Mosquito species diversity and mosquito-associated viruses from the Atrato River valley in Northwest Colombia

Dissertation to obtain the degree of Doctor of Natural Sciences

at the Department of Biology of the

JG|U

JOHANNES GUTENBERG
UNIVERSITÄT MAINZ

David-Joachim Schiemann

born on April 30th, 1981 in Wolfach, Germany

Mainz, 2023

in cooperation with

ROBERT KOCH INSTITUT

UNIVERSIDAD
NACIONAL
DE COLOMBIA
SEDE BOGOTÁ

UNIVERSIDAD
DE ANTIOQUIA
1803

Declaration

This dissertation is the result of my own work and includes nothing, which is the outcome of work done in collaboration except where specifically indicated in the text. It has not been previously submitted, in part or whole, to any university or institution for any degree, diploma, or other qualification.

Signed:____

Date:____

David-Joachim Schiemann
Johannes Gutenberg University Mainz

List of contents

1 List of abbreviations

(-)ssRNA	negative sense single-stranded RNA
(+)ssRNA	positive sense single-stranded RNA
AA	amino acid
Ae.	*Aedes* (genus)
am	*Ante meridiem*
An.	*Anopheles* (genus)
ANLA	National Environmental Licencing Authority *(Autoridad Nacional de Licencias Ambientales)*
arbovirus	arthropod-borne virus
ATP6	mitochondrially encoded ATP synthase membrane subunit 6
ATP8	mitochondrially encoded ATP synthase membrane subunit 8
blastn	Nucleotide to Nucleotide Basic Local Alignment Search Tool
bp	base pairs
cDNA	complementary DNA
CDS	coding region
CD-search	conserved domain search
Cq.	*Coquillettidiae* (genus)
Cx.	*Culex (genus)*
DENV	Dengue virus
DMEM	Dulbecco's Modified Eagle Medium
DNA	deoxyribonucleic acid
DsDNA	double-stranded DNA
ENV	Envelope protein
FCS	fetal calf serum
Gag	group specific antigen
Gly	glycoprotein
Hel	helicase
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
Hg.	*Haemagogus* (genus)
HLC	human landing catch
HMM	Hidden Markov model
ICTV	International Committee on Taxonomy of Virus
IN	integrase
ISV	insect-specific virus
Li.	*Limatus* (genus)
Ma.	*Mansonia* (genus)
maCP	mayor capsid protein
MAFFT	Multiple Alignment using Fast Fourier Transform
MEGA	Molecular Evolutionary Genetics Analysis
miCP	minor capsid protein
Mt	methyltransferase
N	nucleocapsid protein
NaDH5	nicotinamide adenine dinucleotide dehydrogenase 5
NCBI	National Center for Biotechnology Information
NGS	Next Generation Sequencing
NS5	nonstructural protein 5
ORF	open reading frame
ORI	origin of replication

P	phosphoprotein
Penstrep	penicillin streptomycin
Per. Id.	percent identity
PhyML	Phylogenetic Maximum Likelihood
pm	post meridiem
Pol	polymerase
Pro	protease
Ps.	*Psorophora (genus)*
RbP	RNA-binding protein
RdRp	RNA-dependent RNA polymerase
Rep	replication-associated protein
RH	ribonuclease H
RKI	Robert Koch Institute
RT	reverse transcriptase
rtPCR	reverse transcription polymerase chain reaction
s.s.	*sensu sticto*
Sa.	*Sabethes* (genus)
S-Segment	small segment
ssRNA(RT)	reverse transcribing virus
St.	*Stegomyia* (genus)
Tr.	*Trichoprosopon* (genus)
VP	virus particle
WNV	West Nile fever virus
YFV	Yellow fever virus
ZBS1	Division of Highly Pathogenic Viruses, Centre of Biological Threats and Special Pathogens

2 Acknowledgments

I am so grateful to all the people who made this project possible and made one of my life dreams come true. Without you, this study would have been nothing else but a castle in the air.

I would like to express my sincere gratitude for the support and solidarity of everybody who participated in the project, in particular to:

3 Abstract

3.1 Englisch abstract

Colombia is one of the hotspots regarding mosquito biodiversity and mosquito-transmitted diseases. Endemic, tropical diseases like malaria and dengue are major public health issues and novel viruses of zoonotic origin like Chikungunya and Zika are emerging. Nevertheless, little is known about the mosquito vector species circulating in remote Colombian regions and the mosquito-associated viromes.

In a pilot study carried out from August 2014 to December 2016, more than 8000 mosquitoes (Diptera: Culicidae) were collected in the Atrato River valley in Northwest Colombia to identify the mosquito species in the region and get information on the species abundance, biting behaviour, and phylogeny of these mosquito species. In addition, a PCR- and NGS-based virus search was promoted in the most abundantly collected mosquito species to get insight into the virome of each mosquito species and identify yet undescribed mosquito-associated viruses, sequence their genomes, establish their phylogenetic relationships with formerly described viruses and describe their genome organizations. More than 43 mosquito species of 14 genera could be identified by morphology and molecular markers (Cytochrome c oxidase subunit 1, internal transcribed spacer 2), and their biting behaviours and phylogenetic relationships were described. *Mansonia titillans* (17,7%), *Psorophora albipes* (16,5%), *Anopheles darlingi* (15,8%), *Anopheles nuneztovari* (10,9%), *Coquillettidiae venezuelensis* (8,9%), and *Coquillettidiae lynchi* (8,6%) were the most abundantly collected mosquito species, each bearing a species-specific virome. Moreover, 46 human blood samples from patients with suspicion of malaria were screened for viruses. A total of 64 novel viruses were discovered in mosquitoes and human blood samples. The genomes of these viruses were sequenced and annotated, and the viruses were assigned to a taxonomic rank by phylogenetic analyses.

Each of the mosquito species just mentioned has been involved in the transmission of zoonotic diseases. The Atrato River valley thus harbours a high diversity of mosquito species and associated undiscovered viruses, the majority being insect and plant viruses. Systematic monitoring of mosquito species diversity and mosquito-associated viruses in biodiversity hotspots could be a promising approach for the prevention and control of novel zoonotic diseases.

3.2 Deutsches Abstract

Kolumbien ist ein *Hotspot* bezüglich Stechmücken-Biodiversität und der durch Stechmücken übertragenen Krankheiten. Endemische tropische Krankheiten wie Malaria und Dengue stellen ein großes Gesundheitsproblem dar, und neu auftretende Viruserkrankungen zoonotischen Ursprungs wie Chikungunya und Zika sind auf dem Vormarsch. Trotzdem gibt es kaum Informationen darüber, welche Stechmückenarten in den abgelegenen Regionen Kolumbiens verbreitet sind bzw. als Vektoren fungieren und wie sich deren Virome zusammensetzen.

In einer Pilotstudie wurden von August 2014 bis Dezember 2016 mehr als 8000 Stechmücken (Diptera: Culicidae) im Atrato-Flusstal im Nordwesten Kolumbiens gesammelt, um die Mückenarten in der Region erstmals zu bestimmen und Informationen über den Artenreichtum, das Stechverhalten und die Phylogenie dieser Culiciden zu erlangen. Im Anschluss, wurde in den am häufigsten angetroffenen Stechmücken-Arten eine auf PCR und NGS basierende Virensuche durchgeführt, um einen Einblick in die Virome der jeweiligen Stechmücken-Arten zu bekommen. Ziel war es, in den Stechmücken neuartige Viren zu entdecken, ihre Genome zu entschlüsseln und ihre phylogenetischen Verwandtschaftsverhältnisse mit bisher bekannten Viren zu beschreiben. Über 43 Stechmückenarten aus 14 Gattungen konnten mit Hilfe von Bestimmungsschlüsseln und molekularen Markern (Cytochrom-c-Oxidase Untereinheit 1, Interner transkribierter Spacer 2) identifiziert werden, und es wurde Aufschluss über deren phylogenetische Verwandtschaftsverhältnisse sowie das Stechverhalten gewonnen. *Mansonia titillans* (17,7%), *Psorophora albipes* (16,5%), *Anopheles darlingi* (15,8%), *Anopheles nuneztovari* (10,9%), *Coquillettidia venezuelensis* (8,9%) und *Coquillettidia lynchi* (8,6%) gehörten zu den am häufigsten gesammelten Stechmückenarten in der Region, und jede dieser Arten zeichnete sich durch ein artspezifisches Virom aus. Darüber hinaus wurden 46 Blutproben von Patienten mit Verdacht auf Malaria-Infektion auf Viren Präsenz untersucht. Die Genome all dieser Viren konnten sequenziert und annotiert werden, und die Viren wurden mit Hilfe von phylogenetischen Analysen taxonomisch eingeordnet. Insgesamt konnten 64 neuartige Viren in den Stechmücken und Blutproben beschrieben werden.

Jede der hier untersuchten Stechmückenarten ist an der Übertragung zoonotischer Erkrankungen beteiligt. Das Atrato-Flusstal zeichnet sich durch eine außerordentliche Stechmücken-Artenvielfalt aus, die eine ungeahnte Diversität an unbekannten Viren birgt. Bei den meisten handelt es sich um Insekten und Pflanzenviren. Ein kontinuierliches Monitoring der Stechmücken-Diversität und der assoziierten Viren in solchen *Hotspots* der Biodiversität könnte einen vielversprechenden Beitrag zur Prävention und Kontrolle von zoonotischen Krankheiten leisten.

4 Introduction

4.1 Mosquitoes and their role in virus transmission

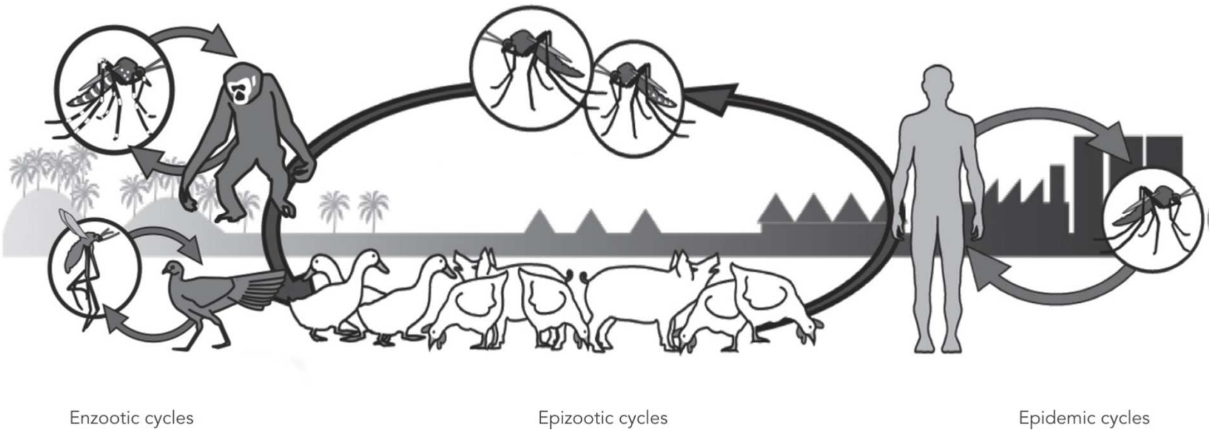


Figure 1: Schematic of mosquito-borne virus transmission
Emerging mosquito-borne viral diseases in humans are mostly of zoonotic origin. Mosquitoes transmit arboviruses from an animal to a human host and a novel transmission cycle is established. (Figure from Ellis and Wilcox 2009)

During the last century, mosquitos (Diptera: Culicidae) have been extensively studied mainly due to their role as vectors for diseases, in particular malaria and yellow fever. Since then, more than 3500 species of Culicidae have been described (Harbach 2013b). At first, mosquitoes were classified into taxonomic groups based on morphological characters, and phylogenetic relationships were interpreted intuitively (Harbach 2007). Later on, morphological keys were elaborated to identify all life stages of neotropical mosquitoes (Zavortink 1979; Sirivanakarn 1982; Lane 1953; González Obando and Carrejo Gironza 2009; Forattini 2002; Sallum and Forattini 1996). Soon, researchers recognized the limits of morphological classification and at the beginning of the 21st century molecular markers became available as an additional tool for species identification and phylogeny (Coleman 2003, Hebert et al. 2003b). Nowadays, the sequences of the ITS2 and COI are commonly used as molecular barcoding regions and markers for phylogenetic studies to classify organisms, particularly mosquitoes (family: *Culicidae*), (Folmer et al. 1994, Hebert et al. 2004, Ashfaq et al. 2014, Djadid et al. 2007).

In the last decades, emerging and re-emerging diseases caused by arthropod-borne viruses (arboviruses), a group of “dual-host” viruses transmitted mainly by mosquitoes to vertebrate hosts, became a threat to public and veterinary health after spillover transmission to humans or domestic animals (Huang et al. 2019). This was favored by globalization, urbanization, climate change, and habitat expansion of mosquito vector species. The majority of the arboviruses of global epidemiological relevance correspond to four genera: The *Flavivirus*, *Alphavirus*, *Orthobunyavirus,* and *Nairovirus*. These include the majority of the most challenging mosquito-borne diseases, such as West

Nile fever, Dengue fever, Yellow fever, Rift Valley fever, Chikungunya and Zika (Forshey et al. 2010, Weaver and Reisen 2010).

At the end of the 19th century, yellow fever virus (YFV) and dengue virus (DENV) were the first arboviruses of the genus *Flavivirus* shown to be transmitted to humans by mosquitoes of the genus *Aedes*, in particular *Ae. aegypti*. In the genus *Mansonia*, in particular *Ma. titillans* has been associated with the transmission of St. Louis virus (genus *Flavivirus*) and viruses of the genus *Orthobunyavirus* (Beranek et al. 2018). *Psorophora* mosquitoes may also play a role in West Nile virus (WNV), (genus *Flavivirus*) transmission (Unlu et al. 2010), and *Cq. venezuelensis* is a natural vector of Bussuquara virus (genus *Flavivirus*), Mayaro virus (genus *Alphavirus*), and Oropouche virus (genus *Orthobunyavirus*) among others. To detect tropical viral diseases and malaria parasites from samples, scientists have developed genus-specific rtPCR protocols (Fontecha et al. 2012, Chew et al. 2012, Fuehrer et al. 2011, Patel et al. 2013, Grywna et al. 2010, Powers and Roehrig 2011, Lambert and Lanciotti 2009, Kramer et al. 2002, Hall et al. 2012, Prado et al. 2005, Bangs et al. 2007, and Michaud et al. 2007).

Another group of viruses that gathered researchers' attention recently consists of insect-specific viruses (ISVs), which are closely related to arboviruses of public and veterinary health interest but are transmitted vertically or horizontally in insect populations. Even though ISVs cannot infect vertebrates, they have been an issue of research as they harbor information on virus evolution, determinants for vertebrate cell infection and host restriction, and the potential as a biological control agent of vectors and arbovirus transmission (Roundy et al. 2017; Junglen and Drosten 2013). Many plant viruses are also transmitted by insect vectors. Different viral transmission strategies have been described for different viruses and vectors, but little information is available on the role of mosquitoes in plant virus propagation, and only a few plant virus–vector systems have been well studied (Bragard et al. 2013, Dietzgen et al. 2016).

4.2 Virus search in mosquitoes

For the discovery of novel viruses in environmental samples and the characterizations of complete genomes by NGS-based metagenomic virus search has proven to be a promising approach. Nowadays, most phylogenetic studies in viruses use the polymerase coding regions (CDS) as molecular markers for establishing phylogenetic and evolutionary relationships, as they are universal genes present in most viruses. During the last decade, numerous protocols for sample preparation and NGS data analysis have been developed. The diversity of recently discovered viruses redefined virus taxonomy and gave insight into the ecology, phylogeny, and evolution of mosquito-associated viruses (Shi et al. 2015*, Shi et al. 2016a, Shi et al. 2017, Shi et al. 2018, Donaldson et al. 2010, Kohl et al. 2015, Stang et al. 2005, Djikeng and Spiro 2009).*

Colombia is a biodiversity hotspot with a great variety of ecosystems and diverse mosquito fauna. More than 324 mosquito species have been reported in the territory (Rozo-Lopez and Mengual 2015). Mosquito-borne viral diseases, especially Dengue, Chikungunya, and Zika have become a major public health issue, and novel arboviruses like WNV, Mayaro virus, Saint Louis encephalitis virus are emerging (Padilla et al. 2017; Berrocal et al. 2006; Hoyos-López et al. 2015). That is why the research in Colombia is focused mainly on these rather neglected tropical diseases. But viral diseases may also harm the agricultural sector. Colombia is a developing country whose economy depends mainly on crop production and trade. Thus, plant viruses could be a menace, and NGS-based methods have been applied to sequence the genomes of plant viruses in major crops (Madroñero et al. 2019). Nevertheless, the use of NGS in Colombia is still scarce and the role of mosquitoes as vectors for plant viruses is still poorly studied. Likewise, insect-specific viruses (ISV) have been scarcely studied, and the genomes of only a few ISVs have been sequenced and characterized (Contreras-Gutiérrez et al. 2018; Contreras-Gutiérrez et al. 2017; Miranda et al. 2019).

In sum, the mosquito fauna in Colombia has been extensively studied, but little is known about the species composition in remote areas, and for many mosquito species no barcoding sequences are available yet. NGS-based virus search is still in the early stages and to date, no metagenomics studies on the mosquito virome have been promoted in Colombia. The Colombian territory harbors a gran diversity of mosquito species, many of which have shown to be vectors and hosts for viruses. Previous studies have demonstrated that emerging and re-emerging mosquito-borne viral diseases constitute a threat to a large proportion of the Colombian population (Mora-Salamanca et al. 2020). Most vector-borne diseases in humans are of zoonotic origin as a result of a spillover event. Plant viruses have been poorly studied despite their impact on crop production and economy, and ISVs harbor information on the evolution of viruses, and virus-host interactions and might be applied in the control of mosquito vectors. Thus, investigating the virome of mosquitoes is a crucial step toward the prevention and control of vector-borne diseases.

4.3 Mosquito and virus classification and taxonomy

Evolutionary biologists believe in the concept of common descent, due to which all living beings are descendants of a universal ancestor. Through natural selection, populations evolve and become distinct species. The evolutionary relationships among species and taxonomic groups may be estimated by measuring and evaluating similarities and differences in their morphology or genetics and are visualized in phylogenetic trees (Lee and Palci 2015, Darwin 1859).

Taxonomy is the science of naming, defining, and classifying organisms into evolutionarily related groups. Biological classification uses taxonomic ranks: Domain, Kingdom, Phylum, Class, Order, Family, Genus, Species, and Strain. Mosquitoes correspond to the family *Culicidae* which currently includes

113 genera with 3,578 extant species (Harbach, R.E. 2013. Mosquito Taxonomic Inventory, http://mosquito-taxonomic-inventory.info/, accessed on February 2021). Mosquito taxonomy follows the binomial nomenclature, a two-term naming system in which each species receives a unique Latinized name that consists of a generic and a specific term (e.g. *Aedes* (genus) *aegypti* (epithet)), (Bauhin 1623, Linnaeus 1753). The biological species concept by Mayr (Mayr 1969) defines species as “groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups”. The concept is widely accepted for mosquitoes, but in practice, mosquito species are commonly identified and distinguished by morphological characteristics via morphological keys and/or comparison of the specimens with the species descriptions available, adopting Cronquist´s morphological species concept (Cronquist 1968). On the other hand, DNA barcoding, a method of species identification using a short section of DNA, and a phyletic approach has been proposed for identifying mosquito species following the phylogenetic species concept (Hebert et al. 2003a, Darwin 1859). Nowadays, the trend is towards integrative taxonomy, a multicharacter approach in which several species concepts are combined to define species and taxa (Aldhebiani 2018, Will et al. 2005).

The identification of mosquitoes with morphological keys is still the gold standard method, but experienced personnel is required, and not always all the life stages are available for proper species identification or specimens are in bad condition. To distinguish members of species complexes or close relatives, molecular methods are necessary, due to interspecific overlapping, and intraspecific variation of morphological characters. DNA-barcoding via specific genes has become a popular method for easy, cheap, and specific identification of mosquito species and phylogenetic analyses (Hebert et al. 2003a). For mosquito identification and phylogeny, the most commonly used barcoding regions are the nucleic acid sequences of the COI and the ITS2 (Beebe 2018).

In the 1960s the *International Committee on Taxonomy of Virus* (ICTV) was created to develop an internationally agreed taxonomy for viruses with internationally agreed names for the taxa and a virus nomenclature that is set out in the International Code of Virus Classification and Nomenclature (ICTV 2018). An official list of all ICTV- approved taxa is available on the ICTV website and is actualized constantly (ICTV 2019). Regarding viruses, the ICTV in the International Code of Virus Classification and Nomenclature defines a virus species as “a monophyletic group of viruses whose properties can be distinguished from those of other species by multiple criteria." Distinguishing criteria may include host range, cell and tissue tropism, pathogenicity, vector specificity, antigenicity, and the degree of relatedness of their genomes or genes among others, and are published in the ICTV report for each taxon (https://ictv.global/taxonomy). “Nomenclature of viruses and sub-viral agents is independent of other biological nomenclature.” “Names for taxa shall be easy to use and easy to remember“, and ranks currently in use are „realm, subrealm, kingdom, subkingdom, phylum, subphylum, class, subclass, order, suborder, family, subfamily, genus and subgenus. The names for these ranks shall be single words ending with the suffixes “-viria”, “-vira”, “-virae”, “-virites”, “-viricota”, “-viricotina”, “viricetes”, “-viricetidae”, “-virales”, “-virineae”, “-viridae”, “-virinae”, “-virus" and “-virus”, respectively. (ICTV-Code 3.23). Furthermore, “a species name shall consist of as few words as practicable but be distinct from names of other taxa “, and provide „an appropriately unambiguous identification of the species (ICTV-Code, 2.2, 3.11, 3.20, 3.21).

In the ambit of virus search, molecular methods like PCR and NGS have proven to be powerful tools. PCR may be applied to detect and identify specific viruses or virus genera in samples, meanwhile NGS is applied to search for unknown viruses, study biomes, make metagenomic analyses and acquire full genomes of organisms directly from a sample (Gu et al. 2019). In both cases, proper sample storage and preparation are crucial for virus detection and genome sequencing. In the case of phylogenetic virus classification, the amino acid (AA) sequence of the polymerase CDS is commonly applied (Koonin et al. 2020).

4.4 Research questions & hypotheses

Despite the importance of mosquitoes as vectors for viral agents in Colombia, the mosquito species diversity in the middle reaches of the Atrato River (Figure 4) and the viruses that might be associated with these mosquito species have never been investigated due to the difficult accessibility of the region and the ongoing armed conflict. The main objective of this study was to identify the mosquito species present in the research region and characterize the viruses associated with the most commonly found mosquito species. Specific research questions to be resolved were:

1. Which are the mosquito species circulating in the Atrato River valley in Northwest Colombia?
2. Which viruses are associated with the mosquito species in the region?
3. Which viruses are associated with blood samples from patients suspected of malaria infection?
4. How are the genomes of these viruses organized, and which proteins do they code for?
5. What are the phylogenetic and evolutionary relationships between these viruses and related viruses?
6. Are there any undiscovered mosquito-borne viral diseases circulating in the region?

