acad Sci. 2017;1399:61–77.
48. Braks MA, Honorio NA, Lourencqo-De-Oliveira R, Juliano SA, Lounibos LP. Convergent habitat segregation of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in southeastern Brazil and Florida. J Med Entomol. 2003;40:785–94.
49. Dos Santos AP, Urbinatti PR, da Rocha CR, de Sa Almeida RMM, Ribeiro SS, de Lima-Camara TN. Parity and gonotrophic discordance of females of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in the city of Sao Paulo, SP, Brazil. J Vector Ecol. 2019;44:233–40.
50. Bonizzoni M, Gasperi G, Chen X, James AA. The invasive mosquito species *Aedes albopictus*: current knowledge and future perspectives. Trends Parasitol. 2013;29:460–8.
51. Ciota AT, Chin PA, Ehrbar DJ, Micieli MV, Fonseca DM, Kramer LD. Differential effects of temperature and mosquito genetics determine transmissibility of arboviruses by *Aedes aegypti* in Argentina. Am J Trop Med Hyg. 2018;99:417–24.
52. Delatte H, Desvars A, Bouetard A, Bord S, Gimonneau G, Vourc'h G, et al. Blood-feeding behavior of *Aedes albopictus*, a vector of Chikungunya on La Reunion. Vector Borne Zoonotic Dis. 2010;10:249–58.
53. Delatte H, Gimonneau G, Triboire A, Fontenille D. Influence of temperature on immature development, survival, longevity, fecundity, and gonotrophic cycles of *Aedes albopictus*, vector of chikungunya and dengue in the Indian Ocean. J Med Entomol. 2009;46:33–41.
54. Coleman M, Hemingway J, Gleave KA, Wiebe A, Gething PW, Moyes CL. Developing global maps of insecticide resistance risk to improve vector control. Malar J. 2017;16:86.

55. Juliano SA, Lounibos LP, O'Meara GF. A field test for competitive effects of *Aedes albopictus* on *A. aegypti* in South Florida: differences between sites of coexistence and exclusion? Oecologia. 2004;139:583–93.
56. Kamgang B, Wilson-Bahun TA, Irving H, Kusimo MO, Lenga A, Wondji CS. Geographical distribution of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) and genetic diversity of invading population of *Ae. albopictus* in the Republic of the Congo. Wellcome Open Res. 2018;3:79.
57. Tedjou AN, Kamgang B, Yougang AP, Njiokou F, Wondji CS. Update on the geographical distribution and prevalence of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae), two major arbovirus vectors in Cameroon. PLoS Negl Trop Dis. 2019;13:e0007137.
58. Lwande OW, Obanda V, Lindstrom A, Ahlm C, Evander M, Naslund J, et al. Globe-trotting *Aedes aegypti* and *Aedes albopictus*: risk factors for arbovirus pandemics. Vector Borne Zoonotic Dis. 2020;20:71–81.
59. Ferreira-de-Lima VH, Camara DCP, Honorio NA, Lima-Camara TN. The Asian tiger mosquito in Brazil: Observations on biology and ecological interactions since its first detection in 1986. Acta Trop. 2020;205:105386.
60. Ruckert C, Weger-Lucarelli J, Garcia-Luna SM, Young MC, Byas AD, Murrieta RA, et al. Impact of simultaneous exposure to arboviruses on infection and transmission by *Aedes aegypti* mosquitoes. Nat Commun. 2017;8:15412.
61. Wilson-Bahun TA, Kamgang B, Lenga A, Wondji CS. Larval ecology and infestation indices of two major arbovirus vectors, *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae), in Brazzaville, the capital city of the Republic of the Congo. Parasit Vectors. 2020;13:492.
62. Camara DC, Codeco CT, Juliano SA, Lounibos LP, Riback TI, Pereira GR, et al. Seasonal differences in density but similar competitive impact of *Aedes albopictus* (Skuse) on *Aedes aegypti* (L.) in Rio de Janeiro, Brazil. PLoS ONE. 2016;11:e0157120.
63. Alonso-Palomares LA, Moreno-Garcia M, Lanz-Mendoza H, Salazar MI. Molecular basis for arbovirus transmission by *Aedes aegypti* mosquitoes. Intervirology. 2018;61:255–64.
64. Cansado-Utrilla C, Zhao SY, McCall PJ, Coon KL, Hughes GL. The microbiome and mosquito vectorial capacity: rich potential for discovery and translation. Microbiome. 2021;9:111.
65. Carrington LB, Seifert SN, Armijos MV, Lambrechts L, Scott TW. Reduction of *Aedes aegypti* vector competence for dengue virus under large temperature fluctuations. Am J Trop Med Hyg. 2013;88:689–97.
66. Viglietta M, Bellone R, Blisnick AA, Failloux AB. Vector specificity of arbovirus transmission. Front Microbiol. 2021;12:773211.
67. Gabrieli P, Caccia S, Varotto-Boccazzi I, Arnoldi I, Barbieri G, Comandatore F, et al. Mosquito trilogy: microbiota, immunity and pathogens, and their implications for the control of disease transmission. Front Microbiol. 2021;12:630438.
68. Gubler DJ. Human arbovirus infections worldwide. Ann N Y Acad Sci. 2001;951:13–24.
69. Ruckert C, Ebel GD. How do virus-mosquito interactions lead to viral emergence? Trends Parasitol. 2018;34:310–21.
70. Lequime S, Paul RE, Lambrechts L. Determinants of arbovirus vertical transmission in mosquitoes. PLoS Pathog. 2016;12:e1005548.
71. Harapan H, Michie A, Sasmono RT. Dengue: a minireview. Viruses. 2020;12:829.
72. da Costa CF, Dos Passos RA, Lima JBP, Roque RA, de Souza SV, Campolina TB, et al. Transovarial transmission of DENV in *Aedes aegypti* in the Amazon basin: a local model of xenomonitoring. Parasit Vectors. 2017;10:249.
73. Boyles SM, Mavian CN, Finol E, Ukhanova M, Stephenson CJ, Hamerlinck G, et al. Under-the-radar dengue virus infections in natural populations of *Aedes aegypti* mosquitoes. MSphere. 2020. https://doi.org/10.1128/mSphere.00316-20.
74. Ferreira-de-Lima VH, Lima-Camara TN. Natural vertical transmission of dengue virus in *Aedes aegypti* and *Aedes albopictus*: a systematic review. Parasit Vectors. 2018;11:77.
75. Gao H, Cui C, Wang L, Jacobs-Lorena M, Wang S. Mosquito microbiota and implications for disease control. Trends Parasitol. 2020;36:98–111.
76. Huang W, Wang S, Jacobs-Lorena M. Use of microbiota to fight mosquito-borne disease. Front Genet. 2020;11:196.
77. Villegas LEM, Campolina TB, Barnabe NR, Orfano AS, Chaves BA, Norris DE, et al. Zika virus infection modulates the bacterial diversity associated with *Aedes aegypti* as revealed by metagenomic analysis. PLoS ONE. 2018;13:e0190352.
78. Strand MR. Composition and functional roles of the gut microbiota in mosquitoes. Curr Opin Insect Sci. 2018;28:59–65.
79. Muturi EJ, Ramirez JL, Rooney AP, Kim CH. Comparative analysis of gut microbiota of mosquito communities in central Illinois. PLoS Negl Trop Dis. 2017;11:e0005377.
80. Scolari F, Sandionigi A, Carlassara M, Bruno A, Casiraghi M, et al. Exploring changes in the microbiota of *Aedes albopictus*: comparison among breeding site water, larvae, and adults. Front Microbiol. 2021;12:624170.
81. Caragata EP, Tikhe CV, Dimopoulos G. Curious entanglements: interactions between mosquitoes, their microbiota, and arboviruses. Curr Opin Virol. 2019;37:26–36.
82. Diaz S, Camargo C, Avila FW. Characterization of the reproductive tract bacterial microbiota of virgin, mated, and blood-fed *Aedes aegypti* and *Aedes albopictus* females. Parasit Vectors. 2021;14:592.
83. Mohlmann TWR, Vogels CBF, Goertz GP, Pijlman GP, Ter Braak CJF, Beest DE, et al. Impact of gut bacteria on the infection and transmission of pathogenic arboviruses by biting midges and mosquitoes. Microb Ecol. 2020;80:703–17.
84. Hegde S, Rasgon JL, Hughes GL. The microbiome modulates arbovirus transmission in mosquitoes. Curr Opin Virol. 2015;15:97–102.
85. Coon KL, Vogel KJ, Brown MR, Strand MR. Mosquitoes rely on their gut microbiota for development. Mol Ecol. 2014;23:2727–39.
86. Guegan M, Zouache K, Demichel C, Minard G, Van Tran V, Potier P, et al. The mosquito holobiont: fresh insight into mosquito-microbiota interactions. Microbiome. 2018;6:49.
87. Chandler JA, Liu RM, Bennett SN. RNA shotgun metagenomic sequencing of northern California (USA) mosquitoes uncovers viruses, bacteria, and fungi. Front Microbiol. 2015;6:185.
88. Ma E, Zhu Y, Liu Z, Wei T, Wang P, Cheng G. Interaction of viruses with the insect intestine. Annu Rev Virol. 2021;8:115–31.
89. Wu P, Sun P, Nie K, Zhu Y, Shi M, Xiao C, et al. A gut commensal bacterium promotes mosquito permissiveness to arboviruses. Cell Host Microbe. 2019;25:101-112.e105.
90. Anglero-Rodriguez YI, Talyuli OA, Blumberg BJ, Kang S, Demby C, Shields A, et al. An *Aedes aegypti*-associated fungus increases susceptibility to dengue virus by modulating gut trypsin activity. Elife. 2017. https://doi.org/10.7554/eLife.28844.
91. Dutra HL, Rocha MN, Dias FB, Mansur SB, Caragata EP, Moreira LA. Wolbachia blocks currently circulating zika virus isolates in Brazilian *Aedes aegypti* mosquitoes. Cell Host Microbe. 2016;19:771–4.
92. Zhang G, Hussain M, O'Neill SL, Asgari S. Wolbachia uses a host microRNA to regulate transcripts of a methyltransferase, contributing to dengue virus inhibition in *Aedes aegypti*. Proc Natl Acad Sci U S A. 2013;110:10276–81.
93. Terradas G, Joubert DA, McGraw EA. The RNAi pathway plays a small part in Wolbachia-mediated blocking of dengue virus in mosquito cells. Sci Rep. 2017;7:43847.
94. Terradas G, McGraw EA. *Wolbachia*-mediated virus blocking in the mosquito vector *Aedes aegypti*. Curr Opin Insect Sci. 2017;22:37–44.
95. Ramirez JL, Short SM, Bahia AC, Saraiva RG, Dong Y, Kang S, et al. Chromobacterium Csp_P reduces malaria and dengue infection in vector mosquitoes and has entomopathogenic and in vitro anti-pathogen activities. PLoS Pathog. 2014;10:e1004398.
96. Saraiva RG, Fang J, Kang S, Anglero-Rodriguez YI, Dong Y, Dimopoulos G. Aminopeptidase secreted by *Chromobacterium* sp. Panama inhibits dengue virus infection by degrading the E protein. PLoS Negl Trop Dis. 2018;12:e0006443.
97. Saraiva RG, Huitt-Roehl CR, Tripathi A, Cheng YQ, Bosch J, Townsend CA, et al. *Chromobacterium* spp. mediate their anti-*Plasmodium* activity through secretion of the histone deacetylase inhibitor romidepsin. Sci Rep. 2018;8:6176.
98. Apte-Deshpande A, Paingankar M, Gokhale MD, Deobagkar DN. *Serratia odorifera* a midgut inhabitant of *Aedes aegypti* mosquito enhances its susceptibility to dengue-2 virus. PLoS ONE. 2012;7:e40401.
99. Apte-Deshpande AD, Paingankar MS, Gokhale MD, Deobagkar DN. *Serratia odorifera* mediated enhancement in susceptibility of *Aedes aegypti* for chikungunya virus. Indian J Med Res. 2014;139:762–8.
100. Minard G, Mavingui P, Moro CV. Diversity and function of bacterial microbiota in the mosquito holobiont. Parasit Vectors. 2013;6:146.
101. Huang J, Jiang E, Yang D, Wei J, Zhao M, Feng J, et al. Metagenomic next-generation sequencing versus traditional pathogen detection in

the diagnosis of peripheral pulmonary infectious lesions. Infect Drug Resist. 2020;13:567–76.
102. Chen K, Pachter L. Bioinformatics for whole-genome shotgun sequencing of microbial communities. PLoS Comput Biol. 2005;1:106–12.
103. Kiselev D, Matsvay A, Abramov I, Dedkov V, Shipulin G, Khafizov K. Current trends in diagnostics of viral infections of unknown etiology. Viruses. 2020;12:211.
104. Altinli M, Schnettler E. Symbiotic interactions between mosquitoes and mosquito viruses. Front Cell Infect Microbiol. 2021. https://doi.org/10.3389/fcimb.2021.694020.
105. Xiao P, Li C, Zhang Y, Han J, Guo X, Xie L, et al. Metagenomic sequencing from mosquitoes in China reveals a variety of insect and human viruses. Front Cell Infect Microbiol. 2018;8:364.
106. Mokili JL, Rohwer F, Dutilh BE. Metagenomics and future perspectives in virus discovery. Curr Opin Virol. 2012;2:63–77.
107. Shi C, Beller L, Deboutte W, Yinda KC, Delang L, Vega-Rua A. Stable distinct core eukaryotic viromes in different mosquito species from Guadeloupe, using single mosquito viral metagenomics. Microbiome. 2019;7:121.
108. 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.
109. He X, Yin Q, Zhou L, Meng L, Hu W, Li F, et al. Metagenomic sequencing reveals viral abundance and diversity in mosquitoes from the Shaanxi-Gansu-Ningxia region, China. PLoS Negl Trop Dis. 2021;15:e0009381.
110. Cholleti H, Hayer J, Fafetine J, Berg M, Blomstrom AL. Genetic characterization of a novel picorna-like virus in *Culex* spp. mosquitoes from Mozambique. Virol J. 2018;15:71.
111. Petit MJ, Shah PS. Mapping arbovirus-vector interactions using systems biology techniques. Front Cell Infect Microbiol. 2018;8:440.
112. Dennison NJ, Jupatanakul N, Dimopoulos G. The mosquito microbiota influences vector competence for human pathogens. Curr Opin Insect Sci. 2014;3:6–13.
113. Ramos-Nino ME, Fitzpatrick DM, Tighe S, Eckstrom KM, Hattaway LM, Hsueh AN, 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.
114. Ramos-Nino ME, Fitzpatrick DM, Eckstrom KM, Tighe S, Hattaway LM, Hsueh AN, et al. Metagenomic analysis of *Aedes aegypti* and *Culex quinquefasciatus* mosquitoes from Grenada, West Indies. PLoS ONE. 2020;15:e0231047.
115. Shi C, Liu Y, Hu X, Xiong J, Zhang B, Yuan Z. A metagenomic survey of viral abundance and diversity in mosquitoes from Hubei province. PLoS ONE. 2015;10:e0129845.
116. Calisher CH, Higgs S. The discovery of arthropod-specific viruses in hematophagous arthropods: an open door to understanding the mechanisms of arbovirus and arthropod evolution? Annu Rev Entomol. 2018;63:87–103.
117. Elrefaey AM, Abdelnabi R, Rosales Rosas AL, Wang L, Basu S, Delang L. Understanding the mechanisms underlying host restriction of insect-specific viruses. Viruses. 2020;12:964.
118. Agboli E, Leggewie M, Altinli M, Schnettler E. Mosquito-specific viruses-transmission and interaction. Viruses. 2019;11:873.
119. Sadeghi M, Popov V, Guzman H, Phan TG, Vasilakis N, Tesh R, et al. Genomes of viral isolates derived from different mosquitos species. Virus Res. 2017;242:49–57.
120. Shi M, Neville P, Nicholson J, Eden JS, Imrie A, Holmes EC. High-resolution metatranscriptomics reveals the ecological dynamics of mosquito-associated RNA viruses in Western Australia. J Virol. 2017. https://doi.org/10.1128/JVI.00680-17.
121. Zakrzewski M, Rasic G, Darbro J, Krause L, Poo YS, Filipovic I, et al. Mapping the virome in wild-caught *Aedes aegypti* from Cairns and Bangkok. Sci Rep. 2018;8:4690.
122. Bolling BG, Weaver SC, Tesh RB, Vasilakis N. Insect-specific virus discovery: significance for the arbovirus community. Viruses. 2015;7:4911–28.
123. Vasilakis N, Tesh RB. Insect-specific viruses and their potential impact on arbovirus transmission. Curr Opin Virol. 2015;15:69–74.
124. Dudas G, Obbard DJ. Are arthropods at the heart of virus evolution? Elife. 2015. https://doi.org/10.7554/eLife.06837.
125. Marklewitz M, Zirkel F, Kurth A, Drosten C, Junglen S. Evolutionary and phenotypic analysis of live virus isolates suggests arthropod origin of a pathogenic RNA virus family. Proc Natl Acad Sci U S A. 2015;112:7536–41.
126. Li CX, Shi M, Tian JH, Lin XD, Kang YJ, Chen LJ, et al. Unprecedented genomic diversity of RNA viruses in arthropods reveals the ancestry of negative-sense RNA viruses. Elife. 2015. https://doi.org/10.7554/eLife.05378.
127. 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. https://doi.org/10.1128/mSystems.00640-20.
128. Thannesberger J, Rascovan N, Eisenmann A, Klymiuk I, Zittra C, Fuehrer HP, et al. Highly sensitive virome characterization of *Aedes aegypti* and *Culex pipiens* complex from central Europe and the Caribbean reveals potential for interspecies viral transmission. Pathogens. 2020. https://doi.org/10.3390/pathogens9090686.
129. Kubacki J, Flacio E, Qi W, Guidi V, Tonolla M, Fraefel C. Viral metagenomic analysis of *Aedes albopictus* mosquitos from Southern Switzerland. Viruses. 2020;12:929.
130. He W, Chen Y, Zhang X, Peng M, Xu D, He H, et al. Virome in adult *Aedes albopictus* captured during different seasons in Guangzhou City, China. Parasit Vectors. 2021;14:415.
131. da Silva Ferreira R, de Toni Aquino da Cruz LC, de Souza VJ, da Silva Neves NA, de Souza VC, Filho LCF, et al: Insect-specific viruses and arboviruses in adult male culicids from Midwestern Brazil. Infect Genet Evol 2020, 85:104561.
132. Coatsworth H, Bozic J, Carrillo J, Buckner EA, Rivers AR, Dinglasan RR, et al. Intrinsic variation in the vertically transmitted core virome of the mosquito *Aedes aegypti*. Mol Ecol. 2022;29:19–577.
133. Frey KG, Biser T, Hamilton T, Santos CJ, Pimentel G, Mokashi VP, et al. Bioinformatic characterization of mosquito viromes within the Eastern United States and Puerto Rico: discovery of novel viruses. Evol Bioinform Online. 2016;12:1–12.
134. Oguzie JU, Nwangwu UC, Oluniyi PE, Olumade TJ, George UE, Kazeem A, et al. Metagenomic sequencing characterizes a wide diversity of viruses in field mosquito samples in Nigeria. Scientifc Reports. 2022;12:7616.
135. Wang L, Rosales Rosas AL, De Coninck L, Shi C, Bouckaert J, Matthijnssens J, et al. Establishment of *Culex modestus* in Belgium and a Glance into the Virome of Belgian mosquito species. MSphere. 2021. https://doi.org/10.1128/mSphere.01229-20.
136. Huang Y, Guo X, Zhang S, Zhao Q, Sun Q, Zhou H, et al. Discovery of two novel totiviruses from *Culex tritaeniorhynchus* classifiable in a distinct clade with arthropod-infecting viruses within the family Totiviridae. Arch Virol. 2018;163:2899–902.
137. Shi M, Zhang YZ, Holmes EC. Meta-transcriptomics and the evolutionary biology of RNA viruses. Virus Res. 2018;243:83–90.
138. Stollar V, Thomas VL. An agent in the *Aedes aegypti* cell line (Peleg) which causes fusion of *Aedes albopictus* cells. Virology. 1975;64:367–77.
139. Zhang G, Asad S, Khromykh AA, Asgari S. Cell fusing agent virus and dengue virus mutually interact in *Aedes aegypti* cell lines. Sci Rep. 2017;7:6935.
140. Parry R, Asgari S. Aedes anphevirus: an insect-specific virus distributed worldwide in *Aedes aegypti* mosquitoes that has complex interplays with *Wolbachia* and Dengue virus infection in cells. J Virol. 2018. https://doi.org/10.1128/JVI.00224-18.
141. Vogels CBF, Ruckert C, Cavany SM, Perkins TA, Ebel GD, Grubaugh ND. Arbovirus coinfection and co-transmission: a neglected public health concern? PLoS Biol. 2019;17:e3000130.
142. Karpf AR, Lenches E, Strauss EG, Strauss JH, Brown DT. Superinfection exclusion of alphaviruses in three mosquito cell lines persistently infected with Sindbis virus. J Virol. 1997;71:7119–23.
143. Goenaga S, Kenney JL, Duggal NK, Delorey M, Ebel GD, Zhang B, et al. Potential for co-infection of a mosquito-specific Flavivirus, Nhumirim virus, to block West Nile virus transmission in mosquitoes. Viruses. 2015;7:5801–12.
144. Nasar F, Erasmus JH, Haddow AD, Tesh RB, Weaver SC. Eilat virus induces both homologous and heterologous interference. Virology. 2015;484:51–8.

Publisher's Note

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Virology

Virology | Full-Length Text

Influence of dengue virus serotypes on the abundance of *Aedes aegypti* insect-specific viruses (ISVs)

Marcela Gómez,[1,2] David Martínez,[1] Luisa Páez-Triana,[1] Nicolás Luna,[1] Angie Ramírez,[1] Julián Medina,[1] Lissa Cruz-Saavedra,[1] Carolina Hernández,[1,3] Sergio Castañeda,[1] Ramiro Bohórquez Melo,[4] Luis Alejandro Suarez,[4] Mónica Palma-Cuero,[4] Luz Mila Murcia,[4] Leonel González Páez,[5] Leonardo Estrada Bustos,[5] Manuel Alfonso Medina,[5] Katiuska Ariza Campo,[6] Holmer David Padilla,[7] Alexander Zamora Flórez,[8] Jorge Luis De las Salas,[8] Marina Muñoz,[1] Juan David Ramírez[1,9]

AUTHOR AFFILIATIONS See affiliation list on p. 13.

ABSTRACT A comprehensive understanding of the virome in mosquito vectors is crucial for assessing the potential transmission of viral agents, designing effective vector control strategies, and advancing our knowledge of insect-specific viruses (ISVs). In this study, we utilized Oxford Nanopore Technologies metagenomics to characterize the virome of *Aedes aegypti* mosquitoes collected in various regions of Colombia, a country hyperendemic for dengue virus (DENV). Analyses were conducted on groups of insects with previous natural DENV infection (DENV-1 and DENV-2 serotypes), as well as mosquito samples that tested negative for virus infection (DENV-negative). Our findings indicate that the *Ae. aegypti* virome exhibits a similar viral composition at the ISV family and species levels in both DENV-positive and DENV-negative samples across all study sites. However, differences were observed in the relative abundance of viral families such as Phenuiviridae, Partitiviridae, Flaviviridae, Rhabdoviridae, Picornaviridae, Bromoviridae, and Virgaviridae, depending on the serotype of DENV-1 and DENV-2. In addition, ISVs are frequently found in the core virome of *Ae. aegypti*, such as *Phasi Charoen-like phasivirus* (PCLV), which was the most prevalent and showed variable abundance in relation to the presence of specific DENV serotypes. Phylogenetic analyses of the L, M, and S segments of the PCLV genome are associated with sequences from different regions of the world but show close clustering with sequences from Brazil and Guadeloupe, indicating a shared evolutionary relationship. The profiling of the *Ae. aegypti* virome in Colombia presented here improves our understanding of viral diversity within mosquito vectors and provides information that opens the way to possible connections between ISVs and arboviruses. Future studies aimed at deepening our understanding of the mechanisms underlying the interactions between ISVs and DENV serotypes in *Ae. aegypti* could provide valuable information for the design of effective vector-borne viral disease control and prevention strategies.

IMPORTANCE In this study, we employed a metagenomic approach to characterize the virome of *Aedes aegypti* mosquitoes, with and without natural DENV infection, in several regions of Colombia. Our findings indicate that the mosquito virome is predominantly composed of insect-specific viruses (ISVs) and that infection with different DENV serotypes (DENV-1 and DENV-2) could lead to alterations in the relative abundance of viral families and species constituting the core virome in *Aedes* spp. The study also sheds light on the identification of the genome and evolutionary relationships of the *Phasi Charoen-like phasivirus* in *Ae. aegypti* in Colombia, a widespread ISV in areas with high DENV incidence.

KEYWORDS Viral metagenomic, mosquito, dengue virus, insect-specific viruses (ISVs), Colombia

Editor Mark T. Heise, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

Address correspondence to Juan David Ramírez, juand.ramirez@urosario.edu.co, juan.ramirezgonzalez@mssm.edu.

The authors declare no conflict of interest.

See the funding table on p. 14.

Received 26 September 2023
Accepted 13 November 2023
Published 14 December 2023

Metagenomic sequencing of *Ae. aegypti* mosquitoes, both naturally infected and uninfected with dengue virus, revealed a stable vector virome composition at the family and species level. However, changes in the abundance of some viral families were observed as a function of DENV serotype (DENV-1 and DENV-2). The results highlight the importance of further exploring ISV-arbovirus interactions, which could have implications for the prevention and control of arboviral (arthropod-borne viral) diseases.

Mosquitoes are important vectors responsible for the transmission of more than one-third of all arboviruses worldwide (1). The *Aedes aegypti* mosquito is the primary transmitter of pathogenic viruses such as dengue virus (DENV), affecting 390 million people in tropical and subtropical regions worldwide annually (2, 3). This virus has four serotypes, namely, DENV-1, DENV-2, DENV-3, and DENV-4, which provide lifelong immunity (4). However, second infections by heterologous serotypes are associated with an increased risk of developing severe and/or fatal forms of the disease (3, 4). Factors such as climate change (5–8), globalization (9, 10), urbanization, and human movements (10, 11) have enabled the worldwide expansion of *Ae. aegypti*, facilitating the rapid spread of arboviral diseases (12).

This species is highly competent due to its strong anthropophilic behavior, resistance to egg desiccation (13), adaptability to urban and natural environments (14), and resistance to insecticides (15). Furthermore, the transovarial and venereal transmission of some viruses by *Ae. aegypti* (4, 16) constitutes important mechanisms to maintain virus circulation even in adverse epidemiological scenarios, such as cold winters or droughts (17). Consequently, this vector species is a significant threat (18), compromising the effectiveness of control measures and management of arboviral diseases worldwide.

In recent years, the intensification of insect vector surveillance and the need to detect emerging pathogens and new viruses (19) have led to the development and application of metagenomic sequencing, a powerful tool for studying virus diversity in a culture-independent manner (20). Although viral metagenomic sequencing can be insensitive, especially in samples with low abundance of viral genomic material relative to host-derived nucleic acid (21), new enrichment strategies allow for the concentration of viruses present in the sample, which increases sensitivity of detection (22). This promising approach is helping to define the mosquito virome, known to be rich and diverse, composed primarily of Insect-Specific Viruses (ISVs) (23–27). ISVs are viruses that naturally infect arthropods but lack the ability to replicate in vertebrate cells or infect humans (28, 29).

Recent studies on the virome of *Aedes* species, carried out in different parts of the world, have shown similarities in the virome composition, formed mainly by viral families such as *Flaviviridae*, *Togaviridae*, *Peribunyaviridae*, *Phenuiviridae*, and *Rhabdoviridae* (28, 30–32). As a result, it is considered that there is a set of viruses that could form a stable species-specific "*core virome*" in mosquito populations (23). This virome is defined as a collection of viral taxa that are consistently detected in various populations and geographic locations of *Aedes* mosquitoes (30, 33). The presence of these viruses can potentially affect the susceptibility of the vector to different pathogenic viruses, including dengue virus, which has important implications for mosquito homeostasis (27, 34–36).

A recent virome analysis of *Ae. aegypti* in urban endemic areas across the world revealed the high abundance of certain ISVs, including *Phasi Charoen-like virus* (PCLV) and *Humaita-Tubiacanga virus* (HTV), which could impact the mosquitoes' ability to transmit DENV and ZIKV to vertebrate hosts (37). Experimental trials have demonstrated decreased infection rates by these arboviruses in cell lines of *Aedes* spp. mosquitoes previously inoculated with *Cell-fusing agent virus* (CFAV) and PCLV (38) and *Nhumirim virus* (*Flaviviridae*) (39). For these reasons, some authors also have suggested the use of ISVs as biological control agents against different arbovirus species (33) or as novel vaccine platforms (32).

Colombia, a country located in the northwestern region of South America, has documented *Ae. aegypti* as the primary vector responsible for transmitting arboviruses such as dengue, Zika, and chikungunya (40, 41). This vector is widespread in peri-urban and urban areas across most of the national territory, increasing the risk of DENV transmission, particularly in areas located below 2,300 meters above sea level (41–43). Recent studies indicate that approximately 80% of the Colombian geography is at risk of dengue, as all four serotypes co-circulate (40, 44, 45). This complexity is more pronounced in regions with high poverty rates, inadequate basic sanitation, and limited

access to healthcare (41), where environmental conditions favor the proliferation of this vector species (46).

Despite the significance of *Ae. aegypti*, our knowledge of the viral communities within this mosquito species in Colombia and the Americas remains limited. Therefore, this study utilizes a metagenomic approach to characterize the virome of *Ae. aegypti* mosquitoes collected from various geographic regions of Colombia. These mosquitoes were identified as positive for dengue virus (DENV-1 and DENV-2 serotypes) as well as uninfected (DENV negative). This research enhances our understanding of the composition and diversity of viral communities within vectors. It serves as a foundational step for future investigations into interactions between ISVs and arboviruses, their potential role in vector competence, and the development of disease control strategies.

RESULTS

Metagenomic analysis of *Ae. aegypti* mosquito virome

Using the results of multiplex PCR, which were obtained in a prior study (41) and focused on detecting dengue virus in *Ae. aegypti* mosquitoes collected from various regions of Colombia, we organized 10 mosquito sample pools for each study location. These pools were further categorized into two groups: DENV-positive (specifically, DENV1 and DENV2 serotypes) and DENV-negative (DENV–) as detailed in Table 1. Thus, a total of 40 pools of *Ae. aegypti* mosquitoes were subjected to metagenomic sequencing using Oxford Nanopore Technologies (ONT), and the data generated were subsequently analyzed with different bioinformatics tools. The quality of the viral metagenomic data filtered using NanoStat software revealed an average of 235,800 raw reads per sample, with an average length of 430 bases and a read quality score of 10 (Table S1). In addition, ViromeQC software detected ~31.75% of sequences per sample that aligned to the LSU rRNA. Once host and prokaryote sequences were removed with the Minimap2 tool, the remaining reads were taxonomically sorted using Centrifuge. PAVIAN was used to visualize the *Ae. aegypti* virome data, revealing 4,219,471 raw reads, with a mean of 105,487 raw reads per sample (range 55,627–216,900). Of these, approximately 3.7% (147,546 reads) were taxonomically classified as viral reads and were assigned to specific viral families or genera (Table S2).

Description of the virome in *Ae. aegypti* mosquito

This study found that the viral sequences primarily belonged to the families Phenuiviridae, Flaviviridae, Partitiviridae, Rhabdoviridae, Picornaviridae, Iflaviridae, PeriBunyaviridae, and Togaviridae. In addition, minor proportions of plant virus families such as Bromoviridae and Virgaviridae were detected in all samples analyzed. The Phenuiviridae family proved to be the most abundant and widespread in the *Ae. aegypti* virome in all

TABLE 1 Summary of samples in *Ae. aegypti* viral metagenomics analysis: pooled samples and mosquito counts with natural DENV-1 and DENV-2 infection (DENV positive) and without infection (DENV negative)[a]

Location (study site)	DENV infection	Number of pools	Serotype of pool		Total pools (DENV+/DENV–)	Number of mosquitoes (individuals)	Total number of mosquitoes (individuals)
			DENV1	DENV2			
Amazonas	Positive	5	2	3	10	25	50
Amazonas	Negative	5	–	–		25	
Boyacá	Positive	5	3	2	10	25	50
Boyacá	Negative	5	–	–		25	
Magdalena	Positive	5	3	2	10	25	50
Magdalena	Negative	5	–	–		25	
Vichada	Positive	5	1	4	10	25	50
Vichada	Negative	5	–	–		25	
Total		40	9	11	40	200	200

[a]–, not applicable.

sample pools analyzed from the study areas in Colombia. These families were detected in both DENV-infected mosquito samples (positive for DENV1 and DENV2 serotypes) and in DENV-negative samples (Fig. 1A) from the locations of Amazonas, Boyacá, Magdalena, and Vichada (Fig. 1B and C).

Metagenomic analysis of viruses revealed abundance of the ISVs PCLV belonging to the family Phenuiviridae of the order Bunyavirales in all mosquito samples analyzed (Fig. 2). PCLV was previously characterized in *Ae. aegypti* mosquitoes in Thailand (48). Other ISVs found in the vector virome, in minor proportions, include HTV, an unclassified virus identified in *Aedes* spp. from Brazil (49); *Guadeloupe mosquito virus* (GMV), an unclassified virus, previously found in the island of Guadeloupe (23); *Hubei mosquito virus 1*, an unclassified virus; and *Aedes aegypti anphevirus* (Xinmoviridae) and *Cell fusing agent virus* (Flaviviridae) (Fig. 2A and B). Other ISVs, such as *Uxmal virus*, a virus belonging to a group (taxon) of viruses Negevirus (50), isolated from *Aedes* mosquitoes from Mexico (51), and *Hanko iflavirus* (Flaviviridae), recently characterized in species of the subgenus *Aedes* in northern Europe (52), were reported for the first time in Colombia (Fig. 2C). In addition, this study also identified potential human pathogens, such as *Corriparta virus* (CORV) recognized within the genus *Orbivirus* (*Reoviridae*) (53) and *Piry vesiculovirus* (PIRYV) (Rhabdoviridae) (54) (Fig. 2D).

Comparison of the relative abundance between viral families of samples positive and negative for natural DENV infection (DENV-1 vs DENV negative and DENV-2 vs DENV negative) revealed no significant changes (Wilcoxon test, $P < 0.05$) (Fig. 3A and B; Table S3). However, comparison of DENV-positive samples by serotype (DENV-1 vs DENV-2) revealed differences in the abundance of some of the families that compose the *Ae. aegypti* virome, including Phenuiviridae, Partitiviridae, Flaviviridae, Rhabdoviridae, Picornaviridae, Bromoviridae, and Virgaviridae (Wilcoxon test, $P < 0.05$) (Fig. 3C; Table S3). In addition, comparing the relative abundance of viral families between different localities, the Phenuiviridae family showed differences between the departments Magdalena-Vichada ($P = 0.014$) and Boyacá-Vichada ($P = 0.029$), using Kruskal-Wallis and a post hoc Dunn's test considering the Benjamini-Hochberg procedure. Other families, such as Flaviviridae, Bromoviridae, and Virgaviridae, also showed significant differences in relative abundance between Magdalena and Vichada ($P < 0.05$) (Fig. 3D). Also, some viral species, such as PCLV and HTV, showed significant differences between the Amazonas-Vichada, Boyacá-Vichada, and Magdalena-Vichada departments ($P < 0.05$).

PCLV genome analysis

In our metagenomic analysis, we observed a dominant presence of PCLV in the *Ae. aegypti* virome, accounting for approximately 70% of the total viral reads that were assembled into PCLV contigs. Using the Genome Detective tool, we successfully reconstructed the complete genomes of PCLV, including the L, M, and S segments

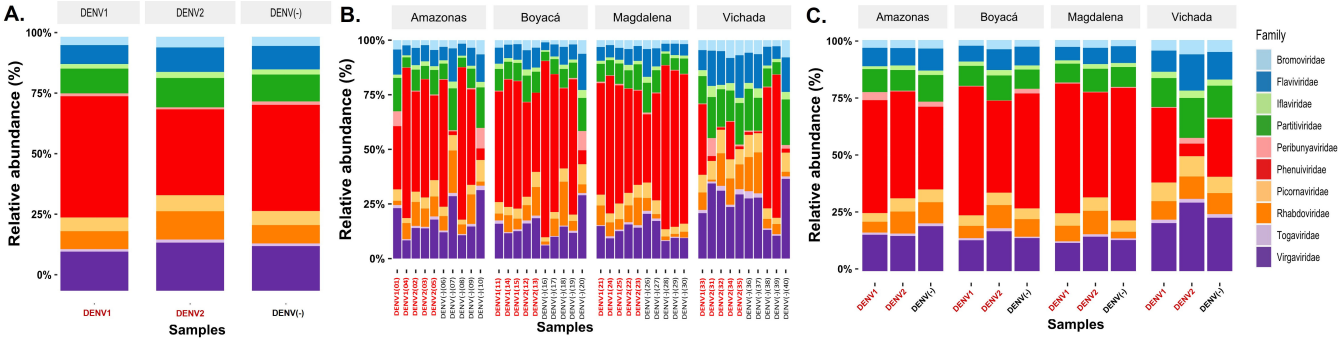

FIG 1 Composition of viral families from field-collected *Ae. aegypti* mosquitoes across various locations in Colombia. Bar graphs depict the relative abundance of major viral families present in *Ae. aegypti* mosquitoes, categorized by samples positive for DENV-1 and DENV-2 serotype infections, as well as negative for DENV (–) infections, as shown in (A) samples from all localities by group. (B) Individual samples by study locality. (C) Samples by study locality in Colombia. Figure created on R studio with ggplot package (47).

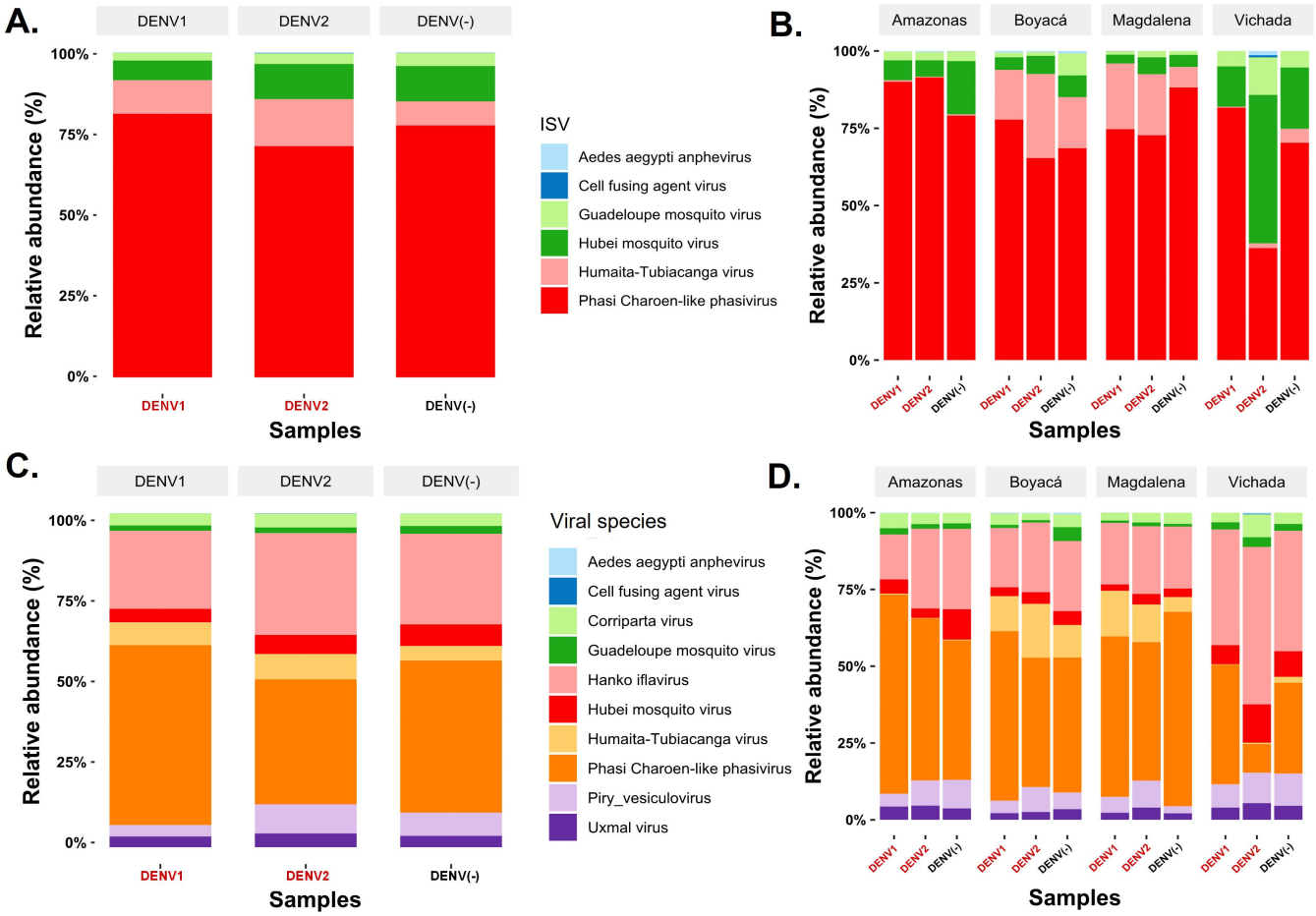

FIG 2 Composition of viral species from field-collected *Ae. aegypti* mosquitoes across various locations in Colombia. Bar graphs show the relative abundance of viral reads for the main viral species categorized by samples positive for DENV-1 and DENV-2 serotype infections, as well as negative for DENV (–) infections, as shown in (A) ISVs reported in *core virome* of *Ae. aegypti* mosquitoes, samples from all localities by group. (B) ISVs reported in core virome, samples by study locality in Colombia. (C) Viruses found in *Ae. aegypti* virome, samples from all localities by group. (D) Viruses found in virome, samples by study locality in Colombia. Figure created on R studio with ggplot package (47).

responsible for encoding RNA-dependent RNA polymerase (RdRp), glycoprotein, and capsid protein, respectively. The nucleotide sequences of these assemblies exhibited an average size of 6,700 nt for the L segment, 3,570 nt for the M segment, and 1,100 nt for the S segment (Table S4). Our comprehensive analysis highlighted the prevalence of PCLV in *Ae. aegypti* mosquitoes on a global scale, underscoring a remarkable conservation in its gene sequences, as determined through BLASTn analysis. Utilizing the Prokka tool for annotation, we identified a coding sequence within each assembled segment for PCLV. The identity levels of each PCLV in comparison to the reference sequences of PCLV-Rio (NC_038262.1 for segment L, NC_038261.1 for segment M, and NC_038263.1 for segment S) ranged from 95.1% to 98.1%, with all three segments exhibiting a coverage exceeding 90% (Table S6).

Phylogenomic analysis of PCLV

The assembled L, M, and S segments of the PCLV genome from the study sites (Amazonas, Boyacá, Magdalena, and Vichada) were analyzed to determine their evolutionary relationship with PCLV sequences identified in *Ae. aegypti* mosquitoes from different regions of the world (Fig. 4). Phylogenetic reconstruction revealed well-supported clustering patterns (Bootstrap ≥ 90) in all three segments (L, M, and S) of PCLV. The Colombian PCLV sequences showed an association with sequences from various regions

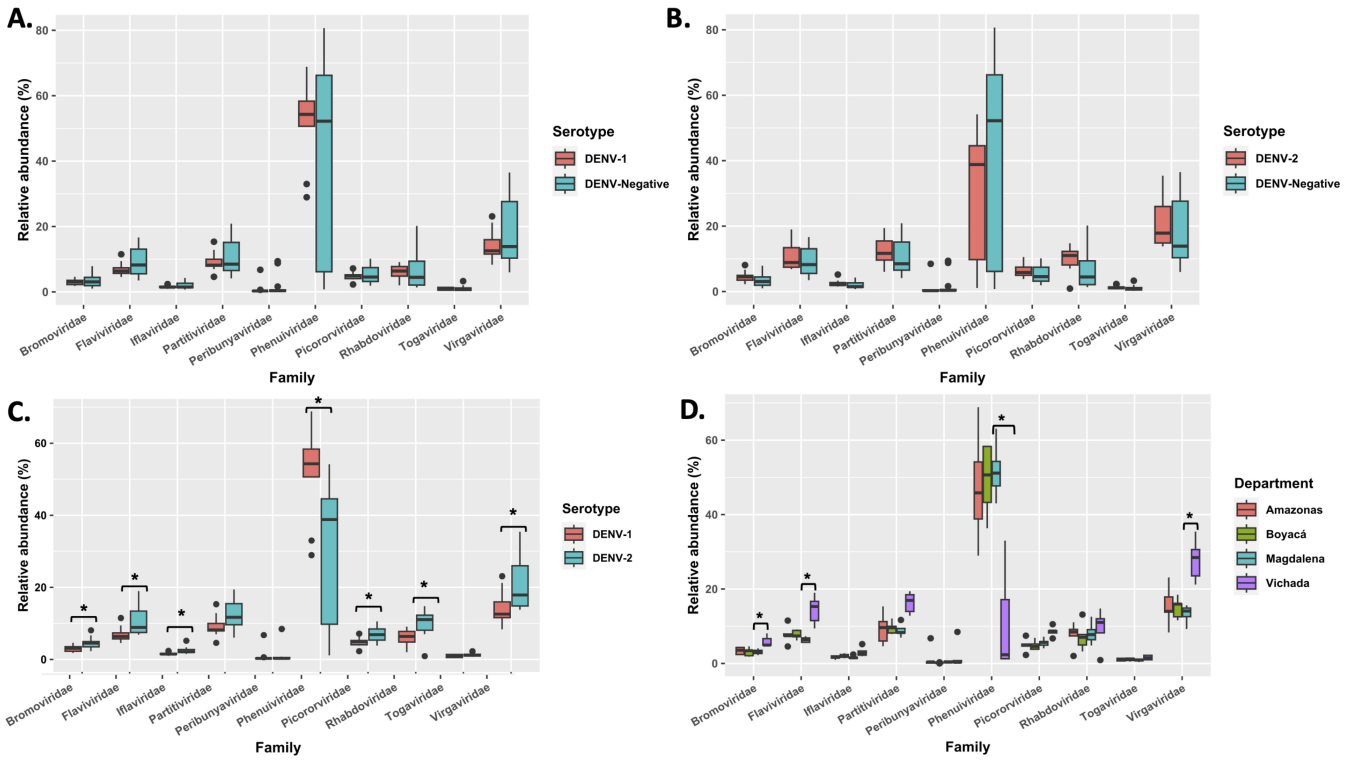

FIG 3 Changes in relative abundance in the viral families found in the *Ae. aegypti* virome. Boxplot showing differences between groups by relative abundances of each family by (A) infection by serotype DENV-1 and DENV negative. (B) infection by serotype DENV-2 and DENV negative. (C) Serotype, DENV1 and DENV2. (D) Department of study in Colombia. Statistical differences (Mann-Whitney-Wilcoxon and Kruskall-Wallis tests; post-hoc: Dunn's test with Benjamini-Hochberg correction at 95% confidence level) ($P < 0.05$) are indicated with an asterisk (*). Figure created in R studio with the ggplot package (55).

worldwide, exhibiting a particularly close evolutionary relationship with sequences from Guadeloupe (2018) (23), Nigeria (2020) (56), and Brazil (2020) (57) (Fig. 4B through D).

DISCUSSION

Surveillance of arboviruses with public health implications from mosquito vector samples is limited. This has hampered efforts to accurately predict the spread of new arboviral strains in human populations and to implement effective management interventions (58). Advances in high-throughput sequencing technologies have made it possible to understand and describe the virome in mosquito vectors, which is considered a promising strategy for epidemiological surveillance (19, 59).

In this study, we performed virome characterization of field-caught *Ae. aegypti* mosquitoes positive and negative for DENV infection by a metagenomic approach using ONT. The mosquito samples analyzed were previously identified by molecular testing (multiplex PCR) as positive for DENV, specifically for serotypes DENV-1 and DENV-2, as well as negative (DENV-negative) in several geographic regions of Colombia (41). In general, the composition of the viral signatures found in *Ae. aegypti*, mainly composed of ISVs, was similar at both the family and species levels across the samples analyzed in all study locations (Fig. 1 and 2). However, we found differences in the abundances of some viral families and species as a function of DENV serotype (Fig. 3). These differences in virome composition could eventually affect the mosquito's ability to transmit DENV to humans, as suggested by some authors (30, 31, 33, 36).

Interestingly, some of the dominant viral families found in *Ae. aegypti*, such as Phenuiviridae (*Bunyavirus*), Flaviviridae, Rhabdoviridae, and Togaviridae (Fig. 1), have been previously documented in mosquito vectors (19, 59) and reported in metataxonomic analysis conducted in a dengue-endemic locality (60) suggesting the relative

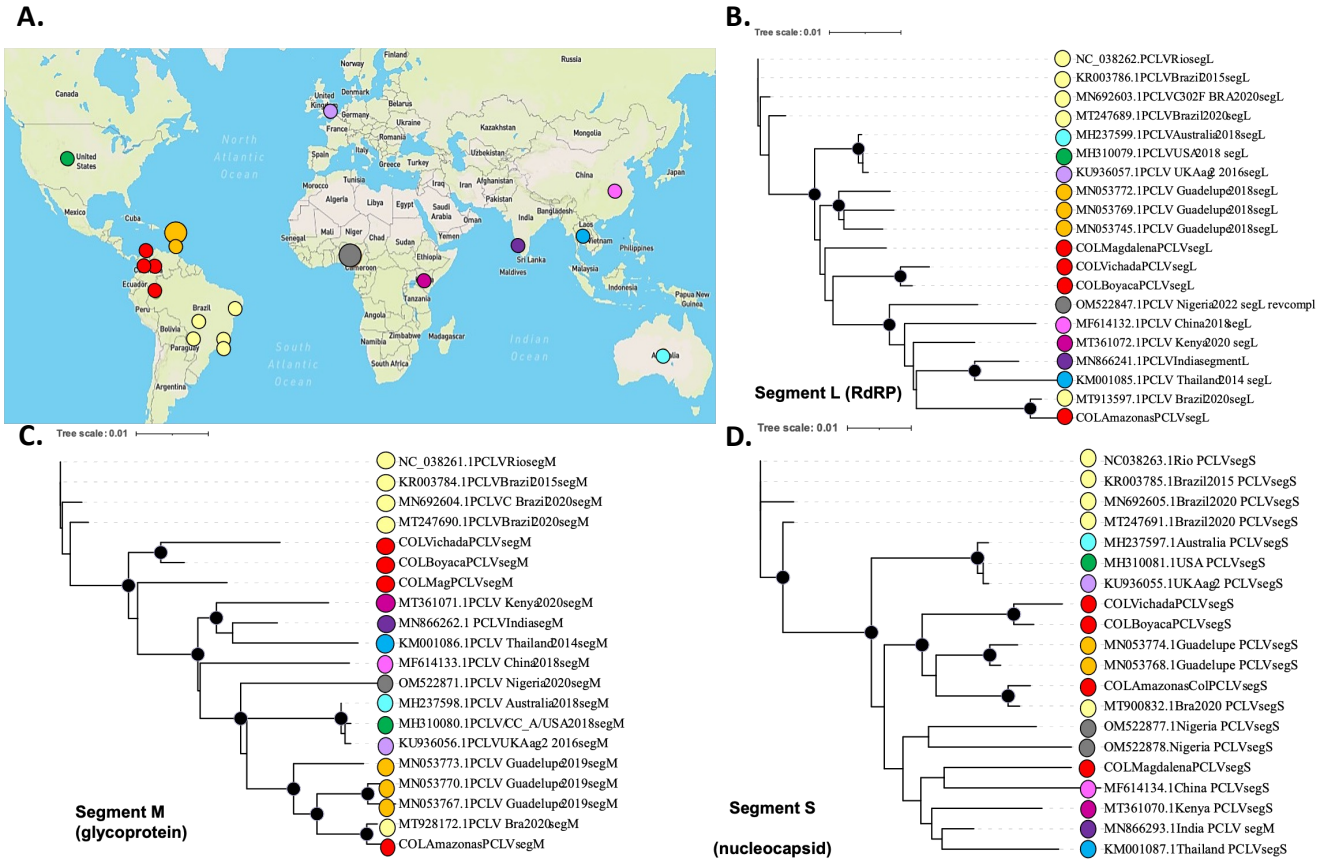

FIG 4 Phylogenetic analysis of the nucleotide sequence of the L, M, and S segments of the *Phasi Charoen-like phasivirus* identified from populations of *Ae. aegypti* mosquitoes from Colombia and other regions of the world. (A) Circles on the map are approximately scaled to the number of genomes of PCLV represented on the tree. Maximum-likelihood (ML) phylogenetic tree showing the evolutionary relationship between PCLV genomic segments from Colombia and those obtained from the NCBI database, respectively. The map was made using Microreact Maps Mapbox (www.mapbox.com/about/maps) and OpenStreetMap (www.openstreetmap.org/about). (B) L segment (RNA-dependent RNA polymerase). (C) M segment (glycoprotein). (D) S segment (nucleocapsid). The black dots represent well-supported nodes (bootstrap support of ≥90), and colored dots indicate sequences obtained in this study and from GenBank. PCLV sequences from Colombia are in red. All reference strains are identified by name and GenBank accession number.

persistence of these viral families in mosquito vectors over time and geographic locations. Additionally, other research points out that viral families such as Flaviviridae, Togaviridae, and Bunyaviridae (Order) include both, arboviruses of medical importance and ISV (19, 27, 33, 61), which could indicate close evolutionary relationships between ISV and arboviruses in the mosquito host (30–32, 36). Thus, further exploration of the mosquito virome can provide valuable insights into their adaptive strategies and the evolutionary history of vector-borne viruses.

Ae. aegypti is known for its high viral abundance and diversity (23, 37, 62), which could be attributed to its high susceptibility to infection and ability to extensively carry viruses (63). Particularly in this study, we found some of the ISV species widespread in the *Ae. aegypti* virome, such as *Phasi Charoen-like virus* (PCLV), *Humaita-Tubiacanga virus* (HTV), GMV, *Hubei mosquito virus 1, Aedes aegypti anphevirus*, and CFAV (1, 64, 65) (Fig. 2A and B). These findings in the virome profile of *Ae. aegypti* from Colombian samples could demonstrate a degree of stability and conservation in the structure described for the *core* virome in *Aedes* spp. in studies conducted worldwide (59, 64, 66).

Interestingly, PCLV and HTV have been identified in *Aedes* mosquitoes from areas with high incidence of flaviviruses such as DENV, particularly in Asia and South America (1, 37, 59). In our findings, we found changes in the abundance of PCLV among

mosquito samples infected with DENV-1 and DENV-2 that could suggest potential specific interactions between this ISV and DENV serotypes (35, 38). Recent transcriptomic studies demonstrate positive association between ISV (PCLV and HTV) and DENV-1 and ZIKV, facilitating replication in the *Ae. aegypti* vector, as well as its ability to transmit to the vertebrate host. Conversely, research conducted in *Aedes* cell lines shows that the interaction between PCLV and CFAV may eventually inhibit ZIKV and DENV-2 replication (38) or not affect the infection and growth of mosquito-borne viruses in *Ae. aegypti* mosquito-derived cell lines (67). Advances in research to understand the dynamics of the complex tripartite interactions between arboviruses, ISVs, and their hosts in different epidemiological scenarios are of great relevance, especially in the absence of prophylactics or therapeutics.

Detection by the metagenomic approach also allowed the detection of other types of ISVs (not part of the *core* virome), such as *Uxmal virus* and *Hanko iflavirus*. These viruses, recently described (51, 52), are related to plant viruses, which could suggest a possible adaptation of plant viruses to insect viruses, which are in constant interaction with the surrounding environment (30, 31, 50). In addition, metagenomic sequencing allowed the detection of CORV (53) and PIRYV, which has demonstrated its potential to cause disease in humans, especially in children (54) (Fig. 2C and D). This important finding warrants further surveillance of potential infectious agents to understand whether they can be transmitted by *Aedes* and its epidemiological implications.

Although no changes were found in the abundance of viral signatures in *Ae. aegypti* in the presence or absence of DENV (Fig. 3A and B), significant changes in the abundance of some viral families were detected as a function of DENV-1 and DENV-2 serotypes (Fig. 3C). These results could be possibly related to the interaction of DENV serotypes with other viruses in the mosquito, as has been suggested previously (36, 39, 68). However, it is crucial to approach this finding with caution. In our study, we conducted qualitative analyses to detect DENV serotypes in *Ae. aegypti* mosquito samples, but we did not perform a comprehensive quantitative assessment of DENV-1 and DENV-2 viral loads per sample or pool (data not available). This omission could introduce potential biases into the data interpretation, particularly when trying to accurately assess how the viral concentration of DENV serotypes influences the structure and composition of specific ISV taxa in the vector virome. Therefore, it is imperative that future studies aimed at investigating the impact of DENV serotypes on virome remodeling consider estimating viral load and how it may or may not subsequently affect ISVs.

In addition, our analyses revealed changes in the relative abundance of certain viral families among the study sites (Fig. 3D). Geographic characteristics possibly have an influence on the shape of the virome in mosquito vectors (41, 44, 66). Validation of the changes that may occur in the *core* virome of *Aedes* mosquitoes, considering DENV infection and geographical factors, would be key to elucidating transmission patterns in an epidemiological context.

Comparative and annotation analysis of PCLV sequences showed >95% similarity between Colombian isolates and reference sequences (49) (Tables S4 and S6) and most of the sequences reported in countries from different continents (23, 24, 56, 57). This result could suggest a low genetic heterogeneity of the PCLV genome, in line with previously published results (24, 34, 56). In addition, phylogenetic analyses using PCLV L (RdRp), M (glycoprotein), and S (Nucleocapsid) segments from *Ae. aegypti* samples together with sequences from different countries showed clustering patterns especially among Colombian isolates with sequences from Guadeloupe (23), Nigeria (56), and Brazil (57) (Fig. 4). These findings could indicate a possible association between PCLV and the genetic structure of *Ae. aegypti* at the geographical level, as previously suggested (24, 34, 56, 65). Also, the coexistence and continuous evolution of PCLV with the vector has also been proposed (24, 56), which would support the close relationships with the host and its potential influence on arboviral transmission dynamics.

Furthermore, we conducted a phylogenetic analysis using the assembled segments L (RdRp), M (glycoprotein), and S (nucleocapsid) of PCLV from *Ae. aegypti* samples, in

conjunction with sequences from various countries. This analysis revealed clustering patterns among Colombian isolates, particularly with sequences from Guadeloupe (23), Brazil (57), and Nigeria (56) (Fig. 4). These findings imply a potential connection between PCLV and the genetic makeup of *Ae. aegypti* on a geographical scale. Previous studies have also proposed a correlation between the genetic variability in the PCLV genome and that observed in host mosquito populations in locations such as Cairns, Australia, and Bangkok, Thailand (65). Moreover, it has been suggested that PCLV coexists and undergoes continuous evolution with its vector (24), further confirming its intimate relationship with the host and its potential impact on the dynamics of arboviral transmission.

Our study does have several important limitations that deserve acknowledgment. First, it is important to note that while the *Ae. aegypti* mosquitoes chosen for viral metagenomic analysis were confirmed as positive for DENV infection, specifically DENV-1 and DENV-2, through conventional multiplex PCR, we did not undertake quantification of DENV via quantitative PCR which could introduce bias in the interpretation of our data. We recognize that including this quantitative data could have provided true evidence for the relationship between the presence and concentration of a specific DENV serotype and alterations in the abundance of ISVs within the vector virome.

Nevertheless, we consider this preliminary study as a foundation for future research aimed at validating and further investigating changes in the composition of the vector virome in the presence or absence of specific DENV serotypes. It is important to highlight that the relatively small sample size of *Ae. aegypti* mosquitoes we tested may have limited our ability to obtain a larger volume of sequencing reads, potentially impacting our ability to detect a broader range of viruses, including arboviruses such as DENV.

We must also acknowledge that viral metagenomic studies can be hindered by incomplete databases, particularly concerning various viral families or genera and the potential existence of undiscovered viruses. This limitation could affect the taxonomic classification of viral sequences. Lastly, it is worth mentioning that the selected sequencing technology, ONT, comes with inherent limitations in terms of depth and accuracy. These limitations could reduce the detection of crucial viral sequences. Furthermore, during metagenomic sequencing of field-collected arthropods, the presence of nucleic acids from nonpathogenic microorganisms, host organisms, and environmental sources could potentially mask viral sequences of interest, resulting in reduced sensitivity in pathogen detection (22, 62, 63).

In summary, our study offers valuable insights into the viral landscape within *Ae. aegypti* mosquitoes in Colombia, a country where DENV is actively circulating. Our data highlights fluctuations in the abundance of certain insect-specific viral species and families (ISVs) in response to DENV1 and DENV2 serotypes. These variations could stem from a multitude of factors, including intricate interactions between ISVs and arboviruses within the mosquito host. However, to deepen and validate these findings, further investigations employing cellular, molecular, and bioinformatics approaches are imperative.

Additionally, our research unveiled a significant presence of ISVs, such as PCLV, which form integral components of the core virome of *Aedes* mosquitoes. Phylogenetic analyses of the L, M, and S segments of the PCLV genome demonstrated associations with sequences from various regions globally, with particularly close clustering with sequences from Brazil and Guadeloupe. This suggests a shared evolutionary relationship.

Furthermore, our findings have brought to light the presence of previously unreported viruses in Colombia and the South American region, including *Uxmal virus* and *Hanko Iflavirus*, as well as the identification of potential pathogens like CORV and PIRYV. This underscores the critical importance of ongoing surveillance and further exploration in this field. Our study serves as a foundational step for future research endeavors, with a focus on unraveling the alterations that could occur in the virome of *Aedes* mosquitoes when exposed to arboviruses. Additionally, it sheds light on the potential contributions

of ISVs, such as PCLV, to the intricate transmission dynamics of the DENV serotype, ultimately influencing vectorial capacity.

MATERIALS AND METHODS

Samples of *Aedes aegypti* mosquitoes selected for metaviromics analysis

In essence, our study involved capturing adult *Ae. aegypti* mosquitoes from December 2020 to August 2021 in four different locations in Colombia, all known for their history of dengue cases (69). Building upon previous research within our group that focused on identifying natural arbovirus infections like DENV in *Aedes* mosquitoes in these same locations (41), we proceeded to conduct metagenomic analyses. This allowed us to comprehensively examine the virome of *Ae. aegypti* mosquitoes.

In our analysis, we included samples from mosquitoes that had tested positive for dengue virus infection, specifically for DENV-1 and DENV-2 serotypes. We also included samples from mosquitoes that tested negative for dengue virus infection. The study was conducted in several locations across Colombia: Leticia (Amazonas) in the southern region, bordering Brazil; Puerto Boyacá and Otanche (Boyacá) in the central Andean region; El Retén (Magdalena) on the Colombian Caribbean coast; and Santa Rosalía and La Primavera (Vichada) to the east, in the plains of the Orinoco region, bordering Venezuela to the east (Fig. 5).

For each collected mosquito, we individually processed RNA extraction, complementary DNA (cDNA) synthesis, and subsequent detection of arboviruses, including DENV. The DENV serotype (DENV 1–4) was determined through multiplex PCR (42), and confirmation of *Aedes* species was achieved by sequencing the subunit I of the cytochrome oxidase (COI) gene using the Sanger method.

Based on the PCR results, we categorized mosquito samples into two groups: those with detected DENV infection, referred to as the "DENV-positive group," and those without infection, labeled as the "DENV-negative/DENV- group," in each study locality. Specifically, we focused on DENV-positive samples with infections of serotypes DENV-1 and DENV-2 due to their higher prevalence in the study areas. Our selection process prioritized RNA samples with higher concentrations (>40 ng/µL) and superior quality based on the A260/280 ratio (1.8–2.0).

Sample processing

To perform the metagenomic analysis, we adopted a method where we combined five RNA samples extracted from *Ae. aegypti* mosquitoes to create a single pool. This approach aligns with a well-established method previously used for metagenomic virological profiling of mosquito populations (23).

As a result, we produced five pools of samples that had tested positive for DENV (specifically, DENV-1 and DENV-2) and an additional five pools of samples that were DENV negative for each study location (Table 1). In total, this process generated 10 pools per location, adding up to a grand total of 40 RNA pools extracted from *Ae. aegypti* mosquitoes. These pooled samples were subsequently subjected to viral metagenomic analysis.

Initially, pools of *Ae. aegypti* mosquitoes were subjected to host RNA removal using a RiboZero Plus rRNA Depletion Kit (Ref 20036696), following the manufacturer's recommendations. Subsequently, to enhance the sensitivity of viral detection, we employed a viral enrichment method, considering the often-limited virus concentrations observed in samples used for insect virome studies. For this purpose, we utilized the Rapid SMART-9N (Rapid Switching Mechanism at the 5′ end of RNA Template)-9n approach for nanopore metagenomics of RNA viruses (22). This approach utilized a random primer method for cDNA synthesis followed by Rapid SMART-9N barcoded PCR primers. In summary, for cDNA synthesis from each pool, 2 µM of RLB RT 9N random oligo-dT primer (TTTTTCGTGCGCCGCTTCAACNNNNNNNNNNNN), 2 mM Deoxynucleotide (dNTP)

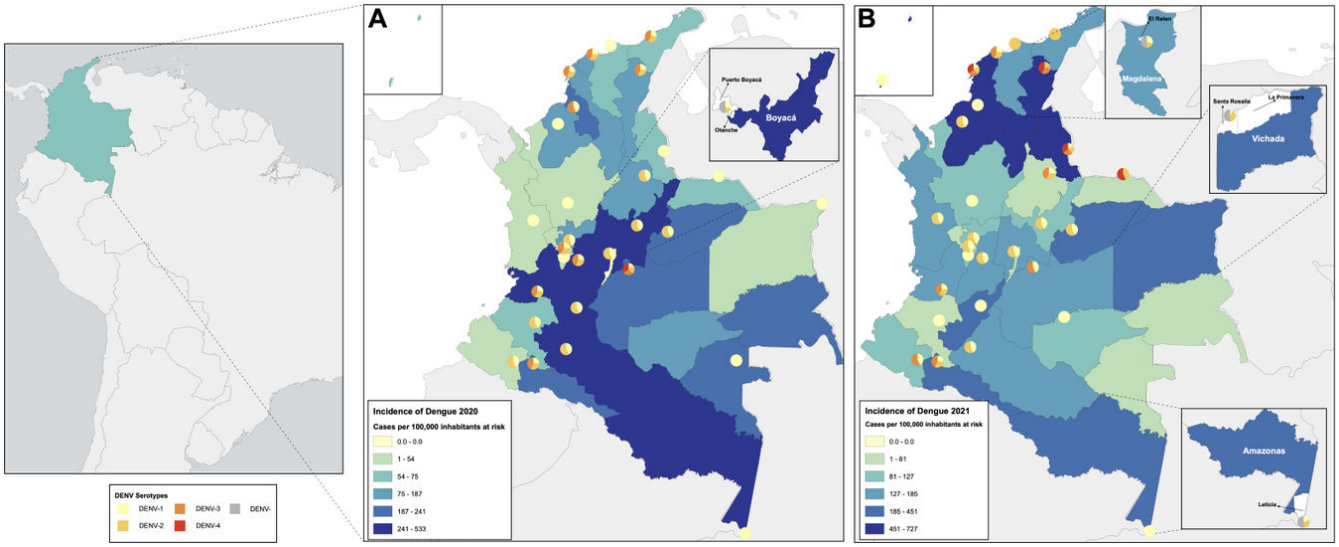

FIG 5 Dengue incidence and circulating serotypes in Colombia (2020–2021) with areas of entomological sampling of *Aedes aegypti* mosquitoes highlighted. This figure illustrates the dengue incidence data and the prevalent dengue serotypes across various locations in Colombia for the years 2020 (A) and 2021 (B). The map provides a visual representation of the areas where entomological sampling of *Aedes aegypti* mosquitoes was conducted. It also emphasizes the incidence of dengue, presented as reported cases per 100,000 population at risk, for the respective years. Furthermore, the map delineates the prevailing dengue serotypes by locality, specifically highlighting the prevalence of serotypes DENV1 and DENV2 in Colombia during the study period. The data used for these maps were obtained from the Colombian Public Health Surveillance System (Sivigila) (National Institute of Health, 2020, 2022) (55, 69). This map was made with QGIS 3.26 [Basemap: World Countries https://hub.arcgis.com/datasets/esri::world-countries-generalized/explore?location=%E2%88%920.208971%2%20C0.000000%2C1.92, sources: Esri and sourced from the National Geographic Society the U.S. Central Intelligence Agency (The World Factbook) and Garmin International Inc]; the map of Colombia's departments and municipalities: https://data.humdata.org/dataset/cod-ab-col? sources: Departamento Administrativo Nacional de Estadística (DANE) and OCHA Latin America and the Caribbean (ROLAC)].

Solution Mix (Biolabs, N0447S), and 10 µM of RNA (ribozero treated), incubated for 5 min at 65°C and on ice, were used. Subsequently, 5X SSIV buffer, 100 mM DTT, 1 µL of Rnase OUT, 200 U/µL of SuperScript IV (Thermo Fisher, 15307696), and 2 µM of RLB TSO (GCTAA TCATGCTTTTTCGTGCGCCGCCGCTTCAACATrGrG) were mixed with 12 µL of annealed RNA before incubating for 90 min at 42°C followed by 10 min at 70°C. The double-labeled cDNA products were then amplified using 2X LongAmp Taq Master Mix enzyme (Biolabs, M0323S), 0.125 µL of RLB-PCR primer (TTTTTCGTGCGTGCGTGCGCGCGCGCTTCA), and 1.25 µL of cDNA, for a final volume of 12.5 µL. The thermal profile used consisted of one cycle at 50°C for reverse transcriptase activation for 10 min, followed by an initial denaturation at 95°C for 45 s, 26 cycles of 95°C for 15 s, 56°C for 15 s, 65°C for 15 s, and a final extension of 65°C for 10 min. The products were quantified by fluorimetry with the Qubit dsDNA High Sensitivity Assay (Cat No. Q32854, Life Technologies, USA) on the Qubit 3.0 instrument (Life Technologies, USA) following the manufacturer's instructions.