As the biogeographic region known as the Chocó on the Pacific coast of Colombia is considered a biodiversity hotspot, it is supposed that the mosquito diversity in the Atrato River region is very high. Most probably each mosquito species bears a specific virome with a variety of undescribed viruses, some of which might be of human health, veterinary, or crop production interest. The mosquito species collected may be identified morphologically and/or through their barcoding regions.

Phylogenetic analyses based on barcoding regions may resolve the evolutionary relationships among the present mosquito species and other genus members and thus update mosquito taxonomy. Furthermore, mosquitoes play a major role in the transmission of many undescribed viruses, and the genomes of these unknown viruses may be sequenced and characterized directly from the mosquito samples. Phylogenetic analyses will resolve the evolutionary relationships of these novel viruses, some of which might belong to yet unassigned taxa. There might be some undiscovered mosquito-borne viruses circulating in the Atrato River valley that cause disease in humans and have not been discovered yet. The work breakdown structure in Figure 2 shows the tasks that were accomplished to achieve the objectives and resolve the research questions.

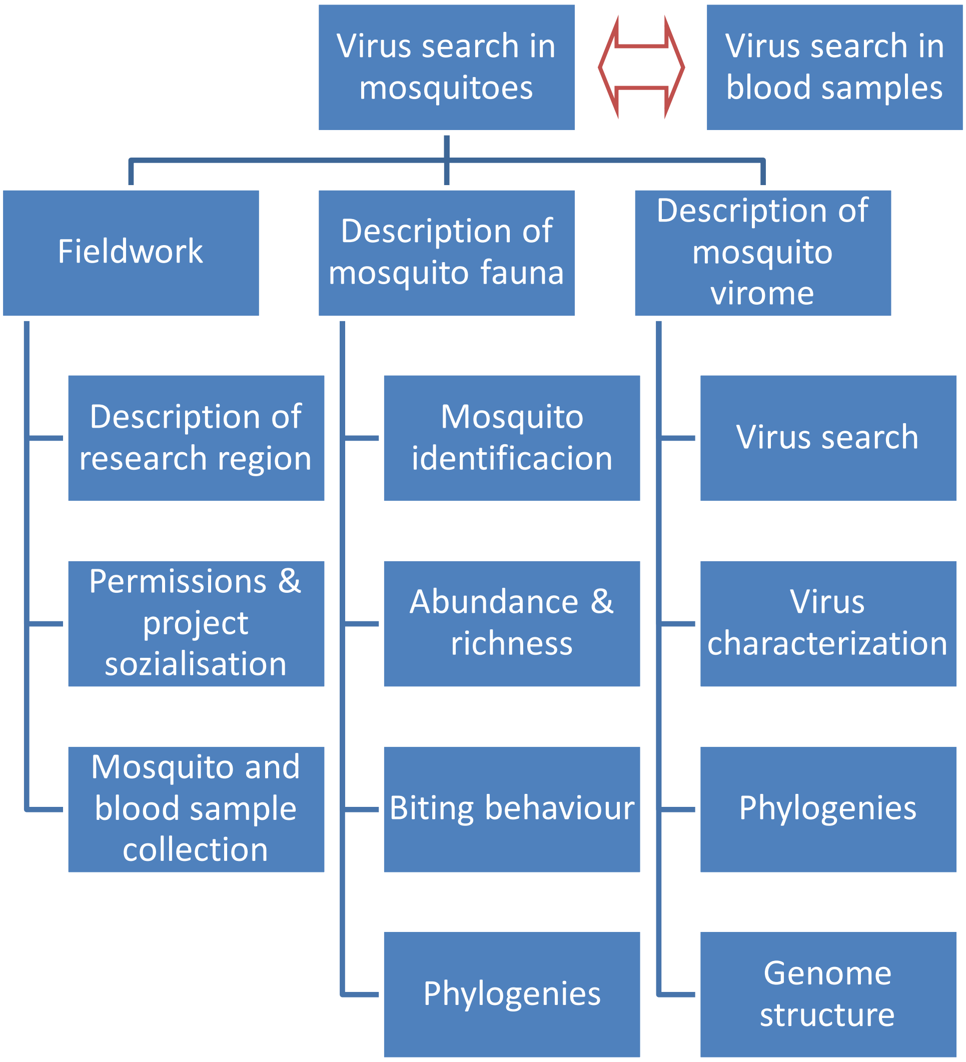


Figure 2: Work breakdown structure for the characterization of the viromes of the mosquito fauna in the Atrato River valley in Colombia. In a descriptive pilot study, mosquitoes and blood samples from the Atrato River region in Northwest Colombia were searched for viruses to get insight into the mosquito species diversity and the mosquito associated viromes, and identify probable threats to humans, livestock, or plant health.

5 Materials and methods

5.1 Description of the research region and population

5.1.1 The Urabá Sur and the Atrato River region

The Urabá is a geographic region in Northwest Colombia near the Panamanian border. The study was carried out in the region of Urabá Sur, which consists of three municipalities: Mutatá, Murindó, and Vigía del Fuerte. It incorporates the subzone known as the "Atrato Medio" in the valley of the river Atrato which is characterized by landscapes of tropical rain forests (Figure 4).

5.1.2 Afro-Colombian communities and Indigenous reserves

Figure 3: Afro-Colombian and Indigenous communities of the Atrato River region in Northwest Colombia Left: Typical Afro-Colombian village on the riverbank of the Atrato River. Right: An Indigenous community of the Embera Eyabida in the mountains.

The communities situated along the Atrato River originated in the pre-Columbian period. The department of the Chocó incorporates an area of 48.353 km^2 that is inhabited by a population of 544.764 people with a population density of 11,27 inhabitants/km^2 (https://citypopulation.de/en/colombia/admin/27__choc%C3%B3/). The population consists of Mestizos, Afro-Colombians, and Indigenous people of the tribe Emberá. In the study region two subgroups are present, the Emberá Dobida (people of the river) and the Emberá Eyabida (people of the mountains). The Afro-Colombian communities are located in the collective territories on the banks of the river Atrato and the lower courses of its tributaries, whereas the Emberá Dobida settle in the reservations on the upper courses of the tributaries. Even though their culture might be different, the Indigenous and Afro-Colombian populations share similar living conditions. In general, settlements are established near a river or stream that serves for drinking water, sewage disposal, and transportation. A family lives in a wooden housing called "Tambo" that is built on stilts near the cultivation areas to avoid flooding and be protected from wild animals. People make their living mainly from farming, livestock production, fishing, lumber exploitation, and in some regions mining. Nowadays some urban centers have a water and sewage system, houses built of concrete or bricks, and infrastructures like electricity, internet, and mobile phone are available.

5.2 Legal affairs and permissions

In 2014, the research project was approved by the ethics committee of the Faculty of Medicine of the National University of Colombia in Bogotá (Evaluation report No. 052-14, 22.05.2014). Permission to carry out the study in the Indigenous reserves and the collective territories of the Afro-Colombian community was given by the Indigenous Organisation of Antioquia (OIA - *Organisación Indígena de Antioquia*), the mayors of the municipality of Murindó, Mutatá and Vigía del Fuerte and the Indigenous and Afro-Colombian municipal governors and leaders of each community.

Mosquitoes were collected in Colombia under the framework permit for the collection of species issued to the National University of Colombia by the National Environmental Licensing Authority (ANLA - *Autoridad Nacional de Licencias Ambientales*, resolutions No. 255 (12.03.2014) and No. 1482 (20.11. 2015)). According to paragraph 2 of article 2 of Resolution 1348 of 2014, and paragraph 5 of article 2 of decree 1376 of 2013 of the Ministry of Environment and Sustainable Development, research activities promoted under the framework permit, involving wildlife of the biological diversity of Colombia for non-commercial purposes, and activities of molecular systematics, molecular ecology, evolution, and biogeography, do not require permission to access the genetic resources and thus are not subject of the Nagoya Protocol on Access to Genetic Resources. Exportation of the specimens from Colombia to the RKI in Berlin, Germany was granted by the ANLA via permit No. 00854 (01.08.2016).

Patients participated voluntarily in the study and were informed about the purpose, objectives, procedures, and related risks. Blood samples were collected after prior consent. The exportation of biological samples of human origin (human blood samples on filter paper) was authorized by the Ministry of Health of Colombia (Document No. 201624001108301, 20.06.2016).

5.3 Human blood sample collection

From September 2014 to December 2016, finger-prick blood samples were taken on Whatman 3mm cellulose chromatography paper (CAT No: 3030-861) from patients with suspected malaria infection at the Hospital San Bartolomé, municipality of Murindó, the Hospital Atrato Medio Antioqueño, municipality of Vigía del Fuerte and a malaria health post in the municipality of Mutatá. Blood spotted filter papers were stored in plastic zipper bags supplied with silica gel at ambient temperature or in 1 ml cryotubes on nitrogen in the dry shipper MVE Doble34. Samples were transported to the laboratory where they were stored at -20 °C and -80 °C respectively.

5.4 Mosquito collection

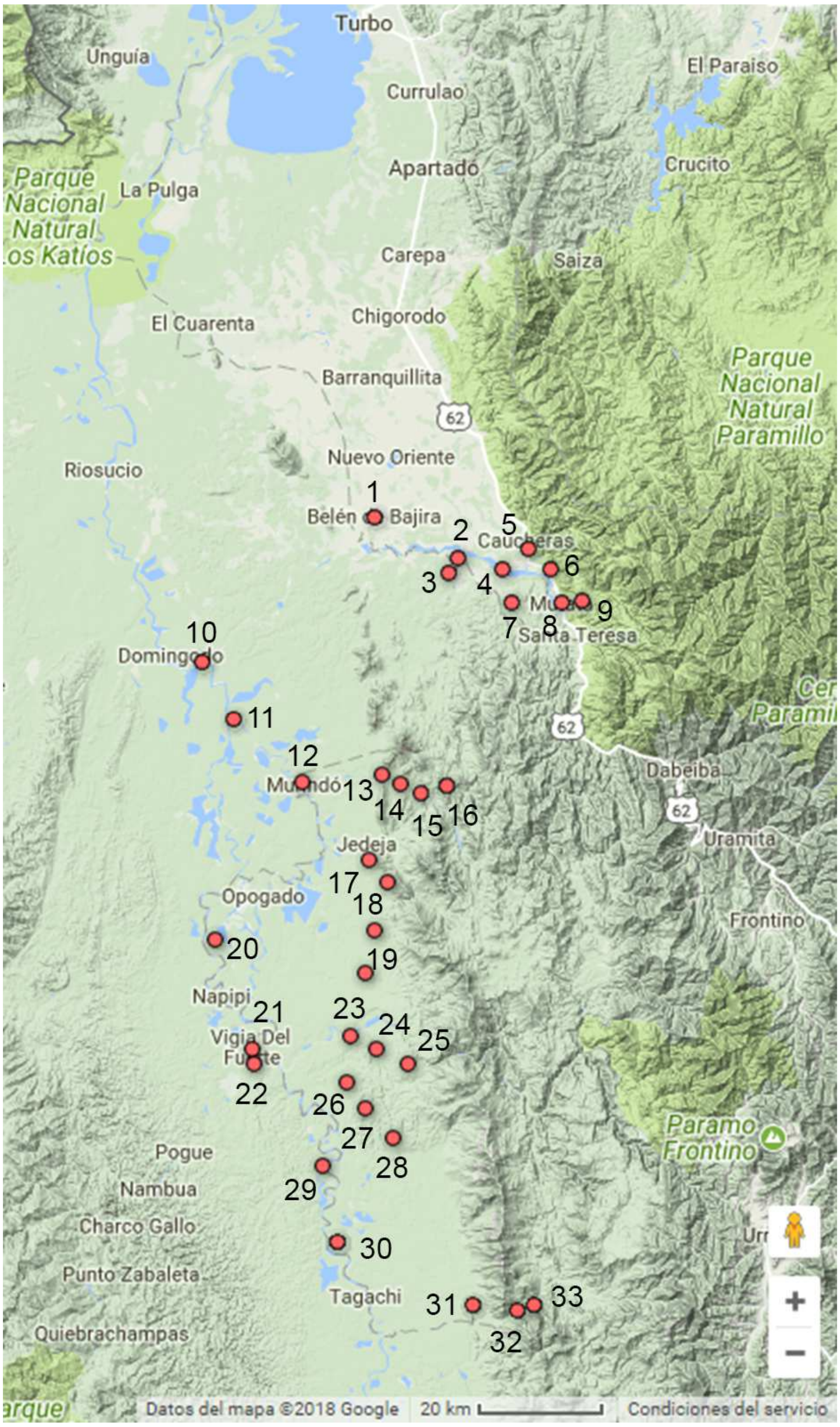


Figure 4: Mosquito collection sites - Indigenous and Afro-Colombian communities of the Mid Atrato River region. Mosquitoes were collected in the following localities and communities: 1. Belén de Bajirá, 2. La Secreta, 3. Los Pisingos, 4. Urada, 5. Caucheras, 6. Caña Verales, 7. Chontadural, 8. Mutatá, 9. Sabaleta, 10. Carmen del Darién, 11. Vigía del Curvaradó, 12. Murindó, 13. Murindó Viejo, 14. Guagua, 15. Isla, 16. Coredó, 17, Ñarangue, 18. Chajeradó, 19. Chibugadó, 20. Puerto Antioquia, 21. Vigía del Fuerte, 22. Bellavista, 23. Playa Murrí, 24. Jarapetó, 25. Turriquitado Llano, 26. Gengadó, 27. Partadó, 28. Salado, 29. Buchadó, 30. San Antonio de Padua, 31. Vidrí, 32. Vegaez, 33. Isleta. Palmiros, Los Cacaos* and Rancho Alegre*) *not on the map. Coordinates of the communities were supplied by the OIA (Organización Indígnea de Antioquia or derived from google maps and visualized with google fusion tables in google maps).*

From September 2014 to December 2016, mosquitoes were collected by the human landing catch method, during daytime in the rainforest and cultivations surrounding the settlements, and during dusk, night, and dawn intra- and peridomiciliary in the settlements by the main investigator and volunteers. Mosquitoes were sacrificed with ethanol (70%), individualized in perforated 0,5 ml Eppendorf tubes, and stored in plastic zipper bags with silica gel at ambient temperature. To conserve the integrity and viability of mosquito-associated viruses, part of the mosquitoes was stored in 1 ml cryotubes and transported to the laboratory on nitrogen in a dry shipper (MVE Doble34), where they were stored at –80°C.

5.5 Morphological identification of mosquitoes

Mosquitoes were identified morphologically with dichotomous keys under a Leica M 80 stereomicroscope (Zavortink 1979; Sirivanakarn 1982; Lane 1953; González Obando and Carrejo Gironza 2009; Forattini 2002; Sallum and Forattini 1996) and identified species were compared to their descriptions. Specimens were photographed with a Keyence VHX-1000D digital microscope using the 20-200x objective and automatic depth composition. Male specimens were identified by the characteristics of their genitals. Therefore, genitals were mounted on glass slides. At first, the last segment of the abdomen was cut off and incubated in 10% KOH at 82°C for 10 min. Then it was transferred to 5% acetic acid (Honeywell, A6283-100ML-D) for 10 min, put in clove oil (Sigma-Aldrich, W232300-SAMPLE-K) for 60 min, and eventually in xylenes histological grade ($C_6H_4(CH_3)_2$), (Honeywell, 534056-500ML-D) for 5 min at ambient temperature. Finally, male genitals were fixed in Canada balsam (Sigma-Aldrich, C1795-25ML) on a glass slide, covered with a cover glass, observed and photographed under the Keyence VHK-1000D and compared to the descriptions (Lane 1953).

5.6 DNA barcoding of mosquitoes

The most commonly used nucleotide sequences for *Culicidae* species barcoding are the mitochondrial cytochrome oxidase subunit I (COI) and the internal transcribed spacer region 2 of the ribosomal DNA (ITS2). For DNA barcoding of the mosquitoes, part of the sequence of the COI was amplified directly from the legs of some representatives of each morphologically identified taxon with primers published by (Folmer et al. 1994) and (Hebert et al. 2004), and PCR conditions indicated by (Ashfaq et al. 2014). A mosquito leg was added to 25 µl of master mix containing 2,5 µl of 10x PCR Buffer, 1,25 µl of MgCL2 (50mM), 0,25 µl of primer mix (LCO 1490, HCO 2198, LepF1, LepR1; 10µM each), 0,1µl of Platinum Taq (Thermofisher) and 20,4 µl of nuclease-free water. Run conditions were as follows: 94°C for 1 min, 5 cycles of 94°C for 30 s, 45°C for 40 s, 72°C for 1 min, 40 cycles of 94°C for 30 s, 51°C for 40 s, 72°C for 1 min and 72°C for 10 min.

Part of the ITS2 was amplified from the mosquitoes with the universal primers and PCR conditions indicated by Djadid et al. 2007. A mosquito leg was added to 25 µl of master mix containing 2,5 µl of 10x High Fidelity PCR Buffer, 1,25 µl of MgCL2 (50mM), 0,25 µl of Primer 5,8s, and 28 s (10µM each) respectively, 0,1µl of Platinum Taq (Thermofisher) and 20,15 µl of nuclease-free water. Run conditions were as follows: 94°C for 5 min, 35 cycles of 94°C for 15 s, 53°C for 1 min, 72°C for 1 min, followed by final elongation for 7 min.

Amplicons (5µl) were run on a 1,5% agarose gel (UltraPure™ Agarose, Thermofisher), stained with GelRed ® Nucleic Acid Gel Stain (Biotium) for 25 min at 90 V. GeneRuler 100 bp Plus DNA Ladder (Thermofisher, SM0322) was used as a marker. The PCR products were cleaned up with MSB® Spin PCRapace (Invitek) and sequenced with BigDye™ Terminator v3.1 Cycle Sequencing Kit. Forward and reverse sequences were assembled in Geneious version R 10 (http://geneious.com, Kearse et al. 2012), primers were trimmed and high-quality sequences were aligned to and uploaded to the NCBI nucleotide database (Table 15, Table 16). There are no universally accepted thresholds for barcoding mosquitoes, as intra-specific variation and inter-specific divergence vary depending on the sequences used for barcoding and the genus or species complex studied. Generally speaking, the mean interspecific genetic distance should be several-fold higher than the mean intraspecific distance resulting in a barcoding gap that allows proper species assignation (Ruiz-Lopez et al. 2012; Laurito et al. 2013). For our purpose, specimens whose COI or ITS2 sequences exceeded 98% sequence identity with COI or ITS2 barcodes available on the GenBank were assigned to that species.

5.6.1 Mosquito phylogeny based on the mitochondrially encoded cytochrome c oxidase I (COI)

A phylogenetic analysis was promoted based on the COI sequences of the mosquitoes found in this study. At first, forward and reverse sequences of the COI region of each sample were assembled in Geneious version R 10 (http://geneious.com, Kearse et al. 2012), primers were trimmed, and consensus nucleotide sequences were extracted. High-quality COI sequences were aligned with MAFFT, a multiple alignment program for amino acid or nucleotide sequences (Galaxy Version 7.221.3), (Katoh and Standley 2013) by using the E-INS-I algorithm. The fasta output file was automatically trimmed with the trimAI v1.2 command line version using the "trimal -in <inputfile> -out <outputfile> -gappyout" command (Capella-Gutierrez et al. 2009). The fasta output file was converted to Relaxed Phylip format (full names and padded) with AliView (version 1.25), (Larsson 2014). Maximum likelihood phylogenies were estimated with PhyML 3.0 (Guindon et al. 2010) using Smart Model Selection (Akaike Information Criterion), (Lefort et al. 2017) for the selection of the best-fit substitution model, subtree pruning and regrafting (SPR), (Hordijk and Gascuel 2005), and bootstrap

(=100 or 1000), (Efron 1979), or likelihood-ratio test (aLRT SH). All COI sequences were compared to the NCBI database nucleotide collection with the megablast algorithm (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and uploaded to the NCBI GenBank (Table 15).

5.6.2 Phylogenetic analysis of closely related mosquito species based on the internal transcribed spacer 2 (ITS2) sequence

The ITS2 is used to distinguish among specimens belonging to species complexes of closely-related species. Species belonging to the subgenus *Protoculex* (formerly: *Serratus* Group) and the subgenus *Ochlerotatus* (formerly *Scapularis* Group) were distinguished based on ITS2 phylogeny. Phylogenetic analyses were carried out as described in 5.6.1.

5.6.3 Genetic distance analyses of COI and ITS2 sequences

Based on the COI and ITS2 sequence alignments (5.6.1 and 5.6.2) the evolutionary divergence between the COI and ITS2 sequences respectively was estimated, and the average and main divergence were calculated for each mosquito species. Analyses were conducted using the Kimura 2-parameter model (K2P2) [1] in MEGA X [2]. The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). All ambiguous positions were removed for each sequence pair (pairwise deletion option).

5.7 Genus-specific rtPCR-based search for *Alpha*-, *Flavi*-, *Orthobunya*- and *Phleboviruses* in mosquitoes and human blood samples

5.7.1 Nucleic acids extraction from mosquitoes

The mosquitoes collected with the dry shipper were pooled by species and region in pools of 10 and tested for *Flavi*-, *Alpha-, Orthobunya*-, and *Phlebovirus* by generic RT-PCRs. Initially, mosquito pools were homogenized in 2ml autoclaved screw-cap tubes (Sarstedt 72.694.006) containing 500 µl of chilled DMEM (5% L-Glutamine, 1% Penstrep, 3% FCS) and about 20 ceramic beads in a SpeedMill PLUS (Analytik Jena) for 2 min. Then the homogenate was centrifuged at 13.000 x g for 5 min. at 4°C, the supernatant was transferred to a new 1,5 ml tube and centrifuged for another 15 min. at 4°C, aliquoted (100µl), and stored at -80°C. Nucleic acids were extracted with QIAamp Viral RNA Mini Kit. At first, 560 µl of AVL buffer (without carrier-RNA) were mixed with 100 µl of supernatant and incubated at room temperature for 30 min. Then, 560 µl of ethanol (100%) was added and the solution was loaded on the QIAamp Mini column and washed with 500 µl of washing buffer AW1 and AW2 as indicated in the manufacturer's manual. Finally, the nucleic acids were eluted in 40 µl of nuclease-free water, aliquoted (4x10µl), and stored at -80°C.