Nanopore library preparation and viral sequencing

MinION libraries were prepared following the manufacturer's instructions for cDNA amplified from the 40 pools of *Ae. aegypti*. In brief, DNA repair, blunt-end preparation, and adapter ligation of amplicons was done using the NEBNext FFPE DNA Repair Mix (M6630) and NEB Next Ultra II end repair/dA-tailing module (NEB, Cat. No. E7546). Next, each DNA sample was subjected to cleanup with AMPure XP beads, and then, the library was formed by binding 10 ng of the amplified DNA to sequencing adapters using the SQK-LSK 109 Kit (ONT) and barcoding was performed using the EXP-NBD104 Native Barcoding Kit (ONT). Adapter-linked libraries were loaded onto FLO-MIN106 flow cells R9.4.1 on the MinION device (ONT, UK) and sequenced using MinKNOW V.3.1.4. program which ran for 48–72 hours.

Bioinformatic data analysis

Bioinformatics analysis was performed on the raw Fast5 files, and low-quality reads with a score below 7 were filtered out to obtain fastq files and then demultiplexed with the Guppy V3.1.5 tool (70) through the MinKNOW software (version 21.11.7, Oxford Nanopore Technologies). NanoStat V 1.1.2 software was employed to evaluate the raw nanopore sequencing data quality, including the number of reads, average read length (bp), average read quality score, and N50 read length. To improve data accuracy, reads related to host sequences were removed. Initially, sequences were aligned to the *Ae. aegypti* reference genome (GenBank accession AaegL5.2, GCF_002204515.2) (71), using Minimap2 software version 2.28.0 (72), with the -ax map-ont parameter for Oxford Nanopore reads, and converted to a BAM file using SAMtools version 1.16 (73). The resulting files were further filtered to eliminate prokaryotic sequence contamination by aligning reads to the SILVA_138.1 database (https://www.arb-silva.de/documentation/release-138.1/), using the same Minimap2 and SAMtools tools. ViromeQC software (74) was used to detect and quantify non-viral contamination, specifically prokaryotic abundance markers in the samples (https://github.com/SegataLab/viromeqc).

Taxonomic assignment and viral identification

The clean sequences obtained after removing host and prokaryotic sequences were taxonomically assigned using the Centrifuge tool v1.0.4 (75). A custom Centrifuge indexing viral database (DBv) was constructed from viral gene and genome sequences available from RefSeq, GenBank, and other NCBI repositories (76) (https://www.ncbi.nlm.nih.gov/labs/virus/vssi/#/). The database contained 12,709 viral sequences that were selected based on completeness, non-redundancy (nr/nt), and no ambiguous characters. To describe the specific species, present in the virome of mosquitoes, a new NCBI-Virus mosquito database (DBm) was constructed by refining the results based on the host option *Culicidae* (Diptera), resulting in a total of 2,899 viral sequences. Taxonomic assignment in Centrifuge was performed using default values. The results were visualized, scanned, and reported using PAVIAN (77). Finally, the taxonomic assignment was confirmed by performing a search of the sequences in Basic Local Alignment Search Tool (BLASTn) considering parameters of percentage identity > 80%, *E*-value greater than 5, and minimum percentage of the coverage length of 70% (78). The number of readings of viral families and species was converted into relative values to estimate their abundance by sample type, positive for DENV serotype infection (DENV-1 and DENV-2) and negative for DENV infection (DENV–), and by study site (Amazonas, Boyacá, Magdalena, and Vichada). Abundance bar graphs were generated using the ggplot2 package in RStudio (47).

Genomic assembly characterization

Using the Genome Detective Virus tool (79), the assembly was generated from the reads. We were able to assemble the complete or nearly complete genomic sequences of the linearly segmented negative-stranded RNA genome, the L segment (RNA-dependent RNA polymerase gene), the M segment (glycoprotein gene), and the S segment (nucleocapsid gene) of PCLV (ISV with the highest abundance of viral reads in *Ae. aegypti* samples). This tool uses a novel alignment method that constructs linkage genomes based on *de novo* contig references by combining amino acid and nucleotide scores. Assemblies with the best quality parameters were selected, including nucleotide identity percentage (>80%), coverage relative to the reference genome (>75%), and alignment match value (>75%). To confirm taxonomic assignment, a BLASTn search was performed with strict criteria (percent identity: >80%, query coverage: >75%, and *E*-value: ≤0.0) using the NCBI BLASTn database. In addition, PCLV genome assembly characterization was performed to identify and annotate important sequence features using the online tool Proksee (80).

Phylogenetic analysis of PCLV

To establish the evolutionary relationship between the PCLV genome sequences obtained in this study and other closely related viral sequences retrieved from NCBI GenBank (Table S5), a phylogenetic analysis was performed (Table S6). Multiple alignments were initially performed with the MAFFT algorithm version 7.450 (81) using Unipro UGENE 3 software (82). Subsequently, a ML phylogenetic tree was reconstructed in IQ-TREE software version 1.6.12 (83), under the nucleotide substitution model (84), which was chosen as the best fitting model according to the BIC information criterion generated by ModelFinder. Bootstrap support values and internal branch reliability were calculated from 1,000 replicates, considering a threshold when its bootstrap value was equal to or greater than 90%. Finally, the phylogenetic trees were visualized in the Itool, together with a global geographic map generated by the Microreact tool (https://microreact.org/) showing the geographic origin of the L, M, and S segments of the PCLV genome from the *Aedes* virome data sets analyzed in this study.

Statistical analysis

Initially, the distribution of the data was assessed using the Shapiro-Wilk test, which indicated that the data were not normally distributed. Subsequently, the non-parametric Wilcoxon-Mann-Whitney test was used to compare samples with and without infection by DENV serotypes (DENV-1 vs DENV negative and DENV-2 vs DENV negative), as well as comparisons between samples infected with DENV1 vs DENV2 serotypes. In addition, to detect differences in the abundance of viral taxa between study sites (Amazonas, Boyacá, Magdalena, and Vichada), a non-parametric Kruskal-Wallis test for multiple comparisons was used, followed by Dunn's test with Benjamini-Hochberg stepwise correction as a post-hoc test at a 95% confidence level. All statistical analyses were performed using R, and $P < 0.05$ was considered statistically significant for all tests (47).

ACKNOWLEDGMENTS

We are grateful to local health authorities from Amazonas, Boyacá, Magdalena, and Vichada for their support and assistance in the collection of mosquitoe samples.

Equally, thanks to the Universidad del Rosario for covering the publication fees and the Universidad de Boyacá for their support in financing M.G.'s doctoral studies.

J.D.R. and M.G. conceived the project. R.B.M., L.A.S., M.P.-C., L.M.M., L.G.P., L.E.B., M.A.M., K.A.C., H.D.P., A.Z.F., and J.L.D. collected the samples. J.D.R., M.G., and M.M. contributed to the writing of the paper. L.C., L.P., D.M., C.H., L.H.P., and M.G. performed laboratory experiments for viral metagenomics. M.M., N.L., A.R., J.M., and S.C. performed bioinformatics analysis. All the authors have read and approved the manuscript for publication.

AUTHOR AFFILIATIONS

[1]Centro de Investigaciones en Microbiología y Biotecnología-UR (CIMBIUR), Facultad de Ciencias Naturales, Universidad del Rosario, Bogota, Colombia
[2]Grupo de Investigación en Ciencias Básicas (NÚCLEO), Facultad de Ciencias e Ingeniería, Universidad de Boyacá, Tunja, Colombia
[3]Centro de Tecnología en Salud (CETESA), Innovaseq SAS, Bogotá, Colombia
[4]Grupo de Estudios en Salud Pública de la Amazonía, Laboratorio de Salud Pública de Amazonas, Leticia, Colombia
[5]Secretaría de Salud de Boyacá, Tunja, Colombia
[6]Secretaría Seccional de Salud de Magdalena, Santa Marta, Colombia
[7]Hospital Local de El Retén, El Retén, Colombia
[8]Secretaría Departamental de Salud del Vichada, Puerto Carreño, Colombia
[9]Department of Pathology, Molecular and Cell-Based Medicine, Molecular Microbiology Laboratory, Icahn School of Medicine at Mount Sinai, New York, New York, USA

AUTHOR ORCIDs

Marina Muñoz https://orcid.org/0000-0002-4216-6928
Juan David Ramírez http://orcid.org/0000-0002-1344-9312

FUNDING

This work was supported by Dirección de Investigación e Innovación from Universidad del Rosario. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. This work was also supported by the Colombian Ministerio de Ciencia, Tecnología e Innovación Minciencias grant number 143889685192-2021.

AUTHOR CONTRIBUTIONS

Marcela Gómez, Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Validation, Visualization, Writing – original draft | David Martínez, Investigation, Methodology, Resources, Writing – review and editing | Luisa Páez-Triana, Data curation, Formal analysis, Investigation, Methodology, Resources, Writing – review and editing | Nicolás Luna, Data curation, Formal analysis, Funding acquisition, Visualization, Writing – review and editing | Julián Medina, Formal analysis, Investigation, Methodology, Resources, Writing – review and editing | Lissa Cruz-Saavedra, Data curation, Formal analysis, Investigation, Methodology, Resources, Validation, Writing – review and editing | Carolina Hernández, Investigation, Methodology, Resources, Writing – review and editing | Sergio Castañeda, Formal analysis, Investigation, Methodology, Resources, Validation, Writing – review and editing | Ramiro Bohórquez Melo, Resources, Writing – review and editing | Luis Alejandro Suarez, Resources, Writing – review and editing | Mónica Palma-Cuero, Resources, Writing – review and editing | Luz Mila Murcia, Resources, Writing – review and editing | Leonel González Páez, Resources, Writing – review and editing | Leonardo Estrada Bustos, Resources, Writing – review and editing | Manuel Alfonso Medina, Resources, Writing – review and editing | Katiuska Ariza Campo, Resources, Writing – review and editing | Holmer David Padilla, Resources, Writing – review and editing | Alexander Zamora Flórez, Resources, Writing – review and editing | Jorge Luis De las Salas, Resources, Writing – review and editing | Marina Muñoz, Conceptualization, Data curation, Formal analysis, Funding acquisition, Supervision, Validation, Writing – review and editing | Juan David Ramírez, Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review and editing.

DATA AVAILABILITY

Data sets generated during the present study are available from NCBI SRA, BioProject number PRJNA995341.

ETHICS APPROVAL

The insects were collected from public land in Colombia using various entomological surveillance techniques by the localities' secretaries of health (Amazonas, Boyacá, Magdalena, and Vichada) as part of routine dengue surveillance in the country. Therefore, ethical approval of permits was not required for this study.

ADDITIONAL FILES

The following material is available online.

Supplemental Material

Figure S1 (JVI01507-23-S0001.pdf). Genome organization and structural protein analysis of *Ae. aegypti* PCLV samples from Colombia.
Table S1 (JVI01507-23-S0002.docx). Summary of metagenomic sequencing results with Oxford Nanopore (MinION) of *Ae. aegypti* mosquito samples collected in the field in different localities of Colombia, with detection of natural infection with DENV positive (DENV1/DENV2) and DENV-negative.
Table S2 (JVI01507-23-S0003.docx). Summary of classification of viral reads after removal of host and bacterial sequences.
Table S3 (JVI01507-23-S0004.docx). Differences between samples positive and negative for natural DENV infection.
Table S4 (JVI01507-23-S0005.docx). Summary of the assembly and classification of Phasi Charoen-like phasivirus (PCLV) genome segments, segment L (RdRp), segment M (glycoprotein), and segment S (capsid), present in the viral long reads produced in the Oxford nanopore sequencing of DENV positive (serotypes DENV1 and DENV2) and DENV negative pooled samples detected in *Ae. aegypti* mosquitoes collected in the field in different departments of Colombia.
Table S5 (JVI01507-23-S0006.docx). Predicted protein-coding genes and putative PCLV assembly products found in *Ae. aegypti* from Colombia.
Table S6 (JVI01507-23-S0007.docx). GenBank accession ID of S, M, and L segments of the *Phasi Charoen-like phasivirus* genome used in the phylogenetic analysis.

REFERENCES

1. de Almeida JP, Aguiar ER, Armache JN, Olmo RP, Marques JT. 2021. The virome of vector mosquitoes. Curr Opin Virol 49:7–12. https://doi.org/10.1016/j.coviro.2021.04.002
2. Weaver SC, Charlier C, Vasilakis N, Lecuit M. 2018. Zika, Chikungunya, and other emerging vector-borne viral diseases. Annu Rev Med 69:395–408. https://doi.org/10.1146/annurev-med-050715-105122
3. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, Drake JM, Brownstein JS, Hoen AG, Sankoh O, Myers MF, George DB, Jaenisch T, Wint GRW, Simmons CP, Scott TW, Farrar JJ, Hay SI. 2013. The global distribution and burden of dengue. Nature 496:504–507. https://doi.org/10.1038/nature12060
4. Harapan H, Michie A, Sasmono RT, Imrie A. 2020. Dengue: a minireview. Viruses 12:829. https://doi.org/10.3390/v12080829
5. Brady OJ, Golding N, Pigott DM, Kraemer MUG, Messina JP, Reiner RC, Scott TW, Smith DL, Gething PW, Hay SI. 2014. Global temperature constraints on *Aedes aegypti* and Ae. albopictus persistence and competence for dengue virus transmission. Parasit Vectors 7:338. https://doi.org/10.1186/1756-3305-7-338
6. Kraemer MUG, Reiner RC, Brady OJ, Messina JP, Gilbert M, Pigott DM, Yi D, Johnson K, Earl L, Marczak LB, Shirude S, et al. 2019. Past and future spread of the arbovirus vectors *Aedes aegypti* and *Aedes* albopictus. Nat Microbiol 4:854–863. https://doi.org/10.1038/s41564-019-0376-y
7. Ryan SJ, Carlson CJ, Mordecai EA, Johnson LR, Han BA. 2019. Global expansion and redistribution of *Aedes*-borne virus transmission risk with climate change. PLoS Negl Trop Dis 13:e0007213. https://doi.org/10.1371/journal.pntd.0007213
8. Goindin D, Delannay C, Ramdini C, Gustave J, Fouque F. 2015. Parity and longevity of *Aedes aegypti* according to temperatures in controlled conditions and consequences on dengue transmission risks. PLoS One 10:e0135489. https://doi.org/10.1371/journal.pone.0135489
9. Morens DM, Folkers GK, Fauci AS. 2004. The challenge of emerging and re-emerging infectious diseases. Nature 430:242–249. https://doi.org/10.1038/nature02759
10. Kraemer MUG, Sinka ME, Duda KA, Mylne A, Shearer FM, Brady OJ, Messina JP, Barker CM, Moore CG, Carvalho RG, Coelho GE, Van Bortel W, Hendrickx G, Schaffner F, Wint GRW, Elyazar IRF, Teng H-J, Hay SI. 2015. The global compendium of *Aedes aegypti* and Ae. albopictus occurrence. Sci Data 2:150035. https://doi.org/10.1038/sdata.2015.35
11. Liu-Helmersson J, Quam M, Wilder-Smith A, Stenlund H, Ebi K, Massad E, Rocklöv J. 2016. Climate change and *Aedes* vectors: 21st century projections for dengue transmission in Europe. EBioMedicine 7:267–277. https://doi.org/10.1016/j.ebiom.2016.03.046
12. Kraemer MUG, Sinka ME, Duda KA, Mylne AQN, Shearer FM, Barker CM, Moore CG, Carvalho RG, Coelho GE, Van Bortel W, et al. 2015. The global distribution of the arbovirus vectors *Aedes aegypti* and Ae. albopictus. Elife 4:e08347. https://doi.org/10.7554/eLife.08347
13. Delatte H, Gimonneau G, Triboire A, Fontenille D. 2009. Influence of temperature on immature development, survival, longevity, fecundity, and gonotrophic cycles of *Aedes* albopictus, vector of Chikungunya and dengue in the Indian ocean. J Med Entomol 46:33–41. https://doi.org/10.1603/033.046.0105
14. Paupy C, Delatte H, Bagny L, Corbel V, Fontenille D. 2009. *Aedes albopictus*, an arbovirus vector: from the darkness to the light. Microbes Infect 11:1177–1185. https://doi.org/10.1016/j.micinf.2009.05.005
15. Coleman M, Hemingway J, Gleave KA, Wiebe A, Gething PW, Moyes CL. 2017. Developing global maps of insecticide resistance risk to improve vector control. Malar J 16:86. https://doi.org/10.1186/s12936-017-1733-z
16. Lequime S, Paul RE, Lambrechts L. 2016. Determinants of arbovirus vertical transmission in mosquitoes. PLoS Pathog 12:e1005548. https://doi.org/10.1371/journal.ppat.1005548
17. Ferreira-de-Lima VH, Lima-Camara TN. 2018. Natural vertical transmission of dengue virus in *Aedes aegypti* and *Aedes* albopictus: a systematic review. Parasit Vectors 11:77. https://doi.org/10.1186/s13071-018-2643-9
18. Conway MJ, Colpitts TM, Fikrig E. 2014. Role of the vector in arbovirus transmission. Annu Rev Virol 1:71–88. https://doi.org/10.1146/annurev-virology-031413-085513
19. Atoni E, Zhao L, Karungu S, Obanda V, Agwanda B, Xia H, Yuan Z. 2019. The discovery and global distribution of novel mosquito-associated viruses in the last decade. Rev Med Virol 29:e2079. https://doi.org/10.1002/rmv.2079
20. Temmam S, Davoust B, Berenger JM, Raoult D, Desnues C. 2014. Viral metagenomics on animals as a tool for the detection of zoonoses prior to human infection Int J Mol Sci 15:10377–10397. https://doi.org/10.3390/ijms150610377
21. Gu W, Miller S, Chiu CY. 2019. Clinical metagenomic next-generation sequencing for pathogen detection. Annu Rev Pathol 14:319–338. https://doi.org/10.1146/annurev-pathmechdis-012418-012751
22. Claro IM, Ramundo MS, Coletti TM, da Silva CAM, Valenca IN, Candido DS, Sales FCS, Manuli ER, de Jesus JG, de Paula A, Felix AC, Andrade PDS, Pinho MC, Souza WM, Amorim MR, Proenca-Modena JL, Kallas EG, Levi

JE, Faria NR, Sabino EC, Loman NJ, Quick J. 2021. Rapid viral metagenomics using SMART-9N amplification and nanopore sequencing. Wellcome Open Res 6:241. https://doi.org/10.12688/wellcomeopenres.17170.2
23. Shi C, Beller L, Deboutte W, Yinda KC, Delang L, Vega-Rúa A, Failloux A-B, Matthijnssens J. 2019. Stable distinct core eukaryotic viromes in different mosquito species from Guadeloupe, using single mosquito viral Metagenomics. Microbiome 7:121. https://doi.org/10.1186/s40168-019-0734-2
24. Ramos-Nino ME, Fitzpatrick DM, Tighe S, Eckstrom KM, Hattaway LM, Hsueh AN, Stone DM, Dragon J, Cheetham S. 2020. High prevalence of phasi charoen-like virus from wild-caught *Aedes aegypti* in Grenada, W.I. as revealed by metagenomic analysis. PLoS One 15:e0227998. https://doi.org/10.1371/journal.pone.0227998
25. Ramos-Nino ME, Fitzpatrick DM, Eckstrom KM, Tighe S, Hattaway LM, Hsueh AN, Stone DM, Dragon JA, Cheetham S. 2020. Metagenomic analysis of *Aedes aegypti* and culex Quinquefasciatus mosquitoes from Grenada, West Indies. PLoS One 15:e0231047. https://doi.org/10.1371/journal.pone.0231047
26. Shi C, Liu Y, Hu X, Xiong J, Zhang B, Yuan Z. 2015. A metagenomic survey of viral abundance and diversity in mosquitoes from hubei province. PLoS One 10:e0129845. https://doi.org/10.1371/journal.pone.0129845
27. Öhlund P, Lundén H, Blomström A-L. 2019. Insect-specific virus evolution and potential effects on vector competence. Virus Genes 55:127–137. https://doi.org/10.1007/s11262-018-01629-9
28. Bolling BG, Vasilakis N, Guzman H, Widen SG, Wood TG, Popov VL, Thangamani S, Tesh RB. 2015. Insect-specific viruses detected in laboratory mosquito colonies and their potential implications for experiments evaluating arbovirus vector competence. Am J Trop Med Hyg 92:422–428. https://doi.org/10.4269/ajtmh.14-0330
29. Calisher CH, Higgs S. 2018. The discovery of arthropod-specific viruses in hematophagous arthropods: an open door to understanding the mechanisms of arbovirus and arthropod evolution Annu Rev Entomol 63:87–103. https://doi.org/10.1146/annurev-ento-020117-043033
30. Vasilakis N, Tesh RB. 2015. Insect-specific viruses and their potential impact on arbovirus transmission. Curr Opin Virol 15:69–74. https://doi.org/10.1016/j.coviro.2015.08.007
31. Carvalho VL, Long MT. 2021. Insect-specific viruses: an overview and their relationship to arboviruses of concern to humans and animals. Virology 557:34–43. https://doi.org/10.1016/j.virol.2021.01.007
32. Roundy CM, Azar SR, Rossi SL, Weaver SC, Vasilakis N. 2017. Insect-specific viruses: a historical overview and recent developments. Adv Virus Res 98:119–146. https://doi.org/10.1016/bs.aivir.2016.10.001
33. Bolling BG, Weaver SC, Tesh RB, Vasilakis N. 2015. Insect-specific virus discovery: significance for the arbovirus community. Viruses 7:4911–4928. https://doi.org/10.3390/v7092851
34. Lole K, Ramdasi A, Patil S, Thakar S, Nath A, Ghuge O, Gangopadhayya A, Sudeep AB, Cherian S. 2022. Abundance of phasi-charoen-like virus in *Aedes aegypti* mosquito populations in different States of India. PLoS One 17:e0277276. https://doi.org/10.1371/journal.pone.0277276
35. Olmo RP, Todjro YMH, Aguiar E, de Almeida JPP, Ferreira FV, Armache JN, de Faria IJS, Ferreira AGA, Amadou SCG, Silva ATS, et al. 2023 Mosquito vector competence for dengue is modulated by insect-specific viruses. Nat Microbiol 8:135–149. https://doi.org/10.1038/s41564-022-01289-4
36. Agboli E, Leggewie M, Altinli M, Schnettler E. 2019. Mosquito-specific viruses-transmission and interaction. Viruses 11:873. https://doi.org/10.3390/v11090873
37. Olmo RP, Todjro YMH, Aguiar E, de Almeida JPP, Ferreira FV, Armache JN, de Faria IJS, Ferreira AGA, Amadou SCG, Silva ATS, Marois E, Imler J-L, Marques JT, et al. 2023. Mosquito vector competence for dengue is modulated by insect-specific viruses. Nat Microbiol 8:135–149. https://doi.org/10.1038/s41564-022-01289-4
38. Schultz MJ, Frydman HM, Connor JH. 2018. Dual insect specific virus infection limits arbovirus replication in *Aedes* mosquito cells. Virology 518:406–413. https://doi.org/10.1016/j.virol.2018.03.022
39. Romo H, Kenney JL, Blitvich BJ, Brault AC. 2018. Restriction of zika virus infection and transmission in *Aedes aegypti* mediated by an insect-specific flavivirus. Emerg Microbes Infect 7:181. https://doi.org/10.1038/s41426-018-0180-4
40. Gutierrez-Barbosa H, Medina-Moreno S, Zapata JC, Chua JV. 2020. Dengue infections in Colombia: epidemiological trends of a hyperendemic country. Trop Med Infect Dis 5:156. https://doi.org/10.3390/tropicalmed5040156
41. Gómez M, Martínez D, Hernández C, Luna N, Patiño LH, Bohórquez Melo R, Suarez LA, Palma-Cuero M, Murcia LM, González Páez L, Estrada Bustos L, Medina MA, Ariza Campo K, Padilla HD, Zamora Flórez A, De las Salas JL, Muñoz M, Ramírez JD. 2022. Arbovirus infection in *Aedes aegypti* from different departments of Colombia. Front Ecol Evol 10. https://doi.org/10.3389/fevo.2022.999169
42. Ruiz-López F F, González-Mazo A A. 2016. Presencia de *Aedes* (Stegomyia) aegypti (Linnaeus, 1762) y su Infección natural con el virus del dengue en alturas no registradas para Colombia. Biomédica 36. https://doi.org/10.7705/biomedica.v36i2.3301
43. Padilla JC, Lizarazo FE, Murillo OL, Mendigaña FA, Pachón E, Vera MJ. 2017. Epidemiología de las principales enfermedades transmitidas por vectores en Colombia. biomedica 37:27. https://doi.org/10.7705/biomedica.v37i0.3769
44. Peña-García VH, Triana-Chávez O, Mejía-Jaramillo AM, Díaz FJ, Gómez-Palacio A, Arboleda-Sánchez S. 2016. Infection rates by dengue virus in mosquitoes and the influence of temperature may be related to different endemicity patterns in three Colombian cities. Int J Environ Res Public Health 13:734. https://doi.org/10.3390/ijerph13070734
45. Gómez-Vargas W, Zapata-Úsuga G. 2019. Presencia de *Aedes aegypti* y *Aedes a*lbopictus (Diptera: Culicidae) en area rural del departamento de. biosalud 18:7–21. https://doi.org/10.17151/biosa.2019.18.1.1
46. Espinal MA, Andrus JK, Jauregui B, Waterman SH, Morens DM, Santos JI, Horstick O, Francis LA, Olson D. 2019. Emerging and reemerging *Aedes*-transmitted arbovirus infections in the region of the Americas: implications for health policy. Am J Public Health 109:387–392. https://doi.org/10.2105/AJPH.2018.304849
47. Team R. 2015. Rstudio: Integrated development environment for R. Boston, MA.
48. Chandler JA, Thongsripong P, Green A, Kittayapong P, Wilcox BA, Schroth GP, Kapan DD, Bennett SN. 2014. Metagenomic shotgun sequencing of a bunyavirus in wild-caught *Aedes aegypti* from Thailand informs the evolutionary and genomic history of the phleboviruses. Virology 464–465:312–319. https://doi.org/10.1016/j.virol.2014.06.036
49. Aguiar ERGR, Olmo RP, Paro S, Ferreira FV, de Faria IJ da S, Todjro YMH, Lobo FP, Kroon EG, Meignin C, Gatherer D, Imler J-L, Marques JT. 2015. Sequence-independent characterization of viruses based on the pattern of viral small RNAs produced by the host. Nucleic Acids Res 43:6191–6206. https://doi.org/10.1093/nar/gkv587
50. Vasilakis N, Forrester NL, Palacios G, Nasar F, Savji N, Rossi SL, Guzman H, Wood TG, Popov V, Gorchakov R, González AV, Haddow AD, Watts DM, da Rosa APAT, Weaver SC, Lipkin WI, Tesh RB. 2013. Negevirus: a proposed new taxon of insect-specific viruses with wide geographic distribution. J Virol 87:2475–2488. https://doi.org/10.1128/JVI.00776-12
51. Charles J, Tangudu CS, Hurt SL, Tumescheit C, Firth AE, Garcia-Rejon JE, Machain-Williams C, Blitvich BJ. 2018. Detection of novel and recognized RNA viruses in mosquitoes from the yucatan peninsula of Mexico using metagenomics and characterization of their in vitro host ranges. J Gen Virol 99:1729–1738. https://doi.org/10.1099/jgv.0.001165
52. Truong Nguyen PT, Culverwell CL, Suvanto MT, Korhonen EM, Uusitalo R, Vapalahti O, Smura T, Huhtamo E. 2022. Characterisation of the RNA virome of nine *Ochlerotatus* species in Finland. Viruses 14:1489. https://doi.org/10.3390/v14071489
53. Belaganahalli MN, Maan S, Maan NS, Nomikou K, Guimera M, Brownlie J, Tesh R, Attoui H, Mertens PPC. 2013. Full genome sequencing of corriparta virus, identifies California mosquito pool virus as a member of the corriparta virus species. PLoS One 8:e70779. https://doi.org/10.1371/journal.pone.0070779
54. Tavares-Neto J, Travassos da Rosa AP, Ataide M, Morais-Souza H, Vasconcelos P, Travassos da Rosa J. 1990. Frequency of neutralizing antibodies to the vesiculovirus piry, in blood donors of Uberaba, Minas Gerais, Brazil. Rev Inst Med Trop Sao Paulo 32:211–214.
55. Instituto Nacional de Salud. 2022. Instituto Nacional de Salud. Boletín Epidemiológico semana 33. In Dirección de Vigilancia y Análisis del Riesgo en Salud Pública Publicación en línea: ISSN 2357-6189 2018
56. Oguzie JU, Nwangwu UC, Oluniyi PE, Olumade TJ, George UE, Kazeem A, Bankole BE, Brimmo FO, Asadu CC, Chukwuekezie OC, et al. 2022.

Metagenomic sequencing characterizes a wide diversity of viruses in field mosquito samples in Nigeria. Sci Rep 12:7616. https://doi.org/10.1038/s41598-022-11797-2
57. Duarte MA, Campos FS, Araújo Neto OF, Silva LA, Silva AB, Aguiar TC, Santos RN, Souza UJB, Alves GB, Melo FL, Ardisson-Araujo DMP, Aguiar RWS, Ribeiro BM. 2022. Identification of potential new mosquito-associated viruses of adult *Aedes aegypti* mosquitoes from Tocantins state, Brazil. Braz J Microbiol 53:51–62. https://doi.org/10.1007/s42770-021-00632-x
58. Golding N, Wilson AL, Moyes CL, Cano J, Pigott DM, Velayudhan R, Brooker SJ, Smith DL, Hay SI, Lindsay SW. 2015. Integrating vector control across diseases. BMC Med 13:249. https://doi.org/10.1186/s12916-015-0491-4
59. Moonen JP, Schinkel M, van der Most T, Miesen P, van Rij RP. 2023. Composition and global distribution of the mosquito virome - a comprehensive database of insect-specific viruses. One Health 16:100490. https://doi.org/10.1016/j.onehlt.2023.100490
60. Calle-Tobón A, Pérez-Pérez J, Forero-Pineda N, Chávez OT, Rojas-Montoya W, Rúa-Uribe G, Gómez-Palacio A. 2022. Local-scale virome depiction in Medellín, Colombia, supports significant differences between *Aedes aegypti* and *Aedes albopictus*. PLoS One 17:e0263143. https://doi.org/10.1371/journal.pone.0263143
61. Halbach R, Junglen S, van Rij RP. 2017. Mosquito-specific and mosquito-borne viruses: evolution, infection, and host defense. Curr Opin Insect Sci 22:16–27. https://doi.org/10.1016/j.cois.2017.05.004
62. Russell JA, Campos B, Stone J, Blosser EM, Burkett-Cadena N, Jacobs JL. 2018. Unbiased strain-typing of arbovirus directly from mosquitoes using nanopore sequencing: a field-forward biosurveillance protocol. Sci Rep 8:5417. https://doi.org/10.1038/s41598-018-23641-7
63. Batovska J, Lynch SE, Rodoni BC, Sawbridge TI, Cogan NO. 2017. Metagenomic arbovirus detection using minion nanopore sequencing. Journal of Virological Methods 249:79–84. https://doi.org/10.1016/j.jviromet.2017.08.019
64. Shi C, Zhao L, Atoni E, Zeng W, Hu X, Matthijnssens J, Yuan Z, Xia H, Lal R. 2020. 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 5:e00640-20. https://doi.org/10.1128/mSystems.00640-20
65. Zakrzewski M, Rašić G, Darbro J, Krause L, Poo YS, Filipović I, Parry R, Asgari S, Devine G, Suhrbier A. 2018. Mapping the virome in wild-caught *Aedes aegypti* from Cairns and Bangkok. Sci Rep 8:4690. https://doi.org/10.1038/s41598-018-22945-y
66. Gómez M, Martinez D, Muñoz M, Ramírez JD. 2022. *Aedes aegypti* and Ae. albopictus microbiome/virome: new strategies for controlling arboviral transmission? Parasit Vectors 15:287. https://doi.org/10.1186/s13071-022-05401-9
67. Fredericks AC, Russell TA, Wallace LE, Davidson AD, Fernandez-Sesma A, Maringer K. 2019. *Aedes aegypti* (Aag2)-derived clonal mosquito cell lines reveal the effects of pre-existing persistent infection with the insect-specific bunyavirus phasi charoen-like virus on arbovirus replication. PLoS Negl Trop Dis 13:e0007346. https://doi.org/10.1371/journal.pntd.0007346
68. Vogels CBF, Rückert C, Cavany SM, Perkins TA, Ebel GD, Grubaugh ND. 2019. Arbovirus coinfection and co-transmission: a neglected public health concern? PLoS Biol 17:e3000130. https://doi.org/10.1371/journal.pbio.3000130
69. Instituto Nacional de Salud. 2020. Instituto Nacional de Salud. Boletín Epidemiológico Semana 41, p 2357–6189. In Dirección de Vigilancia Y Análisis del Riesgo en Salud Pública Publicación en Línea: ISSN
70. Wick RR, Judd LM, Holt KE. 2019. Performance of neural network basecalling tools for oxford nanopore sequencing. Genome Biol 20:129. https://doi.org/10.1186/s13059-019-1727-y
71. Matthews BJ, Dudchenko O, Kingan SB, Koren S, Antoshechkin I, Crawford JE, Glassford WJ, Herre M, Redmond SN, Rose NH, et al. 2018. Improved reference genome of *Aedes aegypti* informs arbovirus vector control. Nature 563:501–507. https://doi.org/10.1038/s41586-018-0692-z
72. Li H, Birol I. 2018. Minimap2: pairwise alignment for nucleotide sequences. Bioinformatics 34:3094–3100. https://doi.org/10.1093/bioinformatics/bty191
73. Danecek P, Bonfield JK, Liddle J, Marshall J, Ohan V, Pollard MO, Whitwham A, Keane T, McCarthy SA, Davies RM, Li H. 2021. Twelve years of SAMtools and BCFtools. Gigascience 10:giab008. https://doi.org/10.1093/gigascience/giab008
74. Zolfo M, Pinto F, Asnicar F, Manghi P, Tett A, Bushman FD, Segata N. 2019. Detecting contamination in Viromes using ViromeQC. Nat Biotechnol 37:1408–1412. https://doi.org/10.1038/s41587-019-0334-5
75. Kim D, Song L, Breitwieser FP, Salzberg SL. 2016. Centrifuge: rapid and sensitive classification of Metagenomic sequences. Genome Res 26:1721–1729. https://doi.org/10.1101/gr.210641.116
76. O'Leary NA NA, Wright MW MW, Brister JR JR. 2016. Reference sequence (Refseq) database at NCBI: current status, taxonomic expansion, and functional annotation. Nucleic acids Res 44:D733. https://doi.org/10.1093/nar/gkv1189
77. Breitwieser FP, Salzberg SL. 2020. Pavian: interactive analysis of metagenomics data for microbiome studies and pathogen identification. Bioinformatics 36:1303–1304. https://doi.org/10.1093/bioinformatics/btz715
78. Sayers EW, Bolton EE, Brister JR, Canese K, Chan J, Comeau DC, Farrell CM, Feldgarden M, Fine AM, Funk K, et al. 2023. Database resources of the national center for biotechnology information in 2023. Nucleic Acids Res 51:D29–D38. https://doi.org/10.1093/nar/gkac1032
79. Vilsker M, Moosa Y, Nooij S, Fonseca V, Ghysens Y, Dumon K, Pauwels R, Alcantara LC, Vanden Eynden E, Vandamme A-M, Deforche K, de Oliveira T. 2019. Genome detective: an automated system for virus identification from high-throughput sequencing data. Bioinformatics 35:871–873. https://doi.org/10.1093/bioinformatics/bty695
80. Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069. https://doi.org/10.1093/bioinformatics/btu153
81. Katoh K, Rozewicki J, Yamada KD. 2019. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Brief Bioinform 20:1160–1166. https://doi.org/10.1093/bib/bbx108
82. Okonechnikov K, Golosova O, Fursov M, UGENE team. 2012. Unipro UGENE: a unified bioinformatics toolkit. Bioinformatics 28:1166–1167. https://doi.org/10.1093/bioinformatics/bts091
83. Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol 32:268–274. https://doi.org/10.1093/molbev/msu300
84. Hasegawa M, Kishino H, Yano T. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. J Mol Evol 22:160–174. https://doi.org/10.1007/BF02101694

Capítulo 3

En respuesta al objetivo específico 3, el capítulo 3 se enfocó en determinar de manera preliminar las especies virales presentes en mosquitos (Díptera: Culicidae) de una zona rural en la Orinoquía de Colombia. En estas áreas, donde conviven humanos, mosquitos y animales tanto domésticos como salvajes, el riesgo de transmisión de enfermedades zoonóticas a humanos aumenta significativamente. Inicialmente, se identificaron las especies de mosquitos circulantes y se evaluó la frecuencia de infección por virus del género Flavivirus. Posteriormente, se profundizó en la caracterización del viroma de las especies predominantes. Durante este proceso, se detectó la infección por un virus de importancia médica, el virus del Nilo Occidental (WNV), en mosquitos de la especie *Culex browni.* Además, se encontró la presencia de virus específicos de insectos (ISV) comunes en el viroma de estas especies de mosquitos. Estos hallazgos representan una importante contribución al conocimiento del viroma en mosquitos vectores neotropicales que se consideran potenciales transmisores de virus patógenos.