5.7.2 Nucleic acid extraction from human blood on filter paper

Nucleic acids were extracted from human blood samples on Whatman 3mm cellulose chromatography paper stored in nitrogen or at -80 °C. At first 3mm^2 were cut out with a biopsy punch. Then extraction protocol from 5.7.1 was applied to blood on filter paper.

5.7.3 Virus genus-specific two-step virus RT-PCRs and melting analysis

The *Flavi*- (Patel et al. 2013), *Alpha*- (Hermanns et al. 2017), *Orthobunya*- and *Phlebovirus* (Lambert and Lanciotti 2009) genus-specific rtPCR assays previously described were adapted to two-step conventional rtPCRs followed by melting analyses. At first, cDNA was synthesized with High-Capacity cDNA Reverse Transcription Kit (Thermofisher) in a 20 µl reaction volume. RNA extract (10 µl) was transcribed with 2 µl of 10X RT Buffer, 2 µl nonribosomal hexamers (10 µM), (Endoh et al. 2005), 0,8 µl of 25x dNTPs Mix (100 mM), 1µl of MultiScribe Reverse Transcriptase (50 U/µL) and 4,2 µl de nuclease-free water under conditions described in the manual: 25 °C for 10 min, 37 °C for 120 °C and 85 °C for 5 min. Next, cDNAs were tested for virus presence by generic PCR reactions. PCRs were run as duplicates in a Rotor-Gene Q thermocycler (Qiagen) in a 10µl reaction volume containing 1 µl of cDNA, 5 µl of MyTaq HS Master Mix (Bioline), 1 µl of SYTO® 9 Green Fluorescent Nucleic Acid Stain (50µM), (Thermofisher), 1 µl of genus-specific primer mix (4µM each) and 2 µl of nuclease-free water, except *Alphavirus* nested PCR mix which contained 0,5 µl of 1st round PCR product, 5 of MyTaq HS Master Mix, 1 µl of SYTO 9 Green Fluorescent Nucleic Acid Stain (50µM), (Thermofisher), 1 µl of 2nd round primer mix (4µM each) and 2,5 µl of nuclease-free water. Run conditions for *Flavivirus* PCR were 95 °C for 30 s, 45 cycles 95°C for 10 s, 60 °C for 25 s, 72°C for 10 s, 79°C for 1 s, and 72°C for 3 min. The cycling protocol for *Alphavirus* PCR consisted of 95°C for 3 min, followed by 10 touchdown cycles of 95 °C for 15s, 55°C (1 °C per cycle) for 20 s, 72 °C for 30 s, 79 °C for 1 s, and 72°C for 5 min. *Orthobunya*- and *Phlebovirus* PCRs were run for 30 s at 95°C, 55 cycles 10 s at 95 °C, 25 s at 55 °C, 10 s at 72 °C, 1s at 79°C, and 3 min at 72°C.

The cDNA positive controls for the generic PCRs were derived from cell culture supernatants provided by the ZBS1. Controls applied for *Flavivirus* PCRs were DENV1 DENV2, DENV3, DENV4, WNV, YFV, and Zika; for *Alphavirus* PCRs Venezuelan equine encephalitis virus, and Chikungunya virus, and *Phlebovirus* PCR Rift valley fever virus and Sandfly virus. Concerning the *Orthobunyaviruses*, positive controls plasmids containing the S- segment of Batai virus (JX846604.1), Wyeomyia virus (FJ235921.1), Oropouche virus (KC759130.1) and Tahyna virus (KJ575082.1) were synthesized by Life Technologies based on the S-Segment sequences available at NCBI GenBank and the primers designed by Lambert and Lanciotti (2009).

RT-PCRs were followed by melting analyses of the PCR products at 95 °C for 1 min, 40 °C for 1 min, and 75 °C for 90 s, increasing the temperature in 0.1 °C increments up to 90 °C, with fluorescence acquisition after 2-s incremental holding periods (Villinger et al. 2017). Melt data was examined and the amplicons of the PCR duplicates showing comparable peaks in melt curve analyses underwent post-PCR analysis and sequencing.

5.7.4 Post-PCR analysis and Sanger sequencing

All PCR products (5µl) were run at 90V for 25 min. on a 1,5% agarose gel (UltraPure™ Agarose, Thermofisher), strained with GelRed® Nucleic Acid Gel Stain (Biotium). GeneRuler 100 bp Plus DNA Ladder (Thermofisher, SM0322) was used as a marker. PCR products were cleaned up with MSB® Spin PCRapace due to the instruction manual, resuspended in 30 µl of nuclease-free water, diluted to a concentration of 10-20 ng/µl, and Sanger-sequenced with BigDye™ Terminator v3.1 Cycle Sequencing Kit. The sequencing mix contained 1 µl of PCR product, 1 µl (10 µM each) forward or reverse primer set, 1,5 µl of 5x Sequencing Buffer, 1 µl of Primer set BigDye® Terminator v3.1 Ready Reaction Mix and 5,5 µl of nuclease-free water, was run at 96°C for 2min, 25 cycles at 96°C for 10s, 51°C for 5 s and 60°C for 4 min. Sequence information was provided by the MF2 Genome Sequencing facility of the RKI.

Forward and reverse sequences of each sample were assembled in Geneious R 10, primers were trimmed and consensus sequences were extracted. Consensus sequences were compared to NCBI database nucleotide collection with megablast and blastn algorithm. (https://blast.ncbi.nlm.nih.gov/Blast.cgi). If the blast search resulted in hits for any virus genera, corresponding mosquito pool cDNA was retested with other genus-specific primers (Vázquez et al. 2012; Grubaugh et al. 2013) as described in 5.7.3.

5.7.5 Virus particle purification from the sample

Virus particles were purified from 100 µl of mosquito homogenate by triple digestion of unprotected nucleic acids (Shi et al. 2015). Mosquito homogenates (100 µl) were digested in 12,5 µl of 10X TURBO DNase buffer, 2, 5 µl TURBO DNase (2 U/µl, Thermofisher, AM2239), 1 µl Benzonase (25 U/µl, Novagen, 70746-3) and 1 µl RnaseI (10 U/µl, Thermofisher, EN0601) and 8 µl nuclease-free water at 37 °C for 90 min, inactivated with 12,5 µl Inactivation Reagent for 5 min, centrifuged at 10.000 x g for 2 min, nucleic acids were extracted from supernatant and cDNA was synthesized as described in 5.7.1.

5.7.6 Virus isolation and NGS sequencing

Mosquito pools that tested positive for *Flavivirus* were inoculated on *Ae. albopictus* cells (C6/36, 89051705, SIGMA) and Vero cells. C6/36 cells were cultivated at 28 °C with 20 ml DMEM (1x, 4,5g/L D-Glucose L Glutamine, Gibco 41965-039), 3 % FCS (Biochrome, S0115), and 1% Penstrep (Gibco, 10.000

U/ml, 15140163), Vero cells at 37°C with DMEM supplemented with 10% heat-inactivated FBS in a 25 cm^2 cell culture bottle until a confluent monolayer was derived. Cells were spitted up into two bottles, one was mixed with 10 µl of the mosquito pool homogenate and cultivated along with the control for 14 days and revised every day for cytopathogenic effect. On day 14, cells and supernatant were removed and stored separately at -80 °C.

Cell culture supernatant was tested for *Flavivirus* with the AgPath-ID One-Step rtPCR Reagents (Thermofisher, 4387391) and the Pan-Flaviassay Primers (Patel et al. 2013) in a 10 µl reaction volume containing 1µl supernatant, 5 µl of 2X RT-PCR Buffer, 0,5 µl Detection Enhancer, 1 µl SYTO 9 Green Fluorescent Nucleic Acid Stain (50µM, Thermofisher), 1 µl Flaviprimermix (4µM each), 0,4 µl of 25X RT-PCR Enzyme Mix and 1,1 µl of nuclease-free water and run on a Rotor-Gene Q thermocycler (Qiagen) at 45°C for 15min, 95 °C for 10 min, and 45 cycles 95 °C for 15 s, 60 °C for 45 s followed by melting analysis as in 5.7.3.

If the cell culture tested positive for *Flavivirus*, 100 µl supernatant of day 14 was purified and cDNA was synthesized as in 5.7.5. DsDNA was synthesized by adding 1 µl of DNA Polymerase I, Large (Klenow) Fragment (3-9 U/µl, Thermofisher, 18012021) to cDNA incubating at 37 °C for 60 min. DsDNA of virus isolate was purified with MSB® Spin PCRapace (Invitek) and diluted in 20 µl of nuclease-free water, the library was created with Nextera XT DNA Library Prep Kit, and virus genome was NGS- sequenced by MF2 Genome Sequencing unit at RKI.

5.8 NGS-based virus search in mosquitoes and human blood samples

5.8.1 Library preparation and NGS

The mosquito homogenates from the mosquito pools in 5.7.1 were pooled by species/ genus in megapools of 4 (100 µl each) or 8 (50µl each). Virus particles were purified by triple nucleic acid digestion (Shi et al. 2015). In brief, 400 µl of megapool homogenate was digested in 50 µl of 10X TURBO DNase buffer, 10 µl TURBO DNase (2 U/µl, Thermofisher, AM2239), 4 µl Benzonase (25 U/µl, Novagen, 70746-3), and 4 µl RnaseI (10 U/µl, Thermofisher, EN0601) and 32 µl nuclease-free water at 37 °C for 90 min, centrifuged at 10.000 x g for 2 min. Then, 400 µl of supernatant were incubated in 2240 µl of AVL buffer (without carrier-RNA) for 30 min, 2240 µl of ethanol (100%) was added and the solution was loaded on the QIAamp Mini column, and washed with 500 µl of washing buffer AW1 and AW2 as indicated in manufacturer’s manual. Finally, the extract was eluted in 30 µl of nuclease-free water, aliquoted (2x15µl), and stored at -80°C.

CDNA was synthesized with Super Script IV Reverse Transcriptase (Thermofisher) in a reaction volume of 20 µl. Extract (12 µl) was mixed with 1 µl of nonribosomal hexamers (50uM), (Endoh et al. 2005), 1µl of dNTPs (100mM), incubated at 65°C for 5 min, put on ice for 1 min. Then 4 µl of 5X RT buffer, 1µl

DTT (100mM), and 1 µl SuperScript IV RT (200 U/µL) were added and run for 10 min at 23 °C, 10 min at 55 °C, and 10 min at 80°C. DsDNA was synthesized and purified as described in 5.7.6. Shotgun libraries were created with Nextera XT DNA Library Preparation Kit and sequenced on an Illumina HiSeq 1500 v3 system (50 bp SR run, single indexed) at MF 2, RKI in Berlin.

5.8.2 De novo reads assembly

The resulting fastq.gz files of each dataset were merged in Windows command prompt with the command “copy /b *.gz combine*.gz” and then uploaded to the Galaxy web platform (Afgan et al. 2018). Public servers at usegalaxy.org and usegalaxy.eu were used to analyze the data. Reads were assembled with the assembly pipeline Unicycler (Galaxy Version 0.4.4.0), (Wick et al. 2017) with normal bridging mode, and contigs shorter than 300bp were excluded from the output fasta file. Output fasta file was depth filtered with Fabox, an online fasta sequence toolbox (Villesen P. 2007).

5.8.3 Virus detection & genome annotation

Nucleotide sequences with depth ≥ 1 were aligned to the nucleotide and protein sequence database (Blastn, blastx) with the online BLAST web interface provided by NCBI (Madden 2003). Query sequences that returned any virus polymerase-related results were searched for open reading frames (ORFs) with NCBI ORF Finder (https://www.ncbi.nlm.nih.gov/orffinder/). Additional segments of segmented virus genomes were identified by aligning the contigs of a sample against the genome segments of the nearest known relative, or by aligning the contigs of two samples that are supposed to contain the same virus against each other. Functions of the coding virus proteins were predicted by searching for conserved domains with Conserved Domains Search (CD-search), (CDD v3.16 database, expect value 0,1-1), (Marchler-Bauer et al. 2015) and homology detection & structure prediction by HMM-HMM comparison (Söding 2005). The genome structures of the viruses found in this study were then visualized with Microsoft PowerPoint 2013.

5.8.4 Phylogenetic virus classification

The amino acid sequences of CD-search hits associated with viral replicases were used for phylogenetic analyses. CDSs that showed homology to any viral replicase (e.g. RNA-dependent RNA polymerase) were translated in Bioedit (version 7.0.5.3), (Hall 1999) and protein sequences were aligned to group/ family/ genus-specific reference sequences derived from NCBI protein database with MAFFT Multiple alignment program for amino acid or nucleotide sequences (Galaxy Version 7.221.3), (Katoh and Standley 2013) by using the E-INS-I algorithm. The fasta output file was automatically trimmed with the trimAl v1.2 command line version using the “trimal -in <inputfile> -out <outputfile> -gappyout” command (Capella-Gutierrez et al. 2009). The fasta output file was converted to relaxed Phylip format (full names and padded) with AliView (version 1.25), (Larsson 2014). Maximum likelihood phylogenies were estimated with PhyML 3.0 (Guindon et al. 2010) using Smart Model Selection (Akaike Information Criterion), (Lefort et al. 2017) for the selection of the best-fit substitution model, subtree pruning, and regrafting (SPR), (Hordijk and Gascuel 2005) and a likelihood-ratio test that relies on a non-parametric, Shimodaira-Hasegawa-like procedure (aLRT SH-like), (Anisimova and Gascuel 2006), or bootstrap (=100 or 1000), (Efron 1979). Based on (1) the estimated phylogenies, (2) the taxonomic and molecular information available on the ICTV (https://talk.ictvonline.org/) and ViralZone (https://viralzone.expasy.org/) websites the novel viruses were classified.

5.9 Visualization of isolated viruses by transmission electron microscopy

First, C6/36 cells were cultured in 75 cm^2 flasks under the conditions described in 5.7.6 until a confluent monolayer was established. Then 3 cell culture flasks were infected with 10 µl of the virus isolate supernatant (5.7.6) and detected virus strains were inoculated for 14 days.

On days 3, 4, and 7, the supernatant was removed from the bottles and fixed with paraformaldehyde (20%) in HEPES (0,05M). The fixation solution was depolymerized at 60 °C for 30 min, cooled to room temperature, mixed 1:9 with supernatant, and incubated for 2 h. Cells were fixed in flasks with 5 ml glutaraldehyde (2,5%) in HEPES (0,05M) at room temperature for 24 h. Afterward, fixed cells and virus particles were stored at 4°C. Electron microscopic pictures were taken of virus particles and infected C6/36 cells at Advanced Light and Electron Microscopy Department (ZBS4) at RKI in Berlin.

6 Results

6.1 Description of the mosquito fauna of the Atrato River region

From August 2014 to December 2016 a total of 7873 mosquitoes were captured in Northwest Colombia by human landing catch in 36 localities belonging to 6 municipalities (Figure 4), 34, 8 % during daytime (6 am-6 pm) and 65,2% during dusk, night and dawn (6 pm-6 am). Mosquitoes were identified to genus/ species level by the morphology of the female, males if available, and/or by aligning the COI and/ or ITS2 barcoding sequence to the NCBI nucleotide database with blastn (Megablast).

6.1.1 Mosquito species identified based on the morphological characteristic of the female

Most of the species could be identified based on the morphological characteristics of the female. The following 33 species could be identified via taxonomic keys without any difficulties (see 5.5):

Table 1: Mosquito species identified by morphology

Aedeomyia squamipennis	*Anopheles punctimacula*	*Haemagogus lucifer*	*Ochlerotatus serratus*	*Trichoprosopon digitatum*
Anopheles apicimacula	*Anopheles shannoni*	*Haemagogus regalis*	*Psorophora albipes*	
Anopheles calderoni	*Anopheles triannulatus*	*Johnbelkinia longipes*	*Psorophora cingulata*	
Anopheles darlingi	*Coquillettidia albicosta*	*Limatus durhamii*	*Psorophora colombiae*	
Anopheles malefactor	*Coquillettidia lynchi*	*Mansonia indubitans*	*Psorophora ferox*	
Anopheles neivai	*Coquillettidia venezuelensis*	*Mansonia titillans*	*Psorophora funiculus*	
Anopheles nuneztovari	*Culex. nigripalpus*	*Ochlerotatus angustivittatus*	*Sabethes tridentatus*	
Anopheles pseudopunctipennis	*Culex. quinquefasciatus*	*Ochlerotatus scapularis*	*Aedes albopictus*	

Species belonging to the genus *Chagasia* were not identified at the species level in this study. Species belonging to the genera *Wyeomyia* and *Culex* (subgenus *Melanoconion*) were not identified morphologically, but COI and ITS2 sequences were compared to the NCBI nucleotide database.

6.1.2 Mosquito species identified based on the shape of the male genitalia

Male genitals were used for mosquito species identification as phallosome structures are species-specific. For *Cx. quinquefasciatus* (Figure 5), *Cq. lynchi* (Figure 6), *Cq. venezuelensis* (Figure 7) and *Ma. titillans* (Figure 8) male specimens were available and genitalia matched the ones available in the literature (Harbach 2012, Lane 1953).

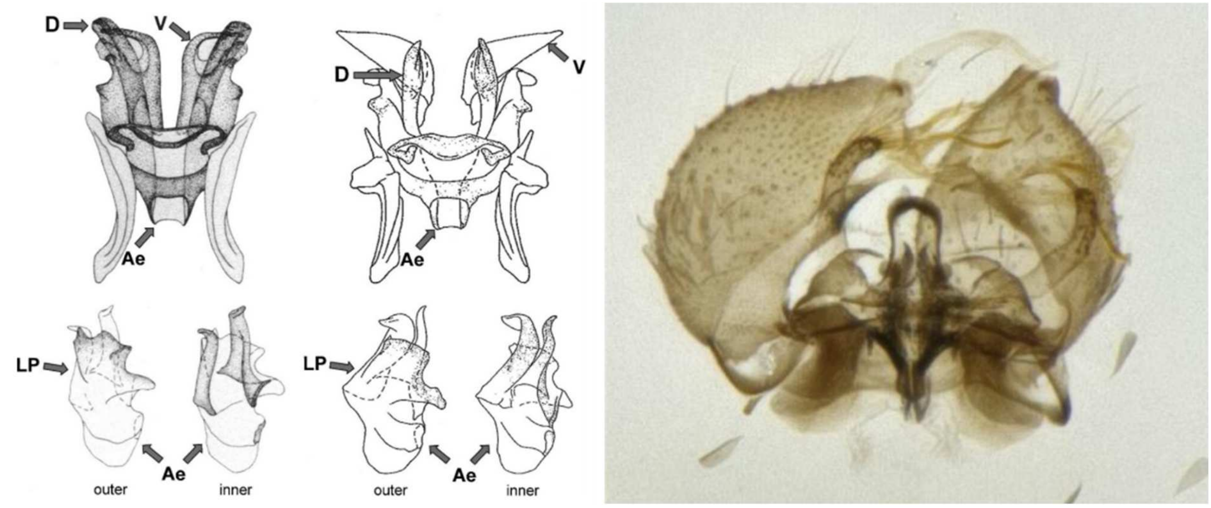


Figure 5: Male genitalia of Cx. quinquefasciatus and Cx. pipiens
Left: Phallosomes of Cx. pipiens (left) and Cx. quinquefasciatus (right). Abbreviations: Ae: Aedeagus, D: Dorsal arm, LP: Lateral plate, V: Ventral arm (Harbach 2012); right: Genitalia of a male Cx. quinquefasciatus collected in Murindó, Antioquia, Colombia.

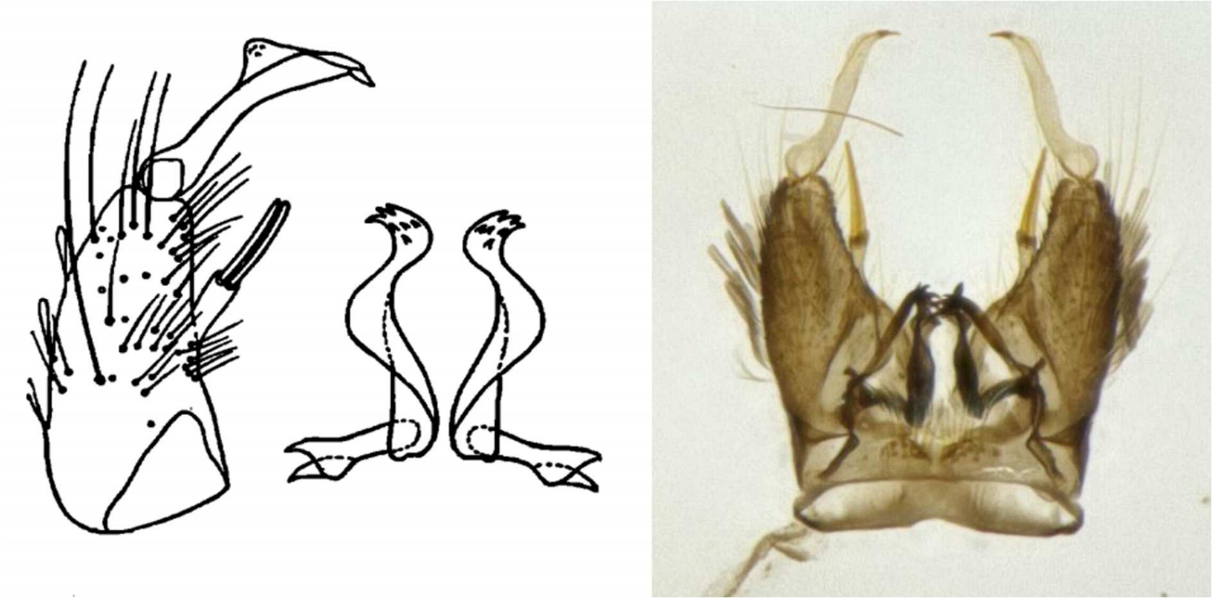


Figure 6: Male genitalia of Cq. lynchi
Left: Male genitalia of Cq. lynchi (Lane 1953); right: Genitalia of a male specimen of Cq. lynchi collected in Vigía del Fuerte, Antioquia, Colombia.

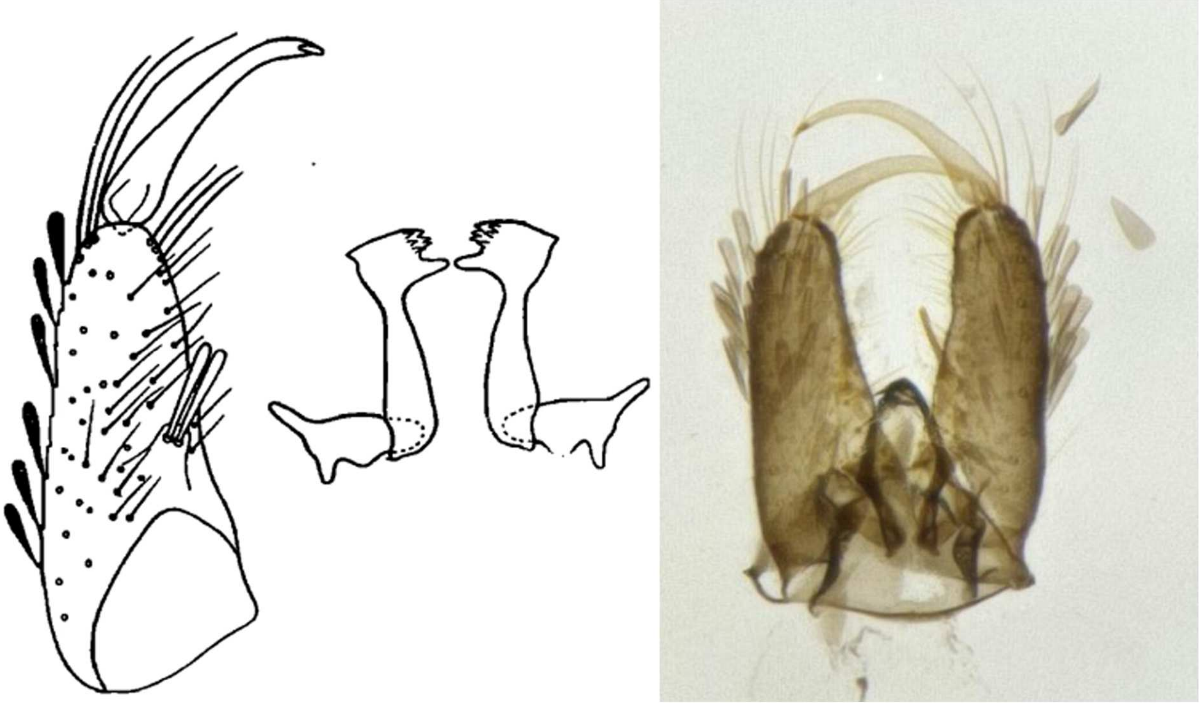


Figure 7: Male genitalia of Cq. venezuelensis
Left: Male genitalia of Cq. venezuelensis (Lane 1953); right: Genitalia of a male Cq. venezuelensis specimen collected in Murindó, Antioquia, Colombia.

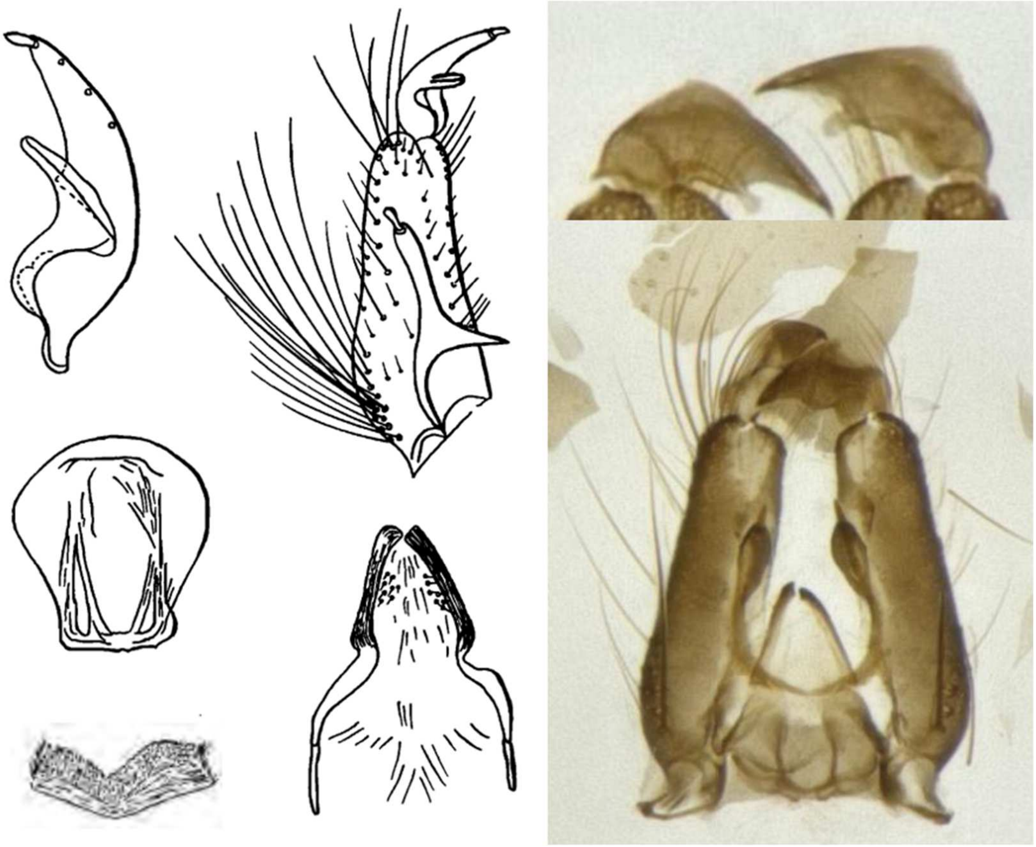


Figure 8: Male genitalia of Ma. titillans
Left: Male genitalia of Ma. titillans (Lane 1953); right: Genitalia of male Ma. titillans collected in Vigía del Fuerte, Antioquia, Colombia

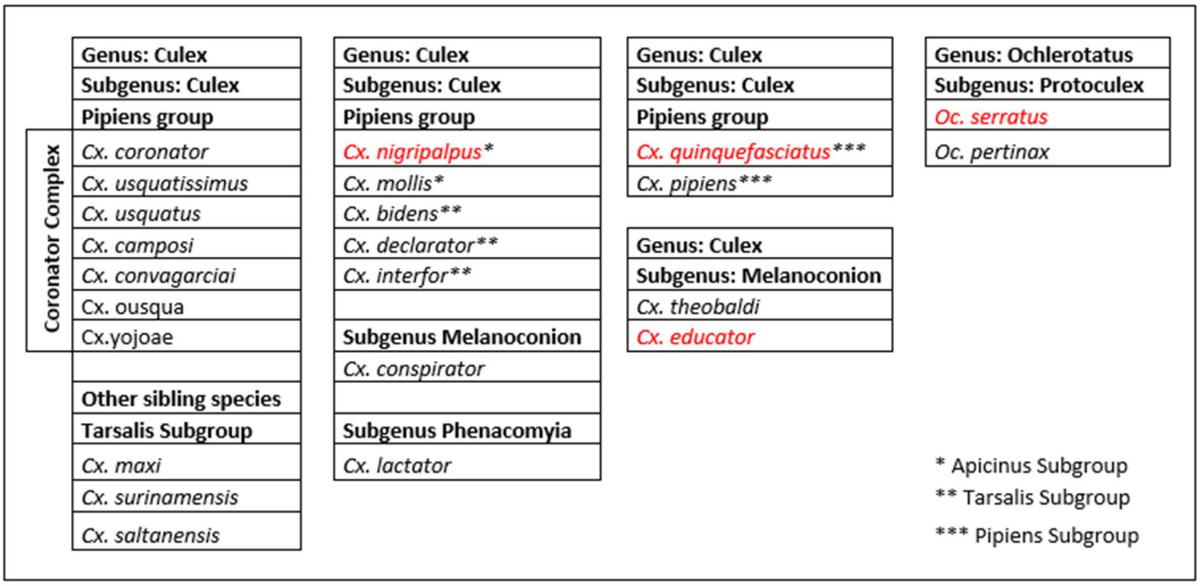
6.1.3 Mosquito species identified by COI barcoding

A total of 298 COI barcoding sequences from mosquitoes that have been identified morphologically previously (6.1.1) or could not be identified via taxonomic keys were aligned to the NCBI nucleotide database for molecular identification. A full list of the blastn results is available in Appendix 11.1.1. A total of 24 species could be identified by COI blastn (Per. Id. >98, for Wy. spp. Per. Id. > 97%). For the following species, the NCBI nucleotide database search resulted in a clear hit:

Anopheles darlingi	*Coquillettidia venezuelensis*	*Ochlerotatus. angustivittatus*	*Trichoprosopon digitatum*
Anopheles neivai	*Culex. coronator*	*Ochlerotatus scapularis*	*Wyeomyia aporonoma*
Anopheles nuneztovari	*Culex. nigripalpus*	*Psorophora cingulata*	*Wyeomyia luteoventralis*
Anopheles pseudopunctipennis	*Culex. rabelloi*	*Psorophora colombiae*	*Wyeomyia melanocephala*
Anopheles punctimacula	*Haemagogus. lucifer*	*Psorophora ferox*	*Wyeomyia pertinans*
Coquillettidia lynchi	*Limatus durhammii*	*Aedes albopictus*	

Table 2: Mosquito species identified by COI blastn in this study.

For five species identified by morphology before as *Cq. lynchi*, *Cq. venezuelensis*, *Ma. indubitans*, *Ps. albipes*, and *Ps. funiculus*[1], no COI sequences were available in the NCBI nucleotide database, thus being the sequences acquired in this study the first ones to be published. The species in Table 3 were identified before by morphology and assigned to *Cx. coronator*, *Cx. nigripalpus*, *Cx. quinquefasciatus*, *Cx. educator*, and *Oc. serratus*, but the NCBI search delivered ambiguous hits.



Genus: Culex
Subgenus: Culex
Pipiens group
Coronator Complex
Cx. coronator
Cx. usquatissimus
Cx. usquatus
Cx. camposi
Cx. convagarciai
Cx. ousqua
Cx.yojoae

Other sibling species
Tarsalis Subgroup
Cx. maxi
Cx. surinamensis
Cx. saltanensis

Genus: Culex
Subgenus: Culex
Pipiens group
Cx. nigripalpus*
Cx. mollis*
Cx. bidens**
Cx. declarator**
Cx. interfor**

Subgenus Melanoconion
Cx. conspirator

Subgenus Phenacomyia
Cx. lactator

Genus: Culex
Subgenus: Culex
Pipiens group
Cx. quinquefasciatus***
Cx. pipiens***

Genus: Culex
Subgenus: Melanoconion
Cx. theobaldi
Cx. educator

Genus: Ochlerotatus
Subgenus: Protoculex
Oc. serratus
Oc. pertinax

* Apicinus Subgroup
** Tarsalis Subgroup
*** Pipiens Subgroup

Table 3: Mosquito species with ambiguous results for COI blastn search.
For some COI sequences, blastn resulted in >99% per. id. with several species. Each box represents a species complex or group of sibling species. In red=species the COI sequence represents due to morphological identification in this study.

---

[1] There is a nucleotide sequence available that shows 99% per. id. with our sequence (KM452781.1), but it is not assigned to species level.

The COI sequences from the specimens that we refer to as *Cx. coronator s.l.* matched with COI sequences that are associated with other species from the *Coronator c*omplex (see Table 15). The *Coronator* group of the subgenus *Culex* includes *Cx. coronator (*Dyar), *Cx. camposi* (Dyar & Knab), *Cx. covagarciai* (Forattini), *Cx. ousqua* (Dyar), *Cx. usquatissimus* (Dyar), *Cx. usquatus* (Dyar) and *Cx. yojoae* (Stickman), and the females of these species cannot be distinguished by morphological characters due to polymorphic or overlapping characteristics (Forattini 1965, Demari-Silva et al. 2014, Demari-Silva et al. 2015). Other sibling species which might be included in the *Coronator* group are *Cx. saltarensis*, *Cx. surenamensis*, and *Cx. maxi* (Laurito et al. 2008, Laurito et al. 2013). The COI has been used as a molecular barcode for species identification in mosquitoes (Hebert et al. 2003b), but is not suitable to distinguish closely related species of the *Coronator* group, a complex of recently diverged species, due to high intraspecific variation and low interspecific divergence (Laurito et al. 2013). In Colombia, *Cx. coronator* (Dyar), *Cx. camposi* (Dyar & Knab), *Cx. ousqua* (Dyar), *Cx. usquatissimus* (Dyar), and *Cx. usquatus* (Dyar) have been registered (Rozo-Lopez and Mengual 2015). The male genitalia permit the identification to species level (Forattini 2002), but male specimens of *Cx. coronator s.l.* were not available in this study. For proper molecular identification and phylogenetic analyses of the members of the *Coronator* group, other mitochondrial genes like the ATP6, ATP8, and NaDH5 have been suggested (Demari-Silva et al. 2015), but were not applied in this study. Therefore, accurate identification of the species of the *Coronator* complex was not possible and the specimens were provisionally named *Cx. coronator s.l.*.

The COI sequences of the specimens we identified by morphology as *Cx. nigripalpus* coincide with GenBank sequences of *Cx. conspirator*, *Cx. nigripalpus*, *Cx. declarator*, *Cx. lactator*, *Cx. bidens*, *Cx. interfor*, and, *Cx. mollis*. Females of *Cx. nigripalpus* may be identified by morphology (Forattini 2002), but cannot be distinguished from the other species by the COI sequence. It is supposed that these species also compose a species complex (Demari-Silva et al. 2011). For the identification of recently diverged taxa and phylogenetic analysis of species complexes in the genus *Culex* the ITS2 has proven to be a more appropriate molecular marker (Vesgueiro et al. 2011). Based on morphological identification and ITS2 sequences available, the specimens we collected correspond to *Cx. nigripalpus*.

The COI sequences we associated with *Cx. quinquefasciatus* matched the ones of *Cx. quinquefasciatus* and *Cx. pipiens that were* available at NCBI GenBank. The *Cx. pipiens* species complex includes at least two species: *Cx. quinquefasciatus* (Say) and *Cx. pipiens* (Coquillet), (Harbach 2012). Other species that might belong to the complex are *Cx. australicus* (Dobrotworsky & Drummond) and *Cx. globocoxitus* (Dobrotworsky)/ (Zittra et al. 2016), but in Colombia only *Cx. quinquefasciatus* has been registered (Rozo-Lopez and Mengual 2015). The species of the complex may not be distinguished based on the COI or ITS2 sequences. Polymorphisms in the second intron of the acetylcholinesterase-2 (*ace-2*), and the microsatellite locus CQ11 allow the identification of the species, subspecies, and hybrid forms, but were not applied in this study (Smith and Fonseca 2004, Bahnck and Fonseca 2006). Anyway, *Cx. quinquefasciatus* may also be distinguished based on the male genitalia (Harbach 2012)/ (see 6.1.2).

The *Educator group* of the subgenus *Melanoconion*, genus *Culex* consists of seven species: *Cx. cristovaoi* (Duret), *Cx, educator* (Dyar & Knab), *Cx. eknomios* (Forattini & Sallum), *Cx inadmirabilis* (Dyar), *Cx. rachoui* (Duret), *Cx. theobaldi* (Lutz), and *Cx. vaxus* (Dyar), (Sallum and Forattini 1996), but only *Cx. educator*, *Cx. theobaldi*, and *Cx. inadmirabilis* have been reported in Colombia (Rozo-Lopez and Mengual 2015). The COI sequences we associated with *Cx. educator* resulted in hits for *Cx. nr theobaldi* (nr=near/affine), (Torres-Gutierrez et al. 2016), and *Cx. educator* (Rozo-Lopez and Mengual 2015). Nevertheless, Torres-Gutierrez et al. in their publication specify, that the specimen with accession number KX779891.1 was mistakenly identified as *Cx. theobaldi* but corresponds to *Cx. educator.* Thus, its COI sequence clusters with the *Cx. educator* sequence of Rozo-Lopez and Mengual. (KM593042.1).

The subgenus *Protoculex*, also known as the *Serratus* group of the genus *Ochlerotatus* currently includes 10 species: *Oc. aenigmaticus* (Cerqueira & Costa), *Oc. atlanticus* (Dyar & Knab), *Oc. dupreei* (Coquillett), *Oc. eucephalaeus* (Dyar), *Oc. hastatus* (Dyar), *Oc. nubilus* (Theobald), *Oc. oligopistus* (Dyar), *Oc. pertinax* (Grabham), *Oc. serratus* (Theobald), and *Oc. tormentor* (Dyar & Knab). In Colombia, *Oc. aenigmaticus*, *Oc. hastatus*, *Oc. nubilus,* and *Oc. serratus* have been registered (Rozo-Lopez and Mengual 2015). As these species cannot be distinguished by their COI sequences, they were distinguished by ITS2 sequence phylogeny (6.1.6.2.1, Figure 9).

For *Ma. titillans* only one COI sequence was available on NCBI (KY859902.1), but it did not match with our sequences from the specimens we identified as *Ma. titillans* by morphology, but with a *Mansonia* specimen, we could not identify. The COI sequences we associated with *Ma. titillans* did not match any sequences available.

Seven species of the genus *Culex*, two of the genus *Mansonia*, two of the genus *Ochlerotatus*, five of the genus *Trichoprosopon*, and one of the genus *Wyeomyia* did not match any sequence available and could not be identified, neither by COI blastn nor morphology. The COI sequences listed in Table 15 were uploaded to NCBI GenBank. (Accession numbers: MN997410-MN997708).

6.1.4 Mosquito species identified by ITS2 barcoding

A total of 82 ITS2 sequences from mosquitoes that have been identified morphologically previously, or could not be identified via taxonomic keys, were aligned to the NCBI nucleotide database. In Appendix 11.1.2 a full list of the blastn search results is available. A total of eight species could be identified by ITS2 blastn (Per. id >98%). For the following species, the NCBI nucleotide database search resulted in a clear hit: *An. apicimacula*, *An. darlingi*, *An. nuneztovari*, *An. pseudopunctipennis*, *An. punctimacula*, *Cx. nigripalpus, Cx. pipiens*/ *Cx. quinquefasciatus*, and *Ps. ferox*.

For nine species identified by morphology and/or COI blastn before as *An. neivai*, *Cq. lynchi*, *Cq. venezuelensis*, *Ma. indubitans*, *Ma. titillans*, *Oc. scapularis*, *Ps. albipes*, *Ps. funiculus*, *Wy. luteoventralis* and *Wy. Pertinans,* no ITS2 sequences were available in the NCBI nucleotide database, being the sequences produced in this study the first ones to be published.

For the specimens identified before by morphology and referred to as *Oc. serratus*, NCBI search delivered ambiguous hits. The ITS2 sequences matched with ITS2 sequences that are associated with *Oc. atlanticus* and *Oc. tormentor*, but for *Oc. serratus* no ITS2 sequences were available. *Oc. atlanticus*, *Oc. tormentor* and *Oc. serratus* belong to the subgenus *Protoculex* also known as the *Serratus* group and are closely related species that may be distinguished by ITS2 phylogeny (6.1.6.2.1, Figure 9). Sequences listed in Table 16 were uploaded to NCBI GenBank (Accession numbers: MT151916-MT152001).

6.1.5 Intra- and interspecific evolutionary divergence of barcoding regions

6.1.5.1 Evolutionary divergence among COI sequences

The genetic distances between the barcoding regions of the specimens that were assigned to a certain “species” may be used as an indicator for conspecifity or heterospecifity. The greater the “intraspecific” distance between the mean and maximum intraspecific K2P distance is, the greater the probability that specimens are not conspecific, but heterospecific. The pairwise genetic distances were estimated for 299 COI nucleotide sequences from 28 mosquito species. For each mosquito species, the mean and maximum intraspecific K2P distances among COI sequences are shown in Table 4. The mean K2P distance observed between specimens considered conspecific varied between 0% and 2,3% and the maximum KP2 distance between 0% and 3,1%. It can be observed that the mean and maximum K2P distances of *Oc. serratus* and *Wy. aporonoma* are elevated. This might be due to the presence of unidentified sibling species or an ongoing speciation process.

Mosquito species	**No. Specimens**	**Mean K2P distance (%)**	**Max K2P distance (%)**	**Mosquito species**	**No. Specimens**	**Mean K2P distance (%)**	**Max K2P distance (%)**
An. darlingi	21	0,2	1	*Cx. educator*	6	0,5	0,8
An. nuneztovari	48	0,1	0,7	*Cx. sp. 8*	7	0,5	1,4
An. punctimacula	8	0,8	2,2	*Ma. indubitans*	4	0,4	0,6
Cq. lynchi	11	0,1	0,6	*Ma. titillans*	3	0,5	0,8
Cq. venezuelensis	22	0,5	1,8	*Oc. serratus*	16	1,5	3,1
Cx. coronator	2	0,2	0,2	*Oc. sp. 1*	2	1,2	1,2
Cx. nigripalpus	12	0,4	0,9	*Ps. albipes*	7	0,9	1,9
Cx. quinquefasciatus	12	0,3	1,9	*Ps. ferox*	5	1,1	1,5
Cx. sp. 1	5	1,6	0,1	*Ps. funiculus*	8	0,8	1,9
Cx. sp. 3	12	0,5	1,7	*Ae. albopictus*	6	0,2	0,3
Cx. sp. 4	5	1,1	1,4	*Tr. sp. 1*	4	0,2	0,7
Cx. sp. 5	3	0,4	0,9	*Wy. aporonoma*	3	2,3	3,1
Cx. sp. 6	2	0,5	0,5	*Wy. Luteoventralis*	2	0	0
Cx. sp. 7	2	0,2	0,2	*Wy. Pertinans*	35	1,3	2,7

Table 4: Estimates of evolutionary divergence among COI sequences of mosquitoes
Average and maximum intraspecific K2P distances observed among COI sequences of mosquito species sequenced in this study are shown. Analyses were conducted using the Kimura 2-parameter model [1] in MEGA X [2]. Elevated values are in red.

6.1.5.2 Evolutionary divergence among ITS2 sequences

Mosquito species	**No. specimens**	**Mean K2P distance (%)**	**Max K2P distance (%)**
An. darlingi	7	0	0
An. nuneztovari	23	0	0
An. punctimacula	3	0,2	0,25
Cq. lynchi	2	1,2	1,2
Cq. venezuelensis	5	6,4	8,8
Cx. sp. 2	2	0	0
Cx. quinquefasciatus	2	0	0
Ma. indubitans	2	0	0
Ma. titillans	9	0	0
Oc. scapularis	4	0,2	0,6
Os. serratus	4	0,8	0,9
Ps. albipes	10	0,4	2,1
Ps. ferox	2	0	0

Table 5: Estimates of evolutionary divergence among ITS2 sequences of mosquitoes
Average and maximum intraspecific K2P distances observed among ITS2 sequences of mosquito specimens sequenced in this study are shown. Analyses were conducted using the K2P model [1] in MEGA X [2]. Elevated values are in red.

The pairwise genetic distances were estimated for 86 ITS2 nucleotide sequences from 13 mosquito species. For each mosquito species, the mean and maximum intraspecific K2P distances among ITS2 sequences are shown in Table 5. The mean K2P distances observed between specimens considered conspecific by morphology varied between 0% and 6,4%, and the maximum K2P distance between 0% and 8,8%. Generally speaking, the values for the intra-specific variations of the ITS2 regions are lower than the ones for the COI regions. For *Cq. venezuelensis* comparatively high K2P distances were observed. Most probably the specimens identified as *Cq. venezuelensis* are heterospecific and correspond to *Cq. venezuelensis* and another sibling species.