Como producto de este capítulo se adjuntan los siguientes artículos científicos:

- **Artículo 4:** Martínez D, Gómez M, De Las Salas JL, Hernández C, Flórez AZ, Muñoz M, Ramírez JD. Employing oxford nanopore technologies (ONT) for understanding the ecology and transmission dynamics of flaviviruses in mosquitoes (Diptera: Culicidae) from Eastern Colombia. Acta Trop. 2023 Sep; 245:106972. Doi: 10.1016/j.actatropica.2023.106972.

- **Artículo 5:** Gómez M, Martínez D, Páez-Triana L, Luna N, De las Salas J, Hernández C, Zamora Flórez A, Muñoz M, Ramírez JD. Characterizing viral species in mosquitoes (Culicidae) in the Colombian Orinoco: insights from a preliminary metagenomic study. Sci Rep 2023.13, 22081. Doi.org/10.1038/s41598-023-49232-9

CAPÍTULO 3

Dinámica de transmisión y composición viral en poblaciones de mosquitos (Díptera: Culicidae) en ecosistemas rurales estratégicos

Acta Tropica 245 (2023) 106972

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Employing oxford nanopore technologies (ONT) for understanding the ecology and transmission dynamics of flaviviruses in mosquitoes (Diptera: Culicidae) from Eastern Colombia

David Martínez [a], Marcela Gómez [a,b], Jorge Luis De las salas [c], Carolina Hernández [a,c], Alexander Zamora Flórez [d], Marina Muñoz [a], Juan David Ramírez [a,e,*]

[a] Centro de Investigaciones en Microbiología y Biotecnología-UR (CIMBIUR), Facultad de Ciencias Naturales, Universidad del Rosario, Bogotá, Colombia
[b] Grupo de Investigación en Ciencias Básicas (NÚCLEO) Facultad de Ciencias e Ingeniería, Universidad de Boyacá, Tunja, Colombia
[c] Centro de Tecnología en Salud (CETESA), Innovaseq SAS, Bogotá, Colombia
[d] Secretaría Departamental de Salud del Vichada, Colombia
[e] Molecular Microbiology Laboratory, Department of Pathology, Molecular and Cell-based Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, USA

ARTICLE INFO

Keywords:
Mosquitoes
Flavivirus
Feeding sources
Insect-specific viruses (ISFVs)
Oxford nanopore technologies (ONT)

ABSTRACT

Studies focused on identifying the viral species of *Flavivirus* in vectors are scarce in Latin America and particularly in Colombia. Therefore, the frequency of infection of the *Flavivirus* genus and its feeding preferences were identified in the mosquito species circulating in the municipality of Puerto Carreño-Vichada, located in the Eastern Plains of Colombia. This was done by sequencing the viral NS5 and vertebrate 12S rRNA genes, respectively, using Oxford Nanopore Technologies (ONT). A total of 1,159 mosquitoes were captured, with the most abundant species being *Aedes serratus* at 73.6% ($n = 853$). All the mosquitoes were processed in 230 pools (2–6 individuals) and 51 individuals, where 37.01% ($n = 104$) were found to be infected with *Flavivirus*. In these samples, infection by arboviruses of epidemiological importance, such as dengue virus (DENV), Zika virus (ZIKV), and chikungunya virus (CHIKV), was ruled out by PCR. However, through sequencing, infection by different insect-specific viruses (ISFVs) and a medically important virus, West Nile virus (WNV), were identified in a mosquito of the *Culex browni* species. Additionally, the feeding patterns showed that most species present a generalist behavior. Given the above, conducting entomovirological surveillance studies is crucial, especially in areas of low anthropogenic intervention, due to the high probability that potentially pathogenic viruses could generate spillover events under deforestation scenarios.

1. Introduction

Arthropod-Borne Viruses are a group of microorganisms mainly transmitted by mosquitoes. Among this group, the Flaviviridae family stands out for harboring virus species that pose a public health problem, particularly those belonging to the genus *Flavivirus*. Based on phylogenetic reconstructions, the species in this genus are grouped into clusters based on the vector: (1) mosquito-borne viruses (dengue virus (DENV), Zika Virus (ZIKV), chikungunya virus (CHIKV), yellow fever virus (YFV) and West Nile virus (WNV)), which can infect a wide range of hosts and cause severe public health problems ; (2) tick-borne viruses (Tick-borne encephalitis viruses); (3) viruses whose the vector is unknown, but with the ability to replicate in vertebrate cells (Kuno et al., 2017); and (4) insect-specific flaviviruses (ISFVs), known as mosquito-specific and unable to replicate in mammalian cells (Bolling et al., 2015). Despite this, only a few studies have focused on determining the circulation of each of these *Flavivirus* viral species and their interaction with insect vectors in endemic areas.

In recent years, molecular techniques such as PCR and/or sequencing have become established as supportive tools in entomovirological surveillance for identifying *Flavivirus* in vectors (de Oliveira Ribeiro et al., 2021). Studies utilizing this approach have characterized the co-circulation of different ISFVs and pathogenic arboviruses in the same area (WNV-Culex *Flavivirus* (CxF)), and have shown that the co-infection of these viruses can modify the vectorial capacity of mosquitoes (Bolling et al., 2012; Newman et al., 2011). Thus, the importance of applying

* Corresponding author.
E-mail address: juand.ramirez@urosario.edu.co (J.D. Ramírez).

https://doi.org/10.1016/j.actatropica.2023.106972
Received 28 March 2023; Received in revised form 23 May 2023; Accepted 15 June 2023
Available online 16 June 2023

these molecular techniques to identify the simultaneous distribution of different circulating flaviviruses in the vectors is highlighted (Fang et al., 2018; Hoyos-López et al., 2016). However, the sequencing techniques used have limitations in their ability to perform highly sensitive identification of viral species. This is due to two factors. Firstly, vectors may be co-infected with different flaviviruses, and sequencing techniques, such as the Sanger method, cannot identify them (Hoyos et al., 2021). Secondly, the next-generation sequencing (NGS) technique, Illumina, commonly used for vector virome characterization, provides sufficient information to assemble viral genomes and identify co-infections, but at the economic level it ends up generating high costs and its accessibility is limited, particularly in endemic areas or areas with restricted resources (Gómez et al., 2022). Recently, third-generation sequencing (3GS) techniques such as Oxford Nanopore Technologies (ONT), have been widely implemented thanks to the SARS-CoV-2 pandemic, with low cost, easy accessbility, high throughput, and allows deep sequencing (Jain et al., 2016; Merhi et al., 2022; Wang et al., 2021).

Studies suggest that *Flavivirus* transmission may be conditioned by the feeding preferences of the mosquito vector (Figueiredo, 2019; Hayes et al., 2005; Huang et al., 2019). Defining these feeding patterns in rural ecosystems with low anthropogenic disturbance is necessary to understand transmission dynamics and to incriminate hosts potentially associated with transmission. This knowledge can help characterize possible changes in pathogen transmission and the risk of generating spillover events. Some mosquito species, like *Aedes aegypti*, are specialists that feed primarily on human hosts, while others, like *Culex*, are highly generalist and often show opportunistic feeding behavior (Harrington et al., 2001; Mendenhall et al., 2012). Therefore, in rural areas where humans, mosquitoes, and animals (domestic and wild) coexist, the risk of zoonotic diseases being transmitted to humans, such as some emerging arboviruses DENV, ZIKV, and CHIKV, increases (Borremans et al., 2019; Patterson et al., 2016). Considering the above, it is of great importance to understand the interactions between mosquitoes and transmitted viruses, which present a significant challenge for the designing prevention and control strategies (Guth et al., 2020).

Most studies have focused on detecting *Flavivirus* in mosquito populations associated with urban cycles, such as *Aedes aegypti* and *Aedes albopictus*. However, other wild species are considered vectors, or their vectorial capacity is unknown or has been little studied. The main wild vectors that transmit mosquito-borne flaviviruses belong to the genera *Aedes* and *Culex* (Subgenera *Culex* and *Melanoconion*), and individuals of the genera *Haemagogus, Sabethes, Mansonia,* and *Psorophora* have been established as potential vectors. In general, different species of the genus *Culex* have been identified as responsible for West Nile Virus (WNV) transmission in Asia, Europe, and the Americas (Hoyos-López et al., 2016; Jiang et al., 2023; Kampen et al., 2021; Rochlin et al., 2019). The species of the genus *Culex* associated with WNV transmission may vary according to geographic regions, changes in climate, and host availability, highlighting the species *Cx. quinquefasciatus, Cx. pipiens and Cx. tarsalis* (Conway et al., 2014). Individuals of the genera *Haemagogus* and *Sabethes* are associated with YFV transmission in the sylvatic cycle (Li et al., 2022), while individuals of the genera *Mansonia* and *Psorophora* have been mainly associated with the transmission of *Mayaro* virus (MAYV) (Gonzalez-Escobar et al., 2021; Langendries et al., 2021).

In Latin America, particularly in Colombia, few studies have focused on identifying flaviviruses in vectors circulating in rural areas. CxFv is the ISFV with the highest number of reports and has mainly been identified in different species of the *Culex* genus in Guatemala (Morales-Betoulle et al., 2008), Mexico (Saiyasombat et al., 2010), Brazil (Machado et al., 2012), and Argentina (Goenaga et al., 2014). In addition, in Guatemala, the co-circulation of CxFv and WNV in individuals of *Cx. quinquefasciatus* was described (Morales-Betoulle et al., 2008). In Colombia, two studies stand out; The first study was conducted by Hoyoz-López, R. et al. (2016), in which they identified flaviviruses of epidemiological importance such as St. Louis encephalitis virus (SLEV), WNV, and ISFV like CxFV, in individuals of *Culex* (Hoyos-López et al., 2016). The second study by Hoyos et al. (2021) found 25 positive samples for the detection of the NS5 gene fragment. However, sequencing of these fragments by Sanger failed to detect known *Flavivirus* sequences (Hoyos et al., 2021). The limitations of Sanger sequencing are highlighted in cases where the presence of multiple viral species of *Flavivirus* may occur.

Therefore, this study initially focused on identifying the mosquito species circulating in the municipality of Puerto Carreño-Vichada (located in the Eastern Plains of Colombia). This municipality is primarily a rural territory that promotes the presence of arboviruses in enzootic cycles. PCR and RT-PCR techniques were employed to detect the arboviruses of primary epidemiological interest, DENV, CHIKV, and ZIKV in mosquitoes. Finally, the viral species of the *Flavivirus* genus and feeding preferences were identified by sequencing the NS5 and 12S markers, respectively. A new methodology was developed and standardized for this purpose, which allowed the sequencing of these markers by ONT, facilitating the identification of different flaviviruses in the same sample and characterization of multiple feeding preferences.

2. Material and methods

2.1. Mosquito sampling and collection area

Mosquito collection was conducted at eight points in different rural areas of Puerto Carreño in the department of Vichada, Colombia as shown in Table S1. The municipality of Puerto Carreño has a total area of 12,409 km^2, with 99.9% of the territory being rural area. It is bordered to the north and east by the Bolivarian Republic of Venezuela and is surrounded by one of the most important rivers in South America, the Orinoco River. Sampling sites were chosen based on strategic points of Sportfishing and ecotourism with mosquito proliferation, nearby human settlements, and low anthropogenic intervention in the area (these areas have not been significantly altered or disturbed by human activity). Sampling point 1 was located near the Juriepe river, point 2 between the Juriepe river and Laguna La Estacada, point three near Laguna Tres Matas, and points 4 to 8 were located in different areas along the Orinoco River (Fig. 1). A non-probabilistic convenience sampling was carried out with the help of the departmental secretary of Vichada on December 8, 10, 11, and 12, 2020 (beginning of the dry season), with a single sampling time per site (Table S1). Mosquitoes were manually captured using mechanical aspirators during the day in the forests. To prevent contamination of the biological material, we maintained the use of gowns and gloves throughout the handling process. In addition, we sterilized the capture materials, as well as those used for storage and transport, beforehand. The mosquitoes of greatest entomovirological importance, belonging to the subfamily Culicinae, were selected at each sampling point and grouped into pools of 2 to 6 individuals according to the species identified (Table S2).

In some cases, the mosquitoes were stored individually to confirm the species using molecular tools. The entomological material was preserved in RNA later (DNA/RNA shield, Zymo. R1100–50) at −4 °C in vials marked with the coordinates and collection site. Finally, the samples were transported to the microbiology laboratory at the Universidad del Rosario in Bogotá, Colombia for processing and molecular analysis.

2.2. Nucleic acids extraction and cDNA synthesis

Pooled and individual entomological material (Table S2) was homogenized at 30 rpm for 20 min using ZR BashingBead™ lysis tubes (Lysis Tubes-ref. S6003–50) with 200 μL of DNA/RNA shield buffer in the TissueLyser II® tissue homogenizer (Qiagen, Hilden, Germany), followed by centrifugation at 10,000 rpm for 2 min and the supernatant was saved. From the supernatant obtained in the previous step, nucleic acid extraction was performed using the Hamilton Microlab Star automated system and the MagBead Quick-DNA/Viral RNA kit (Ref. R2141, Zymo Research) following the manufacturer's recommended

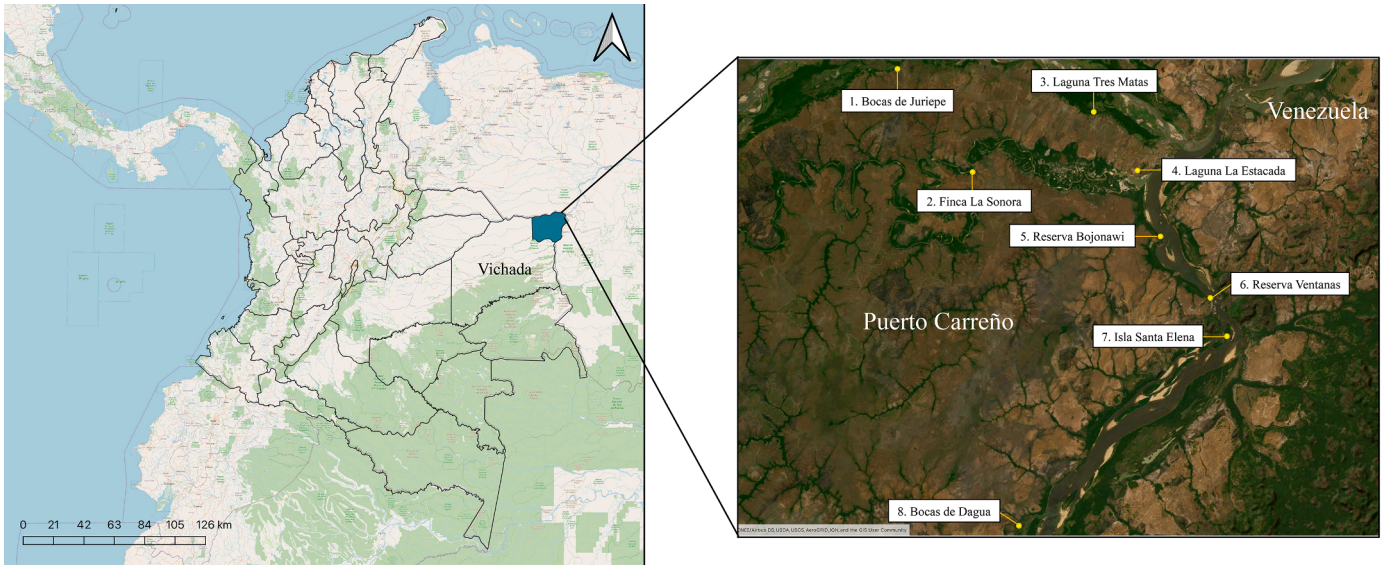

Fig. 1. Geographical distribution of collection sites.
The map shows on the left the geographic location of the municipality of Puerto Carreño in the department of Vichada. On the right side are the georeferenced locations of the eight sampling points. The legend of each point indicates the local name of each sampling point. Built with the tool QGIS 3.22.14 (Basemap: Esri Satellite World_Imagery (MapServer) https://bit.ly/3MRtYCf; Sources: Esri, Maxar, Earthstar Geographics, and the GIS User Community; CC BY-SA 3.0).

instructions. Once the genetic material was obtained, its concentration and quality were quantified using the Nanodrop-2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, United States) and stored at −80 °C. Subsequently, RT-PCR was performed from the genetic material to generate cDNA (complementary DNA) using the LunaScript RT SuperMix Reverse Transcriptase Kit enzyme (NEB #E3010). The cDNA was stored at −30 °C.

2.3. Molecular identification of mosquitoes and analysis of cytochrome c oxidase sequences (Barcoding)

A fragment of the COI (cytochrome oxidase subunit 1) gene was amplified from mosquitoes requiring species confirmation by molecular analysis (Table S2). PCR reactions were performed in final volume of 25 μL containing 2 × GoTaq®Green Master Mix (Promega, # M7123), ten μM of each primer, and 5 μL of the cDNA. The primers used were LCO1490 (5′-GGT CAA ATC ATA AAG ATA TTG G-3′) and HCO2198 (5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′). Thermal profiling consisted of an initial denaturation cycle at 95 °C for 1 min, 45 cycles of 94 °C for 1 min, 60 °C for 1 min, 72 °C for 1 min, and finally, a final extension cycle of 72 °C for 10 min. The gene fragment amplification was confirmed by 2% agarose gel electrophoresis in 1x TBE buffer. As an intercalating agent, 1 μL of SYBR® Safe (Invitrogen®, Carlsbad, CA, USA) was then visualized under UV light to observe a band of ~650 base pairs (bp).

The Sanger sequencing platform sequenced the amplified products after cleanup with the enzyme ExoSAP-IT™ (Applied Biosystems™ #78,205). Sequence cleaning and alignment were performed in Lasergene SeqMan software (DNASTAR, Madison, WI, USA), and each sequence was compared against the GenBank database using the Blastn (Basic Local Alignment Search Tool of nucleotides). The mosquito species were assigned considering the percentage of identity (higher than 95%) and the best e-value result. To confirm the assigned species, a phylogenetic reconstruction was generated on the sequences of each species obtained previously and reference sequences of each species (Table S3). Sequence alignment was initially carried out using MAFFT V.5. With this alignment, a Maximum Likelihood (ML) tree was constructed in iQtree tool version 1.6.12 using the substitution model GTR+F + G4 (Model chosen according to the BIC generated by ModelFinder). Node support was performed using 1000 iterations by the Ultrafast Bootstrap (UFBoot) approach and visualized in iTOL.

2.4. Detection of flaviviruses using PCR and oxford nanopore technologies (ONT) and phylogenetic analysis

As previously described, the samples were initially processed to detect the epidemiologically important arboviruses (DENV, CHIKV, and ZIKV. However, all samples were negative. Therefore *Flavivirus* detection was performed. Detection was performed by PCR using the primers MAMD (5′-AAC ATG GGR AAR AGR GAR AA-3′) and cFD2 (5′-GTG TCC CAG CCG GCG GTG TCA GC-3′). This primer pair amplifies a conserved region of the NS5 gene of the *Flavivirus* genus. PCR reactions were performed in a final volume of 12.5 μL containing 2 × GoTaq®Colorless Master Mix (Promega, # M7133), 10 μM of each primer, and 0.8 μL of the cDNA. The thermal profile consisted of an initial denaturation cycle at 95 °C for 3 min, 40 cycles of 95 °C for 30 s, 59 °C for 1 min, 72 °C for 1 min, and finally, a final extension cycle of 72 °C for 5 min. Gene fragment amplification was verified by 2% agarose gel electrophoresis in 1x TBE buffer and as an intercalating agent 1 μL of SYBR® Safe (Invitrogen®, Carlsbad, CA, USA), then visualized under UV light to observe a band of ~252 base pairs (bp). Culture supernatant from cells infected with the DENV-1 serotype provided by the University of Antioquia (Colombia) was used as a positive control.

Due to economic restrictions, 36 *Flavivirus*-positive samples were randomly selected. The amplified products were sequenced by ONT, for which barcode ligation (assigning one barcode per sample) was initially performed using the ONT Barcode Kit (EXP-NBD196). Subsequently, the library was constructed by joining the amplicons in equal volumes (with their barcode ligated) and proceeded to adapter ligation using the ONT ligation sequencing kit (SQK.LSK109). The constructed library was sequenced in ONT MinION using R.9.4 flow cells and MinKnow V.3.1.4 software. The bioinformatics analysis was performed on the raw Fast5 files, using Super Accuracy base-calling (SUP) (Q>10) to obtain the Fastq files and then demultiplexed with the Guppy V3.1.5 tool. The cleaned sequences were processed with the Centrifuge V1.0.4 tool to perform the taxonomic assignment of reads by global alignments. Te assignment was generated by comparison with a reference dataset using GenBank sequences of the NS5 gene of different viruses belonging to the genus *Flavivirus*. The Centrifuge tool performs a count of the number of reads that map to each taxon and generates a table with that

information. The number of reads in the output table was converted into relative values using the Pavian tool (https://fbreitwieser.shinyapps.io/pavian/) and grouped by sample using RStudio and further used as an approximation of the abundance of the flaviviruses found. Finally, a relative abundance barplot was made using the ggplot2 package.

To confirm the assigned species, a phylogenetic reconstruction was generated on the sequences of each species obtained previously and reference sequences of each species (Fig. 4B). Sequence alignment was initially carried out using MAFFT V.5. With this alignment, a Maximum Likelihood (ML) tree was constructed in iQtree tool version 1.6.12 using the substitution model K2P+R2 (Model chosen according to the BIC generated by ModelFinder). Node support was performed using 1000 iterations by the Ultrafast Bootstrap (UFBoot) approach and visualized in iTOL.

2.5. Molecular characterization of feeding sources

The same 36 previously selected samples were used to amplify a 12S rRNA gene fragment (215 bp) and subsequent sequencing by ONT. Initially, PCR reactions were performed in a final volume of 12.5 μL containing 2 × GoTaq®Colorless Master Mix (Promega, # M7133), 10 μM of each primer, and 1.5 μL of the cDNA. The thermal profile consisted of an initial denaturation cycle at 95 °C for 3 min, 40 cycles of 95 °C for 30 s, 59 °C for 1 min, 72 °C for 1 min, and finally, a final extension cycle of 72 °C for 5 min. The gene fragment amplification was confirmed by 2% agarose gel electrophoresis in 1x TBE buffer. As an intercalating agent, 1 μL of SYBR® Safe (Invitrogen®, Carlsbad, CA, USA) was then visualized under UV light to observe a band of ~215 base pairs (bp).

The amplified products were sequenced by ONT, followed by bioinformatic analysis of the reads obtained as previously described. Taxonomic assignment of reads was also performed by global alignments using the Centrifuge V 1.0.4 tool with the previously reported reference base for feeding sources. First, the Centrifuge V.1.0.4 tool performs a count of the number of reads that map to each taxon and generates a table with that information. Second, the output table's values are normalized and converted to relative values using the Pavian tool (https://fbreitwieser.shinyapps.io/pavian/). These values by sample are used to estimate the feeding sources based on the calculated relative abundances. Finally, the calculation of relative values and the abundance barplot were performed as previously described.

2.6. Statistical analysis

Descriptive analyses of the frequency of each species were carried out based on percentages, abundance analyses of the flaviviruses identified, and feeding preferences. Subsequently, the Chi-square test was used to establish possible associations between the different sampling points, vector species, and *Flavivirus* infection frequency. To perform multiple comparisons between variables, post hoc tests were implemented in R software (RStudio Team, 2019) using the Rcmdr and chisq.posthoc.test packages, performing pairwise comparisons using the Bonferroni adjustment method. Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. Mosquito diversity and geographic distribution

During the days 8, 10–12 December 2020 a total of 1159 individuals were captured at the eight sampling points, of which 1108 were processed in pools ($n = 230$) and 51 individually (Table S2). Twelve mosquito species representing seven genera were identified, and 11 were confirmed from COI sequence analysis. The phylogenetic reconstruction obtained from the sequences shows 11 well-supported clusters (Bootstrap ≥90) corresponding to each of the confirmed species (Fig. 2A). *Aedes* (*Ochlerotatus*) *serratus* (Theobald, 1901) was the species found in the highest abundance (74.1% $n = 859$) and was identified in all eight sampling points (Fig. 2B and S1). *Culex* was the most diverse genus, with five species identified; four were reported simultaneously at sampling point 6 (Figs. 2B and S1). This same sampling point has the highest diversity of mosquitoes captured, with eight species identified. Individuals identified as *Culex* (*Melanoconion*) *theobaldi* (Lutz, 1904), *Cx.* (Mel.)

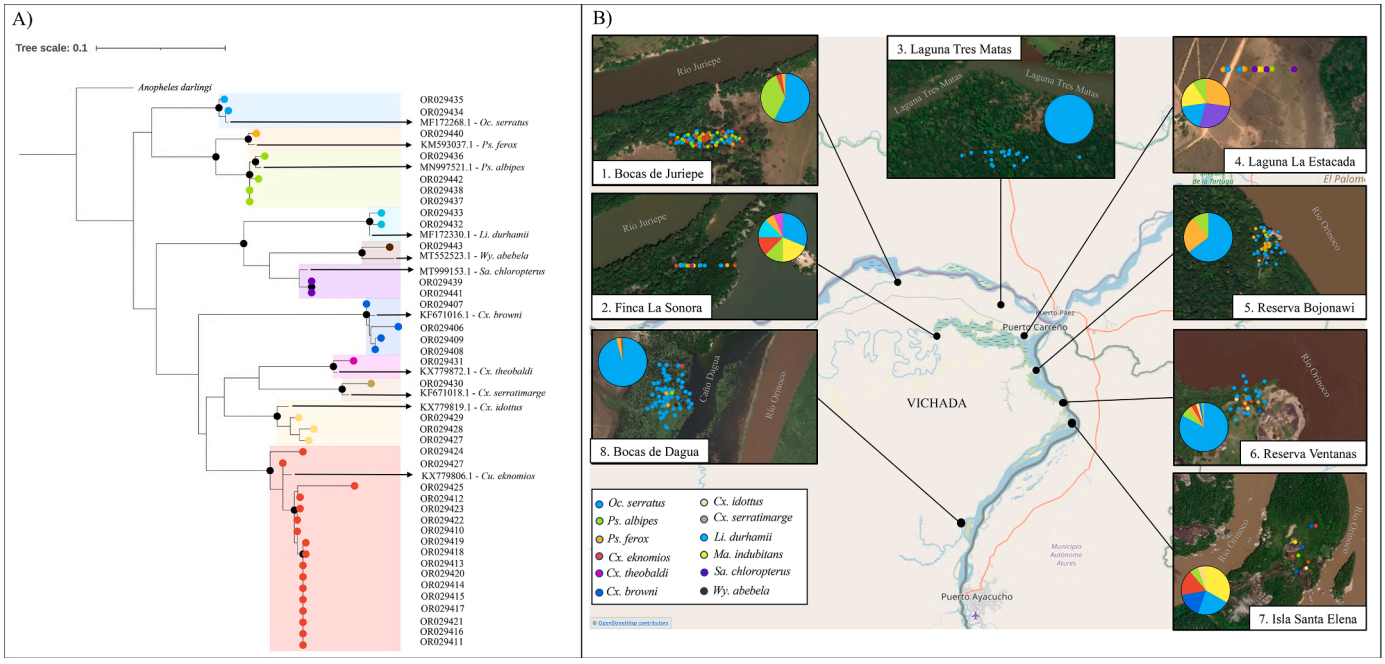

Fig. 2. Vector diversity and abundance.
A) Maximum likelihood phylogenetic reconstruction based on the GTR+*F* + G4 substitution model using COI sequences obtained in this study and GenBank. Black dots indicate bootstrap ≥90, colored dots indicate sequences obtained in this study and arrows indicate sequences obtained from GenBank. B) Shows the abundance of mosquito species captured per sampling point.

serratimarge (Root, 1927), *Sabethes* (*Sabethoides*) *chloropterus* (von Humboldt, 1819), and *Wyeomya* (*Wyeomyia*) *abebela* (Dyar & Knab, 1908) were found only at sampling points 2, 6, 4 and 8, respectively.

3.2. Flavivirus detection

Of the 281 pools processed, the *Flavivirus* genus's overall infection rate was 37.01% (104/281). A higher rate of *Flavivirus* infection was observed in the *Ochlerotatus serratus* species (Table 1), most of which were found at sampling point 8. According to the chi-square test, no associations were found between the frequency of *Flavivirus* infection and sampling point and vector species (X-squared=14.242, df=10, p-value=0.1622). Identification of the viral species of the genus *Flavivirus* was achieved using ONT amplicon sequencing. We could estimate the relative abundance (calculated with the number of reads per taxon) of the different viral species identified in each sample (Fig. 3). In all the samples, sequences that could not be assigned to known viral species were found, and their taxonomic assignment was achieved up to the genus *Flavivirus*. In the case of *Mansonia* (*Mansonia*) *indubitans* (Dyar & Shannon, 1925) none of the sequences could be assigned.

The viral species found in the highest abundance was the *Guapiacu virus* (GUAPV), present in 6 mosquito species (Fig. 3). On the other hand, CxFv was found only in *Cx.* (*Mel.*) *eknomios* (Forattini & Sallum, 1992) species and *Aedes* Flavivirus (AeFV) were only identified in *Oc. Serratus* species. One individual of the species *Cx.* (*Anoedioporpa*) *browni* (Komp, 1936) was found infected with WNV. It is worth mentioning that some of the samples analyzed were found to be simultaneously infected with three or more viruses (Fig. S2). The phylogenetic reconstruction obtained from the sequences shows six well-supported clusters (Bootstrap ≥90) corresponding to each of the confirmed species (Fig. 4B).

Table 1
Number and percentage of species per sampling site positive for *Flavivirus*.

Species	Sampling point	Positive	Negative	Total
Oc. serratus		72 (40)	108 (60)	180
	1.Bocas de Juriepe	16	30	46
	2.Finca la Sonora	0	2	2
	3.Laguna Tres Matas	6	14	20
	4. Laguna La Estacada	0	1	1
	5.Reserva Bojonawi	15	13	28
	6.Reserva Ventanas	10	7	17
	7. Isla Santa Elena	0	1	1
	8.Bocas de Dagua	25	40	65
Cx. browni		1 (25)	3 (75)	4
	6.Reserva Ventanas	0	1	1
	7. Isla Santa Elena	1	2	3
Cx. eknomios		4 (17)	20 (83)	24
	1.Bocas de Juriepe	4	10	14
	2.Finca la Sonora	0	2	2
	6.Reserva Ventanas	0	3	3
	7. Isla Santa Elena	0	3	**3**
	8.Bocas de Dagua	0	2	2
Ma. indubitans		1 (33)	2 (67)	3
	2.Finca la Sonora	0	1	1
	4. Laguna La Estacada	1	0	1
	7. Isla Santa Elena	0	1	1
Ps. albipes		19 (49)	20 (51)	39
	1.Bocas de Juriepe	15	14	29
	2.Finca la Sonora	0	1	1
	4. Laguna La Estacada	0	1	1
	5.Reserva Bojonawi	4	1	5
	6.Reserva Ventanas	0	1	1
	7. Isla Santa Elena	0	1	1
	8.Bocas de Dagua	0	1	1
Ps. ferox		**6 (32)**	**13 (68)**	**19**
	1.Bocas de Juriepe	0	3	3
	2.Finca la Sonora	0	1	1
	4. Laguna La Estacada	0	1	1
	5.Reserva Bojonawi	6	5	11
	6.Reserva Ventanas	0	1	1
	8.Bocas de Dagua	0	2	2
Wy. abebela		**1 (100)**	**0 (0)**	**1**
	8.Bocas de Dagua	1	0	1

3.3. Feeding sources

ONT sequencing of the 12S rRNA gene allowed the characterization of feeding sources in six of the 12 mosquito species collected. The vertebrate species identified comprised five mammals, including humans, two birds, and one reptile (Fig. 4A). Humans (*Homo sapiens*) and pigs (*Sus scrofa domesticus*) were found to be the primary food sources for the six mosquito species. *Oc. serratus* was the mosquito species that presented the greatest range of food sources with eight identified sources and *Wy. abbela* was the species that presented the most significant restriction in its diet because it presents only three sources, among them individuals of the genus *Procyonidae*. We found that *Cx. eknomios* was the only species that managed to feed on the *Iguana iguana* (Fig. 4A). To evaluate whether there was a relationship between the number of flaviviruses identified and the number of feeding sources, a Shapiro-Wilk normality test was performed. The two variables do not have a normal distribution ($p < 0.05$). Consequently, a Spearman correlation test was performed to compare the number of feeding sources against the number of flaviviruses identified, but no statistically significant correlations were found ($p > 0.05$).