6.1.6 Phylogenies of neotropical mosquitoes

6.1.6.1 Mosquito phylogeny based on the COI nucleotide sequence

The sequences from Appendix 11.1.1, Table 15 were used to estimate the COI phylogenies between the described mosquito species. Fifty taxa could be identified, of which 26 could be assigned to a described species by COI blastn at a sequence identity of >97%. Eight taxa could not be assigned by COI blastn, because the COI nucleotide sequences showed less than 97% sequence identity with GenBank entries. These specimens could be identified by morphology only. For specimens identified morphologically and assigned to *Ps. albipes*, *Cq. venezuelensis*, *Ma. titillans*, *Ma. indubitans*, *Oc. scapularis*, *Cq. lynchi*, *Oc. serratus* and *Ps. funiculus*, no COI sequences were available in the NCBI database. Another 16 taxa could only be assigned at the genus level. Seven *Culex* species of the subgenus *Melanoconion*, two of the genus *Mansonia*, two of the genus *Ochlerotatus*, one of the genus *Wyeomyia,* and five of the genus *Trichoprosopon* could be identified neither by COI blastn nor by morphology and might correspond to species described formerly that have no COI sequences available in the GenBank, or yet undescribed species. In Table 2 the species that could be identified by COI blastn are listed. The complete phylogenetic tree is available in Table 10.

6.1.6.2 *Phylogeny of species complexes of closely related mosquito species*

6.1.6.2.1 Phylogeny of the subgenus *Protoculex*, genus *Ochlerotatus* based on the ITS2 nucleotide sequence

The ITS2 sequences from mosquitoes identified in this study as *Oc. serratus* by morphology were aligned to all the ITS2 sequences available at NCBI for the genus *Ochlerotatus*, subgenus *Protoculex*. Only for the species *Oc. pertinax*, *Oc. tormentor*, and *Oc. atlanticus* ITS2 sequences were available at GenBank. These were aligned to the ITS2 sequences from the mosquitos we identified as *Oc. serratus* by morphology and a phylogenetic analysis was conducted. Even though ITS2 blastn results were ambiguous (Table 16), phylogenetic analysis indicates clear branching for the four species.

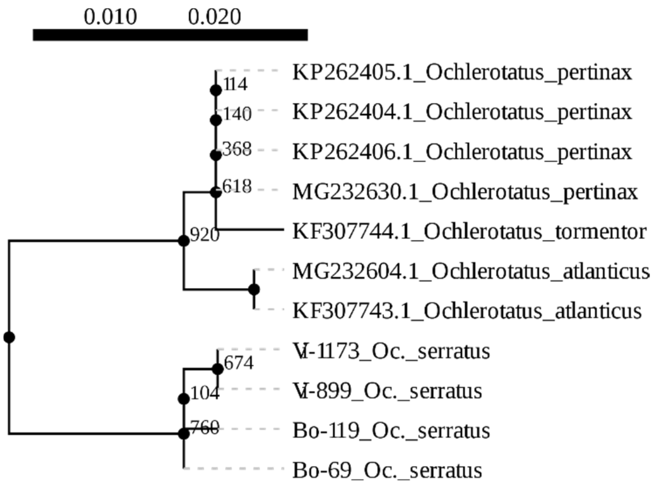


Figure 9: Phylogeny of the subgenus Protoculex, genus Ochlerotatus estimated based on the nucleotide sequence of the ITS2 region (Best model: GTR). The midrooted maximum likelihood tree including all available ITS2 sequences available at NCBI GenBank for the subgenus is displayed in a rectangular view. Nodes show bootstrap values (bootstrap=1000) and branch length is shown in the upper left corner.

6.1.6.2.2 Phylogeny of the genus *Ochlerotatus* based on the COI region

The COI sequences from mosquitoes identified in this study as *Oc. angustivittatus*, *Oc. scapularis, Oc. serratus,* and *Oc. spp.* by morphology were aligned to all the COI sequences available at NCBI for the genus *Ochlerotatus*, and a phylogenetic analysis was performed. Our COI sequences of *Oc. angustivittatus*, *Oc. scapularis* and *Oc. serratus* clustered with sequences of conspecific specimens available on NCBI and thus confirm our COI blastn results. The COI sequences of our unidentified *Oc.* specimens clustered with the COI sequences of specimens belonging to the subgenus *Protoculex*. Only four species of the subgenus *Protoculex* are registered in Colombia: *Oc. aenigmaticus*, *Oc. hastatus*, *Oc. nubilus,* and *Oc. serratus*. Most probably, our unidentified *Ochlerotatus* specimens correspond to *Oc. aenigmaticus*, *Oc. hastatus* or *Oc. nubilus*, but they might also belong to the other species of the Subgenus *Protoculex* that have not been registered yet in Colombia, or yet undescribed novel species of the subgenus. The species *Oc. flavifrons* currently is assigned to the subgenus *Ochlerotatus* but due to COI phylogeny should be reassigned to the subgenus *Rusticoides.* For *Oc. diantaeus*, two sequences were available, but they did not cluster, and branching indicated, that at least one of the two specimens should correspond to another species.

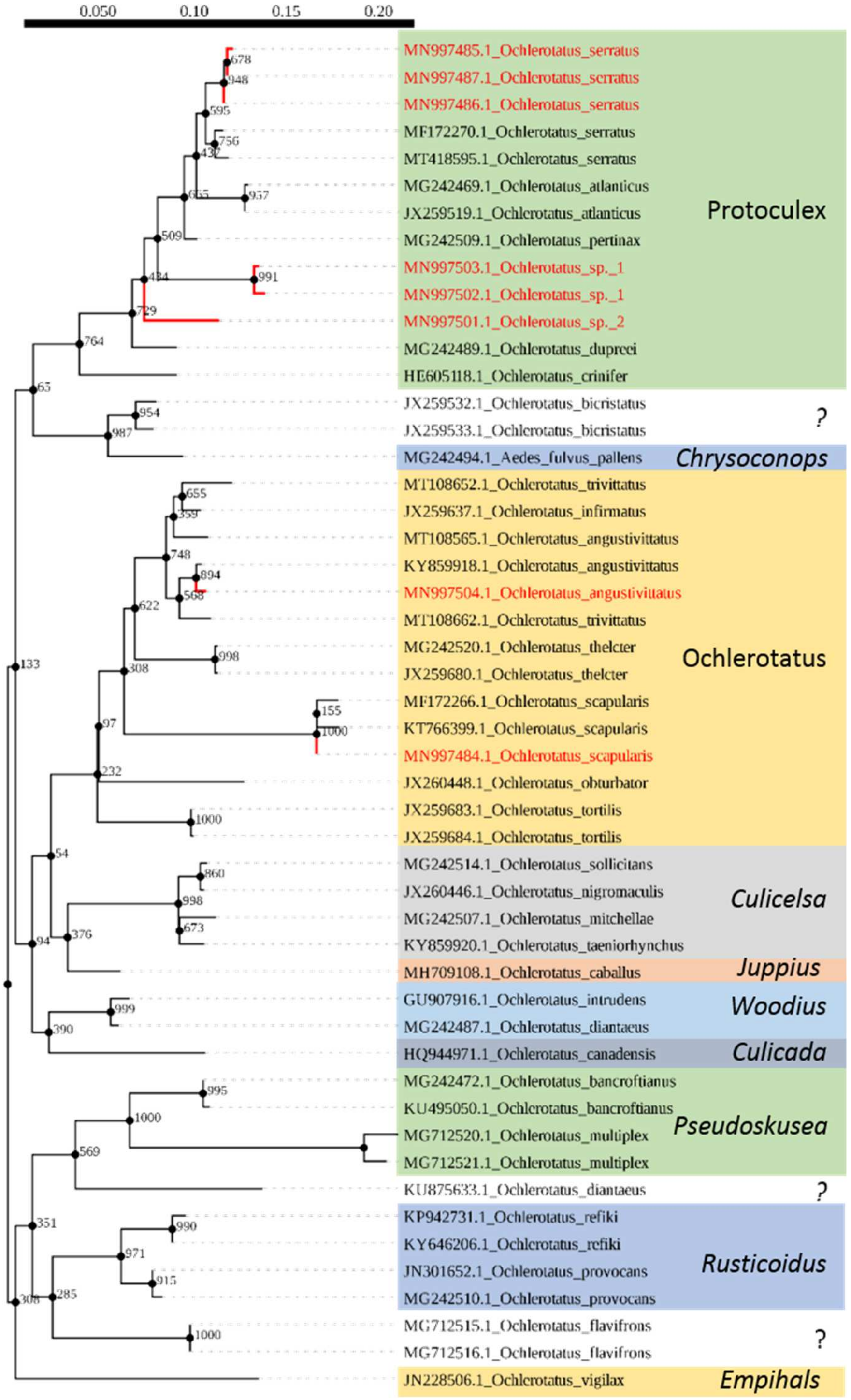
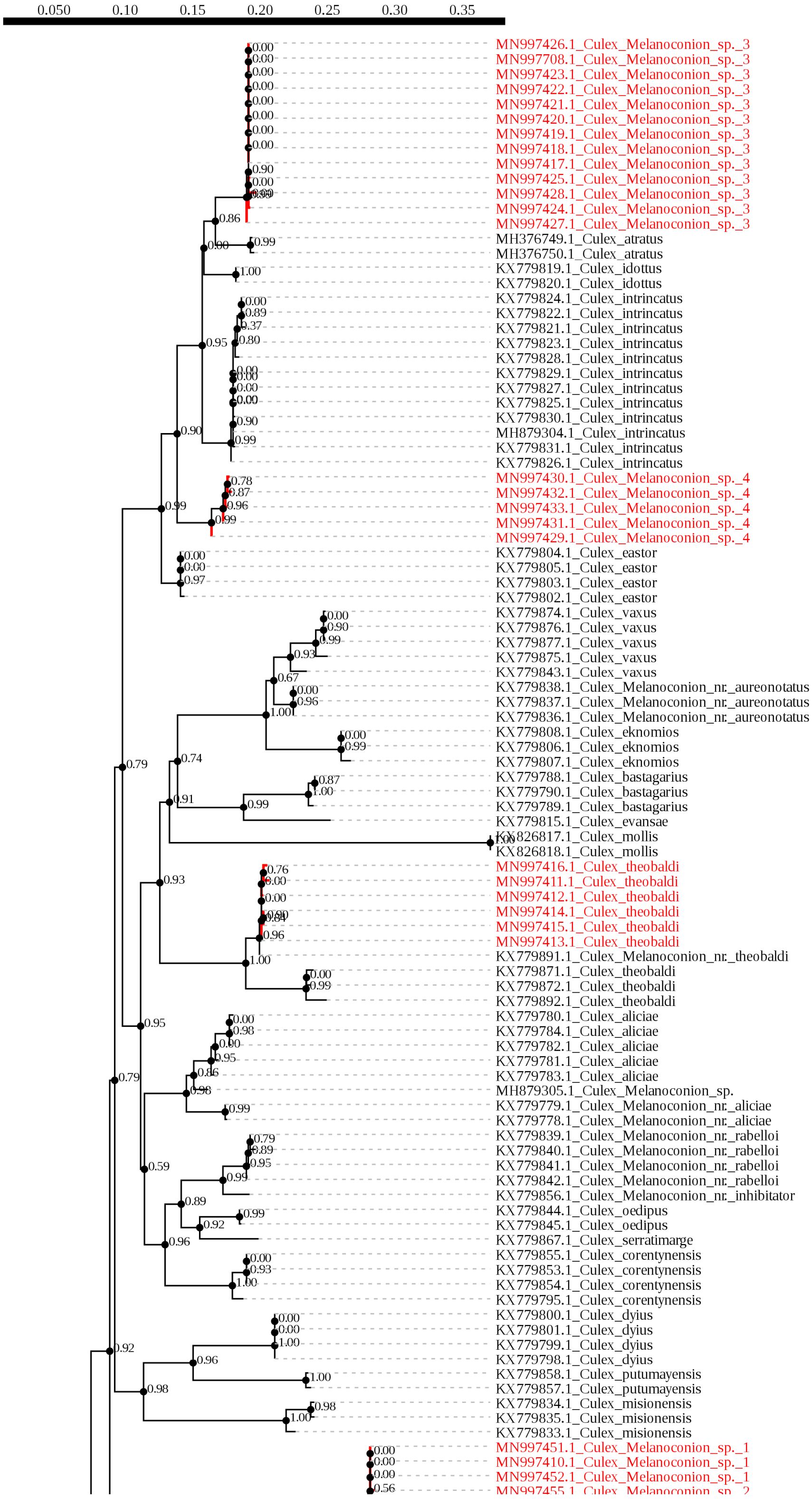


Table 6: Phylogeny of the genus Ochlerotatus estimated based on the nucleotide sequence of the COI region (Best model: GTR+G+I). The midrooted maximum likelihood tree including all available COI sequences available at NCBI GenBank for the genus is displayed in a rectangular view. Nodes show bootstrap values (bootstrap=1000) and branch length is shown in the upper left corner. Specimens found in this study are in red.

6.1.6.2.3 Phylogeny of the genus *Culex*, subgenus *Melanoconion based on the COI region*

The neotropical subgenus *Melanoconion* (*Mel*.) is one of the biggest subgenera of the genus *Culex* and includes more than 166 species (Harbach 2013a). Morphological identification of the species belonging to the subgenus *Melanoconion* is not always possible if not all life stages are available, as some species may only be distinguished by the morphology of the male genitalia or larval traits. Molecular identification with markers, particularly the COI gene, has proven to be a good tool for species identification for this subgenus if the species has been barcoded correctly (Torres-Gutierrez et al. 2016), but for most of the species of the subgenus, no sequences are available. Due to the lack of male and immature life stages, morphological identification of *Melanoconion* specimens from the Atrato River region was not possible in most cases. Therefore, we intended to identify the subgenus members by their COI sequences. Their COI sequences were aligned to the available sequences and COI phylogeny was estimated (Figure 10), but many specimens could not be assigned on the species level as the species had not been barcoded before. The COI phylogeny branching pattern gave evidence for thirteen species of the subgenus in the research region, but only two could be assigned at the species level. The sequences of *Cx. educator* and *Cx. rabelloi* from this study clustered with COI sequence available at NCBI Genbank that made the molecular identification possible.

The COI sequences of *Cx. (Mel.) sp. 3* clustered on a sister glade to *Cx. atratus* but shared only 95% of nucleic acid identity. *Cx. atratus* is the type species of the subgenus *Melanoconion* and belongs to the *Atratus* group that has been revised recently (Sá et al. 2020). Currently, it consists of 14 species, and revised descriptions and taxonomical keys for the identification of all species and life stages are available, but they were not taken into account in this study, as the mosquitoes were identified before the keys were published. Most probably, the sequences of *Cx. sp. 3* correspond to one of the species of the *Atratus* group. The other sequences could not be assigned as no similar sequences were available, or because the sequences available were not assigned on the species level.


0.050
0.10
0.15
0.20
0.25
0.30
0.35
MN997426.1_Culex_Melanoconion_sp._3
MN997708.1_Culex_Melanoconion_sp._3
MN997423.1_Culex_Melanoconion_sp._3
MN997422.1_Culex_Melanoconion_sp._3
MN997421.1_Culex_Melanoconion_sp._3
MN997420.1_Culex_Melanoconion_sp._3
MN997419.1_Culex_Melanoconion_sp._3
MN997418.1_Culex_Melanoconion_sp._3
MN997417.1_Culex_Melanoconion_sp._3
MN997425.1_Culex_Melanoconion_sp._3
MN997428.1_Culex_Melanoconion_sp._3
MN997424.1_Culex_Melanoconion_sp._3
MN997427.1_Culex_Melanoconion_sp._3
MH376749.1_Culex_atratus
MH376750.1_Culex_atratus
KX779819.1_Culex_idottus
KX779820.1_Culex_idottus
KX779824.1_Culex_intrincatus
KX779822.1_Culex_intrincatus
KX779821.1_Culex_intrincatus
KX779823.1_Culex_intrincatus
KX779828.1_Culex_intrincatus
KX779829.1_Culex_intrincatus
KX779827.1_Culex_intrincatus
KX779825.1_Culex_intrincatus
KX779830.1_Culex_intrincatus
MH879304.1_Culex_intrincatus
KX779831.1_Culex_intrincatus
KX779826.1_Culex_intrincatus
MN997430.1_Culex_Melanoconion_sp._4
MN997432.1_Culex_Melanoconion_sp._4
MN997433.1_Culex_Melanoconion_sp._4
MN997431.1_Culex_Melanoconion_sp._4
MN997429.1_Culex_Melanoconion_sp._4
KX779804.1_Culex_eastor
KX779805.1_Culex_eastor
KX779803.1_Culex_eastor
KX779802.1_Culex_eastor
KX779874.1_Culex_vaxus
KX779876.1_Culex_vaxus
KX779877.1_Culex_vaxus
KX779875.1_Culex_vaxus
KX779843.1_Culex_vaxus
KX779838.1_Culex_Melanoconion_nr._aureonotatus
KX779837.1_Culex_Melanoconion_nr._aureonotatus
KX779836.1_Culex_Melanoconion_nr._aureonotatus
KX779808.1_Culex_eknomios
KX779806.1_Culex_eknomios
KX779807.1_Culex_eknomios
KX779788.1_Culex_bastagarius
KX779790.1_Culex_bastagarius
KX779789.1_Culex_bastagarius
KX779815.1_Culex_evansae
KX826817.1_Culex_mollis
KX826818.1_Culex_mollis
MN997416.1_Culex_theobaldi
MN997411.1_Culex_theobaldi
MN997412.1_Culex_theobaldi
MN997414.1_Culex_theobaldi
MN997415.1_Culex_theobaldi
MN997413.1_Culex_theobaldi
KX779891.1_Culex_Melanoconion_nr._theobaldi
KX779871.1_Culex_theobaldi
KX779872.1_Culex_theobaldi
KX779892.1_Culex_theobaldi
KX779780.1_Culex_aliciae
KX779784.1_Culex_aliciae
KX779782.1_Culex_aliciae
KX779781.1_Culex_aliciae
KX779783.1_Culex_aliciae
MH879305.1_Culex_Melanoconion_sp.
KX779779.1_Culex_Melanoconion_nr._aliciae
KX779778.1_Culex_Melanoconion_nr._aliciae
KX779839.1_Culex_Melanoconion_nr._rabelloi
KX779840.1_Culex_Melanoconion_nr._rabelloi
KX779841.1_Culex_Melanoconion_nr._rabelloi
KX779842.1_Culex_Melanoconion_nr._rabelloi
KX779856.1_Culex_Melanoconion_nr._inhibitator
KX779844.1_Culex_oedipus
KX779845.1_Culex_oedipus
KX779867.1_Culex_serratimarge
KX779855.1_Culex_corentynensis
KX779853.1_Culex_corentynensis
KX779854.1_Culex_corentynensis
KX779795.1_Culex_corentynensis
KX779800.1_Culex_dyius
KX779801.1_Culex_dyius
KX779799.1_Culex_dyius
KX779798.1_Culex_dyius
KX779858.1_Culex_putumayensis
KX779857.1_Culex_putumayensis
KX779834.1_Culex_misionensis
KX779835.1_Culex_misionensis
KX779833.1_Culex_misionensis
MN997451.1_Culex_Melanoconion_sp._1
MN997410.1_Culex_Melanoconion_sp._1
MN997452.1_Culex_Melanoconion_sp._1
MN997455.1_Culex_Melanoconion_sp._2

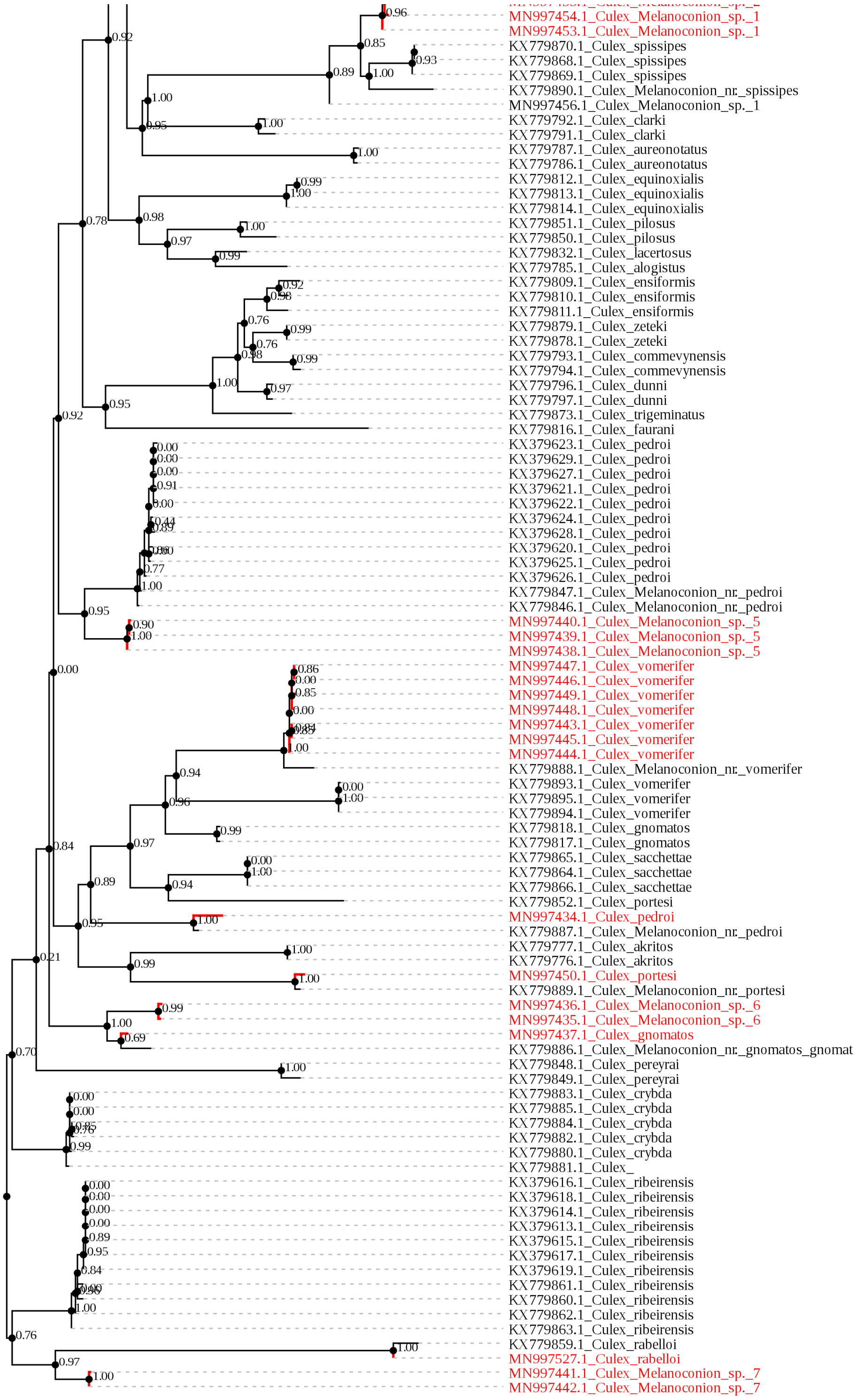


Figure 10: Phylogeny of the genus Culex, subgenus Melanoconion estimated based on the nucleotide sequence of the COI gene (selected model GTR+G+I). The midrooted maximum likelihood tree of all the COI sequences available at NCBI GenBank for the subgenus Melanoconion (03.06.2021) and our sequences is displayed in a rectangular view. Nodes show aLRT SH-like support values and branch length is shown in the upper left corner. Specimens found in this study are in red.