Additionally, a graph integrating information on mosquito species, relative abundances of identified flaviviruses, and feeding sources per sampling point were constructed (Fig. 3). In general, it can be observed that the species *Oc. serratus* (present in all sampling points) has a variable behavior in terms of its feeding sources since it can be present from three feeding sources (Point 8) to eight sources (Point 1). Finally, to evaluate whether there is a relationship between the sampling points and the number of feeding sources found, a Shapiro-Wilk normality test was initially performed for the number of feeding sources, showing a normal distribution ($p>0.05$). Accordingly, a one-factor ANOVA test was performed, and it was found that there are significant differences between the number of feeding sources per sampling point. This indicates a relationship between feeding sources and sampling point, showing that the mosquitoes at point 1 have the highest number of feeding sources and point 8 has the lowest number of sources.

4. Discussion

Strategies for arbovirus prevention and vector control rely on understanding the dynamics and interactions between mosquitoes, transmitted viruses, and hosts. Entomovirological surveillance plays a crucial role in studying these dynamics and providing valuable information on local arbovirus transmission. Therefore, in this study, we evaluated these dynamics by identifying flaviviruses and feeding sources in mosquitoes circulating in rural areas of the municipality of Puerto Carreño-Vichada, an area with low anthropogenic disturbance and low fragmentation. For this, we initially identified the species of mosquitoes captured, where we found that 51 individuals presented confusing morphological characters that did not allow the corresponding taxonomic assignment. However, using COI gene sequences, we successfully classified 11 mosquito species out of the 51 individuals. Among them, 60.7% (31/51) belonged to the genus *Culex* with five species identified, representing many individuals we found with problems with taxonomic identification. Similar studies have reported such challenges due to the diversity and taxonomic revisions gaps in the *Culex* genus. (Hoyos et al., 2021; Torres-Gutierrez and Sallum, 2015). Therefore, in these cases, the COI gene has been established to classify mosquito species based on inter- and intraspecific genetic distances (Torres-Gutierrez et al., 2016). Our phylogenetic reconstruction (Fig. 2A) supported this classification, showing distinct clusters with robust node support (>90) for each species. Notably, this study reports the first occurrence of *Sa. chloropterus* species in the country. While the COI gene fragment was used for classification, further confirmation using other molecular markers is

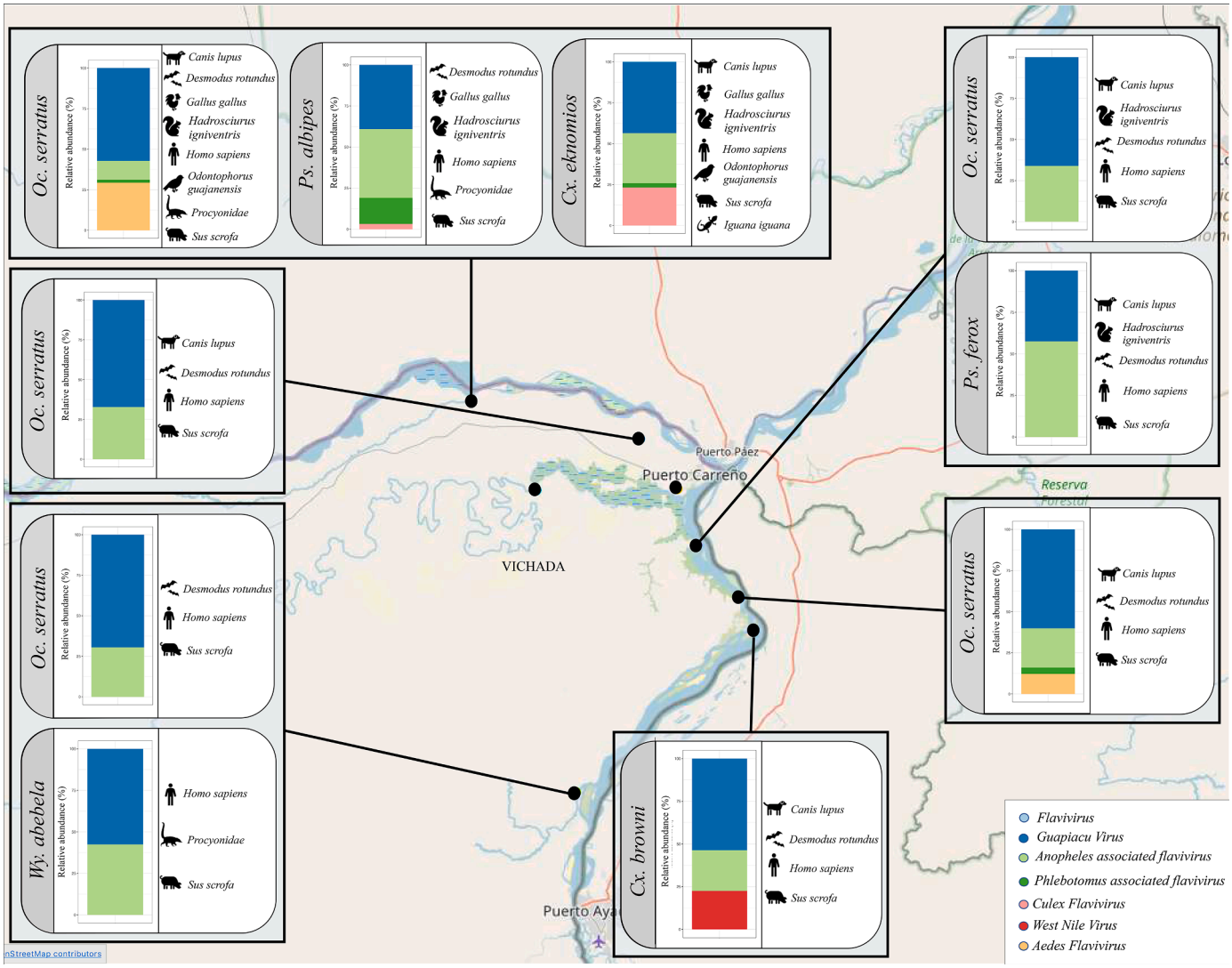

Fig. 3. Relative abundance of identified flaviviruses.
A) Graphical representation of the abundance of *Flavivirus* identified, as well as the food sources for each of the mosquito species found by sampling point. The barplot shows the relative abundance of flaviviruses identified in each of the mosquito species by sampling point.

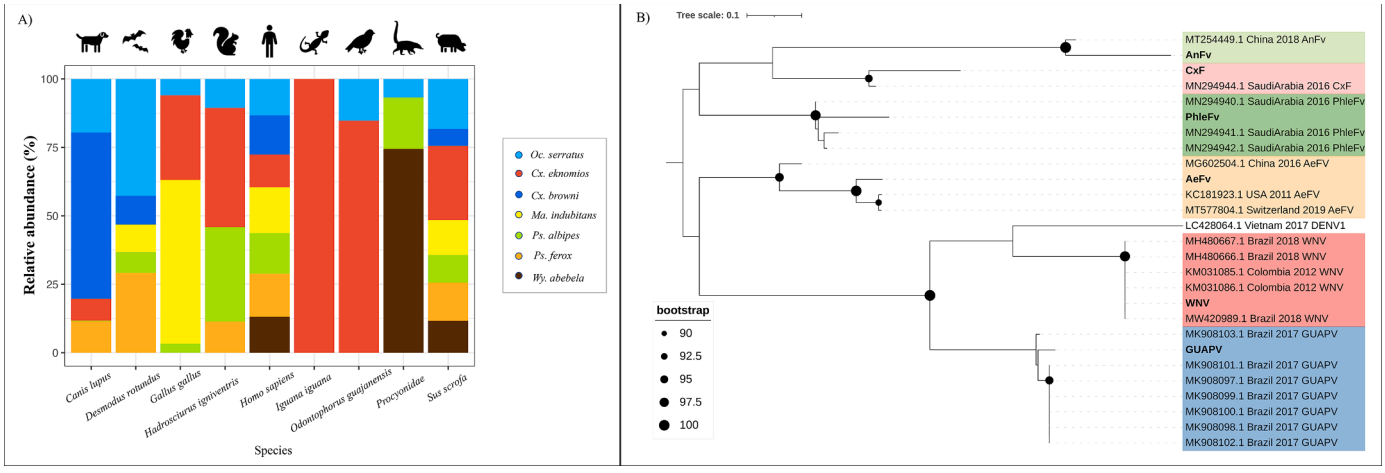

Fig. 4. Relative abundance of food sources and Phylogenetic analyzes of *Flavivirus*.
A) The barplot shows the relative abundance of the number of reads found for each vertebrate by mosquito species. B) Maximum likelihood phylogenetic reconstruction based on the K2P+R2 substitution model using NS5 fragment sequences obtained in this study and GenBank. Black dots indicate bootstrap ≥90, labels names in bold indicate sequences obtained in this study.

neccesary given its importance as a vector for YFV.

The correct identification of mosquito species is crucial in studies such as ours that aim to understand the ecology of vectors and the transmission dynamics of viruses of importance for public health. In contrast, incorrect identification can lead to erroneous conclusions and invalid associations. Additionally, knowing the vector species specifically provides a priori information on the transmission dynamics that may occur locally. Previous studies have identified specific relationships between virus transmission, mosquito species, and their feeding preferences. For example, *Cx. pipiens* has been associated with active WNV transmission in the northeastern United States, with a preference for avian hosts (Nasci et al., 2001). However, molecular techniques are too costly for the massive use of vector identification. Therefore, we emphasize to highlight the importance of their use as a complementary tool for surveillance under the specific situations described above (Martínez et al., 2020).

Our results show *Flavivirus* infection in seven of the 12 species identified (Table 1), of which, in only three species (*Oc. serratus, Psorophora* (*Janthinosoma*) *albipes* (Theobald, 1907), *Ps.* (*Jan.*) *ferox* (von Humboldt, 1819) *Flavivirus* infection has been previously reported (Auguste et al., 2021; Ramos et al., 2022; Saiyasombat et al., 2010). As for the flaviviruses identified, we found GUAPV and *Anopheles associated Flavivirus* (AnFv) present in six species; these results are inconsistent with previous reports since GUAPV had only one in Brazil during 2021 in *Ae. terrens* and *Ae. scapularis* species (Fig. 4B) (de Oliveira Ribeiro et al., 2021). Therefore, this study presents the first report of GUAPV circulation in mosquitoes circulating in Colombia, specifically in these species. This new report and its high prevalence in our study may be due to the failure of previous studies to identify this virus adequately. This is since the sequences of this ISFV were included in the databases recently (less than two years ago); then, previous studies, when performing the assignment by alignments (global and/or local), could not find this new virus. In addition, the sequencing methodology we propose allowed us to perform an approximate calculation of the relative abundance of the flaviviruses we identified. However, future studies should be conducted to understand the impact of GUAPV circulation in mosquitoes, whether it affects the development of other viruses of public health importance, and their cryptic circulation in arbovirus endemic areas.

One of the most important results was the identification of WNV in *Cx. browni*, the first report of WNV infection in this species. Previously, only *Cx. pipiens, Cx. quinquefasciatus* and *Cx. torrentium* species have been implicated in WNV transmission in the northern United States and central Europe (Jansen et al., 2019; Rochlin et al., 2019). In Latin America, specifically in Colombia, the circulation of WNV has been previously reported in *Culex* mosquitoes (Fig. 4B). However, the authors were unable to identify the mosquito species of the infected individuals (Hoyos-López et al., 2016). This information has public health relevance since it exposes the species *Cx. browni* as a potential vector of WNV in the municipality of Puerto de Carreño. However, it is essential to conduct vector capacity studies to clarify the current situation of WNV transmission dynamics in the country, again highlighting the importance of using molecular techniques as a complementary tool for the taxonomic identification of mosquitoes since there is information on the circulation of the virus, but not precisely the species that are maintaining its transmission.

On the other hand, a relationship between the simultaneous infection of WNV and CxFV, an ISFV, has been described in some cases, where an *in vitro* study initially identified that WNV replication is suppressed when the abundance of CxFv is 10 to 100 times higher (Goenaga et al., 2020). However, another study suggests that CxFv infection cannot exclude secondary infection by genetically distinct flaviviruses such as WNV and that these two viruses may be present simultaneously in the same mosquito (Newman et al., 2011). In our study, the WNV-infected *Cx. browni* individual was not found to be co-infected with CxFv. Additionally, the *Cx. eknomios* and *Ps. albipes* species were found to be infected with CxFv (Fig. 3). Therefore, the data obtained are highly variable, and the exact reason for these patterns has yet to be discovered since no clear relationships have been found between these two viruses. Therefore, it is necessary to continue studies that focus on determining the relationships between these two viruses and the interaction between other ISFVs and viruses of public health importance. This is remarkable, given recent evidence of how ISVs can modulate vector competence in *Aedes* and, thus, DENV transmission *in vitro* (Olmo et al., 2023). Ultimately, a better understanding of this pattern will allow generating future control strategies that permit preventive intervention to stop the transmission of flaviviruses causing human diseases.

The present study identified *Homo sapiens* and *Sus scrofa* as the primary feeding sources for the seven mosquito species (Fig. 4A). The preferences of *Oc. serratus* and *Ps. albipes* species for *Homo sapiens* have been previously reported (Hoyos et al., 2021). This shows that, despite the low fragmentation of the area, it is evident that anthropogenic disturbances facilitate human blood as a potential food source, with the possibility of adaptation to this source and, thus, the potential emergence of arboviruses in the region. Thanks to our analyses, we found that the number of feeding sources identified for our study was associated with the sampling points (Fig. 3). This is related to the availability of food sources that can be found in each area and added that regular interaction with specific hosts results in a robust vector-vertebrate relationship which in turn can lead to enhanced virus transmission (Scott and Takken, 2012). In addition, it is essential to highlight that although the species *Oc. serratus* belongs to a rural cycle; its opportunistic behavior can occasionally lead this species to feed on human blood, as previously reported in Colombia (Hoyos et al., 2021). This is of great importance since, in Brazil, this species has been incriminated as a possible secondary vector of YFV (Cardoso et al., 2010).

Finally, our study shows that entomovirological surveillance using sequencing techniques such as ONT is a useful tool that can identify the early circulation of pathogenic viruses. Accordingly, the design of approaches that integrate different objectives, such as 1) the molecular identification of mosquito species using molecular markers as a complementary tool, 2) the identification of viral species of the genus *Flavivirus* present in mosquitoes in rural areas, and 3) the characterization of the food sources of these mosquitoes, can become a potential tool for understanding the dynamics associated with the ecology and transmission of pathogenic viruses and early detection of emerging viruses. Therefore, it is proposed to use this tool as an early warning system that identifies the transmission dynamics of different emerging and re-emerging viruses by monitoring potential vectors and hosts. The above, with the support of the sequencing capacity that was acquired during the pure contingency of the Coronavirus disease 2019 (COVID-19) (Álvarez-Díaz et al., 2021). The use of these sequencing tools, together with approaches similar to our study, has allowed the identification of epidemiologically essential viruses such as Japanese encephalitis virus (JEV) in mosquitoes from Xinjiang, China (Hameed et al., 2021), ZIKV in patients from the Brazilian Amazon (M. L. G. de Figueiredo et al., 2022) and in Colombia with the recent identification of Oropuche virus (OROV) associating it to the cause of emerging febrile illness in patients (Ciuoderis et al., 2022).

However, it is important to recognize certain limitations in our study. Firstly, the sample size of the mosquitoes captured at the sampling points, which may impact the generalizability of our findings. Additionally, the identification of flaviviruses was based on Amplicon Based Sequencing rather than a comprehensive virome characterization conducted in previous studies. This limited approach restricted our ability to perform a deeper analysis, including the identification of interactions among different viral families. Furthermore, a more extensive sampling strategy encompassing wider spatio-temporal scales would provide a more comprehensive understanding of the dynamics and distribution of these viruses. Moreover, the lack of inclusion of negative controls throughout the sample collection to rule out human contamination that could be observed during the sequencing process. Lastly, we believe that our findings from entomovirological surveillance in the municipality of

Puerto Carreño-Vichada contribute to elucidating the ecological and transmission dynamics of flaviviruses in the local area. Likewise, our results highlight the importance of continuing studies to determine the dynamics of virus transmission in our country and to understand the importance of ISFVs in these dynamics.

5. Conclusions

This study reports on the co-circulation of ISFVs (CxFv, GUAPV, AnFv, and AeFv) in mosquitoes circulating in rural areas of the Puerto Carreño-Vichada. The high abundance of ISFVs may be the result of the new sampling technique employed, which allowed us to identify the co-infection of two to four flaviviruses in the same individual and estimate their relative abundance. The identification of WNV circulation generates an alert about the incipient transmission in the municipality and the possible incrimination of the *Cx. browni*. Additionally, our analysis of food sources demonstrated the opportunistic behavior of the *Oc. Serratus* and diverse feeding hosts, including humans. These findings underscore the potential impact of anthropogenic disturbances in arbovirus outbreaks. Our findings highlight the importance of including ONT in entomovirological surveillance to better understand the dynamics associated with the ecology and transmission of viruses. We suggest that health and governmental entities take advantage of the installed capacity for health containment by COVID-19 to adopt these complementary tools. We also recommend that future studies focus on determining the importance of ISFVs in the transmission dynamics of pathogenic viruses. Finally, we consider that our results on *Flavivirus* circulation and food sources in circulating mosquitoes in the municipality of Puerto Carreño provide valuable information that supports institutional capacities for the prevention and control of viral agents in our country and the region.

Funding

This study was funded by Dirección de Investigación e Innovación from Universidad del Rosario.

CRediT authorship contribution statement

David Martínez: Conceptualization, Data curation, Formal analysis, Methodology, Software, Validation, Visualization, Writing – review & editing. **Marcela Gómez:** Data curation, Investigation, Writing – review & editing. **Jorge Luis De las salas:** Resources, Writing – review & editing. **Carolina Hernández:** Resources, Writing – review & editing. **Alexander Zamora Flórez:** Resources, Writing – review & editing. **Marina Muñoz:** Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – review & editing. **Juan David Ramírez:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Visualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

We thank the local health authorities from the Vichada department for their support and assistance with the fieldwork. Also, we thank Sergio Castañeda for his expertise in statistical analysis.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.actatropica.2023.106972.

Fig. S1. Mosquito abundance. The barplot shows the relative abundance of mosquito species identified by sampling point.

References

Álvarez-Díaz, D.A., Valencia-Álvarez, E., Rivera, J.A., Rengifo, A.C., Usme-Ciro, J.A., Peláez-Carvajal, D., Lozano-Jiménez, Y.Y., Torres-Fernández, O., 2021. An updated RT-qPCR assay for the simultaneous detection and quantification of chikungunya, dengue and zika viruses. Infect. Genet. Evol. 93, 104967 https://doi.org/10.1016/j.meegid.2021.104967.

Auguste, A.J., Langsjoen, R.M., Porier, D.L., Erasmus, J.H., Bergren, N.A., Bolling, B.G., Luo, H., Singh, A., Guzman, H., Popov, V.L., Travassos da Rosa, A.P.A., Wang, T., Kang, L., Allen, I.C., Carrington, C.V.F., Tesh, R.B., Weaver, S.C., 2021. Isolation of a novel insect-specific flavivirus with immunomodulatory effects in vertebrate systems. Virology 562, 50–62. https://doi.org/10.1016/j.virol.2021.07.004.

Bolling, B.G., Olea-Popelka, F.J., Eisen, L., Moore, C.G., Blair, C.D., 2012. Transmission dynamics of an insect-specific flavivirus in a naturally infected Culex pipiens laboratory colony and effects of co-infection on vector competence for West Nile virus. Virology 427 (2), 90–97. https://doi.org/10.1016/j.virol.2012.02.016.

Bolling, B.G., Weaver, S.C., Tesh, R.B., Vasilakis, N., 2015. Insect-specific virus discovery: significance for the arbovirus community. Viruses 7 (9), 4911–4928. https://doi.org/10.3390/v7092851.

Borremans, B., Faust, C., Manlove, K.R., Sokolow, S.H., Lloyd-Smith, J.O., 2019. Cross-species pathogen spillover across ecosystem boundaries: mechanisms and theory. Philos. Trans. R. Soc. B Biol. Sci. 374 (1782), 20180344 https://doi.org/10.1098/rstb.2018.0344.

Cardoso, J., da, C., de Almeida, M.A.B., dos Santos, E., da Fonseca, D.F., Sallum, M.A.M., Noll, C.A., Monteiro, H.A., de, O., Cruz, A.C.R., Carvalho, V.L., Pinto, E.V., Castro, F. C., Neto, J.P.N., Segura, M.N.O., Vasconcelos, P.F.C., 2010. Yellow fever virus in haemagogus leucocelaenus and aedes serratus mosquitoes, Southern Brazil, 2008. Emerg. Infect. Dis. 16 (12), 1918–1924. https://doi.org/10.3201/eid1612.100608.

Ciuoderis, K.A., Berg, M.G., Perez, L.J., Hadji, A., Perez-Restrepo, L.S., Aristizabal, L.C., Forberg, K., Yamaguchi, J., Cardona, A., Weiss, S., Qiu, X., Hernandez-Ortiz, J.P., Averhoff, F., Cloherty, G.A., Osorio, J.E., 2022. Oropouche virus as an emerging cause of acute febrile illness in Colombia. Emerg. Microbes Infect. 11 (1), 2645–2657. https://doi.org/10.1080/22221751.2022.2136536.

Conway, M.J., Colpitts, T.M., Fikrig, E., 2014. Role of the vector in arbovirus transmission. Annu. Rev. Virol. 1 (1), 71–88. https://doi.org/10.1146/annurev-virology-031413-085513.

de Figueiredo, M.L.G., Williams, E.P., Jonsson, C.B., Khan, M.J., Nunes, M.R.T., de Lima, C.P.S., Figueiredo, L.T.M., Costa, M.R.F., Mourão, M.P.G., Lacerda, M.V.G., Aquino, V.H., 2022. Screening of febrile patients with suspected malaria from the Brazilian Amazon for virus infection. Arch. Virol. 167 (11), 2151–2162. https://doi.org/10.1007/s00705-022-05514-7.

de Oliveira Ribeiro, G., da Costa, A.C., Gill, D.E., Ribeiro, E.S.D., Rego, M.O.da S., Monteiro, F.J.C., Villanova, F., Nogueira, J.S., Maeda, A.Y., de Souza, R.P., Tahmasebi, R., Morais, V.S., Pandey, R.P., Raj, V.S., Scandar, S.A.S., da Silva Vasami, F.G., D'Agostino, L.G., Maiorka, P.C., Deng, X., Cunha, M.S., 2021. Guapiaçu virus, a new insect-specific flavivirus isolated from two species of Aedes mosquitoes from Brazil. Sci. Rep. 11, 4674. https://doi.org/10.1038/s41598-021-83879-6.

Fang, Y., Zhang, Y., Zhou, Z.B., Shi, W.Q., Xia, S., Li, Y.Y., Wu, J.T., Liu, Q., Lin, G.Y., 2018. Co-circulation of Aedes flavivirus, Culex flavivirus, and Quang Binh virus in Shanghai, China. Infect. Dis. Poverty 7 (1), 75. https://doi.org/10.1186/s40249-018-0457-9.

Figueiredo, L.T.M., 2019. Human urban arboviruses can infect wild animals and jump to sylvatic maintenance cycles in South America. Front. Cell. Infect. Microbiol. 9. https://www.frontiersin.org/articles/10.3389/fcimb.2019.00259.

Goenaga, S., Fabbri, C.M., García, J.B., Rondán, J.C., Gardenal, N., Calderón, G.E., Enria, D.A., Levis, S.M.C., 2014. New Strains of Culex flavivirus Isolated in Argentina. J. Med. Entomol. 51 (4), 900–906. https://doi.org/10.1603/ME13172.

Goenaga, S., Goenaga, J., Boaglio, E.R., Enria, D.A., Levis, S.del C., 2020. Superinfection exclusion studies using West Nile virus and Culex flavivirus strains from Argentina. Mem. Inst. Oswaldo Cruz 115. https://doi.org/10.1590/0074-02760200012.

Gómez, M., Martinez, D., Muñoz, M., Ramírez, J.D., 2022. Aedes aegypti and Ae. albopictus microbiome/virome: new strategies for controlling arboviral transmission? Parasites Vectors 15 (1), 287. https://doi.org/10.1186/s13071-022-05401-9.

Gonzalez-Escobar, G., Churaman, C., Rampersad, C., Singh, R., Nathaniel, S., 2021. Mayaro virus detection in patients from rural and urban areas in Trinidad and Tobago during the Chikungunya and Zika virus outbreaks. Pathog. Glob. Health 115 (3), 188–195. https://doi.org/10.1080/20477724.2021.1878445.

Guth, S., Hanley, K.A., Althouse, B.M., Boots, M., 2020. Ecological processes underlying the emergence of novel enzootic cycles: arboviruses in the neotropics as a case study. PLoS Negl. Trop. Dis. 14 (8), e0008338 https://doi.org/10.1371/journal.pntd.0008338.

Hameed, M., Khan, S., Xu, J., Zhang, J., Wang, X., Di, D., Chen, Z., Naveed Anwar, M., Wahaab, A., Ma, X., Nawaz, M., Liu, K., Li, B., Shao, D., Qiu, Y., Wei, J., Ma, Z., 2021. Detection of Japanese encephalitis virus in mosquitoes from Xinjiang during next-generation sequencing arboviral surveillance. Transbound. Emerg. Dis. 68 (2), 467–476. https://doi.org/10.1111/tbed.13697.

Harrington, L.C., Edman, J.D., Scott, T.W., 2001. Why do female Aedes aegypti (Diptera: culicidae) feed preferentially and frequently on human blood? J. Med. Entomol. 38 (3), 411–422. https://doi.org/10.1603/0022-2585-38.3.411.

Hayes, E.B., Komar, N., Nasci, R.S., Montgomery, S.P., O'Leary, D.R., Campbell, G.L, 2005. Epidemiology and transmission dynamics of West Nile Virus disease. Emerging Infect. Dis. 11 (8), 1167–1173. https://doi.org/10.3201/eid1108.050289a.

Hoyos, J., Carrasquilla, M.C., León, C., Montgomery, J.M., Salyer, S.J., Komar, N., González, C., 2021. Host selection pattern and flavivirus screening of mosquitoes in a disturbed Colombian rainforest. Sci. Rep. 11 (1), 1 https://doi.org/10.1038/s41598-021-98076-8.

Hoyos-López, R., Suaza-Vasco, J., Rúa-Uribe, G., Uribe, S., Gallego-Gómez, J.C., 2016. Molecular detection of flaviviruses and alphaviruses in mosquitoes (Diptera: culicidae) from coastal ecosystems in the Colombian Caribbean. Mem. Inst. Oswaldo Cruz 111, 625–634. https://doi.org/10.1590/0074-02760160096.

Huang, Y.J.S., Higgs, S., Vanlandingham, D.L., 2019. Arbovirus-mosquito vector-host interactions and the impact on transmission and disease pathogenesis of arboviruses. Front. Microbiol. 10. https://www.frontiersin.org/articles/10.3389/fmicb.2019.00022.

Jain, M., Olsen, H.E., Paten, B., Akeson, M., 2016. The oxford nanopore MinION: delivery of nanopore sequencing to the genomics community. Genome Biol. 17 (1), 239. https://doi.org/10.1186/s13059-016-1103-0.

Jansen, S., Heitmann, A., Lühken, R., Leggewie, M., Helms, M., Badusche, M., Rossini, G., Schmidt-Chanasit, J., Tannich, E., 2019. Culex torrentium: a potent vector for the transmission of West Nile virus in Central Europe. Viruses 11 (6), 6. https://doi.org/10.3390/v11060492.

Jiang, S., Xing, D., Li, C., Dong, Y., Zhao, T., Guo, X., 2023. Replication and transmission of West Nile virus in simulated overwintering adults of Culex pipiens pallens (Diptera: culicidae) in China. Acta Trop. 237, 106720 https://doi.org/10.1016/j.actatropica.2022.106720.

Kampen, H., Tews, B.A., Werner, D., 2021. First evidence of West Nile virus overwintering in mosquitoes in Germany. Viruses 13 (12), 12. https://doi.org/10.3390/v13122463.

Kuno, G., Mackenzie, J.S., Junglen, S., Hubálek, Z., Plyusnin, A., Gubler, D.J., 2017. Vertebrate reservoirs of arboviruses: myth, synonym of amplifier, or reality? Viruses 9 (7), 185. https://doi.org/10.3390/v9070185.

Langendries, L., Abdelnabi, R., Neyts, J., Delang, L., 2021. Repurposing drugs for mayaro virus: identification of eidd-1931, favipiravir and Suramin as Mayaro Virus Inhibitors. Microorganisms 9 (4), 4. https://doi.org/10.3390/microorganisms9040734.

Li, S.L., Acosta, A.L., Hill, S.C., Brady, O.J., Almeida, M.A.B.de, Cardoso, J.da C., Hamlet, A., Mucci, L.F., Deus, J.T.de, Iani, F.C.M., Alexander, N.S., Wint, G.R.W., Pybus, O.G., Kraemer, M.U.G., Faria, N.R., Messina, J.P, 2022. Mapping environmental suitability of Haemagogus and Sabethes spp. Mosquitoes to understand sylvatic transmission risk of yellow fever virus in Brazil. PLoS Negl. Trop. Dis. 16 (1), e0010019 https://doi.org/10.1371/journal.pntd.0010019.

Machado, D.C., Mondini, A., dos Santos Santana, V., Yonamine, P.T.K., Chiaravalloti Neto, F., Zanotto, P.M.de A., Nogueira, M.L., 2012. First identification of Culex flavivirus (Flaviviridae) in Brazil. Intervirology 55 (6), 475–483. https://doi.org/10.1159/000337166.

Martínez, D., Hernández, C., Muñoz, M., Armesto, Y., Cuervo, A., Ramírez, J.D., 2020. Identification of aedes (diptera: culicidae) species and arboviruses circulating in arauca, Eastern Colombia. Front. Ecol. Evol. 8. https://www.frontiersin.org/articles/10.3389/fevo.2020.602190.

Mendenhall, I.H., Tello, S.A., Neira, L.A., Castillo, L.F., Ocampo, C.B., Wesson, D.M., 2012. Host preference of the arbovirus vector culex erraticus (diptera: culicidae) at Sonso Lake, Cauca valley department, Colombia. J. Med. Entomol. 49 (5), 1092–1102. https://doi.org/10.1603/me11260.

Merhi, G., Koweyes, J., Salloum, T., Khoury, C.A., Haidar, S., Tokajian, S., 2022. SARS-CoV-2 genomic epidemiology: data and sequencing infrastructure. Future Microbiol. 17 (13), 1001–1007. https://doi.org/10.2217/fmb-2021-0207.

Morales-Betoulle, M.E., Monzón Pineda, M.L., Sosa, S.M., Panella, N., López, M.R.B., Cordón-Rosales, C., Komar, N., Powers, A., Johnson, B.W, 2008. Culex flavivirus isolates from mosquitoes in Guatemala. J. Med. Entomol. 45 (6), 1187–1190. https://doi.org/10.1603/0022-2585(2008)45[1187:cfifmi]2.0.co;2.

Nasci, R.S., Savage, H.M., White, D.J., Miller, J.R., Cropp, B.C., Godsey, M.S., Kerst, A.J., Bennett, P., Gottfried, K., Lanciotti, R.S., 2001. West Nile virus in overwintering Culex mosquitoes, New York City, 2000. Emerg. Infect. Dis. 7 (4), 742–744.

Newman, C.M., Cerutti, F., Anderson, T.K., Hamer, G.L., Walker, E.D., Kitron, U.D., Ruiz, M.O., Brawn, J.D., Goldberg, T.L., 2011. Culex flavivirus and west nile virus mosquito coinfection and positive ecological association in Chicago, United States. Vector Borne Zoonotic Dis. 11 (8), 1099–1105. https://doi.org/10.1089/vbz.2010.0144.

Olmo, R.P., Todjro, Y.M.H., Aguiar, E.R.G.R., de Almeida, J.P.P., Ferreira, F.V., Armache, J.N., de Faria, I.J.S., Ferreira, A.G.A., Amadou, S.C.G., Silva, A.T.S., de Souza, K.P.R., Vilela, A.P.P., Babarit, A., Tan, C.H., Diallo, M., Gaye, A., Paupy, C., Obame-Nkoghe, J., Visser, T.M., Marques, J.T., 2023. Mosquito vector competence for dengue is modulated by insect-specific viruses. Nat. Microbiol. 8 (1), 1 https://doi.org/10.1038/s41564-022-01289-4.

Patterson, J., Sammon, M., Garg, M., 2016. Dengue, Zika and chikungunya: emerging arboviruses in the new world. West. J. Emerg. Med. 17 (6), 671–679. https://doi.org/10.5811/westjem.2016.9.30904.

Ramos, B.A., Chagas, L.L.D., de Arruda e Silva, F., Santos, E.B.dos, Chiang, J.O., Neto, J.P.N., Vieira, D.B.R., Junior, J.W.R., da Silva, E.V.P., Freitas, M.N.O., Santos, M.M., Pantoja, J.A.de S., Gonçalves, E.de J., Barros, L.J.L., Silva, S.P., Aragão, C.F., Cruz, A.C.R., Casseb, L.M.N., Souto, L.C.D.S., Martins, L.C., 2022. Arboviruses in free-ranging birds and hematophagous arthropods (diptera, nematocera) from forest remnants and urbanized areas of an environmental protection area in the Amazon Biome. Viruses 14 (10), 10. https://doi.org/10.3390/v14102101.

Rochlin, I., Faraji, A., Healy, K., Andreadis, T.G., 2019. West Nile virus mosquito vectors in North America. J. Med. Entomol. 56 (6), 1475–1490. https://doi.org/10.1093/jme/tjz146.

Saiyasombat, R., Dorman, K.S., Garcia-Rejon, J.E., Loroño-Pino, M.A., Farfan-Ale, J.A., Blitvich, B.J., 2010. Isolation and sequence analysis of culex flavivirus from culex interrogator and culex quinquefasciatus in the yucatan Peninsula of Mexico. Arch. Virol. 155 (6), 983–986. https://doi.org/10.1007/s00705-010-0665-5.

Scott, T.W., Takken, W., 2012. Feeding strategies of anthropophilic mosquitoes result in increased risk of pathogen transmission. Trends Parasitol. 28 (3), 114–121. https://doi.org/10.1016/j.pt.2012.01.001.

Torres-Gutierrez, C., Bergo, E.S., Emerson, K.J., de Oliveira, T.M.P., Greni, S., Sallum, M.A.M., 2016. Mitochondrial COI gene as a tool in the taxonomy of mosquitoes culex subgenus melanoconion. Acta Trop. 164, 137–149. https://doi.org/10.1016/j.actatropica.2016.09.007.

Torres-Gutierrez, C., Sallum, M.A.M., 2015. Catalog of the subgenus melanoconion of culex (diptera: culicidae) for South America. Zootaxa 4028 (1), 1–50. https://doi.org/10.11646/zootaxa.4028.1.1.