6.1.7 Abundance and richness of mosquito species in the Atrato River region

A total of 43 species of 14 genera could be identified by morphology and/or COI and/or ITS2 sequencing. In Appendix 11.3, Table 17 all the species identified are listed and the mode of identification is indicated. The relative abundances of the most frequently collected mosquito species (> 1%) are shown in Figure 11. The six most abundant species belonged to only four genera and made up 78,4 % of the total number of specimens collected: *Ma. titillans* (17,7%), *Ps. albipes* (16,5%), *An. darlingi* (15,8%), *An. nuneztovari* (10,9%), *Cq. venezuelensis* (8,9%), and *Cq. lynchi* (8,6%). Less frequently captured species were *Oc. serratus* (3,7%), *Cx. quinquefasciatus* (2,5%), *Ps. funiculus* (1,6%), *Ps. ferox* (1,1%) and Oc. scapularis (1,0%). Other species collected in the research region (<1%) are listed in Table 7. The exact numbers of specimens collected of each species at each location are shown in Appendix 11.3, Table 18 (see map Figure 4 for locations). Heatmaps in Figure 40 visualize the relative abundances with which each of the twelve most abundantly collected species were collected in each locality.

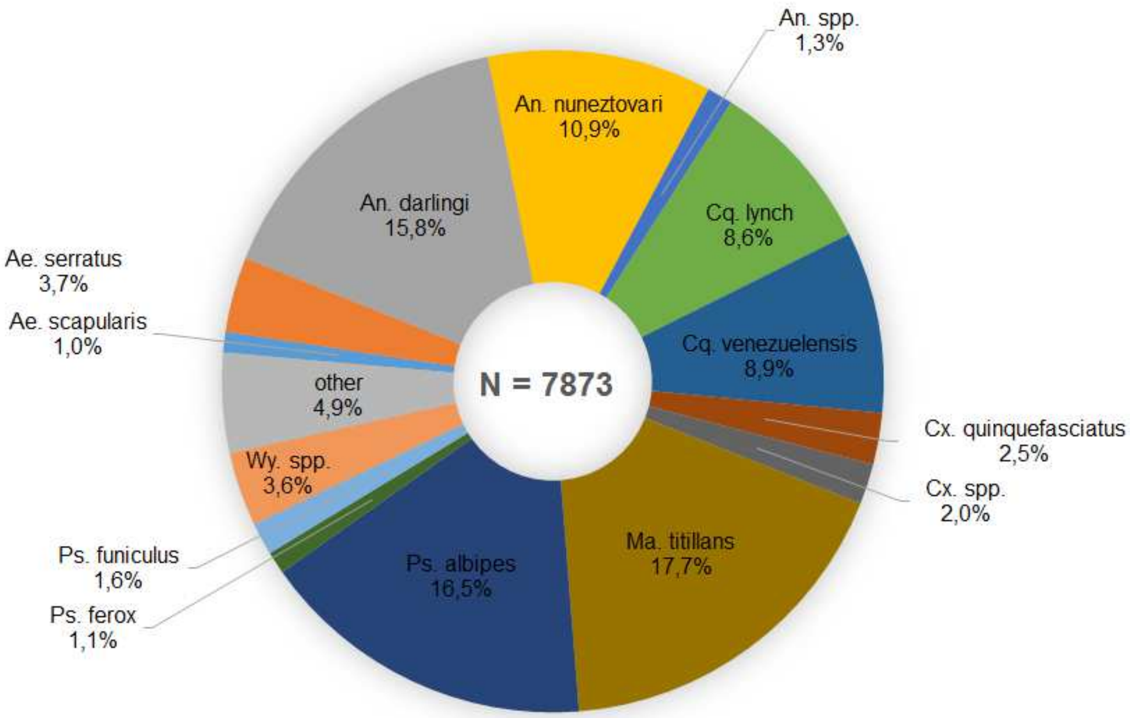


Figure 11: Relative abundance of mosquito species collected in the Atarto River valley Only main species/genus >1% are shown. Other species collected are listed in Table 7.

Other species	No.	%	Other species	No.	%
Ad. squamipennis	2	0,03%	*Cx. sp. 2*	1	0,01%
Ae. angustivittatus	57	0,73%	*Cx. rabelloi*	1	0,01%
An. apicimacula	3	0,04%	*Cx. educator*	6	0,08%
An. calderoni	1	0,01%	*Cx. sp. 8*	4	0,05%
An. malefactor	6	0,08%	*Hg. lucifer*	1	0,01%
An. neivai	1	0,01%	*Hg. regalis*	1	0,01%
An. pseudopunctipennis	5	0,06%	*Jb. longipes*	4	0,05%
An. punctimacula	18	0,23%	*Li durhammii*	2	0,03%
An. shannoni	1	0,01%	*Ma. indubitans*	24	0,31%
An. triannulatus	2	0,03%	*Ps. cingulata*	3	0,04%
Cq. albicosta	1	0,01%	*Ps. columbiae*	1	0,01%
Ch. spp.	3	0,04%	*Sa. tridentatus*	1	0,01%
Cx. coronator	7	0,09%	*Ae. albopictus*	30	0,38%
Cx. sp. 10	2	0,03%	*Tr. digitatum*	1	0,01%
Cx. nigripalpus	54	0,69%			

Table 7: Mosquito species collected in the Atrato River valley with low frequencies.

6.1.8 Biting behavior

Most of the mosquito species identified in this study have been associated with the transmission of diseases to humans or animals in former studies. *An. nuneztovari* and *An. darlingi* for instance are the main vectors of malaria in Colombia (Montoya-Lerma et al. 2011), and *Ps. albipes* and *Ma. titillans* are involved in VEEV transmission (Navarro et al. 2005) Measures to prevent transmission are often related to the biting behavior of each mosquito species. Therefore, the biting behavior of the most abundantly collected mosquito species was studied to get insight into their daily biting rhythms. Mosquitoes were collected by human landing catch and thus show anthropophilic biting behavior. The most abundantly collected mosquito species were categorized into two groups due to their biting behavior: (1) Diurnal (6 am-6 pm) and (2) crepuscular/nocturnal (6 pm-6 am). The species *Oc. scapularis*, *Oc. serratus*, *Oc. angustivittatus*, *Cq. lynchi*, *Ps. albipes*, *Ps. ferox*, *Ps. funiculus* and *Ae. albopictus* presented mainly diurnal biting behavior, while *An. darlingi*, *An. nuneztovari*, *Cq. venezuelensis*, *Cx. quinquefasciatus*, and *Ma. titillans* were rather crepuscular/nocturnal. *An. nuneztovari* and *An. darlingi,* two of the main malaria vectors in the Americas, showed a crepuscular/nocturnal biting behavior and almost exclusively bit between 20 - 23 h. *Oc. scapularis* was found with increased incidence from, 3 - 5 pm, *Oc. serratus* from 9 - 10 am and 2 - 4 pm, *Oc. angustivittatus* from 8 - 10 am, *Ps. albipes* and *Ps. ferox* from 9 - 10 am and 3 - 5 pm, and *Ps. funiculus* from 10 am - 1pm. Concerning the genus *Coquillettidiae,* the two species identified showed the opposite behavior. While *Cq. lynchi* is a diurnal species biting mainly from 9 am to 6 pm, *Cq. venezuelensis* prefers to hunt at dusk and night, mostly between 6 and

9 pm. *Cx. quinquefasciatus* and *Ma. titillans* were caught almost exclusively during dusk/ night with increased abundance from 7-10 pm and 6-10 pm respectively. The local and temporal biting behavior of the main mosquito species is summed up in Figure 12.

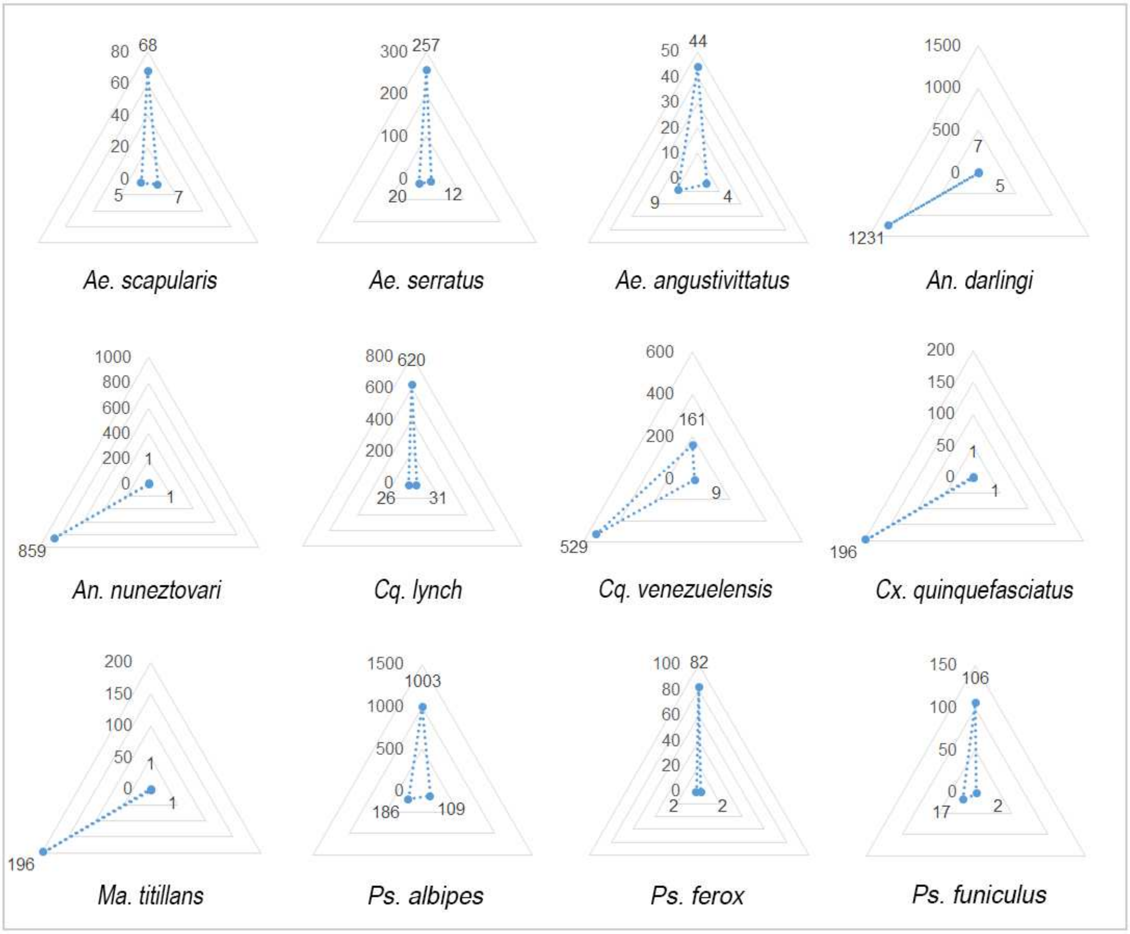


Figure 12: Local and temporal biting behavior of main mosquito species found in the Atrato River region. For each species, the total number of mosquitoes collected in all localities during the day (6 am-6 pm) and at night (6 pm- 6 am) are shown. Each corner of the triangle corresponds to the combination of two variables. The bottom left corner corresponds to the number of mosquitoes collected at night in the settlements, the bottom right corner to the number collected during the day in the settlements, and the upper corner to the number collected by day in the surroundings of the villages (rainforest, fields, etc.).

6.2 Characterization of the mosquito viromes

A systematic virus search was promoted in mosquitoes from the Atrato River valley in Colombia to get insight into the virome of the most frequently occurring mosquito species. By the means of virus genus-specific (rt)PCRs and an NGS-based approach, 52 novel virus species could be characterized in association with mosquitoes.

6.2.1 Two novel *Flaviviruses* detected in mosquitoes by genus-specific rtPCR

A total of 2042 mosquitoes were pooled in 206 pools (approx. 10 specimens per pool) by genus, species, and locality and tested by genus-specific rtPCRs for *Flavi*-, *Alpha*-, *Phlebo*-, and *Orthobunyaviruses*. Mosquito species (no. of pools) tested were *An. darlingi* (29), *An. nuneztovari* (7), *An. spp.* (2), *Cq. lynchi* (8), *Cq. venezuelensis* (12), *Cx. nigripalpus* (1), *Cx. quinquefasciatus* (3), Cx. spp. (4), *Ma. titillans* (16), *Oc. serratus* (8), *Ps. albipes* (93), *Ps. ferox* (5), *Ps. funiculus* (7), *Ae. albopictus* (2), and *Wy. spp.* (9).

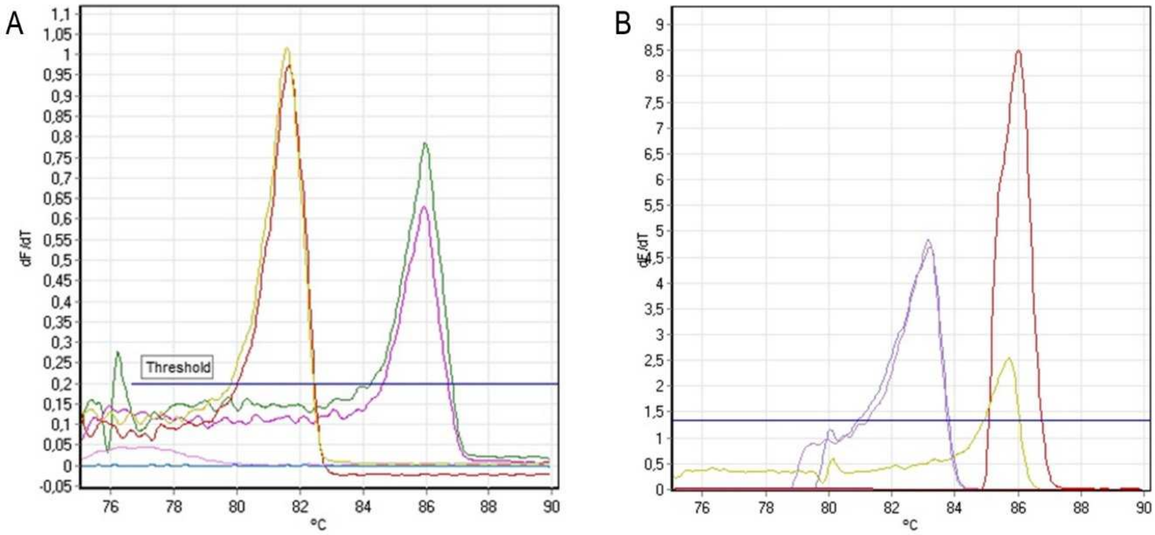


Figure 13: RT-PCR melting analysis profiles from mosquito pools infected with a novel Flavi-like virus. Part of the conserved region of the NS5 gene (about 260bp) was amplified with primers from the Pan-Flavi assay (Patel et al. 2013) and melting curve analysis was done with intercalating SYBR Green dye. Melting curves for (A) Ps. albipes pool tested positive for novel Flavi-like virus (green/purple: Atrato Flavi-like virus 1, Tm = 86,0 °C; red/yellow: DENV 1 cDNA from cell culture supernatant diluted 1:100, Tm= 81,6 °C; blue/pink: negative control. (B) Oc. serratus pool tested positive for novel Flavi-like virus (red/yellow: Atrato Flavi-like virus 1, Tm = 85,9 °C; purple: DENV 4 cDNA from cell culture supernatant diluted 1:100, Tm = 83,2 °C)

Two *Ps. albipes* pools and one *Oc. serratus* pool tested positive for two novel Flavi-like viruses with primers targeting a conserved region of the RdRp coding NS5 gene (Patel et al. 2013, Vázquez et al. 2012). Rt-PCR melting curve analyses are shown in Figure 13. A short description of Atrato Flavi-like virus 2 (AFlv2) and Atrato Flavi-like virus 3 (AFlv3) and the GenBank accession numbers for the partly sequenced genomes are available in Table 9. Phylogenetic analyses based on the coding region of the RdRp are shown in appendix 11.6.1.6, Figure 61, Figure 62, and genome structure in Figure 87. Two attempts were launched to sequence AFlv2 and AFlv3, one from a pool and one from a cell culture supernatant, but unfortunately, it was impossible to assemble the full genomes.

The detected virus strains were inoculated in C6/36 cells and Vero cells for 14 days. Even though no cytopathic effect was noticed, high viral loads could be detected in the C6/36 cell culture by one-step RT-PCR, and electron micrographing of infected cells revealed the virus typical structures (Figure 14).

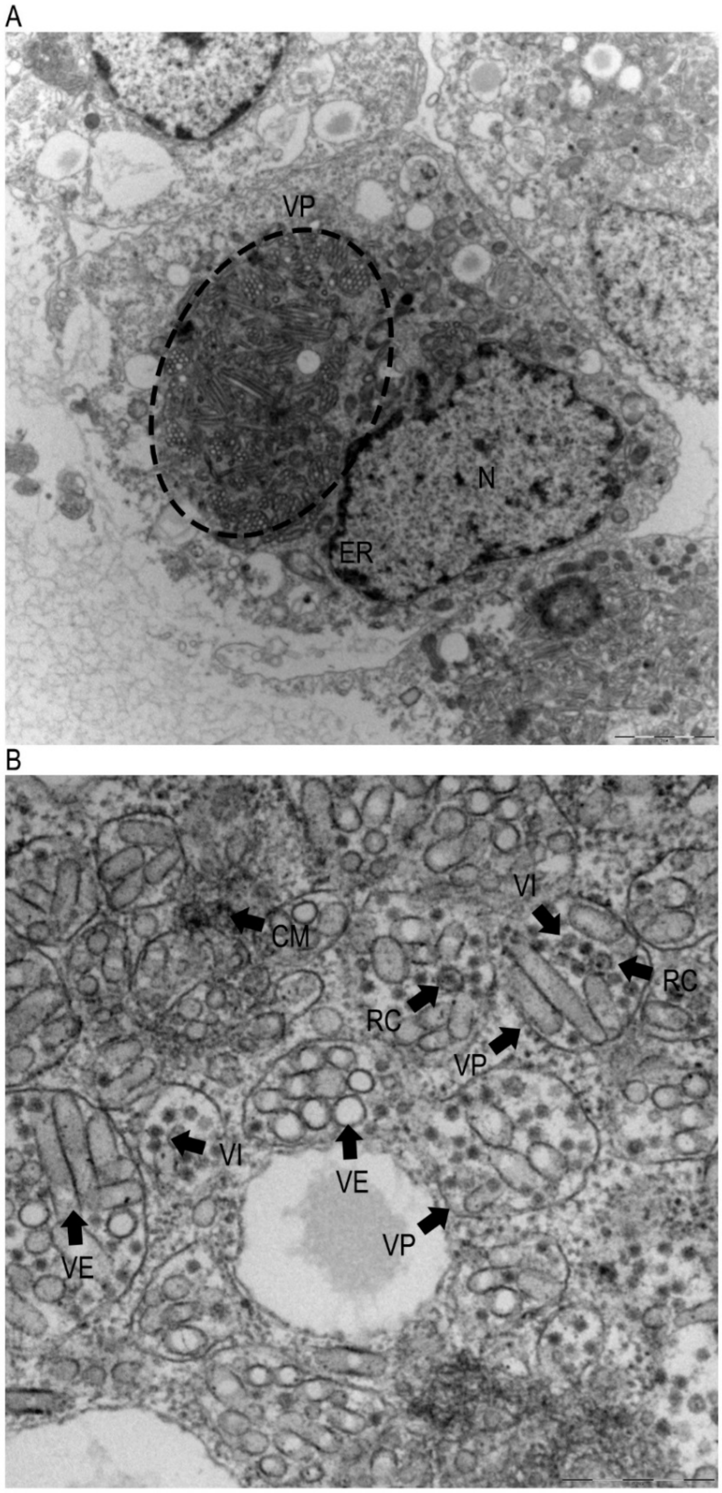


Figure 14: C6/36 cells infected with AFlv; a novel insect-specific flavi-like virus isolated from Oc. serratus. Electron micrographs show chemically fixed C6/36 cells 5 days post-infection. (A) Virus factory: Virus replication takes place on the endoplasmatic reticulum (ER) and derived membrane vesicles (VE) that are arranged in vesicle packages (VP) and occupy the majority of the cytoplasm. (B) Virus particles (VI) are produced by the replication complex (RC) in the spherules. Convoluted membranes (CM) were also observed.

6.2.2 Description of novel viruses found by NGS-based virus search in mosquitoes

An NGS-based virus search was performed on mosquitos from the Atrato River region in Colombia. A total of 2000 mosquitoes were tested for viruses. The mosquito homogenate pools from 6.2.1 that contained approximately 10 specimens were pooled in megapools with up to 50-100 specimens. At first nucleic acids were extracted from megapools, virus particles were purified and cDNA was synthesized. Then shotgun libraries were created, Illumina sequenced, and reads were de novo-assembled.

6.2.2.1 Identification of viruses and annotation of their genomes

De novo-assembled contigs, with nucleotide sequence lengths larger than 300 bp that showed a sequencing coverage equal to or greater than one, were examined. Preselected contigs were aligned to the NCBI database via blastx algorithm (default parameters, expect threshold 0,05) and the Conserved Domain Database (default parameters, E-value cutoff 0,1-1), and HHpred protein homology detection and structure prediction (default parameters, E-value cutoff 0,001) was applied to identify virus-related genes and proteins. Then the CDSs of the viral polymerases were predicted with NCBI ORF finder and translated to AA sequences for phylogenetic analyses.

6.2.2.2 Viral polymerase coding segments identified in mosquitoes by NCBI blastx

Contigs that resulted in hits for viral polymerases are listed in Table 8. The complete annotated virus genomes are available at NCBI Genbank under the accession numbers listed in Table 9, and the genome structures are visualized in appendix 11.6.2. Pools from megapools that tested positive for a certain virus were retested individually by virus species-specific rtPCRs designed based on the conserved regions of the polymerase coding segment and PCR products were sequenced for confirmation (Förster 2019).

Virus/Polymerase coding segment	NCBI blastx Hit Description (Top hit)	Query Cover	Per Ident	Accession
Atrato virus	RdRp [Aedes camptorhynchus toti-like virus 1]	90%	33.99%	YP_009388611.1
Atrato Chu-like virus 1	RNA-directed RNA polymerase [Chuvirus Mos8Chu0]	99%	59.33%	API61887.1
Atrato Chu-like virus 2	polymerase [Tacheng Tick Virus 4]	92%	31.41%	YP_009177716.1
Atrato Chu-like virus 4	RNA-dependent RNA polymerase [Scaldis River bee virus]	54%	36.21%	APT68159.1
Atrato Chu-like virus 5	RNA-dependent RNA polymerase [Scaldis River bee virus]	93%	37.25%	APT68159.1
Atrato Denso-like virus	nonstructural protein 1 [Haematobia irritans densovirus]	93%	26.63%	QCH41361.1
Atrato Denso-like virus 1	nonstructural protein 1 [Haematobia irritans densovirus]	75%	30.77%	QCH41361.1
Atrato Flavi-like virus 1	putative polyprotein [Lampyris noctiluca flavivirus 1]	35%	26.49%	QBP37018.1

Virus/Polymerase coding segment	NCBI blastx Hit Description (Top hit)	Query Cover	Per Ident	Accession
Atrato Gouko-like virus 1	RNA-dependent RNA polymerase-like protein [uncultured virus]	99%	47.49%	AGW51765.1
Atrato human blood virus	putative viral replication protein [Circoviridae sp.]	43%	54.15%	AXH75487.1
Atrato Nege-like virus 1	ORF1 [Tanay virus]	99%	55.99%	AHX42605.1
Atrato Partiti-like virus 1	RdRp [Hubei tetragnatha maxillosa virus 8]	98%	66.98%	YP_009337885.1
Atrato Partiti-like virus 2	RdRp [Partitivirus-like 1]	95%	82.33%	AOR51388.1
Atrato Partiti-like virus 3	RdRp [Vespa velutina partiti-like virus 1]	97%	54.76%	ATY36109.1
Atrato Partiti-like virus 4	RdRp [Hubei partiti-like virus 39]	99%	45.85%	APG78233.1
Atrato Partiti-like virus 5	RdRp [Hubei partiti-like virus 19]	96%	49.49%	APG78260.1
Atrato Partiti-like virus 6	RdRp [Hubei partiti-like virus 20]	71%	26.97%	APG78253.1
Atrato Picorna-like virus	hypothetical protein [Hubei picorna-like virus 41]	98%	51.98%	YP_009337750.1
Atrato Picorna-like virus 1	putative polyprotein [Yongsan picorna-like virus 2]	99%	90.41%	AXV43882.1
Atrato Reo-like virus 1	RdRp [Aedes camptorhynchus reo-like virus]	99%	60.97%	YP_009389547.1
Atrato Retro-like virus	hypothetical protein B5V51_13913 [Heliothis virescens]	90%	39.82%	PCG74073.1
Atrato Rhabdo-like virus 2	RdRp [Grenada mosquito rhabdovirus 1]	99%	53.63%	AVP26977.1
Atrato Rhabdo-like virus 3	RNA-dependent RNA polymerase [Wuhan Ant Virus]	95%	39.33%	YP_009304559.1
Atrato Sobemo-like virus 1	putative RNA-dependent RNA polymerase [Medway virus]	82%	63.47%	AWA82252.1
Atrato Sobemo-like virus 2	putative RNA-dependent RNA polymerase [Medway virus]	95%	52.65%	AWA82252.1
Atrato Sobemo-like virus 3	RdRp [Culex luteo-like virus]	94%	65.40%	ASA47392.1
Atrato Sobemo-like virus 4	hypothetical protein [Culex mosquito virus 6]	91%	64.86%	AXQ04826.1
Atrato Sobemo-like virus 5	hypothetical protein 2 [Wenzhou sobemo-like virus 4]	100%	79.01%	YP_009337376.1
Atrato Sobemo-like virus 6	RdRp [Hubei partiti-like virus 20]	71%	26.97%	APG78253.1
Atrato Virga-like virus 1	RdRp [Hubei virga-like virus 2]	87%	51.66%	YP_009337412.1
Atrato Virga-like virus 2	RdRp [Hubei virga-like virus 2]	91%	52.21%	YP_009337412.1
Atrato Virga-like virus 3	RdRp [Hubei virga-like virus 2]	85%	55.60%	YP_009337412.1
Atrato Virga-like virus 4	RdRp [Hubei virga-like virus 2]	69%	54.34%	YP_009337412.1
Atrato Virga-like virus 5	replicase [Culex pipiens associated Tunisia virus]	100%	41.23%	YP_009553255.1
Atrato Virga-like virus 6	hypothetical protein [Hubei virga-like virus 21]	99%	40.85%	YP_009337659.1
Atrato Virga-like virus 7	hypothetical protein [Hubei virga-like virus 21]	99%	40.80%	YP_009337659.1
Cacaos virus	putative RNA-dependent RNA polymerase [Solenopsis invicta virus 2]	21%	31.19%	AXL96895.1
Chajerado virus	hypothetical protein [Wuhan insect virus 13]	90%	36.46%	YP_009342321.1
Chibugado virus	pol-like protein [Ceratitis capitata]	99%	45.04%	AAC28743.1
Coredo virus	RNA-dependent RNA polymerase [Scaldis River bee virus]	93%	37.25%	APT68159.1
CulexPhasma-like virus	RdRp [Culex phasma-like virus]	100%	99.66%	ASA47278.1
Embera virus	RdRp [Anopheles totivirus]	84%	43.77%	AOR51364.1
Gengado virus	polyprotein [Switchgrass mosaic virus]	97%	44.35%	YP_004464920.1
Guagua virus	RNA-dependent RNA polymerase [Ferak virus]	100%	60.03%	YP_009664550.1
Hubei mosquito virus 4	hypothetical protein 1 [Hubei mosquito virus 4]	100%	96.85%	APG76308.1

Virus/Polymerase coding segment	NCBI blastx Hit Description (Top hit)	Query Cover	Per Ident	Accession
Hubei Virga-like virus 2	RdRp [Hubei virga-like virus 2]	100%	93.43%	YP_009337412.1
Isla virus	hypothetical protein [Wuhan insect virus 13]	91%	36.01%	YP_009342321.1
Murindo virus	hypothetical protein [Hubei macula-like virus 2]	98%	42.58%	YP_009337794.1
Murri virus	hypothetical protein 2 [Hubei toti-like virus 10]	39%	36.89%	YP_009336493.1
Narangue virus	RNA-dependent RNA polymerase [Zhee Mosquito virus]	98%	40.65%	AJG39275.1
Pisingos virus	RdRp [Australian Anopheles totivirus]	100%	69.46%	ASU43981.1
Sabaleta virus	hypothetical protein [Wuhan insect virus 13]	92%	35.45%	YP_009342321.1

Table 8: Viral polymerase coding segments identified by NCBI blastx in denovo assembled contigs from mosquito pools.

6.2.2.3 Short description of novel viruses from mosquitoes

In total, 52 virus genomes could be characterized of which 13 were assigned to negative-sense single-strand RNA viruses ((-)ssRNA), 23 to positive sense single-strand RNA viruses ((+)ssRNA), 12 to double-strand RNA (dsRNA) viruses, 2 to the single strand DNA (ssDNA) viruses and 2 to reverse transcribing viruses (ssRNA(RT)). Basic information on the viruses detected in this study is summed up in Table 9. Phylogenetic trees based on the AA sequence of the polymerase coding region for each virus are available in 11.6.1. and genomic structures in 11.6.2. The nucleic acid sequences of the assembled viral genomes and the amino acid sequences of the associated coding proteins are available at NCBI GenBank under the accession numbers listed in Table 9.

*Table 9: Short description of the virus strains detected in mosquito samples. Taxonomic classification was done based on the phylogenetic analysis of the AA sequence of the polymerase coding segment ([1] genome composition, [2] genome/ segment size in base pairs, [3] number of genome segments, * incomplete, ° circular). Host/vector association was approximated based on the phylogenetic trees and available publications. Unassigned and new genera proposed are in red.*

gcom[1]	**Order**	**Family**	**Genus**	**Species**	**strain**	**Seqs**	**depth**	**bps[2]**	**Accession**	**host/vector**
ssRNA(-)	*Bunyavirales*	*Phasmaviridae*	*Feravirus*	Guagua virus	Mati1755-7	3	2-3	6634 4166 1529	MN661015 MN661016 MN661017	insects
ssRNA(-)	*Bunyavirales*	*Phasmaviridae*	*Orthophasmavirus*	Coredo virus	Mati1754-5	3	1-10	6428 2538 2001	MN661021 MN661022 MN661023	insects
ssRNA(-)	*Bunyavirales*	*Phasmaviridae*	*Orthophasmavirus*	Culex Phasma-like virus	Cx1773-14	3	1-7	6365 2198 1982	MN661018 MN661019 MN661020	insects
ssRNA(-)	*Bunyavirales*	*Phenuiviridae*	*Zheemosvirus*	Atrato Gouko-like virus 1	Cx1773-10	3	1-678	7418 983*, 743* 1383	MN661032	insects
ssRNA(-)	*Bunyavirales*	*Phenuiviridae*	*Zheemosvirus*	Narangue virus	Mati1754-2	3	2-128	7394 1931 1492	MN661012 MN661013 MN661014	insects
ssRNA(-)	*Jingchuvirales*	*Chuviridae*	*Doliuvirus*	Atrato Chu-like virus 1	Cqvz1753-8	2	61-277	6700 4098	MN661024 MN661025	insects
ssRNA(-)	*Jingchuvirales*	*Chuviridae*	*Doliuvirus*	Atrato Chu-like virus 1	Cqvz1759-4	2	30-65	6940 2896*	MN661026 MN661027	insects
ssRNA(-)	*Jingchuvirales*	*Chuviridae*	*Atchuvirus*	Atrato Chu-like virus 2	An1771-2	2	2-4	6335 3856	MN661028 MN661029	insects
ssRNA(-)	*Jingchuvirales*	*Aliusviridae*	*Rachuvirus*	Atrato Chu-like virus 4	Wy1731-1	1?	2	7890* 2980*	MN661030 MN661031	insects
ssRNA(-)	*Jingchuvirales*	*Aliusviridae*	*Obscuruvirus*	Atrato Chu-like virus 5	Psal1739-1	1	2	13163	MN661033	insects
ssRNA(-)	*Mononegavirales*	*Rhabdoviridae*	*Atratorhabdovirus*	Atrato Rhabdo-like virus 2	Aese1749-7	1	61-83	7194* 3029* 2363*	MN661109 MN661110 MN661111	insects
ssRNA(-)	*Mononegavirales*	*Rhabdoviridae*	*Alphahymrhabdovirus*	Atrato Rhabdo-like virus 3	Cx1773-3	1	2	12076	MN661034	insects
ssRNA(+)	*Picornavirales*	*Iflaviridae*	*Adelivirus*	Atrato-Picorna-like virus	Mati1755-5	1	2	8422*	MN661041	insects
ssRNA(+)	*Picornavirales*	*Iflaviridae*	*Iflavirus*	Chajerado virus	Cx1773-7	1	8	9328	MN661038	insects
ssRNA(+)	*Picornavirales*	*Iflaviridae*	*Iflavirus*	Isla virus	Cx1773-5	1	13	9466	MN661039	insects
ssRNA(+)	*Picornavirales*	*Iflaviridae*	*Iflavirus*	Sabaleta virus	Cx1773-6	1	35	9358	MN661040	insects
ssRNA(+)	*Picornavirales*	*Marnaviridae*	*Unassigned*	Atrato Picorna-like virus 1	Psal1743-6	1	2	9404	MN661036	insects, plants
ssRNA(+)	*Picornavirales*	*Marnaviridae*	*Unassigned*	Atrato Picorna-like virus 1	An1771-1	1	5	9579	MN661035	insects, plants
ssRNA(+)	*Picornavirales*	*Polycipiviridae*	*Caosvirus*	Cacaos virus	Psal1743-3	1	60	12080	MN661037	insects

gcom[1]	Order	Family	Genus	Species	strain	Seqs	depth	bps[2]	Accession	host/vector
ssRNA(RT)	*Ortervirales*	*Metaviridae*	*Errantivirus*	Chibugado virus	Psal1744-4	1	2	6912	MN661043	plants, fungi, animals
ssRNA(RT)	*Ortervirales*	*Pseudoviridae*	*Hemivirus*	Atrato Retro-like virus	Anda1769-2	1	14	4307	MN661044	fungi, algae, insects
ssRNA(RT)	*Ortervirales*	*Pseudoviridae*	*Hemivirus*	Atrato Retro-like virus	Psal1734-4	1	17	4307	MN661042	fungi, algae, insects
ssRNA(+)	*Tymovirales*	*Tymoviridae*	*Gendovirus*	Gengado virus	Anda1734-1	1	1	5902	MN661045	plants, insects
ssRNA(+)	*Tymovirales*	*Tymoviridae*	*Mudovirus*	Murindó virus	Anda1750-5	1	3	5748	MN661046	
dsRNA	*Ghabrivirales*	*Chrysoviridae*	*Chrysovirus*[5]	Salado virus	Wy1731-12	4	57-31	3490 3166 2964 3063	MN661047 MN661048 MN661049 MN661050	fungus
ssRNA(+)	*Amarillovirales*	*Flaviviridae*	*Atratovirus*	Atrato Flavi-like virus 1	Mati1755-1	1	4	21837	MN661083	plants, insects
ssRNA(+)	*Amarillovirales*	*Flaviviridae*	*Flavivirus*	Atrato Flavi-like virus 2	Psal6	1	1-2	2516* 1771* 1129* 1062*	MT513089 MT513091 MT513088 MT513090	insects
ssRNA(+)	*Amarillovirales*	*Flaviviridae*	*Flavivirus*	Atrato Flavi-like virus 2	Psal16	1	1-4	4165* 2441* 1334* 902*	MT513092 MT513093 MT513094 MT5130955	insects
ssRNA(+)	*Amarillovirales*	*Flaviviridae*	*Flavivirus*	Atrato Flavi-like virus 3	Ae1	1	1-2	1299* 1174* 827* 566* 549*	MT513096 MT513097 MT513099 MT513100 MT513098	insects
ssDNA	*Piccovirales*	*Parvoviridae*	*Adelivirus*	Atrato Denso-like virus	Mati1755-12	1	5	4939	MN661135	insects
ssDNA	*Piccovirales*	*Parvoviridae*	*Adelivirus*	Atrato Denso-like virus 1	Psal1745-6	1	50	5315	MN661133	insects
ssDNA	*Piccovirales*	*Parvoviridae*	*Adelivirus*	Atrato Denso-like virus 1	Psal1747-3	1	77	5260	MN661134	insects
ssRNA(+)	*Amarillovirales*	*Flaviviridae*	*Atratovirus*	Atrato Flavi-like virus 1	Cx1773-1	1	1	20446	MN661082	plants, insects
ssRNA(+)	*Unassigned*	*Unassigned*	*Negevirus*	Atrato Nege-like virus 1	Cqvz1753-5	1	791	8872	MN661084	insects
dsRNA	*Durnavirales*	*Partitiviridae*	*Unassigned*	Atrato Partiti-like virus 1	Mati1738-218	2?	>518	1410* 978*	MN661051 MN661052	insects
dsRNA	*Durnavirales*	*Partitiviridae*	*Unassigned*	Atrato Partiti-like virus 1	Mati1755-173	4	23-52	1610* 1564 1432 1396*	MN661053 MN661054 MN661055 MN661056	insects
dsRNA	*Durnavirales*	*Partitiviridae*	*Unassigned*	Atrato Partiti-like virus 2	Anda1746-25	2	65-146	1657 1498	MN661057 MN661058	insects

gcom[1]	Order	Family	Genus	Species	strain	Seqs	depth	bps[2]	Accession	host/vector
dsRNA	*Durnavirales*	*Partitiviridae*	*Unassigned*	Atrato Partiti-like virus 3	Psal1745-39	2	>1084	1509 1293	MN661061 MN661062	insects
dsRNA	*Durnavirales*	*Partitiviridae*	*Unassigned*	Atrato Partiti-like virus 3	Anda1771-64	2	2-6	1201* 1155	MN661059 MN661060	insects
dsRNA	*Durnavirales*	*Partitiviridae*	*Unassigned*	Atrato Partiti-like virus 4	Cx1773-110	2	678	1379*	MN661063	insects
dsRNA	*Durnavirales*	*Partitiviridae*	*Unassigned*	Atrato Partiti-like virus 5	Mati1755-132	1?	3	1780	MN661064	insects
dsRNA	*Durnavirales*	*Partitiviridae*	*Atrapavirus*	Atrato Partiti-like virus 6	Psal1743-56	1?	41	1853	MN661065	protist
dsRNA	*Reovirales*	*Reoviridae*	*Relicuvirus*	Atrato Reo-like virus 1	Psal1733-6	11?	1-4	4240 3774 3113 2478 1978 1457 1322 1256 1200 1129 1003	MN661066 MN661067 MN661068 MN661069	plants, insects
dsRNA	*Reovirales*	*Reoviridae*[6]	*Relicuvirus*	Atrato Reo-like virus 1	Psal1761-8	11?	1-6	4266 3904 3116 2466 1971 1876 1453 1311 1143 1190 1013	MN661070 MN661071 MN661072 MN661073	plants, insects
ssRNA(+)	*Sobelivirales*	*Solemoviridae*	*Unassigned*	Atrato Sobemo-like virus 1	Mati1755-46	2	>336	2675 1522	MN661087 MN661088	plants, insects
ssRNA(+)	*Sobelivirales*	*Solemoviridae*	*Unassigned*	Atrato Sobemo-like virus 1	Aese1768-21	2	>760	2617 1531	MN661085 MN661086	plants, insects
ssRNA(+)	*Sobelivirales*	*Solemoviridae*	*Unassigned*	Atrato Sobemo-like virus 2	Cx1773-43	2	6-8	2647 1502	MN661089 MN661090	plants, insects
ssRNA(+)	*Sobelivirales*	*Solemoviridae*	*Unassigned*	Atrato Sobemo-like virus 3	Cqvz1753-66	2	1-3	2679 1376	MN661091 MN661092	plants, insects
ssRNA(+)	*Sobelivirales*	*Solemoviridae*	*Unassigned*	Atrato Sobemo-like virus 3	Cqvz1772-141	2	7-21	2767 1446	MN661093 MN661094	plants, insects
ssRNA(+)	*Sobelivirales*	*Solemoviridae*	*Unassigned*	Atrato Sobemo-like virus 4	Psal1758-7	2	31-40	2639 1370	MN661095 MN661096	plants, insects

gcom[1]	Order	Family	Genus	Species	strain	Seqs	depth	bps[2]	Accession	host/vector
ssRNA(+)	*Sobelivirales*	*Solemoviridae*	*Unassigned*	Atrato Sobemo-like virus 5	Cqvz1759-15	2	52-75	2925 1531	MN661107 MN661108	plants, insects
ssRNA(+)	*Sobelivirales*	*Solemoviridae*	*Unassigned*	Atrato Sobemo-like virus 5	Cqvz1772-143	2	1-2	2733 1577	MN661099 MN661100	plants, insects
ssRNA(+)	*Sobelivirales*	*Solemoviridae*	*Unassigned*	Atrato Sobemo-like virus 5	Cqvz1753-174	2	>164	1649 748*	MN661097 MN661098	plants, insects
ssRNA(+)	*Sobelivirales*	*Solemoviridae*	*Unassigned*	Atrato Sobemo-like virus 6	Wy1731-20	2?	46	3013	MN661101	plants, insects
ssRNA(+)	*Martellivirales*	*Virgaviridae*	*Unassigned*	Atrato Virga-like virus 1	Cx1773-8	1	15	9006	MN661103	plants, insects
ssRNA(+)	*Martellivirales*	*Virgaviridae*	*Unassigned*	Atrato Virga-like virus 2	Cqvz1772-6	1	76	8650	MN661106	plants, insects
ssRNA(+)	*Martellivirales*	*Virgaviridae*	*Unassigned,*	Atrato Virga-like virus 2	Cqvz1753-6	1	70	8775	MN661104	plants, insects
ssRNA(+)	*Martellivirales*	*Virgaviridae*	*Unassigned*	Atrato Virga-like virus 2	Cqvz1759-3	1	48	8822	MN661105	plants, insects
ssRNA(+)	*Martellivirales*	*Virgaviridae*	*Unassigned*	Atrato Virga-like virus 3	Mati1775-5	1	110	10309	MN661112	plants, insects
ssRNA(+)	*Martellivirales*	*Virgaviridae*	*Unassigned*	Atrato Virga-like virus 4	Psal1733-2	1	1	9638*	MN661113	plants, insects
ssRNA(+)	*Martellivirales*	*Virgaviridae*	*Unassigned*	Atrato Virga-like virus 4	Psal1757-2	1	7	10038	MN661114	plants, insects
ssRNA(+)	*Martellivirales*	*Virgaviridae*	*Unassigned*	Atrato Virga-like virus 4	Psal1761-1	1	7	10265	MN661115	plants, insects
ssRNA(+)	*Martellivirales*	*Virgaviridae*	*Unassigned*	Atrato Virga-like virus 5	Mati1738-4	1	720	7137*	MN661132	insects
ssRNA(+)	*Martellivirales*	*Virgaviridae*	*Unassigned*	Atrato Virga-like virus 5	Mati1755-3	1	380	10862	MN661116	insects
ssRNA(+)	*Martellivirales*	*Virgaviridae*	*Unassigned*	Atrato Virga-like virus 5	Mati1756-1	1	423	10891	MN661117	insects
ssRNA(+)	*Martellivirales*	*Virgaviridae*	*Unassigned*	Atrato Virga-like virus 6	Psal1741-2	1	9	9388*	MN661119	plants, insects
ssRNA(+)	*Martellivirales*	*Virgaviridae*	*Unassigned*	Atrato Virga-like virus 6	Ps1775-6	1	45	10224	MN661118	plants, insects
ssRNA(+)	*Martellivirales*	*Virgaviridae*	*Unassigned*	Atrato Virga-like virus 7	Psal1744-1	1	73	10263	MN661122	plants, insects
ssRNA(+)	*Martellivirales*	*Virgaviridae*	*Unassigned*	Atrato Virga-like virus 7	Anda1746-1	1	2	10266	MN661120	plants, insects
ssRNA(+)	*Martellivirales*	*Virgaviridae*	*Unassigned*	Atrato Virga-like virus 7	Psal1757-1	1	32	10268	MN661128	plants, insects
ssRNA(+)	*Martellivirales*	*Virgaviridae*	*Unassigned*	Atrato Virga-like virus 7	Psal1748-2	1	2	9906	MN661126	plants, insects
ssRNA(+)	*Martellivirales*	*Virgaviridae*	*Unassigned*	Atrato Virga-like virus 7	Psal1751-1	1	2	10312	MN661127	plants, insects