Wang, Y., Zhao, Y., Bollas, A., Wang, Y., Au, K.F., 2021. Nanopore sequencing technology, bioinformatics and applications. Nat. Biotechnol. 39 (11), 11 https://doi.org/10.1038/s41587-021-01108-x.

scientific reports

Check for updates

OPEN

Characterizing viral species in mosquitoes (Culicidae) in the Colombian Orinoco: insights from a preliminary metagenomic study

Marcela Gómez[1,2], David Martínez[1], Luisa Páez-Triana[1], Nicolás Luna[1], Jorge Luis De las salas[3], Carolina Hernández[1], Alexander Zamora Flórez[3], Marina Muñoz[1] & Juan David Ramírez[1,4]✉

Mosquitoes (Diptera: Culicidae) are primary vectors of arthropod-borne viruses (arboviruses) that pose significant public health threats. Recent advances in sequencing technology emphasize the importance of understanding the arboviruses and insect-specific viruses (ISVs) hosted by mosquitoes, collectively called the "virome". Colombia, a tropical country with favorable conditions for the development and adaptation of multiple species of Culicidae, offers a favorable scenario for the transmission of epidemiologically important arboviruses. However, entomovirological surveillance studies are scarce in rural areas of the country, where humans, mosquitoes, and animals (both domestic and wild) coexist, leading to a higher risk of transmission of zoonotic diseases to humans. Thus, our study aimed to perform a preliminary metagenomic analysis of the mosquitoes of special relevance to public health belonging to the genera *Ochlerotatus*, *Culex*, *Limatus*, *Mansonia*, *Psorophora*, and *Sabethes*, within a rural savanna ecosystem in the Colombian Orinoco. We employed third-generation sequencing technology (Oxford Nanopore Technologies; ONT) to describe the virome of mosquitoes samples. Our results revealed that the virome was primarily shaped by insect-specific viruses (ISVs), with the Iflaviridae family being the most prevalent across all mosquito samples. Furthermore, we identified a group of ISVs that were common in all mosquito species tested, displaying the highest relative abundance concerning other groups of viruses. Notably, *Hanko iflavirus-1* was especially prevalent in *Culex eknomios* (88.4%) and *Ochlerotatus serratus* (88.0%). Additionally, other ISVs, such as *Guadalupe mosquito virus* (GMV), *Hubei mosquito virus1* (HMV1), *Uxmal virus*, *Tanay virus*, *Cordoba virus*, and *Castlerea virus* (all belonging to the *Negevirus* genus), were found as common viral species among the mosquitoes, although in lower proportions. These initial findings contribute to our understanding of ISVs within mosquito vectors of the Culicidae family in the Eastern Plains of Colombia. We recommend that future research explore deeper into ISV species shared among diverse vector species, and their potential interactions with arboviruses. In addition, we also showed the need for a thorough exploration of the influence of local rural habitat conditions on the shape of the virome in mosquito vectors.

Mosquitoes (Diptera, Culicidae) are globally recognized vectors of medically and veterinary significant pathogens, contributing to millions of disease cases annually[1]. Hematophagous mosquito species play a crucial role in transmitting various epidemic arboviruses, particularly those belonging to the Flaviviridae and Togaviridae

[1]Centro de Investigaciones en Microbiología y Biotecnología-UR (CIMBIUR), Facultad de Ciencias Naturales, Universidad del Rosario, Bogotá, Colombia. [2]Grupo de Investigación en Ciencias Básicas (NÚCLEO), Facultad de Ciencias e Ingeniería, Universidad de Boyacá, Tunja, Colombia. [3]Secretaría Departamental de Salud del Vichada, Puerto Carreño, Colombia. [4]Molecular Microbiology Laboratory, Department of Pathology, Molecular and Cell-Based Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, USA. ✉email: jdramirez85@gmail.com

families[2–4]. In tropical and subtropical regions, mosquitoes capable of transmitting pathogenic viruses in both urban and rural cycles are primarily found within the *Aedes* and *Culex* genera[5–7]. These vectors serve as primary carriers of viruses such as dengue virus (DENV), Zika virus (ZIKV) West Nile virus (WNV)[8], St. Louis encephalitis (SLEV) and Japanese encephalitis (JEV) from the Flaviviridae family, as well as chikungunya virus (CHIKV) from the Togaviridae family. The biological characteristics of *Aedes and Culex* mosquitoes facilitate their rapid adaptation to new habitats and their wide geographic expansion, often associated with anthropogenic interventions in forested areas,[9–11] unplanned urbanization[12,13], global trade, and climate change[14,15]. Additionally, other members of the Culicidae family, belonging to the genera *Haemagogus, Sabethes, Mansonia* and *Psorophora,* have been implicated in the transmission of viruses such as yellow fever virus (YFV), Mayaro virus (MAYV)[16,17] and Venezuelan equine encephalitis virus (VEEV)[8,18] in sylvatic cycles. However, in rural areas at risk of zoonotic disease transmission to humans (spillover), the vectors endemic to these areas remain poorly studied.

The importance of developing new entomovirological surveillance strategies to monitor (re)emerging viruses and discover novel ones has been propelled by advancements in next-generation sequencing (NGS) technologies[19,20]. These breakthroughs have driven the progress of viral metagenomic studies in mosquitoes, leading to the identification of numerous viruses. This development has expanded our understanding of the diversity, taxonomy, and wide range of environmental conditions in which viral agents exist in mosquito vectors[19,20]. In addition to detecting arboviruses, a representative group in the mosquito virome, known as insect-specific viruses (ISVs)[21], has been found. ISVs are described as viruses that naturally infect arthropods but cannot replicate in vertebrate cells or infect humans[20,22,23] suggesting their long-term symbiotic interactions with their hosts[24]. Indeed, it has been proposed that ISVs play an important role in modulating vectorial competence[21,22,25,26], and could be a key component in the development of new strategies for arbovirus control and the development of vaccines or diagnostic platforms[23,25,27].

Current explorations into the mosquito virome have shed light on the concept of a "core virome," which comprises a group of viruses (ISVs) shared among mosquito vector populations of the same species[20,24,28]. However, viruses shared between different mosquito species have also been detected[29–31], probably due to environmental factors such as food resources and breeding sites[5,32]. In both cases, ISVs could directly affect the ecology and evolution of the mosquito host, as well as the rest of the mosquito's microbiota[24]. Analyses of the global composition and distribution of the mosquito virome suggest that the environment, modes of virus transmission, and genetic factors in specific mosquito species or genera are factors that shape the virome composition[5]. Similarly, other studies show that viral dynamics depends primarily on interactions between mosquitoes and their environment, where feeding is considered, a critical factor affecting virus composition[29,30] However, the factors influencing the complex associations of virome composition remain largely unknown, especially in mosquitoes associated with sylvatic cycles, in environmentally conserved natural areas.

Colombia, a world-renowned megadiverse nation, has a rich variety of mosquito species[33].This diversity, coupled with the diversity of ecosystems, creates favorable conditions in approximately 85% of the country for the migration, adaptation, and proliferation of mosquitoes, which contributes to the increased prevalence of mosquito-borne diseases in the country[34–36]. The high richness of mosquitoes in different regions of the country is probably associated with landscape modifications, deforestation and changes in temperature[8,34] favoring the proximity of wildlife vectors to humans, which increases the risk of transmission of known and emerging zoonotic arboviruses[37,38]. In the country, entomovirological surveillance efforts have focused mainly on viral species of public health importance[36,38], especially in urban centers, while exploration of other viral species remains limited. In addition, a considerable portion of the viral composition in vectors and other insects associated with the family Culicidae remains unknown, highlighting the need for comprehensive research and studies in this field[34]. Findings on the virome of mosquito vectors in the country are an emerging and recently developed topic. A pioneering study employed a meta-taxonomic approach in *Aedes aegypti* and *Aedes albopictus* mosquitoes captured in urban areas in the center of the country, revealing significant differences in the presence, diversity, and abundance of viruses between these two mosquito species[39]. Another study conducted in a rural area in the northern (coastal) part of the country discovered a putative new virus called *Guachaca virus* (family Tymoviridae) in *Culex* spp. Phylogenetically related to plants, suggesting an ecological relationship between plant viruses and plant-feeding insects, as well as a selection pressure driving the adaptation of the virus in the mosquito vector[40].

The Colombian Orinoco region, commonly known as the Eastern Plains, holds significant strategic importance owing to its exceptional biodiversity and extensive water resources. Despite documented cases of mosquito-borne diseases such as dengue, chikungunya, Zika, and yellow fever infections, this area has been relatively underexplored from an entomovirological perspective[35,36]. Rural areas in this region have experienced minimal human interference, with limited anthropogenic disturbance. This circumstance raises the possibility that potentially pathogenic viruses generate spillover events under deforestation scenarios[41]. Based on the results of a recent study focused on understanding the ecology and transmission dynamics of flaviviruses in mosquitoes (Diptera: Culicidae) from the Eastern Plains of Colombia[41], the circulation of West Nile Virus (WNV) in *Culex browni* was revealed, highlighting the potential impact of human activities in these anthropogenically sensitive ecosystems[41]. Therefore, the objective of this study was to characterize the virome of mosquito vector species such as *Ochlerotatus serratus, Culex eknomios, Cx. browni, Limatus durhamii, Mansonia indubitans, Psorophora albipes, Psorophora ferox* and *Sabethes chloropterus*, previously identified in the ecosystem of the Orinoco River basin. The results obtained underline the importance of insect-specific viruses (ISVs), given their abundance and the presence of shared viral species among different mosquito hosts. We propose that habitat-related factors could shape mosquito virome composition, especially among mosquitoes coexisting in a local natural ecosystem with similar ecological characteristics.

Results

Metagenomic analysis of mosquitoes virome

From the genetic material (RNA) of adult vector mosquitoes identified (based on the combination of morphology and DNA barcoding) in a savanna ecosystem in the Orinoco region (Colombia)[41], we selected eight of the most abundant and epidemiologically significant mosquito species belonging to six genera: *Ochlerotatus, Culex, Limatus, Mansonia, Psorophora and Sabethes*. The samples were organized into 15 pools, subjected to viral enrichment using SMRT-9 methodology and sequenced using Oxford Nanopore technology (Table 1). Subsequently, the viral metagenomic data was analyzed using different bioinformatics tools, revealing a total of 1,439,457 reads with an average of approximately 95,964 reads per sample. After removing bacterial sequences using the Minimap2 tool against the Silva 16S bacterial reference database, a total of 1,161,300 clean reads were obtained, showing a length of 375 bp and an average quality score of 9.5. Among them, 1408 sequences (1.88% of the total reads) were identified as viral per sample (Table 1).

Characterization of viral families

To obtain a comprehensive understanding of the virome present in the mosquitoes under study, we initially analyzed the identified viral families. For direct taxonomic assignment of the viral reads, we used the metagenomic sequence classifier Centrifuge, together with a high-quality virus database obtained from GenBank via NCBI. The metagenomic classification results were analyzed and visualized using the Pavian package. The virome in mosquitoes captured in the Orinoco region were mainly represented by RNA virus families known to contain ISVs and/or arboviruses. These included the families Iflaviridae, Baculoviridae, Mesoniviridae, Partitiviridae and Flaviridae (Fig. 1). Iflaviridae was the most abundant family in all mosquito vector pools analyzed. In addition, viral families infecting host plants were identified, such as Virgaviridae, Bromoviridae, Polydnaviridae and Secoviridae, as well as viral families infecting host bacteria, such as Herelleviridae, Siphoviridae and Myoviridae. Families associated with vertebrate viruses were also identified, the most common taxonomic group being Herpesviridae. Another representative family in the mosquito vector virome was Mimiviridae, mainly associated with infection of the protist host (Fig. 1).

Mosquito virome includes viruses from a diverse range of hosts

Based on the data obtained from the taxonomic assignment of viral families, we performed a verification of the respective host species in the NCBI database. Our results revealed that mosquitoes of the species *Och. serratus, Cx. eknomios, Cx. browni, Li. durhamii, Ma. indubitans, Ps. albipes, Ps. ferox,* and *Sa. chloropterus* carried viruses belonging to various host groups. These included insect, vertebrate, plant, fungal (mycovirus), bacterial (bacteriophage), algal and protist viruses. Insect-specific viruses (ISVs) were the most abundant in all mosquito species, with 73.8% of the viruses identified. After ISVs, plant viruses accounted for 11.4% of the total, while bacterial viruses accounted for 5.5%, fungal viruses for 4.87%, vertebrate viruses for 2.7%, and viruses with algal and protozoan hosts for 1.34% (Fig. 2a). Among the mosquito species, *Och. serratus, Ps. albipes*, and *Sa. chloropterus* mosquitoes exhibited the highest percentages of insect-specific viruses (ISVs). On the other hand, mosquitoes belonging to the *Culex* genus (*Cx. eknomios* and *Cx. browni)* and *Li. durhamii* showed the highest proportion of plant viruses (Fig. 2b).

Pool (Sample)	Mosquito Species	Number of raw reads	Classified reads		Unclassified reads		Viral reads		Mean read length	Mean read quality
			Number	%	Number	%	Number	%		
Pool1	*Ochlerotatus serratus*	68,106	1563	2.29	66,543	97.70	1563	2.29	366.1	9.7
Pool2	*Ochlerotatus serratus*	56,280	1343	2.39	54,937	97.60	1343	2.39	370.8	9.7
Pool3	*Ochlerotatus serratus*	54,250	1501	2.77	52,749	97.20	1501	2.77	364	9.8
Pool4	*Ochlerotatus serratus*	77,745	2173	2.80	75,572	97.20	2173	2.80	368	9.8
Pool5	*Ochlerotatus serratus*	79,786	1825	2.29	77,961	97.70	1825	2.29	368.6	9.7
Pool6	*Ochlerotatus serratus*	74,223	1626	2.19	72,597	97.80	1626	2.19	382.6	9.7
Pool7	*Ochlerotatus serratus*	75,060	2115	2.82	72,945	97.20	2115	2.82	373	9.8
Pool8	*Culex eknomios*	59,225	815	1.38	58,410	98.60	815	1.38	400.3	9.7
Pool9	*Culex browni*	76,593	921	1.20	75,672	98.80	921	1.20	372.5	9.7
Pool10	*Limatus durhamii*	84,940	1007	1.19	83,933	98.80	1007	1.19	385.6	9.7
Pool11	*Mansonia indubitans*	119,658	1501	1.25	118,157	98.70	1501	1.25	386.7	9.7
Pool12	*Psorophora albipes*	64,698	951	1.47	63,747	98.50	951	1.47	380.5	9.8
Pool13	*Psorophora ferox*	89,835	1165	1.30	88,670	98.70	1165	1.30	382.9	9.8
Pool14	*Psorophora ferox*	90,583	1344	1.48	89,239	98.50	1344	1.48	374.4	9.8
Pool15	*Sabethes chloropterus*	90,318	1265	1.40	89,053	98.60	1265	1.40	345	9.6

Table 1. Summary of quality data and analysis of metagenomic sequencing results detected in field-collected mosquitoes from a local ecosystem in the Orinoco region of Colombia.

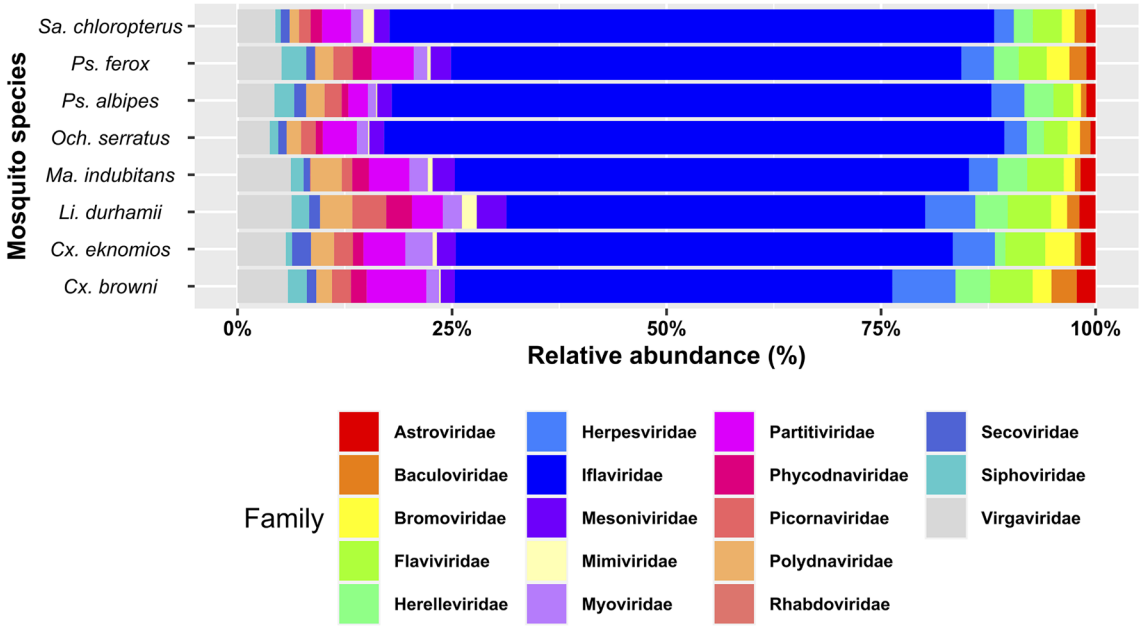

Figure 1. Relative abundance of viral families in each mosquito species. Bar graph showing virus families in the virome of each mosquito species *Och. serratus, Cu. eknomios, Cu. browni, Li. durhamii, Ma. indubitans, Ps. albipes, Ps. ferox, Sa. chloropterus.* The abundance of viral families was estimated by transforming the number of reads into relative values, providing an assessment of their presence in each mosquito species. Figure was created in R studio.

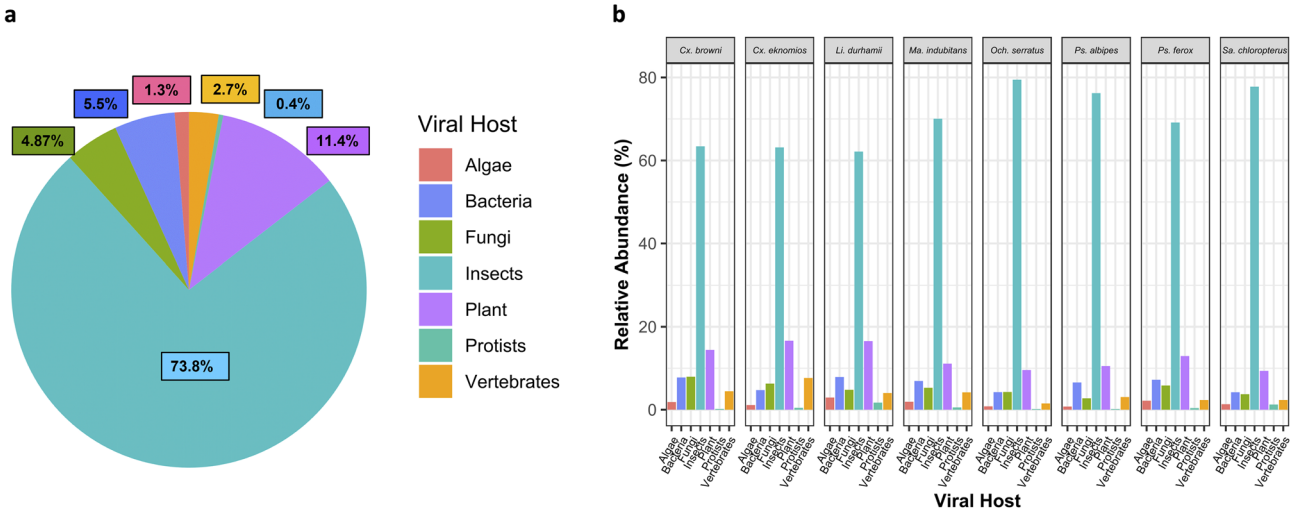

Figure 2. Hosts of viruses in the viromes of mosquitoes (Culicidae) in a rural ecosystem in Vichada, Colombia. (**a**) Proportions of viruses in different host groups from mosquitoes. (**b**) Relative abundance of viruses grouped by host organism in each vector species. To estimate the abundance, the number of reads was transformed into relative values, providing an assessment of their presence in each mosquito species. Figure was created in R studio.

Shared ISVs among different mosquito species

To enhance the precision of taxonomic assignments for viruses in the studied mosquito vectors, we established a new NCBI-Mosquito virus database. This database was curated specifically for Culicidae (Diptera) hosts, incorporating all relevant sequences accessible on NCBI. The Centrifuge program and visual representation followed the same specifications described above for viral families. The analysis of viral species abundance involved the transformation of reads for each ISV into relative values for individual mosquitoes. This approach facilitated the assessment of viral presence in vector mosquitoes collected from the Orinoco River basin, revealing a common set of ISVs across all studied vector mosquitoes. Metaviromes were predominantly composed of *Hanko iflavirus 1*, which showed the highest abundance in all mosquito species. This ISV presented a high abundance in *Cx. eknomios* (88.4%) and *Och. serratus* (88.0%), whereas its abundance was lower in *Sa. chloropterus* (63.5%). In

addition, *Enontekio iflavirus* ranked second in abundance in the mosquito virome, especially in *L. durhamii* (21%) and *Sa. chloropterus* (18%). Another important virus, *Corriparta virus*, showed the highest prevalence in *Sa. chloropterus* (16%) and the lowest in *Cx. browni* (3.5%) (Fig. 3).

In addition, other ISVs were identified in mosquito samples in smaller proportions, accounting for 1.3% of the total number of viruses identified. Although these ISVs were common to most of the groups evaluated, their abundance varied. *Uxmal virus, Guadalupe mosquito virus* and *Tanay virus* were the most prevalent in all mosquito samples. In contrast, *Tesano Aedes virus, Cordoba virus, Culex tritaeniorhynchus Negev-like virus, Castlerea virus, Hubei mosquito virus1, Pedersore iflavirus* and *Alphamesonivirus 1* showed lower abundance in the mosquito virome (Fig. 3). Statistical analysis, which employed the Kruskal–Wallis test followed by Dunn's post hoc test with Benjamini–Hochberg correction (p *value* > 0.05), showed no apparent differences in viral composition among the mosquito species studied. However, a notable distinction in the relative abundance of *Hanko iflavirus 1* was observed when compared to other viral species by this test (p value = 0.00019) (Figure S1).

Discussion

Exploring the mosquito virome and unraveling how its composition influences arbovirus transmission is paramount to understanding the emergence of arboviral diseases and the dynamics of outbreaks[20,22,25]. In the present study, we performed metagenomic sequencing to characterize the virome of some epidemiologically important mosquito species associated with arbovirus transmission in rural and sylvatic cycles. The study was carried out in a local savanna ecosystem of the Colombian Orinoco region, an area with limited anthropogenic activity, which could be susceptible to spillover events under scenarios of anthropogenic intervention[41]. Specifically, we have focused on eight vector species, including *Och. serratus, Cx. eknomios, Cx. browni, Li. durhamii, Ma. indubitans, Ps. albipes, Ps. ferox,* and *Sa. chloropterus,* with potential to transmit viral agents causing diseases of public health importance[5,8,19]. These species were selected based on a previous study that identified them as representative mosquito species in terms of abundance and importance in the study area[41].

Our investigations have revealed a predominance of insect-specific viruses (ISVs) in the virome composition of all mosquito species studied. The ISVs, well-documented as nonpathogenic viruses exclusive to insect hosts[20], demonstrate no transmission to vertebrates, including humans[23,25]. Recent studies have underscored the significant potential of ISVs as biological control agents against vector-borne diseases. This influence is demonstrated through various mechanisms, encompassing both the enhancement and suppression of arbovirus replication within the mosquito vector. These mechanisms include acceleration of the extrinsic incubation period[42], competitive exclusion[43], induction of antiviral immune responses[26], interference with transmission pathways[44], cellular resistance, and modulation of insect behavior[45]. The high prevalence of common ISVs in the virome profile in all vector species studied highlights the importance of continuing to study this group of ISV (Figs. 2 and 3), especially their adaptations and potential applications as biological control agents against vector-borne disease as suggested by several authors[24,25,45].

In addition, our research uncovered a diverse representation within the virome of the eight mosquito vector species, including viruses associated with plants, fungi, bacteria, vertebrates, algae, and protists (Fig. 2). These findings suggest that the virome of field-collected mosquitoes, especially in rural settings in tropical areas (with a low degree of anthropogenic intervention), could reflect intricate interactions with their natural environment[27,29,30]. Possibly this phenomenon is likely to be influenced by some factors, such as breeding and

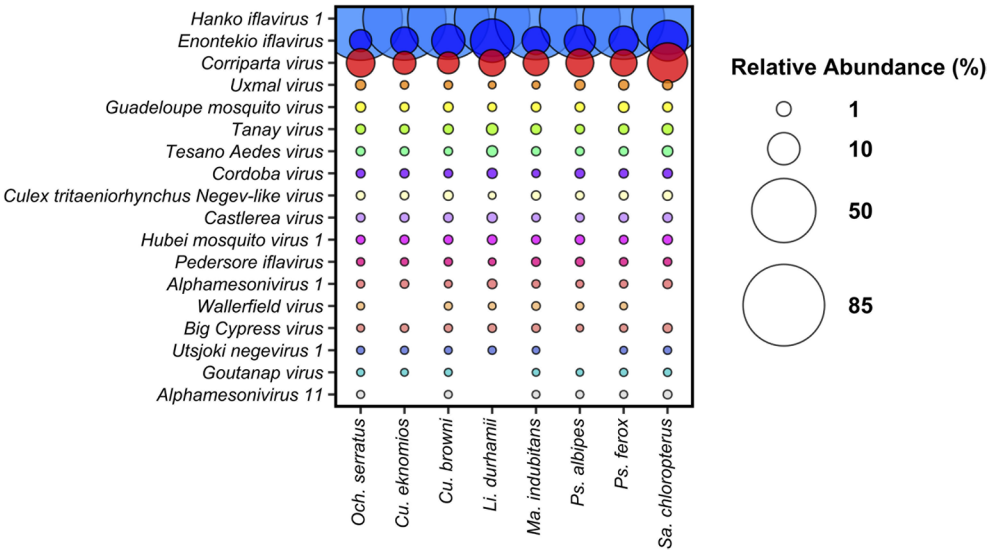

Figure 3. The relative abundance of Insect-Specific Virus (ISVs) in mosquito species. Bubble plot showing the relative abundance of ISVs. The abundance of viral species was estimated by transforming the number of reads into relative values, providing an assessment of their presence in each mosquito species. Figure was created in R studio.

feeding sites as has been suggested by previous studies[30,46,47]. However, future research could systematically explore the influence of local environmental conditions, encompassing factors such as temperature, humidity, rainfall, vegetation, nutrition, and mosquito breeding water sources. This exploration would yield crucial information on viral habitat-related factors that may shape the transient or permanent composition of the mosquito virome[29,32]. Unfortunately, we have not collected comprehensive data on the environmental and ecological characteristics of the ecosystem studied, which lays the groundwork for future research on the relationship between specific habitat variables and the virome of mosquito populations.

In the present study, conducted in a rural ecosystem of Colombian Orinoco, viromes of mosquito species belonging to *Ochlerotatus, Culex, Limatus, Mansonia, Psorophora* and *Sabethes* genera showed the presence of common ISV species. The specific conditions of the shared environment could explain the similarities observed in the viromes of different mosquito species co-occurring in the same habitat. These conditions could potentially act as a selective pressure on mosquitoes, a concept proposed in a recent study in Colombia[40]. These findings are in line with metatranscriptomic analyses of the virome of *Culex* mosquitoes (*Cx. pipiens* and *Cx. torrentium*) in northern Europe[7], which showed similar levels of viral diversity and numerous shared viruses between the two vector species. They are also consistent with the Metaviromic study in Greece, where viral similarity was observed between different species within the same genus and even between different mosquito genera[31].Therefore, in our study, conducted in a savanna ecosystem, with specific ecological characteristics, including low anthropogenic activity, could offer similar conditions that can potentially favor the adaptation and persistence of certain viral species, among mosquitoes that coexist within the same endemic habitat.

These common ISVs between mosquitoes are objects of study have been frequently detected in *Culex* and *Aedes* mosquitoes[5] and were recently described in a viral metagenomics study in *Ochlerotatus* species in Europe[48]. Additionally, another dominant virus was *Corriparta virus*, classified within the *Orbivirus* genus[49,50] which includes arboviruses with a wide range of hosts[50]. Interestingly, ISVs found in lower abundance, such as *Guadeloupe mosquito virus* (GMV) and *Hubei mosquito virus1* (HMV1), have been described as part of the "core virome" in *Aedes* species[27–29], indicating a broad global distribution. Meanwhile, other ISVs with low representation (Fig. 3), such as *Uxmal virus, Tanay virus, Cordoba virus,* and *Castlerea virus*, from the *Negevirus* genus, are phylogenetically related to plant viruses[51,52], suggesting the adaptation of plant viruses in insects that feed on plant fluids, possibly through selection pressure processes[40]. It is noteworthy that the ISVs detected in this study have been reported with a wide geographic distribution and a broad range of mosquito host species, including *Aedes, Culex, Ochlerotatus,* and *Mansonia*[27,45,52,53]. It is crucial to continue studies that help elucidate the complex interactions involving virome shape in mosquitoes, especially the persistence of these common ISVs in vector species over time and geographic space. New insights in this area of study could reveal the critical role that some ISVs play in host fitness, especially in the presence and absence of viral pathogens.

In conclusion, our study provides a comprehensive insight into the viral composition within mosquitoes, collected from a local savanna ecosystem in the Colombian Orinoco. Despite the relatively stable nature of this ecosystem, the potential for spillover events increases significantly under scenarios involving anthropogenic intervention. Leveraging a metagenomic approach and advanced third-generation sequencing technologies, we identified the presence of insect-specific viruses (ISVs) common in the virome of these vector species. Furthermore, the virome of all mosquitoes also exhibited representation of viruses associated with plants, fungi, bacteria, vertebrates, algae, and protists. This broad viral representation underscores the intricate interactions of mosquitoes with their natural habitat. We propose further exploration of environmental and geographical conditions to enhance our understanding of the ecological dynamics of mosquito-vectors.

Finally, it is necessary to recognize some limitations of our study. First, the small number of samples could have limited the information on viral composition in the vector species studied. In addition, our investigation was based on a single sampling event of entomological material, and we did not collect detailed information on the environmental and ecological characteristics of the ecosystem studied, which could have provided valuable information on host-local environment interactions and their influence on virome.

We consider our findings to be an important contribution to the knowledge of the virome in Neotropical mosquito vectors described as potentially transmitting pathogenic viruses of the genera *Ochlerotatus, Culex, Limatus, Mansonia, Psorophora* and *Sabethes*. The presence of common ISVs in the virome of different vector species suggests that ecological information from the study site emerges as an essential component for understanding the determinants of virome in vectors. It highlights the need for further investigation of shared viruses among mosquito species, which would provide a more complete representation of diversity, distribution, and potential applications as control agents.

Methods

Study area and mosquito collection

Based on the identification of mosquito species circulating in the rural area of the Eastern Plains of Colombia from a previous study conducted by our research group[41], we selected eight adult mosquitoes of the species *Ochlerotatus (Aedes) serratus, Culex eknomios, Culex browni, Limatus durhamii, Mansonia indubitans, Psorophora albipes, Psorophora ferox, Sabethes chloropterus, Culex eknomios, Culex browni, Limatus durhamii, Mansonia indubitans, Psorophora albipes, Psorophora ferox,* and *Sabethes chloropterus*, for metagenomic sequencing belonging. Species selection was based on their abundance in the study area and per sampling site[41] (Fig. 4), as well as their epidemiological importance for the transmission of arboviruses of public health importance[3,17,18]. Briefly, the collection of adult mosquitoes in the field was carried out at the beginning of the dry season (low water) in December 2020, in the rural area of the municipality of Puerto Carreño, in the department of Vichada. The study site was located in the Eastern Plains of the Orinoco region, bordering Venezuela. This ecosystem is characterized by an extensive grassland plain (savannah), composed mainly of grasses, with the presence of forest

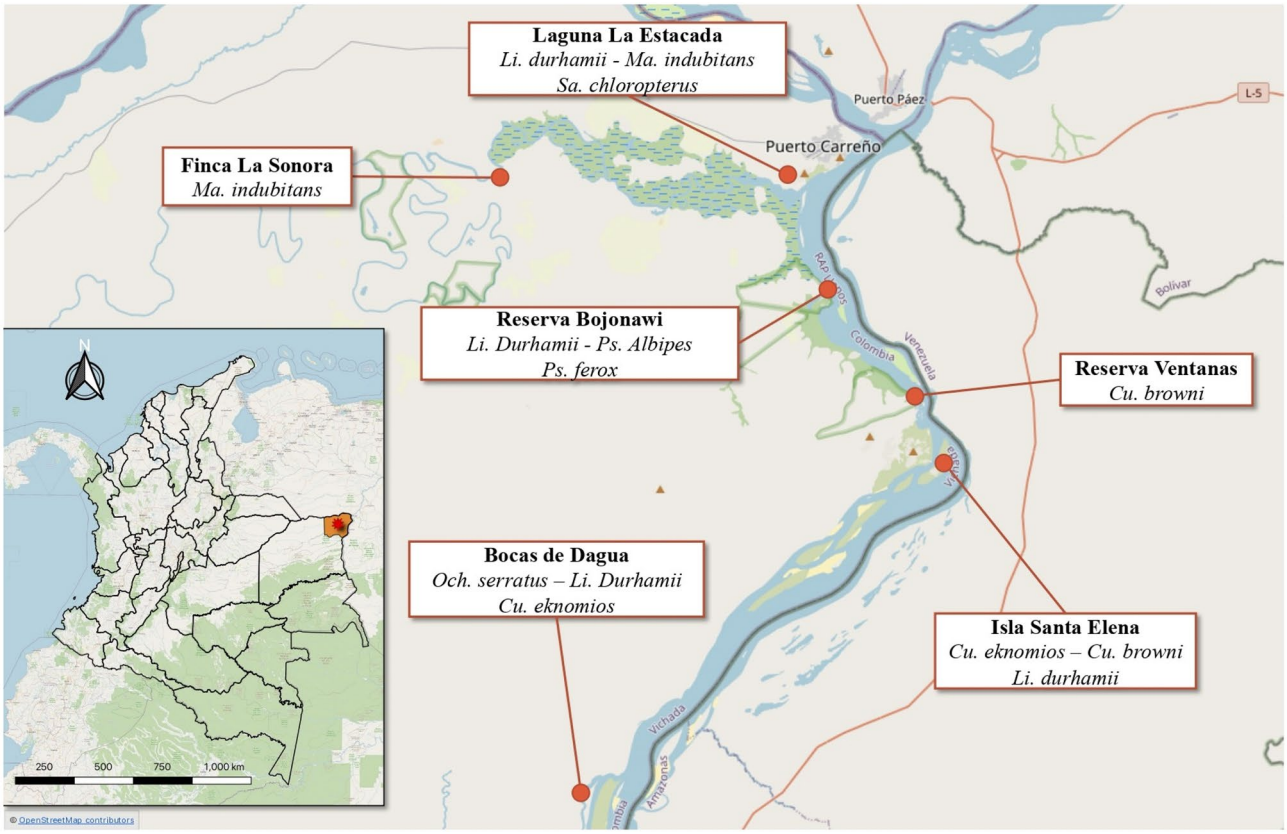

Figure 4. The map shows the geographic location of the Culicidae mosquito collection sites in the municipality of Puerto Carreño, located in the department of Vichada in Colombia. The georeferenced sampling points in different areas along the Orinoco River are highlighted, and each point is labeled with the local name of the corresponding sampling site and the mosquito species selected for viral metagenomic analysis. This map was produced using the QGIS 3.22.14 tool and different data sources were used, including Basemap: Esri Satellite World_Imagery (MapServer) available at https://bit.ly/3MRtYCf, as well as sources from Esri, Maxar, Earthstar Geographics, and the GIS user community. This map is licensed under CC BY-SA 3.0.

patches. Geographically, it is located at 6° 11′ 16″ north latitude and 67° 28′ 57″ west longitude, with an altitude of 51 m.a.s.l. (meters above sea level). The weather is tropical, with an average maximum temperature of 39.5 °C, rainfall of 2688 mm and an annual relative humidity of 70%. Sampling localities were chosen in proximity to the Orinoco River (Fig. 4), with shared ecological characteristics, particularly in a region influenced by recreational fishing and ecotourism, which create favorable conditions for mosquito breeding[41]. These areas also have nearby human settlements and have experienced minimal human interference, with limited disturbance or disruption due to human activity. This is of particular concern in view of possible future anthropogenic activities, which could facilitate the spread of potentially pathogenic viruses.

For mosquito collection, entomology professionals from the Department of Vichada Health Secretary provided support. A single sampling per site was carried out, lasting one hour per site, using mechanical vacuums during the day. In the forest patches, all mosquitoes were collected in the same field season. To avoid contamination of the biological material, gowns and gloves were worn throughout the handling process. In addition, trapping, storage, and transport material was pre-sterilized. Only adult mosquitoes were collected (males and females) and subsequently morphologically identified to species level using taxonomic keys. Mosquitoes of major entomovirological importance, belonging to the subfamily Culicinae, were selected at each sampling point, and grouped into pools of 2–6 individuals according to the species identified. In some cases, mosquitoes were stored individually for subsequent confirmation based on mitochondrial cytochrome oxidase I (COI) sequencing. Entomological material was subsequently preserved in RNA (DNA/RNA shield, Zymo. R1100-50) at −4 °C. Finally, the samples were analyzed at the microbiology laboratory of the Universidad del Rosario in Bogotá, Colombia. The Hamilton Microlab Star automated system and the MagBead Quick-DNA/Viral RNA kit (Ref. R2141, Zymo Research) were used to extract RNA from pooled and individual entomological material, after homogenization of the entomological material using the TissueLyser II® tissue homogenizer (Qiagen, Hilden, Germany). Subsequently, RNA was quantified using the NanoDrop™ spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) stored at − 80 °C.

Sample preparation and viral enrichment

Initially, samples of the selected mosquito species were checked for RNA quality and concentration. Then, 15 pools were chosen to reflect the abundance of mosquitoes (Diptera) in the study area. Thus, the species *Och. serratus* species, due to its high prevalence, was represented with the highest number of pools, followed by *Ps. ferox* (Table 1). The RNA used for sequencing was obtained from the 2020 RNA stock. In this regard, most of the pools analyzed corresponded to RNA from mosquitoes that were previously grouped by species and subsequently subjected to RNA extraction. For each pool, we ensured that it was composed of RNA from five individuals of the same species. Subsequently, the Ribo-Zero Plus rRNA Depletion kit (Illumina, REF: 20036696) was used in the RNA pools, following the manufacturer's instructions. Next, viral enrichment was carried out using the Rapid-SMART9n methodology (Switching Mechanism at the 5′ end of RNA Template), according to Claro et al.[54] with a minor modification. In summary, cDNA synthesis involved 5 µl of RNA, 0.5 µl of RLB-RT9N primer (TTTTTCGTGCGCCGCTTCAACNNNNNNNNN, 2 µM), and 0.5 µl of dNTPs (10 mM) (New England BioLabs, USA), followed by incubation at 65 °C for 5 min. A mix containing 2 µl of SuperScript IV First-strand Buffer, 0.5 µL of 0.1 M DTT, 0.5 µl RNase OUT, 0.5 µl RLB TSO (GCTAATCATTGCTTTTTCGTGCGCCGCTTCAACATrGrGrG, 2 µM), and 0.5 µL SuperScript IV (Invitrogen, Carlsbad, CA, USA) was then added to the annealed RNA. The mixture underwent an incubation at 42 °C for 90 min followed by 10 min at 70 °C to yield the cDNA. Next, the cDNA product was amplified using 6.25 µl of LongAmp Taq 2X master mix (New England BioLabs, USA), 4.875 µl of NFW, 0.125 µl of RLB primer (TTTTTCGTGCGCCGCTTCA, 20 µM), and 1.25 µl of cDNA, employing the following conditions: 98 °C for 45 s, 30 cycles of 98 °C for 15 s, 62 °C for 15 s, and 65 °C for 5 min, with a final step at 65 °C for 10 min. During both the cDNA construction and post-viral enrichment steps, all samples were quantified to ensure the proper execution of the process. This quantification was performed using the Qubit dsDNA High Sensitivity Assay (Life Technologies, USA) on the Qubit 3.0 instrument (Life Technologies, USA), following the manufacturer's instructions.

Nanopore library preparation and viral sequencing

Third-generation technology was used for sequencing. Initially, the End Prep procedure was employed using the commercial NEBNext® Ultra™ II End Repair/dA-Tailing Module kit for end preparation. For barcode ligation, the NEBNext® Ultra™ II Ligation Module kit was used. Finally, the adapters were ligated with a NEBNext® Quick Ligation Module kit together with the Oxford Nanopore Technologies ligation kit (SQK-LSK109). To ensure removal of unligated barcodes and adapters, a cleaning step was performed with Beckman Coulter AMPure XP paramagnetic beads. The original library was sequenced on a MinION device from Oxford Nanopore Technologies using R.9.4 flow cells. The sequencing process followed the standard 48–72 h script with MinKNOW 1.15.1 software, allowing for accurate and efficient data generation.

Bioinformatic analysis

For bioinformatics analysis, raw Fast5 files were subjected to base calling and demultiplexing using the Guppy V3.1.5 tool from Oxford Nanopore Technologies[55]. Low quality reads with a score below 7 were filtered out. The obtained set of long reads was then subjected to statistical analysis using the NanoStat V 1.1.2 tool (https://github.com/wdecoster/nanostat) to determine the average length and quality scores. Host filtering was not performed, as no reference genome is available for most of the mosquito vector species studied. To improve data accuracy, files were filtered to remove prokaryotic sequence contamination (bacterial and archaeal ribosomal reads) using Minimap 2.24 software[56] (https://github.com/lh3/minimap2). This software was specifically designed to align long genomic reads obtained from Oxford Nanopore sequencing. Alignment was performed against the prokaryotic database SILVA_138.1 (https://www.arb-silva.de/documentation/release-1381/). Finally, the aligned reads were then converted into a sorted BAM file using SAMtools[57](https://github.com/samtools/samtools) and Bam2fastq tools (https://github.com/jts/bam2fastq).

The clean sequences obtained after filtering were taxonomically assigned using the metagenomic sequence classifier Centrifuge tool v1.0.4[58]. The Centrifuge tool is used to count the number of reads that align to each taxon and produces a table with this information. To assign reads to specific viruses a custom Centrifuge indexing viral database was constructed from viral gene and genome sequences available from GenBank via NCBI Virus repositories (74) (https://www.ncbi.nlm.nih.gov/labs/virus/vssi/#/), until the most recent accession date in October 2022. The database contained 12,709 viral sequences that were selected based on completeness, non-redundancy (nr/nt), and no ambiguous characters. This viral database was initially used to get an overall representation of virus families in vector mosquitoes and verify the viral hosts. To deepen the taxonomic assignment results for the viruses present in the mosquito virome (ISV/arbovirus), a new NCBI-Mosquito virus database was constructed by refining the results based on the Culicidae (Diptera) host option, resulting in a total of 2899 viral sequences. In both cases, the database was selected based on completeness, non-redundancy (nr/nt) and absence of ambiguous characters. The taxonomic assignment in Centrifuge was performed utilizing a minimum length for partial hits (–min-hitlen) of 95 and a k classification parameter of 1. The resulting outputs were converted to Kraken-Report format using the Centrifuge-kreport function. The results of the metagenomic classification were further analyzed and visualized using the Pavian package[59] (https://fbreitwieser.shinyapps.io/pavian/). To verify the assigned sequences, they were aligned against the non-redundant (nr) nucleotide database using the Basic Local Alignment Search Tool (BLASTn)[60] considering parameters of percentage identity > 80%, E-value greater than 5 and minimum percentage of the coverage length of 60%. The number of reads assigned to viral families and species were converted into relative values to estimate their abundance within each mosquito species. Abundance barplots were generated using the ggplot2 package in RStudio[61].

Statistical analysis

To assess the normality of the data, the Shapiro–Wilk test was used, which indicated a non-normal distribution. To compare the abundance of viral taxa among the different mosquito species in the study, a non-parametric Kruskal–Wallis test for multiple comparisons was employed. For post-hoc analysis, Dunn's test with Benjamini–Hochberg stepwise correction was applied. This test was also used to compare viral species. Statistical analyses and data visualization were conducted using R software[61]. A significance level of p-value < 0.05 was considered statistically differential for all tests.

Data availability

The raw sequence reads generated in this study are available at the NCBI Sequence Read Archive (SRA) database under BioProject PRJNA98633 (https://www.ncbi.nlm.nih.gov/bioproject/986331). BioSamples SAMN35839862-SAMN35839876.

Received: 15 June 2023; Accepted: 6 December 2023
Published online: 12 December 2023

References

1. Kraemer, M. U. *et al.* The global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. albopictus*. *Elife* **4**, e08347. https://doi.org/10.7554/eLife.08347 (2015).
2. Bhatt, S. *et al.* The global distribution and burden of dengue. *Nature* **496**, 504–507. https://doi.org/10.1038/nature12060 (2013).
3. Weaver, S. C., Charlier, C., Vasilakis, N. & Lecuit, M. Zika, chikungunya, and other emerging vector-borne viral diseases. *Annu. Rev. Med.* **69**, 395–408. https://doi.org/10.1146/annurev-med-050715-105122 (2018).
4. Girard, M., Nelson, C. B., Picot, V. & Gubler, D. J. Arboviruses: A global public health threat. *Vaccine* **38**, 3989–3994. https://doi.org/10.1016/j.vaccine.2020.04.011 (2020).
5. Moonen, J. P., Schinkel, M., van der Most, T., Miesen, P. & van Rij, R. P. Composition and global distribution of the mosquito virome—A comprehensive database of insect-specific viruses. *One Health* **16**, 100490. https://doi.org/10.1016/j.onehlt.2023.100490 (2023).
6. Espinal, M. A. *et al.* Emerging and reemerging *Aedes*-transmitted arbovirus infections in the region of the Americas: Implications for health policy. *Am. J. Public Health* **109**, 387–392. https://doi.org/10.2105/AJPH.2018.304849 (2019).
7. Pettersson, J. H., Shi, M., Eden, J. S., Holmes, E. C. & Hesson, J. C. Meta-transcriptomic comparison of the RNA viromes of the mosquito vectors *Culex pipiens* and *Culex torrentium* in Northern Europe. *Viruses* https://doi.org/10.3390/v11111033 (2019).
8. Hoyos-Lopez, R., Suaza-Vasco, J., Rua-Uribe, G., Uribe, S. & Gallego-Gomez, J. C. Molecular detection of flaviviruses and alphaviruses in mosquitoes (Diptera: Culicidae) from coastal ecosystems in the Colombian Caribbean. *Mem. Inst. Oswaldo Cruz* **111**, 625–634. https://doi.org/10.1590/0074-02760160096 (2016).
9. Gould, E., Pettersson, J., Higgs, S., Charrel, R. & de Lamballerie, X. Emerging arboviruses: Why today?. *One Health* **4**, 1–13. https://doi.org/10.1016/j.onehlt.2017.06.001 (2017).
10. Kraemer, M. U. G. *et al.* Past and future spread of the arbovirus vectors *Aedes aegypti* and *Aedes albopictus*. *Nat. Microbiol.* **4**, 854–863. https://doi.org/10.1038/s41564-019-0376-y (2019).
11. Kauffman, E. B. & Kramer, L. D. Zika virus mosquito vectors: Competence, biology, and vector control. *J. Infect. Dis.* **216**, S976–S990. https://doi.org/10.1093/infdis/jix405 (2017).
12. Kraemer, M. U. *et al.* The global compendium of *Aedes aegypti* and *Ae. albopictus* occurrence. *Sci. Data* **2**, 150035. https://doi.org/10.1038/sdata.2015.35 (2015).
13. Rodriguez-Morales, A. J., Villamil-Gomez, W. E. & Franco-Paredes, C. The arboviral burden of disease caused by co-circulation and co-infection of dengue, chikungunya and Zika in the Americas. *Travel Med. Infect. Dis.* **14**, 177–179. https://doi.org/10.1016/j.tmaid.2016.05.004 (2016).
14. Papa, A. Emerging arboviral human diseases in Southern Europe. *J. Med. Virol.* **89**, 1315–1322. https://doi.org/10.1002/jmv.24803 (2017).
15. Mweya, C. N., Kimera, S. I., Stanley, G., Misinzo, G. & Mboera, L. E. Climate change influences potential distribution of infected *Aedes aegypti* co-occurrence with dengue epidemics risk areas in Tanzania. *PLoS One* **11**, e0162649. https://doi.org/10.1371/journal.pone.0162649 (2016).
16. Gonzalez-Escobar, G., Churaman, C., Rampersad, C., Singh, R. & Nathaniel, S. Mayaro virus detection in patients from rural and urban areas in Trinidad and Tobago during the Chikungunya and Zika virus outbreaks. *Pathog. Glob. Health* **115**, 188–195. https://doi.org/10.1080/20477724.2021.1878445 (2021).
17. Li, S. L. *et al.* Mapping environmental suitability of *Haemagogus* and *Sabethes* spp. mosquitoes to understand sylvatic transmission risk of yellow fever virus in Brazil. *PLoS Negl. Trop. Dis.* **16**, e0010019. https://doi.org/10.1371/journal.pntd.0010019 (2022).
18. Weaver, S. C. & Reisen, W. K. Present and future arboviral threats. *Antiviral Res.* **85**, 328–345. https://doi.org/10.1016/j.antiviral.2009.10.008 (2010).
19. Atoni, E. *et al.* The discovery and global distribution of novel mosquito-associated viruses in the last decade (2007–2017). *Rev. Med. Virol.* **29**, e2079. https://doi.org/10.1002/rmv.2079 (2019).
20. de Almeida, J. P., Aguiar, E. R., Armache, J. N., Olmo, R. P. & Marques, J. T. The virome of vector mosquitoes. *Curr. Opin. Virol.* **49**, 7–12. https://doi.org/10.1016/j.coviro.2021.04.002 (2021).
21. Vasilakis, N. & Tesh, R. B. Insect-specific viruses and their potential impact on arbovirus transmission. *Curr. Opin. Virol.* **15**, 69–74. https://doi.org/10.1016/j.coviro.2015.08.007 (2015).
22. Roundy, C. M., Azar, S. R., Rossi, S. L., Weaver, S. C. & Vasilakis, N. Insect-specific viruses: A historical overview and recent developments. *Adv. Virus Res.* **98**, 119–146. https://doi.org/10.1016/bs.aivir.2016.10.001 (2017).
23. Agboli, E., Leggewie, M., Altinli, M. & Schnettler, E. Mosquito-specific viruses-transmission and interaction. *Viruses* https://doi.org/10.3390/v11090873 (2019).
24. Altinli, M., Schnettler, E. & Sicard, M. Symbiotic interactions between mosquitoes and mosquito viruses. *Front. Cell Infect. Microbiol.* **11**, 694020. https://doi.org/10.3389/fcimb.2021.694020 (2021).
25. Bolling, B. G., Weaver, S. C., Tesh, R. B. & Vasilakis, N. Insect-specific virus discovery: Significance for the arbovirus community. *Viruses* **7**, 4911–4928. https://doi.org/10.3390/v7092851 (2015).
26. Ohlund, P., Lunden, H. & Blomstrom, A. L. Insect-specific virus evolution and potential effects on vector competence. *Virus Genes* **55**, 127–137. https://doi.org/10.1007/s11262-018-01629-9 (2019).
27. Gomez, M., Martinez, D., Munoz, M. & Ramirez, J. D. *Aedes aegypti* and *Ae. albopictus* microbiome/virome: New strategies for controlling arboviral transmission?. *Parasit. Vectors* **15**, 287. https://doi.org/10.1186/s13071-022-05401-9 (2022).
28. Shi, C. *et al.* Stable distinct core eukaryotic viromes in different mosquito species from Guadeloupe, using single mosquito viral metagenomics. *Microbiome* **7**, 121. https://doi.org/10.1186/s40168-019-0734-2 (2019).

29. Shi, C. *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* https://doi.org/10.1128/mSystems.00640-20 (2020).
30. Liu, Q. *et al.* Association of virome dynamics with mosquito species and environmental factors. *Microbiome* **11**, 101. https://doi.org/10.1186/s40168-023-01556-4 (2023).
31. Konstantinidis, K. *et al.* Defining virus-carrier networks that shape the composition of the mosquito core virome of a local ecosystem. *Virus Evol.* **8**, veac036. https://doi.org/10.1093/ve/veac036 (2022).
32. Guegan, M. *et al.* The mosquito holobiont: Fresh insight into mosquito-microbiota interactions. *Microbiome* **6**, 49. https://doi.org/10.1186/s40168-018-0435-2 (2018).
33. Rozo-Lopez, P. & Mengual, X. Updated list of the mosquitoes of Colombia (Diptera: Culicidae). *Biodivers. Data J.* https://doi.org/10.3897/BDJ.3.e4567 (2015).
34. Rozo-Lopez, P. & Mengual, X. Mosquito species (Diptera, Culicidae) in three ecosystems from the Colombian Andes: Identification through DNA barcoding and adult morphology. *Zookeys* https://doi.org/10.3897/zookeys.513.9561 (2015).
35. Martínez, D. *et al.* Identification of Aedes (Diptera: Culicidae) species and arboviruses circulating in Arauca, Eastern Colombia. *Front. Ecol. Evol.* https://doi.org/10.3389/fevo.2020.602190 (2020).
36. Gómez, M. *et al.* Arbovirus infection in *Aedes aegypti* from different departments of Colombia. *Front. Ecol. Evol.* https://doi.org/10.3389/fevo.2022.999169 (2022).
37. Mantilla-Granados, J. S. *et al.* Multidisciplinary approach for surveillance and risk identification of yellow fever and other arboviruses in Colombia. *One Health* **15**, 100438. https://doi.org/10.1016/j.onehlt.2022.100438 (2022).
38. Gutierrez-Barbosa, H., Medina-Moreno, S., Zapata, J. C. & Chua, J. V. Dengue infections in Colombia: Epidemiological trends of a hyperendemic country. *Trop. Med. Infect. Dis.* https://doi.org/10.3390/tropicalmed5040156 (2020).
39. Calle-Tobon, A. *et al.* Local-scale virome depiction in Medellin, Colombia, supports significant differences between *Aedes aegypti* and Aedes albopictus. *PLoS One* **17**, e0263143. https://doi.org/10.1371/journal.pone.0263143 (2022).
40. Laiton-Donato, K. *et al.* Novel Putative tymoviridae-like virus isolated from culex mosquitoes in Colombia. *Viruses* https://doi.org/10.3390/v15040953 (2023).
41. Martinez, D. *et al.* Employing oxford nanopore technologies (ONT) for understanding the ecology and transmission dynamics of flaviviruses in mosquitoes (Diptera: Culicidae) from Eastern Colombia. *Acta Trop.* **245**, 106972. https://doi.org/10.1016/j.actatropica.2023.106972 (2023).
42. Olmo, R. P. *et al.* Mosquito vector competence for dengue is modulated by insect-specific viruses. *Nat. Microbiol.* **8**, 135–149. https://doi.org/10.1038/s41564-022-01289-4 (2023).
43. Nasar, F., Erasmus, J. H., Haddow, A. D., Tesh, R. B. & Weaver, S. C. Eilat virus induces both homologous and heterologous interference. *Virology* **484**, 51–58. https://doi.org/10.1016/j.virol.2015.05.009 (2015).
44. Schultz, M. J., Frydman, H. M. & Connor, J. H. Dual Insect specific virus infection limits Arbovirus replication in *Aedes* mosquito cells. *Virology* **518**, 406–413. https://doi.org/10.1016/j.virol.2018.03.022 (2018).
45. Carvalho, V. L. & Long, M. T. Insect-specific viruses: An overview and their relationship to arboviruses of concern to humans and animals. *Virology* **557**, 34–43. https://doi.org/10.1016/j.virol.2021.01.007 (2021).
46. Thannesberger, J. *et al.* Highly sensitive virome characterization of *Aedes aegypti* and *Culex pipiens* complex from Central Europe and the Caribbean reveals potential for interspecies viral transmission. *Pathogens* https://doi.org/10.3390/pathogens9090686 (2020).
47. Atoni, E. *et al.* Metagenomic virome analysis of culex mosquitoes from Kenya and China. *Viruses* https://doi.org/10.3390/v10010030 (2018).
48. Truong Nguyen, P. T. *et al.* Characterisation of the RNA virome of nine ochlerotatus species in Finland. *Viruses* https://doi.org/10.3390/v14071489 (2022).
49. Belaganahalli, M. N. *et al.* Full genome sequencing of Corriparta virus, identifies California mosquito pool virus as a member of the Corriparta virus species. *PLoS One* **8**, e70779. https://doi.org/10.1371/journal.pone.0070779 (2013).
50. Harrison, J. J. *et al.* A new orbivirus isolated from mosquitoes in North-Western Australia shows antigenic and genetic similarity to corriparta virus but does not replicate in vertebrate cells. *Viruses* https://doi.org/10.3390/v8050141 (2016).
51. da Silva Ribeiro, A. C. *et al.* Negeviruses isolated from mosquitoes in the Brazilian Amazon. *Virol. J.* **19**, 17. https://doi.org/10.1186/s12985-022-01743-z (2022).
52. Vasilakis, N. *et al.* Negevirus: A proposed new taxon of insect-specific viruses with wide geographic distribution. *J. Virol.* **87**, 2475–2488. https://doi.org/10.1128/JVI.00776-12 (2013).
53. Charles, J. *et al.* Detection of novel and recognized RNA viruses in mosquitoes from the Yucatan Peninsula of Mexico using metagenomics and characterization of their in vitro host ranges. *J. Gen. Virol.* **99**, 1729–1738. https://doi.org/10.1099/jgv.0.001165 (2018).
54. Claro, I. *et al.* Rapid viral metagenomics using SMART-9N amplification and nanopore sequencing [version 1; peer review: 2 approved with reservations. *Wellcome Open Res.* **6**, 241. https://doi.org/10.12688/wellcomeopenres.17170.1 (2021).
55. Wick, R. R., Judd, L. M. & Holt, K. E. Performance of neural network basecalling tools for Oxford Nanopore sequencing. *Genome Biol.* **20**, 129. https://doi.org/10.1186/s13059-019-1727-y (2019).
56. Li, H. Minimap2: Pairwise alignment for nucleotide sequences. *Bioinformatics* **34**, 3094–3100. https://doi.org/10.1093/bioinformatics/bty191 (2018).
57. Danecek, P. *et al.* Twelve years of SAMtools and BCFtools. *Gigascience* https://doi.org/10.1093/gigascience/giab008 (2021).
58. Kim, D., Song, L., Breitwieser, F. P. & Salzberg, S. L. Centrifuge: Rapid and sensitive classification of metagenomic sequences. *Genome Res.* **26**, 1721–1729. https://doi.org/10.1101/gr.210641.116 (2016).
59. Breitwieser, F. P. & Salzberg, S. L. Pavian: Interactive analysis of metagenomics data for microbiome studies and pathogen identification. *Bioinformatics* **36**, 1303–1304. https://doi.org/10.1093/bioinformatics/btz715 (2020).
60. Sayers, E. W. *et al.* Database resources of the national center for biotechnology information in 2023. *Nucleic Acids Res.* **51**, D29–D38. https://doi.org/10.1093/nar/gkac1032 (2023).
61. Wickham, H. *ggplot2: Elegant Graphics for Data Analysis*. (Springer-Verlag, New York, 2016). ISBN 978-3-319-24277-4. https://ggplot2.tidyverse.org.

Acknowledgements

We extend our sincere thanks to the local health authorities of the Vichada departments for their valuable assistance in sample collection. We are also grateful for the financial support provided by the Dirección de Investigación e Innovación at the Universidad del Rosario. Additionally, we acknowledge the University of Boyacá for their support in financing MG's doctoral studies.

Author contributions

J.D.R., M.M., M.G., L.P. conceived and designed the experiments; J.L.D.S., A.Z.F. collected the entomological material; D.M., M.G. and L.P. performed the experiments; M.G., C.H., N.L. analyzed the data; M.G. wrote and prepared the original draft; J.D.R., M.M., reviewed and edited the manuscript.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-023-49232-9.

Correspondence and requests for materials should be addressed to J.D.R.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

10. CONCLUSIONES

Capítulo 1

- La vigilancia entomovirológica emerge como una herramienta esencial para comprender la dinámica epidemiológica del dengue y otras arbovirosis. La identificación temprana de la presencia y propagación de virus en los vectores proporciona una base sólida para la para comprender de manera integral la dinámica de transmisión de arbovirus y evaluar el riesgo potencial para la población

- La detección de infecciones naturales por arbovirus en mosquitos *Aedes aegypti* en diversas regiones de Colombia refleja el patrón epidemiológico de los arbovirus en el país, y resalta su importancia como vector principal de enfermedades virales. Estos hallazgos destacan la importancia de mantener una vigilancia continua del vector para controlar la propagación de estos virus y proteger la salud pública mediante la implementación de prevención y de control.

- El predominio del Linaje I de *Aedes aegypti* en Colombia, que se encuentra ampliamente distribuido en América y ha sido previamente vinculado con áreas de alta incidencia de dengue, sugiere que la epidemiología de esta enfermedad podría estar influenciada por la dinámica poblacional del vector. Este hallazgo subraya la importancia de comprender y monitorear la población de mosquitos para evitar la transmisión del dengue de manera efectiva.

Capítulo 2

- Se resalta el potencial de las técnicas de secuenciación de próxima generación (NGS) para explorar el viroma de los mosquitos vectores. Esto subraya la importancia de incorporar estas herramientas moleculares en la vigilancia virológica en tiempo real ante la (re)emergencia de patógenos arbovirales.

- El análisis del viroma de *Aedes aegypti* en Colombia mediante metagenómica viral contribuye a una mejor comprensión de la diversidad de virus presentes en los mosquitos vectores de relevancia epidemiológica. Además, esta investigación ofrece información crucial que podría ayudar a identificar posibles conexiones entre los virus insecto-específicos (ISV) y los arbovirus.

- El análisis de la composición de las comunidades virales en *A. aegypti* reveló que la infección con diferentes serotipos de DENV (DENV-1 y DENV-2) podría provocar alteraciones en la abundancia relativa de las familias y especies virales que constituyen el viroma central en *Aedes* spp.

- La abundancia diferencial de ISV, como el PCLV, en el "*core virome*" o viroma central de *A. aegypti* en muestras de Colombia con infección natural por DENV-1 y DENV-2, indica su potencial implicación en la biología del vector y en la dinámica de transmisión de arbovirus.

Capítulo 3

- Se resalta la importancia de incluir ONT en la vigilancia entomovirológica para comprender mejor la dinámica asociada a la ecología y transmisión de los virus en vectores.

- La ecología y dinámica de transmisión de flavivirus en mosquitos (Diptera: Culicidae) de los Llanos Orientales de Colombia, reveló la circulación del Virus del Nilo Occidental (WNV) en *Culex browni*, resaltando el impacto potencial de las actividades humanas en estos ecosistemas antropogénicamente sensibles.

- El análisis de las fuentes de alimentación en mosquitos Culiciade en una demostró el comportamiento oportunista del *Oc. Serratus* y diversos hospedadores de alimentación, incluidos los humanos.

- La caracterización preliminar de metagenómica viral en mosquitos (Culicidae) en la Orinoquia colombiana, amplía el entendimiento sobre las especies virales en mosquitos vectores del neotrópico de los géneros *Ochlerotatus, Culex, Limatus, Mansonia, Psorophora* y *Sabethes*. Este estudio destaca la presencia y relevancia de ISV comunes en estos vectores, sugiriendo su posible relación con la competencia vectorial y su influencia en la transmisión de enfermedades arbovirales.

- Los factores asociados al hábitat pueden influir en la composición del viroma de manera significativa, especialmente en áreas donde coexisten múltiples especies de mosquitos. Por lo tanto, se destaca la importancia del entorno de desarrollo del hospedero en la configuración de las comunidades virales en el mosquito.

11. PERSPECTIVAS

- Se sugiere ampliar los estudios de vigilancia entomovirológica en diversas áreas de Colombia para mejorar la comprensión de la transmisión de enfermedades virales por vectores y facilitar el diseño de estrategias de intervención integrales.

- Se propone continuar con estudios de infección natural en mosquitos *Aedes* spp, e incluir la identificación de otros posibles vectores de enfermedades virales en el país, como los mosquitos del género *Culex* spp. Ampliar el monitoreo entomológico y los virus que portan permitiría una comprensión más completa de la epidemiología de las enfermedades transmitidas por vectores en Colombia y facilitaría el diseño de estrategias de control más efectivas.

- Se sugiere seguir desarrollando investigaciones sobre la diversidad genética y estructura poblacional de *Aedes aegypti* en Colombia, así como su relación con la epidemiología del dengue. Estos estudios podrían proporcionar información valiosa y robusta para mejorar los sistemas integrales de vigilancia y control vectorial.

- Se propone, especialmente a las entidades de salud y de gobierno, aprovechar la capacidad instalada frente a la contención sanitaria por COVID-19, para monitorear la circulación de serotipos y genotipos del DENV tanto en muestras de pacientes humanos, como en mosquitos-vectores, como estrategia de manejo integral de enfermedades causadas por arbovirus.

- Se alienta a desarrollar análisis sobre genómica de arbovirus, utilizando muestras de los hospederos humanos, y de mosquitos vectores. Esto permitirá una comprensión más completa e integral del ciclo de transmisión de estos agentes virales, lo que contribuirá al fortalecimiento de las capacidades institucionales para el control e intervención ante la (re)emergencia de patógenos arbovirales.

- Se sugiere profundizar en los los virus insecto-específicos (ISV) que constituyen el "viroma central" de *A. aegypti*, con un enfoque especial en comprender la dinámica de las complejas interacciones entre los serotipos de DENV, ISV y sus hospederos-mosquitos, en diversos contextos epidemiológicos. Esto podría proporcionar información esencial para dilucidar la dinámica viral en mosquitos.

- Se recomienda que en futuras investigaciones se profundice en la comprensión de los mecanismos subyacentes a las interacciones entre PCLV y los serotipos de DENV en *A. aegypti,* utilizando enfoques celulares, moleculares y bioinformáticos.

- Se subraya la importancia de incluir en la vigilancia entomovirologica a partir de secuenciación próxima generación (ONT) para comprender mejor las dinámicas asociadas

con la ecología y transmisión de arbovirus de importancia en saud publica en diversos hábitats-ecositemas de Colombia, especialmente en áreas geográficas rurales.

- Se propone en análisis próximos de metagenómica viral incluir las características ecológicas relacionadas con el hábitat, y genéticas asociadas con la especie vectorial, especialmente entre mosquitos que coexisten en un mismo ecosistema. Estos datos aportarían información crucial sobre los factores determinantes en la composición transitoria o permanente del viroma de mosquitos vectores en ecosistemas conservados sensibles a intervenciones antrópicas.

- Se recomienda que en estudios posteriores se profundice en las especies de virus insecto-específicos (ISV) compartidas entre diversas especies de vectores, lo que proporcionaría una representación más completa de la diversidad y la distribución, así como las posibles aplicaciones como agentes de control.

12. PRODUCTOS DE LA TESIS

Los artículos se encuentran a lo largo del documento, los anexos correspondientes a la información suplementaria se encuentran en la carpeta de anexos de artículos divididos en carpetas por artículo. Todos los productos que se mencionarán a continuación se encuentran en la carpeta de anexos de productos de la tesis divididos según los numerales a continuación:

12.1 Artículos Científicos

- **Artículo 1:** Gómez M, Martínez D, Hernández C, Luna N, Patiño LH, Bohórquez Melo R, Suarez LA, Palma-Cuero M, Murcia LM, González Páez L, Estrada Bustos L, Medina MA, Ariza Campo K, Padilla HD, Zamora Flórez A, De las Salas JL, Muñoz M and Ramírez JD. Arbovirus infection in *Aedes aegypti* from different departments of Colombia. Front. Ecol. Evol. 2022. 10:999169. doi: 10.3389/fevo.2022.999169.

- **Artículo 2:** Gómez M, Martínez D, Muñoz M, Ramírez JD. *Aedes aegypti* and *Ae. albopictus* microbiome/virome: new strategies for controlling arboviral transmission? Parasit Vectors. 2022 Aug 9;15(1):287. doi: 10.1186/s13071-022-05401-9.

- **Artículo 3:** Gómez M, Martínez D, Páez-Triana L, Luna N, Ramírez A, Medina J, Cruz-Saavedra L, Hernández C, Castañeda S, Bohórquez Melo R, Suarez LA, Palma-Cuero M, Murcia LM, González Páez L, Estrada Bustos L, Medina MA, Ariza Campo K, Padilla HD, Zamora Flórez A, De Las Salas JL, Muñoz M, Ramírez JD. Influence of dengue virus serotypes on the abundance of *Aedes aegypti* insect-specific viruses (ISVs). J Virol. 2023 Dec 14:e0150723. doi: 10.1128/jvi.01507-23.

- **Artículo 4:** Martínez D, Gómez M, De Las Salas JL, Hernández C, Flórez AZ, Muñoz M, Ramírez JD. Employing oxford nanopore technologies (ONT) for understanding the ecology and transmission dynamics of flaviviruses in mosquitoes (Diptera: Culicidae) from Eastern Colombia. Acta Trop. 2023 Sep; 245:106972. doi: 10.1016/j.actatropica.2023.106972.

- **Artículo 5:** Gómez M, Martínez D, Páez-Triana L, Luna N, De las Salas J, Hernández C, Zamora Flórez A, Muñoz M, Ramírez JD. Characterizing viral species in mosquitoes (Culicidae) in the Colombian Orinoco: insights from a preliminary metagenomic study. Sci Rep 2023.13, 22081. doi.org/10.1038/s41598-023-49232

12.2 Presentación En Eventos Científicos

- **2022.** Presentación oral en el XVIII Congreso Colombiano de Parasitología y Medicina Tropical. Ponente. Título del trabajo: "Vigilancia entomovirológica de *Aedes aegypti* en Colombia".

- **2022.** Presentación oral en el V Congreso Internacional de Ciencias Básicas e Ingeniería 'CICI 2022' - Unillanos. Título del trabajo: "Infección natural por arbovirus en mosquitos *Aedes aegypti* en diferentes departamentos de Colombia".

- **2022.** Presentación oral en el Seminario virtual internacional. El desarrollo sostenible: el papel de las mujeres en el cumplimiento de la Agenda 2030. Diálogos entre México y Colombia. Título del trabajo: "Virus emergentes: retos para la sostenibilidad".

- **2023.** Presentación oral en el X Simposio Colombiano y VI Congreso Latinoamericano de Virología. Título del trabajo: "El perfil metagenómico del viroma de *Aedes aegypti* revela alteraciones específicas en respuesta a la infección por los serotipos dengue"

- **2023.** Presentación poster en el X Simposio Colombiano y VI Congreso Latinoamericano de Virología. Título del trabajo "La Metagenómica Viral Revela Especies Virales Compartidas en Mosquitos (Culicidae) de un Ecosistema Local en la Orinoquía Colombiana".

12.3 Cursos

- **2020.** Metagenómica aplicada a la caracterización de la diversidad virológica con potencial emergente. Instituto Nacional de Salud - Universidad Cooperativa de Colombia.

- **2021.** Curso internacional Estrategias bioinformáticas para el estudio de Enfermedades Tropicales Desatendidas (ETDs). CABANA

- **2021.** Curso Fundamentos de Genómica para la Investigación en Salud. Universidad del Valle.

- **2021.** Perspectivas para el fortalecimiento de laboratorios nacional de referencia y Laboratorios de Salud Pública fronterizos de referencia territorial.

- **2021.** Curso internacional Data Science con R.

- **2022.** Viral Genomics and Bioinformatics (Virtual) (LAC). Wellcome Genome Campus, Hinxton, Cambridge. Institute Sanger.

- **2023.** ABSL2 University of Texas Medical Branch.

- **2023.** Biología Molecular de Virus. I Curso De Herramientas Biotecnológicas para la Vigilancia y Control De Enfermedades transmitidas por Vectores (ETVs). Ministerio de Ciencia Tecnología e Innovación-MinCiencias, Centro de Investigaciones en Biotecnología y Microbiología - CIMBIUR, Centro Argentino y Brasileño de Biotecnología – CABBIO.

- **2023.** Papel de los virus en las ETVs. I Curso De Herramientas Biotecnológicas para la Vigilancia y Control De Enfermedades transmitidas por Vectores (ETVs). Biología Molecular de Virus. Ministerio de Ciencia Tecnología e Innovación-MinCiencias, Centro de Investigaciones en Biotecnología y Microbiología - CIMBIUR, Centro Argentino y Brasileño de Biotecnología – CABBIO.

- **2023.** Entomovirología, métodos de muestreo, taxonomía de mosquitos vectores y detección de arbovirus. Curso Pre-Congreso X Simposio Colombiano y VI Congreso Latinoamericano de Virología.

12.4 Pasantía Internacional

2023. World Reference Center for Emerging Viruses and Arboviruses (WRCEVA) – University of Texas Medical Branch. Texas - Estados Unidos. Duración 4 meses.

En el segundo semestre de 2023, realicé una pasantía internacional como parte de mi doctorado en Ciencias Biomédicas y Biológicas en la Universidad del Rosario. La estancia tuvo lugar en el World Reference Center for Emerging Viruses and Arboviruses (WRCEVA) de la University of Texas Medical Branch, bajo la supervisión de los doctores Scott Weaver, Ph.D., y Kenneth Plante, Ph.D. El objetivo principal de la pasantía fue profundizar en el conocimiento sobre la detección, identificación y caracterización de arbovirus y otros virus emergentes.

Durante mi tiempo en el WRCEVA, participé activamente en una serie de actividades y entrenamientos cuidadosamente diseñados para mejorar mis habilidades técnicas y científicas. Estas actividades incluyeron:

- Formación en biocontención y manejo de bioseguridad en laboratorios de nivel 2.

- Entrenamiento en técnicas de cultivo celular para el aislamiento y propagación de virus.

- Realización de ensayos de placas y ensayos de formación de focos para la caracterización viral.

- Aprendizaje de técnicas de inmunotinción para la identificación de antígenos virales.

- Participación en la extracción y secuenciación de material genético viral.

- Manejo básico de animales de laboratorio para estudios relacionados con enfermedades virales.

Durante la pasantía, se resalta la oportunidad de interactuar con científicos de renombre internacional y de nivel académico excepcional. Sus conocimientos y experiencia en el campo de la virología fueron una fuente invaluable de aprendizaje y motivación. Los conocimientos y experiencias obtenidos durante la estancia en el WRCEVA son considerados de gran utilidad para la carrera investigativa futura y para el avance del conocimiento científico en el campo de la virología.

12.5 Becas

2018. Beca Programas de Formación Postgradual Avanzada con apoyo institucional, Programa DIA (Doctorado para la Investigación y la Academia) otorgado por la Universidad de Boyacá. Tunja-Colombia.

2022. Beca Pasantía estudiantes doctorales, otorgada por el Comité de Becas y Apoyos para el Fortalecimiento Académico. Dirección Académica de la Universidad del Rosario. Bogotá-Colombia.

13. BIBLIOGRAFÍA

1. Gould E, Pettersson J, Higgs S, Charrel R, de Lamballerie X. Emerging arboviruses: Why today? One Health. 2017;4:1-13.
2. Young PR. Arboviruses: A Family on the Move. Adv Exp Med Biol. 2018;1062:1-10.
3. Golding N, Wilson AL, Moyes CL, Cano J, Pigott DM, Velayudhan R, et al. Integrating vector control across diseases. BMC Med. 2015;13:249.
4. World Health Organization. Global vector control response 2017–2030 2017.
5. Mboera LE, Mweya CN, Rumisha SF, Tungu PK, Stanley G, Makange MR, et al. The Risk of Dengue Virus Transmission in Dar es Salaam, Tanzania during an Epidemic Period of 2014. PLoS Negl Trop Dis. 2016;10(1):e0004313.
6. Chauhan L, Matthews E, Piquet AL, Henao-Martinez A, Franco-Paredes C, Tyler KL, et al. Nervous System Manifestations of Arboviral Infections. Curr Trop Med Rep. 2022;9(4):107-18.
7. Patterson J, Sammon M, Garg M. Dengue, Zika and Chikungunya: Emerging Arboviruses in the New World. West J Emerg Med. 2016;17(6):671-9.
8. Paixao ES, Teixeira MG, Rodrigues LC. Zika, chikungunya and dengue: the causes and threats of new and re-emerging arboviral diseases. BMJ Glob Health. 2018;3(Suppl 1):e000530.
9. Murray NE, Quam MB, Wilder-Smith A. Epidemiology of dengue: past, present and future prospects. Clin Epidemiol. 2013;5:299-309.
10. Weaver SC, Charlier C, Vasilakis N, Lecuit M. Zika, Chikungunya, and Other Emerging Vector-Borne Viral Diseases. Annu Rev Med. 2018;69:395-408.
11. Kraemer MUG, Reiner RC, Jr., Brady OJ, Messina JP, Gilbert M, Pigott DM, et al. Past and future spread of the arbovirus vectors Aedes aegypti and Aedes albopictus. Nat Microbiol. 2019;4(5):854-63.
12. Kauffman EB, Kramer LD. Zika Virus Mosquito Vectors: Competence, Biology, and Vector Control. J Infect Dis. 2017;216(suppl_10):S976-S90.
13. de Souza WM, Weaver SC. Effects of climate change and human activities on vector-borne diseases. Nat Rev Microbiol. 2024.
14. Kraemer MU, Sinka ME, Duda KA, Mylne A, Shearer FM, Brady OJ, et al. The global compendium of Aedes aegypti and Ae. albopictus occurrence. Sci Data. 2015;2:150035.
15. Rodriguez-Morales AJ, Villamil-Gomez WE, Franco-Paredes C. The arboviral burden of disease caused by co-circulation and co-infection of dengue, chikungunya and Zika in the Americas. Travel medicine and infectious disease. 2016;14(3):177-9.
16. Papa A. Emerging arboviral human diseases in Southern Europe. J Med Virol. 2017;89(8):1315-22.
17. Mweya CN, Kimera SI, Stanley G, Misinzo G, Mboera LE. Climate Change Influences Potential Distribution of Infected Aedes aegypti Co-Occurrence with Dengue Epidemics Risk Areas in Tanzania. PLoS One. 2016;11(9):e0162649.
18. Conway MJ, Colpitts TM, Fikrig E. Role of the Vector in Arbovirus Transmission. Annu Rev Virol. 2014;1(1):71-88.
19. Harapan H, Michie A, Sasmono RT, Imrie A. Dengue: A Minireview. Viruses. 2020;12(8).

20. Guzman MG, Gubler DJ, Izquierdo A, Martinez E, Halstead SB. Dengue infection. Nat Rev Dis Primers. 2016;2:16055.
21. Alonso-Palomares LA, Moreno-Garcia M, Lanz-Mendoza H, Salazar MI. Molecular Basis for Arbovirus Transmission by Aedes aegypti Mosquitoes. Intervirology. 2018;61(6):255-64.
22. Gomez M, Martinez D, Munoz M, Ramirez JD. Aedes aegypti and Ae. albopictus microbiome/virome: new strategies for controlling arboviral transmission? Parasit Vectors. 2022;15(1):287.
23. Salud INd. Boletín epidemiológico semana 33. Dirección de vigilancia y análisis del riesgo en salud pública publicación en línea. Bogota, CO: Ministerio de Salud y Protección Social. . 2023.
24. Messina JP, Brady OJ, Golding N, Kraemer MUG, Wint GRW, Ray SE, et al. The current and future global distribution and population at risk of dengue. Nat Microbiol. 2019;4(9):1508-15.
25. Lessa CLS, Hodel KVS, Goncalves MS, Machado BAS. Dengue as a Disease Threatening Global Health: A Narrative Review Focusing on Latin America and Brazil. Trop Med Infect Dis. 2023;8(5).
26. Pollett S, Melendrez MC, Maljkovic Berry I, Duchene S, Salje H, Cummings DAT, et al. Understanding dengue virus evolution to support epidemic surveillance and counter-measure development. Infect Genet Evol. 2018;62:279-95.
27. Salud OPdl. Actualización Epidemiológica Dengue, chikunguña y Zika. 2023.
28. Salud OMdl. Partes sobre brotes epidémicos; dengue: situación mundial. Disponible en: https://www.who.int/es/emergencies/disease-outbreak-news/item/2023-DON498. 2023.
29. Gutierrez-Barbosa H, Medina-Moreno S, Zapata JC, Chua JV. Dengue Infections in Colombia: Epidemiological Trends of a Hyperendemic Country. Trop Med Infect Dis. 2020;5(4).
30. Mantilla-Granados JS, Sarmiento-Senior D, Manzano J, Calderon-Pelaez MA, Velandia-Romero ML, Buitrago LS, et al. Multidisciplinary approach for surveillance and risk identification of yellow fever and other arboviruses in Colombia. One Health. 2022;15:100438.
31. Padilla J, Lizarazo F, Murillo O, Mendigaña F, Pachón E, Vera M. Epidemiología de las principales enfermedades transmitidas por vectores en Colombia 1990-2016. 2017;37.
32. Morgan J, Strode C, Salcedo-Sora JE. Climatic and socio-economic factors supporting the co-circulation of dengue, Zika and chikungunya in three different ecosystems in Colombia. PLoS Negl Trop Dis. 2021;15(3):e0009259.
33. Salud OPdl. Plataforma de Información en Salud de las Américas (PLISA), Indicadores dengue. 2019-2024 en Colombia. https://www3.paho.org/data/index.php/es/temas/indicadores-dengue/dengue-nacional/9-dengue-pais-ano.html. 2024.
34. Martínez D, Gómez M, Campo-Palacio S, González-Robayo M, Montilla M, Pavas-Escobar N, et al. Cryptic transmission and novel introduction of Dengue 1 and 2 genotypes in Colombia. Virus Evol (Sometido). 2024.
35. Instituto Nacional de Salud. Informe Técnico Entomológico de arbovirus - Colombia, 2018. 2019.
36. Padilla J, Lizarazo F, Murillo O, Mendigaña F, Pachón E, Vera M. Epidemiología de las principales enfermedades transmitidas por vectores en Colombia, 1990-20162017 [cited 2017; 37:[27-40 pp.].

37. Ministerio de Salud y Protección Social. Lineamiento para la gestion y operación del programa de enfermedades transmitidas por vectores y zoonosis y otras consideraciones para la ejecución de sus transferencias nacionales de funcionamiento. Dirección de Promoción y Prevención. In: Endemoepidémicas. SdETG, editor. Combia: Ministerio de Salud y Protección Social. República de Colmbia; 2020. p. 166.
38. Kraemer MU, Sinka ME, Duda KA, Mylne AQ, Shearer FM, Barker CM, et al. The global distribution of the arbovirus vectors Aedes aegypti and Ae. albopictus. Elife. 2015;4:e08347.
39. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global distribution and burden of dengue. Nature. 2013;496(7446):504-7.
40. Moonen JP, Schinkel M, van der Most T, Miesen P, van Rij RP. Composition and global distribution of the mosquito virome - A comprehensive database of insect-specific viruses. One Health. 2023;16:100490.
41. Espinal MA, Andrus JK, Jauregui B, Waterman SH, Morens DM, Santos JI, et al. Emerging and Reemerging Aedes-Transmitted Arbovirus Infections in the Region of the Americas: Implications for Health Policy. Am J Public Health. 2019;109(3):387-92.
42. Hoyos-Lopez R, Suaza-Vasco J, Rua-Uribe G, Uribe S, Gallego-Gomez JC. Molecular detection of flaviviruses and alphaviruses in mosquitoes (Diptera: Culicidae) from coastal ecosystems in the Colombian Caribbean. Mem Inst Oswaldo Cruz. 2016;111(10):625-34.
43. Weaver SC, Reisen WK. Present and future arboviral threats. Antiviral Res. 2010;85(2):328-45.
44. Viglietta M, Bellone R, Blisnick AA, Failloux AB. Vector Specificity of Arbovirus Transmission. Front Microbiol. 2021;12:773211.
45. Li SL, Acosta AL, Hill SC, Brady OJ, de Almeida MAB, Cardoso JDC, et al. Mapping environmental suitability of Haemagogus and Sabethes spp. mosquitoes to understand sylvatic transmission risk of yellow fever virus in Brazil. PLoS Negl Trop Dis. 2022;16(1):e0010019.
46. Gonzalez-Escobar G, Churaman C, Rampersad C, Singh R, Nathaniel S. Mayaro virus detection in patients from rural and urban areas in Trinidad and Tobago during the Chikungunya and Zika virus outbreaks. Pathog Glob Health. 2021;115(3):188-95.
47. Houe V, Bonizzoni M, Failloux AB. Endogenous non-retroviral elements in genomes of Aedes mosquitoes and vector competence. Emerg Microbes Infect. 2019;8(1):542-55.
48. Santiago GA, Vergne E, Quiles Y, Cosme J, Vazquez J, Medina JF, et al. Analytical and clinical performance of the CDC real time RT-PCR assay for detection and typing of dengue virus. PLoS Negl Trop Dis. 2013;7(7):e2311.
49. Alvarez-Diaz DA, Valencia-Alvarez E, Rivera JA, Rengifo AC, Usme-Ciro JA, Pelaez-Carvajal D, et al. An updated RT-qPCR assay for the simultaneous detection and quantification of chikungunya, dengue and zika viruses. Infect Genet Evol. 2021;93:104967.
50. Kirstein OD, Ayora-Talavera G, Koyoc-Cardena E, Chan Espinoza D, Che-Mendoza A, Cohuo-Rodriguez A, et al. Natural arbovirus infection rate and detectability of indoor female Aedes aegypti from Merida, Yucatan, Mexico. PLoS Negl Trop Dis. 2021;15(1):e0008972.
51. Cevallos V, Ponce P, Waggoner JJ, Pinsky BA, Coloma J, Quiroga C, et al. Zika and Chikungunya virus detection in naturally infected Aedes aegypti in Ecuador. Acta Trop. 2018;177:74-80.

52. Monteiro FJC, Mourao FRP, Ribeiro ESD, Rego M, Frances P, Souto RNP, et al. Prevalence of dengue, Zika and chikungunya viruses in Aedes (Stegomyia) aegypti (Diptera: Culicidae) in a medium-sized city, Amazon, Brazil. Rev Inst Med Trop Sao Paulo. 2020;62:e10.
53. Pena-Garcia VH, Triana-Chavez O, Mejia-Jaramillo AM, Diaz FJ, Gomez-Palacio A, Arboleda-Sanchez S. Infection Rates by Dengue Virus in Mosquitoes and the Influence of Temperature May Be Related to Different Endemicity Patterns in Three Colombian Cities. Int J Environ Res Public Health. 2016;13(5).
54. Scarpassa VM, Cardoza TB, Cardoso Junior RP. Population genetics and phylogeography of Aedes aegypti (Diptera: Culicidae) from Brazil. Am J Trop Med Hyg. 2008;78(6):895-903.
55. Paupy C, Le Goff G, Brengues C, Guerra M, Revollo J, Barja Simon Z, et al. Genetic structure and phylogeography of Aedes aegypti, the dengue and yellow-fever mosquito vector in Bolivia. Infect Genet Evol. 2012;12(6):1260-9.
56. Jimenez-Silva CL, Carreno MF, Ortiz-Baez AS, Rey LA, Villabona-Arenas CJ, Ocazionez RE. Evolutionary history and spatio-temporal dynamics of dengue virus serotypes in an endemic region of Colombia. PLoS One. 2018;13(8):e0203090.
57. Rozo-Lopez P, Mengual X. Mosquito species (Diptera, Culicidae) in three ecosystems from the Colombian Andes: identification through DNA barcoding and adult morphology. Zookeys. 2015(513):39-64.
58. Rozo-Lopez P, Mengual X. Updated list of the mosquitoes of Colombia (Diptera: Culicidae). Biodivers Data J. 2015(3):e4567.
59. Ruiz-López F, González-Mazo A, A. V-M, Gómez G, L Z, Uribe S, et al. Presencia de Aedes (Stegomyia) aegypti (Linnaeus, 1762) y su infección natural con el virus del dengue en alturas no registradas para Colombia. Biomédica. 2016;36.
60. Perez-Castro R, Castellanos JE, Olano VA, Matiz MI, Jaramillo JF, Vargas SL, et al. Detection of all four dengue serotypes in Aedes aegypti female mosquitoes collected in a rural area in Colombia. Mem Inst Oswaldo Cruz. 2016;111(4):233-40.
61. Velandia-Romero ML, Coronel-Ruiz C, Castro-Bonilla L, Camacho-Ortega S, Calderon-Pelaez MA, Castellanos A, et al. Prevalence of dengue antibodies in healthy children and adults in different Colombian endemic areas. Int J Infect Dis. 2020;91:9-16.
62. Carrasquilla MC, Ortiz MI, Leon C, Rondon S, Kulkarni MA, Talbot B, et al. Entomological characterization of Aedes mosquitoes and arbovirus detection in Ibague, a Colombian city with co-circulation of Zika, dengue and chikungunya viruses. Parasit Vectors. 2021;14(1):446.
63. Rúa-Uribe G, Suárez-Acosta C, Rojo R. Implicaciones epidemiológicas de Aedes albopictus (Skuse) en Colombia. Rev Fac Nac Salud Pública. 2012;30.
64. Echeverry-Cardenas E, Lopez-Castaneda C, Carvajal-Castro JD, Aguirre-Obando OA. Potential geographic distribution of the tiger mosquito Aedes albopictus (Skuse, 1894) (Diptera: Culicidae) in current and future conditions for Colombia. PLoS Negl Trop Dis. 2021;15(5):e0008212.
65. Instituto Nacional de Salud. Instituto Nacional de Salud. Boletín Epidemiológico semana 41. Dirección de Vigilancia y Análisis del Riesgo en Salud Pública Publicación en línea: ISSN 2357-6189 2018. 2020.
66. Gómez W, P ZG. Presencia de Aedes aegypti y Aedes albopictus (Diptera: culicidae) en área rural del departamento de Santander, Colombia Revista Biosalud. 2019.

67. Miranda J, Mattar S, Gonzalez M, Hoyos-Lopez R, Aleman A, Aponte J. First report of Culex flavivirus infection from Culex coronator (Diptera: Culicidae), Colombia. Virol J. 2019;16(1):1.
68. Velandia-Romero M OV, Coronel-Ruiz C, Cabezas L, Calderón-Peláez M, Castellanos E, Matiz L Detección del virus del dengue en larvas y pupas de Aedes aegypti recolectadas en áreas rurales del municipio de Anapoima, Cundinamarca, Colombia. Biomedica. 2017;37.
69. Hoyos-Lopez R, Soto SU, Rua-Uribe G, Gallego-Gomez JC. Molecular identification of Saint Louis encephalitis virus genotype IV in Colombia. Mem Inst Oswaldo Cruz. 2015;110(6):719-25.
70. Lopez RH, Soto SU, Gallego-Gomez JC. Evolutionary relationships of West Nile virus detected in mosquitoes from a migratory bird zone of Colombian Caribbean. Virol J. 2015;12:80.
71. Mattar S, Edwards E, Laguado J, Gonzalez M, Alvarez J, Komar N. West Nile virus antibodies in Colombian horses. Emerg Infect Dis. 2005;11(9):1497-8.
72. Ferro C, De Las Salas J, Gonzalez M, Diaz A, Cabrera C, Florez Z, et al. [Do some conditions contribute to the reemergence of the Venezuelan equine encephalitis virus in the Colombian Alta Guajira?]. Biomedica. 2015;35(1):62-72.
73. Osorio JE, Ciuoderis KA, Lopera JG, Piedrahita LD, Murphy D, Levasseur J, et al. Characterization of West Nile viruses isolated from captive American Flamingoes (Phoenicopterus ruber) in Medellin, Colombia. Am J Trop Med Hyg. 2012;87(3):565-72.
74. Mayer SV, Tesh RB, Vasilakis N. The emergence of arthropod-borne viral diseases: A global prospective on dengue, chikungunya and zika fevers. Acta Trop. 2017;166:155-63.
75. Souza-Neto JA, Powell JR, Bonizzoni M. Aedes aegypti vector competence studies: A review. Infect Genet Evol. 2019;67:191-209.
76. Gao H, Cui C, Wang L, Jacobs-Lorena M, Wang S. Mosquito Microbiota and Implications for Disease Control. Trends Parasitol. 2020;36(2):98-111.
77. Huang W, Wang S, Jacobs-Lorena M. Use of Microbiota to Fight Mosquito-Borne Disease. Front Genet. 2020;11:196.
78. Strand MR. Composition and functional roles of the gut microbiota in mosquitoes. Curr Opin Insect Sci. 2018;28:59-65.
79. Hegde S, Rasgon JL, Hughes GL. The microbiome modulates arbovirus transmission in mosquitoes. Curr Opin Virol. 2015;15:97-102.
80. Guegan M, Zouache K, Demichel C, Minard G, Tran Van V, Potier P, et al. The mosquito holobiont: fresh insight into mosquito-microbiota interactions. Microbiome. 2018;6(1):49.
81. Cansado-Utrilla C, Zhao SY, McCall PJ, Coon KL, Hughes GL. The microbiome and mosquito vectorial capacity: rich potential for discovery and translation. Microbiome. 2021;9(1):111.
82. Scolari F, Casiraghi M, Bonizzoni M. Aedes spp. and Their Microbiota: A Review. Front Microbiol. 2019;10:2036.
83. Scolari F, Sandionigi A, Carlassara M, Bruno A, Casiraghi M, Bonizzoni M. Exploring Changes in the Microbiota of Aedes albopictus: Comparison Among Breeding Site Water, Larvae, and Adults. Front Microbiol. 2021;12:624170.
84. Patterson EI, Villinger J, Muthoni JN, Dobel-Ober L, Hughes GL. Exploiting insect-specific viruses as a novel strategy to control vector-borne disease. Curr Opin Insect Sci. 2020;39:50-6.

85. Chen K, Pachter L. Bioinformatics for whole-genome shotgun sequencing of microbial communities. PLoS Comput Biol. 2005;1(2):106-12.
86. Kiselev D, Matsvay A, Abramov I, Dedkov V, Shipulin G, Khafizov K. Current Trends in Diagnostics of Viral Infections of Unknown Etiology. Viruses. 2020;12(2).
87. Gu W, Miller S, Chiu CY. Clinical Metagenomic Next-Generation Sequencing for Pathogen Detection. Annu Rev Pathol. 2019;14:319-38.
88. Liu YX, Qin Y, Chen T, Lu M, Qian X, Guo X, et al. A practical guide to amplicon and metagenomic analysis of microbiome data. Protein Cell. 2021;12(5):315-30.
89. Greninger AL, Naccache SN, Federman S, Yu G, Mbala P, Bres V, et al. Rapid metagenomic identification of viral pathogens in clinical samples by real-time nanopore sequencing analysis. Genome Med. 2015;7:99.
90. Kumar A, Murthy S, Kapoor A. Evolution of selective-sequencing approaches for virus discovery and virome analysis. Virus Res. 2017;239:172-9.
91. Thomson E, Ip CL, Badhan A, Christiansen MT, Adamson W, Ansari MA, et al. Comparison of Next-Generation Sequencing Technologies for Comprehensive Assessment of Full-Length Hepatitis C Viral Genomes. J Clin Microbiol. 2016;54(10):2470-84.
92. Mlakar J, Korva M, Tul N, Popovic M, Poljsak-Prijatelj M, Mraz J, et al. Zika Virus Associated with Microcephaly. N Engl J Med. 2016;374(10):951-8.
93. Li T, Mbala-Kingebeni P, Naccache SN, Theze J, Bouquet J, Federman S, et al. Metagenomic Next-Generation Sequencing of the 2014 Ebola Virus Disease Outbreak in the Democratic Republic of the Congo. J Clin Microbiol. 2019;57(9).
94. de Almeida JP, Aguiar ER, Armache JN, Olmo RP, Marques JT. The virome of vector mosquitoes. Curr Opin Virol. 2021;49:7-12.
95. Atoni E, Zhao L, Karungu S, Obanda V, Agwanda B, Xia H, et al. The discovery and global distribution of novel mosquito-associated viruses in the last decade (2007-2017). Rev Med Virol. 2019;29(6):e2079.
96. Shi C, Beller L, Deboutte W, Yinda KC, Delang L, Vega-Rua A, et al. Stable distinct core eukaryotic viromes in different mosquito species from Guadeloupe, using single mosquito viral metagenomics. Microbiome. 2019;7(1):121.
97. Ramos-Nino ME, Fitzpatrick DM, Tighe S, Eckstrom KM, Hattaway LM, Hsueh AN, 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(1):e0227998.
98. Ramos-Nino ME, Fitzpatrick DM, Eckstrom KM, Tighe S, Hattaway LM, Hsueh AN, et al. Metagenomic analysis of Aedes aegypti and Culex quinquefasciatus mosquitoes from Grenada, West Indies. PLoS One. 2020;15(4):e0231047.
99. Shi C, Liu Y, Hu X, Xiong J, Zhang B, Yuan Z. A metagenomic survey of viral abundance and diversity in mosquitoes from Hubei province. PLoS One. 2015;10(6):e0129845.
100. Ohlund P, Lunden H, Blomstrom AL. Insect-specific virus evolution and potential effects on vector competence. Virus Genes. 2019;55(2):127-37.
101. Bolling BG, Vasilakis N, Guzman H, Widen SG, Wood TG, Popov VL, et al. Insect-specific viruses detected in laboratory mosquito colonies and their potential implications for experiments evaluating arbovirus vector competence. Am J Trop Med Hyg. 2015;92(2):422-8.
102. Calisher CH, Higgs S. The Discovery of Arthropod-Specific Viruses in Hematophagous Arthropods: An Open Door to Understanding the Mechanisms of Arbovirus and Arthropod Evolution? Annu Rev Entomol. 2018;63:87-103.

103. 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.
104. He X, Yin Q, Zhou L, Meng L, Hu W, Li F, et al. Metagenomic sequencing reveals viral abundance and diversity in mosquitoes from the Shaanxi-Gansu-Ningxia region, China. PLoS Negl Trop Dis. 2021;15(4):e0009381.
105. Cholleti H, Hayer J, Fafetine J, Berg M, Blomstrom AL. Genetic characterization of a novel picorna-like virus in Culex spp. mosquitoes from Mozambique. Virol J. 2018;15(1):71.
106. Petit MJ, Shah PS. Mapping Arbovirus-Vector Interactions Using Systems Biology Techniques. Front Cell Infect Microbiol. 2018;8:440.
107. Sadeghi M, Popov V, Guzman H, Phan TG, Vasilakis N, Tesh R, et al. Genomes of viral isolates derived from different mosquitos species. Virus Res. 2017;242:49-57.
108. Shi M, Neville P, Nicholson J, Eden JS, Imrie A, Holmes EC. High-Resolution Metatranscriptomics Reveals the Ecological Dynamics of Mosquito-Associated RNA Viruses in Western Australia. J Virol. 2017;91(17).
109. Zakrzewski M, Rasic G, Darbro J, Krause L, Poo YS, Filipovic I, et al. Mapping the virome in wild-caught Aedes aegypti from Cairns and Bangkok. Sci Rep. 2018;8(1):4690.
110. Altinli M, Schnettler E, M S. Symbiotic Interactions Between Mosquitoes and Mosquito Viruses. Front Cell Infect Microbiol. 2021.
111. Roundy CM, Azar SR, Rossi SL, Weaver SC, Vasilakis N. Insect-Specific Viruses: A Historical Overview and Recent Developments. Adv Virus Res. 2017;98:119-46.
112. Nouri S, Matsumura EE, Kuo YW, Falk BW. Insect-specific viruses: from discovery to potential translational applications. Curr Opin Virol. 2018;33:33-41.
113. Halbach R, Junglen S, van Rij RP. Mosquito-specific and mosquito-borne viruses: evolution, infection, and host defense. Curr Opin Insect Sci. 2017;22:16-27.
114. 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(5).
115. Olmo RP, Todjro YMH, Aguiar E, de Almeida JPP, Ferreira FV, Armache JN, et al. Mosquito vector competence for dengue is modulated by insect-specific viruses. 2023. p. 135--49.
116. Kubacki J, Flacio E, Qi W, Guidi V, Tonolla M, Fraefel C. Viral Metagenomic Analysis of Aedes albopictus Mosquitos from Southern Switzerland. Viruses. 2020;12(9).
117. Xiao P, Li C, Zhang Y, Han J, Guo X, Xie L, et al. Metagenomic Sequencing From Mosquitoes in China Reveals a Variety of Insect and Human Viruses. Front Cell Infect Microbiol. 2018;8:364.
118. Parry R, James ME, Asgari S. Uncovering the Worldwide Diversity and Evolution of the Virome of the Mosquitoes Aedes aegypti and Aedes albopictus. Microorganisms. 2021;9(8).
119. Xia H, Wang Y, Shi C, Atoni E, Zhao L, Yuan Z. Comparative Metagenomic Profiling of Viromes Associated with Four Common Mosquito Species in China. Virol Sin. 2018;33(1):59-66.
120. Liu Q, Cui F, Liu X, Fu Y, Fang W, Kang X, et al. Association of virome dynamics with mosquito species and environmental factors. Microbiome. 2023;11(1):101.

121. Zhao L, Yu P, Shi C, Jia L, Evans A, Wang X, et al. Global mosquito virome profiling and mosquito spatial diffusion pathways revealed by marker-viruses. Biorxiv, preprint. 2022.
122. Romo H, Kenney JL, Blitvich BJ, Brault AC. Restriction of Zika virus infection and transmission in Aedes aegypti mediated by an insect-specific flavivirus. Emerg Microbes Infect. 2018;7(1):181.
123. Goenaga S, Kenney JL, Duggal NK, Delorey M, Ebel GD, Zhang B, et al. Potential for Co-Infection of a Mosquito-Specific Flavivirus, Nhumirim Virus, to Block West Nile Virus Transmission in Mosquitoes. Viruses. 2015;7(11):5801-12.
124. Schultz MJ, Frydman HM, Connor JH. Dual Insect specific virus infection limits Arbovirus replication in Aedes mosquito cells. Virology. 2018;518:406-13.
125. Vogels CBF, Ruckert C, Cavany SM, Perkins TA, Ebel GD, Grubaugh ND. Arbovirus coinfection and co-transmission: A neglected public health concern? PLoS Biol. 2019;17(1):e3000130.
126. Karpf AR, Lenches E, Strauss EG, Strauss JH, Brown DT. Superinfection exclusion of alphaviruses in three mosquito cell lines persistently infected with Sindbis virus. J Virol. 1997;71(9):7119-23.
127. Jones R, Kulkarni MA, Davidson TMV, Team R-LR, Talbot B. Arbovirus vectors of epidemiological concern in the Americas: A scoping review of entomological studies on Zika, dengue and chikungunya virus vectors. PLoS One. 2020;15(2):e0220753.
128. Christofferson RC. Zika Virus Emergence and Expansion: Lessons Learned from Dengue and Chikungunya May Not Provide All the Answers. Am J Trop Med Hyg. 2016;95(1):15-8.
129. Girard M, Nelson CB, Picot V, Gubler DJ. Arboviruses: A global public health threat. Vaccine. 2020;38(24):3989-94.
130. Mancini MV, Damiani C, Accoti A, Tallarita M, Nunzi E, Cappelli A, et al. Estimating bacteria diversity in different organs of nine species of mosquito by next generation sequencing. BMC Microbiol. 2018;18(1):126.
131. Olmo RP, Todjro YMH, Aguiar E, de Almeida JPP, Ferreira FV, Armache JN, et al. Mosquito vector competence for dengue is modulated by insect-specific viruses. Nat Microbiol. 2023;8(1):135-49.
132. Batovska J, Lynch SE, Rodoni BC, Sawbridge TI, Cogan NO. Metagenomic arbovirus detection using MinION nanopore sequencing. J Virol Methods. 2017;249:79-84.
133. Claro I, Ramundo S, Coletti T, da Silva C, Valenca I, Candido D, et al. Rapid viral metagenomics using SMART-9N amplification and nanopore sequencing [version 1; peer review: 2 approved with reservations. Wellcome Open Res. 2021;6:241.
134. Ko HY, Salem GM, Chang GJ, Chao DY. Application of Next-Generation Sequencing to Reveal How Evolutionary Dynamics of Viral Population Shape Dengue Epidemiology. Front Microbiol. 2020;11:1371.
135. Altinli M, Schnettler E, Sicard M. Symbiotic Interactions Between Mosquitoes and Mosquito Viruses. Front Cell Infect Microbiol. 2021;11:694020.
136. Bolling BG, Weaver SC, Tesh RB, Vasilakis N. Insect-Specific Virus Discovery: Significance for the Arbovirus Community. Viruses. 2015;7(9):4911-28.
137. Li C, Liu S, Zhou H, Zhu W, Cui M, Li J, et al. Metatranscriptomic Sequencing Reveals Host Species as an Important Factor Shaping the Mosquito Virome. Microbiol Spectr. 2023;11(2):e0465522.
138. Agboli E, Leggewie M, Altinli M, Schnettler E. Mosquito-Specific Viruses-Transmission and Interaction. Viruses. 2019;11(9).

139. Arbeláez-Cortés E. Knowledge of Colombian biodiversity: published and indexed. Biodiversity and Conservation 2013;22(12):2875-906.