gcom[1]	Order	Family	Genus	Species	strain	Seqs	depth	bps[2]	Accession	host/vector
ssRNA(+)	*Martellivirales*	*Virgaviridae*	*Unassigned*	Atrato Virga-like virus 7	Psal1739-2	1	1	10258	MN661121	plants, insects
ssRNA(+)	*Martellivirales*	*Virgaviridae*	*Unassigned*	Atrato Virga-like virus 7	Psal1747-1	1	10	10275	MN661125	plants, insects
ssRNA(+)	*Martellivirales*	*Virgaviridae*	*Unassigned*	Atrato Virga-like virus 7	Psal1745-3	1	23-30	8138* 1853*	MN661123 MN661124	plants, insects
ssRNA(+)	*Martellivirales*	*Virgaviridae*	*Unassigned*	Atrato Virga-like virus 7	Psal1761-6	1	4-12	4992* 2744* 1885* 524*		plants, insects
ssRNA(+)	*Martellivirales*	*Virgaviridae*	*Unassigned*	Atrato Virga-like virus 7	Psal1736-1	1	1-3	7684*		plants, insects
ssRNA(+)	*Martellivirales*	*Virgaviridae*	*Unassigned*	Atrato Virga-like virus 7	Psal1766-2	1	62	10342	MN661129	plants, insects
ssRNA(+)	*Martellivirales*	*Virgaviridae*	*Unassigned*	Atrato Virga-like virus 7	Psal1772-3	1	11	10267	MN661130	plants, insects
dsRNA	*Ghabrivirales*	*Totiviridae*	*Muratovirus*	Atrato virus	Mati-1738-6	1	25	5586	MN661074	insects
dsRNA	*Ghabrivirales*	*Totiviridae*	*Muratovirus*	Atrato virus	Mati1756-3	1	7	7119	MN661075	insects
ssRNA(+)	*Tolivirales*	*Tolicuviridae*	*Unassigned*	Hubei mosquito virus 4	Cx1773-22	1	45	4805*	MN661131	insects
dsRNA	*Ghabrivirales*	*Totiviridae*	*Totcuvirus*	Embera virus	Wy1731-4	1	11	6158	MN661076	insects
ssRNA(+)	*Martellivirales*	*Virgaviridae*	*Unassigned*	Hubei Virga-like virus 2	Cx1773-4	1	14	10309	MN661102	insects
dsRNA	*Ghabrivirales*	*Totiviridae*	*Muratovirus*	Murri virus	Cqvz1772-8	1	18	6973	MN661077	insects
dsRNA	*Ghabrivirales*	*Totiviridae*	*Totcuvirus*	Pisingos virus	Psfu1741-8	1	20	5998*	MN661081	insects
dsRNA	*Ghabrivirales*	*Totiviridae*	*Totcuvirus*	Pisingos virus	Cqly1752-1.2	1	10	6094	MN661079	insects
dsRNA	*Ghabrivirales*	*Totiviridae*	*Totcuvirus*	Pisingos virus	Cqly1752-1.1	1	10	6000	MN661078	insects
dsRNA	*Ghabrivirales*	*Totiviridae*	*Totcuvirus*	Pisingos virus	Ps1775-23	1	14	3818	MN661080	insects

6.2.2.4 *Taxonomies, phylogenies, and genome structures of novel viruses from mosquitoes*

A total of 52 viruses were discovered in the mosquito pools and assigned to a taxonomic level. Based on the information acquired by NCBI blastx, Conserved Domain Search, HMM-HMM comparison, and ORF finder the genomes were annotated. Most of the genomes could be annotated completely. Then, a phylogenetic analysis based on the AA sequence of the CDS of the polymerase coding genome segment was promoted. There are no established universal criteria for the novelty of viruses. For each taxon distinguishing criteria are different and normally include sequence identity, phylogenetic analyses, and host restriction among others. In this study, the novelty was determined by sequence identity and clustering in the phylogenetic analyses based on the AA sequence of the polymerase coding regions.

6.2.2.4.1 Negative-stranded RNA viruses

To date, the phylum *Negarnaviricota* consists of two subphyla, six classes, seven orders, 29 families, 131 genera, and 779 species (https://ictv.global/taxonomy/). Negative strand RNA viruses descend from dsRNA viruses and are closely related to arthropods. Two subphyla are distinguished: The *Haploviricotina* which are mostly unsegmented and the *Polyploviricotina* which are segmented. Novel viruses found in this study belong to the orders *Mononegavirales*, *Jingchuvirales,* and *Bunyavirales*.

6.2.2.4.1.1 *Bunyavirales*

Bunyaviruses are segmented, negative-strand RNA viruses with a bipartite or tripartite genome consisting of the L-segment that encodes the RdRp, the M-segment that encodes the glycoproteins (Glys), and the S-segment that codes for the nucleocapsid protein (N). A total of five virus species belonging to the order *Bunyavirales* could be detected in the mosquito pools, of which three were assigned to the family *Phasmaviridae*, and two to the family *Phenuiviridae* by RdRp phylogeny (Figure 41 and Figure 42).

6.2.2.4.1.1.1 *Phasmaviridae*

The *Phasmaviruses* form an insect-associated clade in the order *Bunyavirales*. Three virus species of the family *Phasmaviridae* were found in *Culex* and *Mansonia* mosquitoes. Two were assigned to the genus *Orthophasmavirus* and one to the genus *Feravirus* by phylogeny based on the AA sequences of the L-segment and their genome structures (Figure 15). A novel *Orthophasmavirus* that was exclusively detected in *Ma. titillans* pools was named Coredo virus[2]. In addition, a strain of a formerly described *Orthophasmavirus* species, Culex-phasma-like virus (Cx1773-14 strain) was detected in *Cx. quinquefasciatus* and *Cx. spp.* pools. All three segments showed 99% AA sequence similarity to Culex-

---

[2] Referring to Coredó, a village in the research region.

phasma-like virus (ASA47278.1, ANW72255.1, ANW72255.1)/ (Shi et al. 2017). A novel virus species of the genus *Feravirus* was detected in *Ma. titillans* and named Guagua virus[3]. All three segments showed 68-70% AA sequence similarity with Ferak virus genome segments (KP710246.1, KP710268.1, KP710266.1).

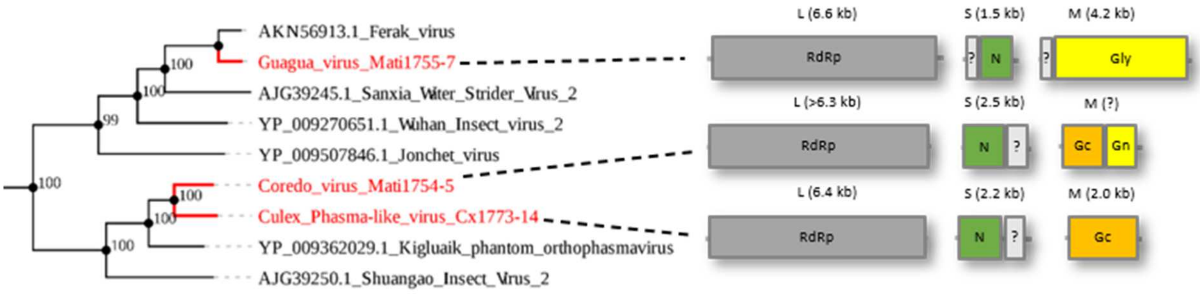


Figure 15: ML tree based on the AA sequence of the RdRp of members of the family Phasmaviridae. The names of the viruses discovered in this study are in red and their genome structures are shown on the right. See Figure 43, appendix 11.6.1.1.1 for the fully labeled Phasmaviridae phylogenetic tree.

6.2.2.4.1.1.2 Phenuiviridae

The *Phenuiviruses* form another clade of the order *Bunyavirales*. Two undescribed viruses of the family *Phenuiviridae* were detected in *Culex* and *Mansonia* mosquitoes and named Narangue virus[4] and Atrato Gouko-like virus 1[5]. These novel viruses are in the same clade as Mothra virus, the first species described of the genus *Mobuvirus* but the AA sequences of the CDS of the L-segment show very low similarity. RdRp phylogeny branching patterns (Figure 16) and low AA sequence similarities indicate, that Narangue virus and Atrato Gouko-like virus 1 and its close relatives belong to a new, yet unassigned genus we designated *Zheemovirus* [6].

Figure 16: ML tree based on the AA sequence of the RdRp of members of the family Phenuiviridae. The names of the viruses discovered in this study are in red and their genome structures are shown on the right. See Figure 43, appendix 11.6.1.1.2 for the fully labeled Phenuiviridae phylogenetic tree.

---

[3] Referring to Guagua, a village in the research region.
[4] Referring to Ñarangue, a village in the research region.
[5] Referring to the Atrato River and the genus Goukovirus.
[6] Zheemovirus: Referring to **Zhee mos**quito virus the first described genus member.

6.2.2.4.1.2 Mononegavirales and Jingchuvirales

The orders *Mononegavirales* and *Jingchuvirales* group together in the class *Monjiviricetes*. Whereas the *Mononegavirales* typically have linear and nonsegmented genomes, members of the recently established order *Jingchuvirales* have a variable genome organization including nonsegmented and segmented, linear, or circular genomes (Figure 82), (Virus Taxonomy: 2018b Release EC 50, Washington, DC, July 2018). A total of four virus species belonging to the order *Jingchuvirales* and two species belonging to the order *Mononegavirales* could be detected in the mosquito pools. For phylogenetic trees of the orders *Mononegavirales* and *Jingchuvirales* see Figure 45 and *Figure 46*.

6.2.2.4.1.2.1 Jingchuvirales

By now, the *Jingchuvirales* compromise five families and 19 genera. Two novel species of the family *Chuviridae and two of the family Aliusviridae* were detected. They were named Atrato Chu-like virus 1, 2, 4, and 5[7]. The closest known relative of Atrato Chu-like virus 1 is Chuvirus Mos8Chu0 (Figure 17) sharing 59,3% AA identity and 99% query cover with the RdRp CDS of the L-segment (API61887.1) and 60,6% AA identity and 96% query cover with the Gly CDS located on the S-segment (API61888.1). Atrato Chu-like virus 2, 4, and 5 show low AA sequence similarity with any known virus and could not

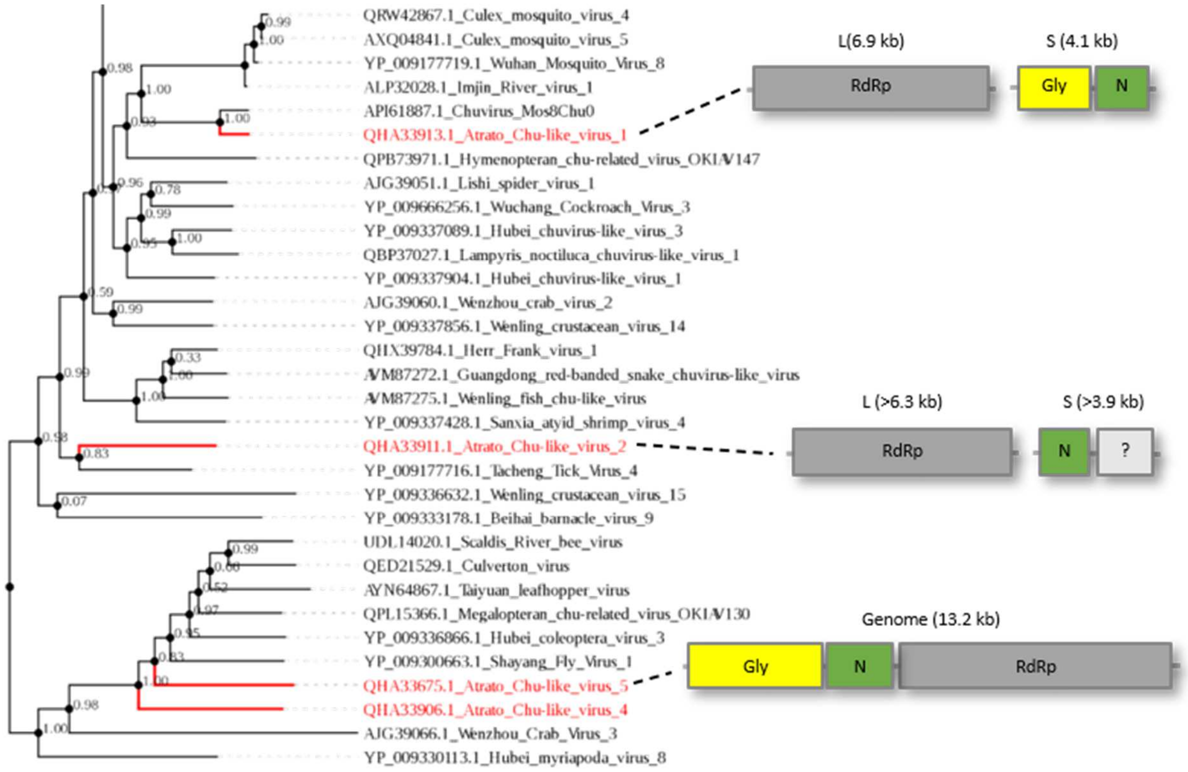


Figure 17: ML tree based on the AA sequence of the RdRp of members of the order Jingchuvirales.
The names of the viruses discovered in this study are in red and their genome structures are shown on the right. See Figure 47, appendix 11.6.1.2.1 for the fully labeled Jingchuvirales phylogenetic tree.

---

[7] Referring to the **Atrato** River and the family ***Chu**viridae*.

be assigned to any existing genus based on RdRp phylogeny. Therefore novel genera were proposed. Atrato Chu-like virus 2 (AClv2) is in the same clade as Tacheng Tick virus 4 but the RdRp AA sequences show only 31.4% similarity (Table 12). Therefore, AClv2 composes the first species of a yet unassigned genus we refer to as *Atchuvirus*[8]. *Jingchuvirales* RdRp phylogenetic tree (Figure 17) shows that Atrato Chu-like virus 4 and 5 respectively form completely new clades that give rise to two new genera. Thus, Atrato Chu-like virus 4 is considered the first known species of the genus designated *Rachuvirus*[9] , and Atrato Chu-like virus 5 of the genus designated *Oscuruvirus* (Di Paola et al. 2020).

6.2.2.4.1.2.2 Rhabdoviridae

Concerning the order of *Mononegavirales*, two novel members of the family *Rhabdoviridae* have been detected in the mosquito samples. *Rhabdoviruses* normally carry a linear monopartite, negative-strand RNA genome with at least 5 ORFs coding an L-protein (RdRP) an N, a phosphoprotein (P), and a matrix protein (M). The new family members were designated Atrato Rhabdo-like virus 2 and Atrato Rhabdo-like virus 3[10] and were derived from an *Oc. serratus* pool and a *Culex* pool respectively. Phylogenetic analysis based on the AA sequence of the L-segment (Figure 38) supports the inclusion of these novel viruses in the family *Rhabdoviridae* and the creation of two new genera. Both viruses resemble the family characteristic genome organization (N-P-M-G-L), but Atrato Rhabdo-like virus 2 has an additional ORF probably coding for an additional protein (Figure 18). Atrato Rhabdo-like virus 2 was assigned to the proposed genus *Atratorhabdovirus*[11].

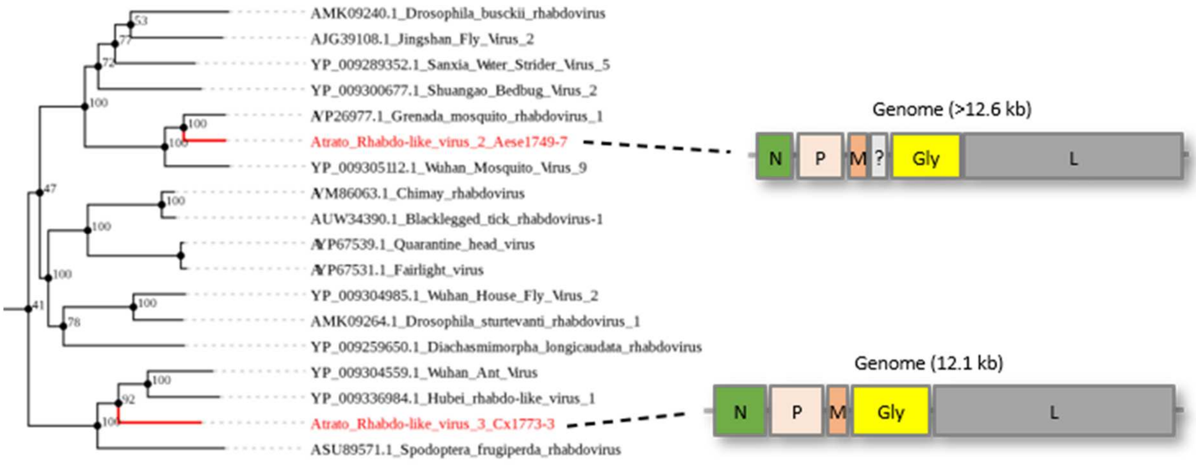


Figure 18: ML tree based on the AA sequence of the L-segment of members of the family Rhabdoviridae.
The names of the viruses discovered in this study are in red and their genome structures are shown on the right. See Figure 38, chapter 7.5.10 for the fully labeled Rhabdoviridae phylogenetic tree and 11.6.1.2., Figure 45 and Figure 46 *for phylogeny of the order Mononegavirales.*

---

[8] Atchuvirus: from the type species **At**rato **Chu**-like virus 2

[9] Rachuvirus: from type species Atr**a**to Chu-like virus 4

[10] Atrato Rhabdo-like virus 1 and 2: Referring to the **Atrato** River and the family **Rhabdo**viridae.

[11] ***Atratorhabdo****virus (genus)*: From **Atrato Rhabdo**-like virus 3.

6.2.2.4.2 Positive-stranded RNA viruses

Positive-strand RNA viruses are divided into three phyla: *Kitrinoviricota*, *Lenarviricota*, and *Pisuviricota* and form the kingdom *Orthornavirae* in the realm *Riboviria*. Viruses found in this study belong to the phyla *Kitrinoviricota* and *Pisuviricota.* The phylum *Kitrinoviricota* compromises four classes, six orders, 22 families, five subfamilies, 83 genera, 679 species, and the phylum *Pisuviricota* has three classes, six orders, eight suborders, 32 families, 26 subfamilies, 172 genera, 68 subgenera, and 835 species (Walker et al. 2020). Viruses found in this study belong to the orders *Tymovirales*, *Martellivirales*, *Picornavirales*, *Tolivirales*, *Amarillovirales,* and *Sobelivirales*.

6.2.2.4.2.1 *Tymovirales*

Viruses of the order *Tymovirales* mostly infect plants and have a monopartite linear positive sense RNA genome coding a replication-associated polyprotein (Mt-P-Hel-RdRp) and a capsid protein (CP). Currently, five families, two subfamilies, 25 genera, and 209 species are accepted (King 2011, Walker et al. 2020). Viruses found in this study were assigned to the family *Tymoviridae* by phylogeny based on the polyprotein AA sequence (Figure 48, Figure 49).

6.2.2.4.2.1.1 Tymoviridae

Two novel members of the family *Tymoviridae* were encountered in *An. darlingi* mosquitoes and named Murindo virus[12] and Gengado virus[13]. Both viruses resemble the characteristic genome structure of the order *Tymovirales* (Mt-P-Hel-RdRp), (Figure 19), but share only little AA sequence similarity with any known *Tymoviruses* and each other. Polyprotein AA sequence phylogeny shows that they form completely new clades and therefore should be the first species of two new genera designated *Mudovirus*[14] and *Gendovirus*[15] (Figure 19).

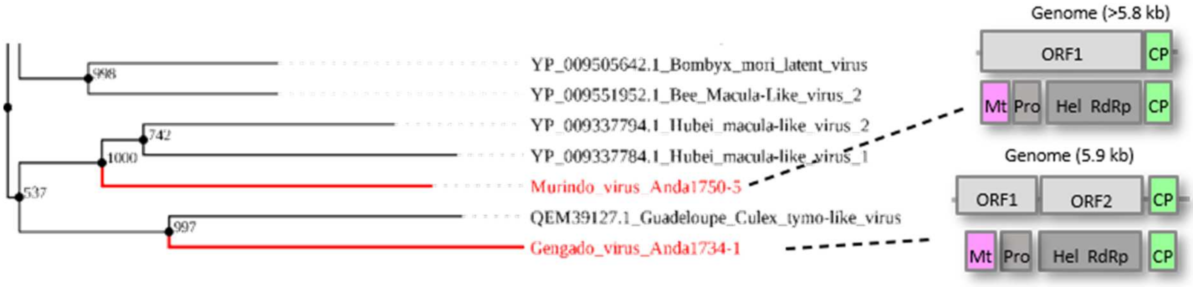


Figure 19: ML tree based on the AA sequence of the RdRp containing polyprotein of members of the family Tymoviridae. The names of the viruses discovered in this study are in red and their genome structures are shown on the right. See Figure 50, appendix 11.6.1.3.1 for the fully labeled phylogenetic tree of the family Tymoviridae, and Figure 48 and Figure 49 for the phylogeny of the orders Hepevirales, Martellivirales and Tymovirales.

---

[12] Murindo virus: From Murindó a municipality in the department of Antioquia, Colombia (Figure 4).

[13] Gengado virus From Gengadó an indigenous village (Figure 4).

[14] Mudovirus: From **Mu**rin**do** virus, type species of a novel genus.

[15] Gendovirus (genus): From **Gen**ga**do** virus, type species of a novel genus. Gengado is an indigenous village.

6.2.2.4.2.2 Martellivirales

The order *Martellivirales* to date consists of seven families, 25 genera, and 222 species of plant-related viruses (Walker et al. 2020). A total of eight Virga-like virus species could be distinguished in the mosquito samples, of which seven were new and named Atrato Virga-like virus (AVlv) 1-7[16] , and one has been described formerly (Hubei Virga-like virus 2). AVlv1 was detected in *Culex spp*., AVlv2 in *Cq. venezuelensis*, AVlv3 in *Ma. titillans*, AVlv4 in *Ps. albipes*, AVlv5 in *Ma. titillans*, AVlv6 in *Ps. funiculus* and AVlv7 in *An. darlingi*, *Cq. venezuelensis* and *Ps. albipes* pools. None of the detected viruses were classified for the assigned *Virgaviridae* genera. They all cluster on different branches of unassigned

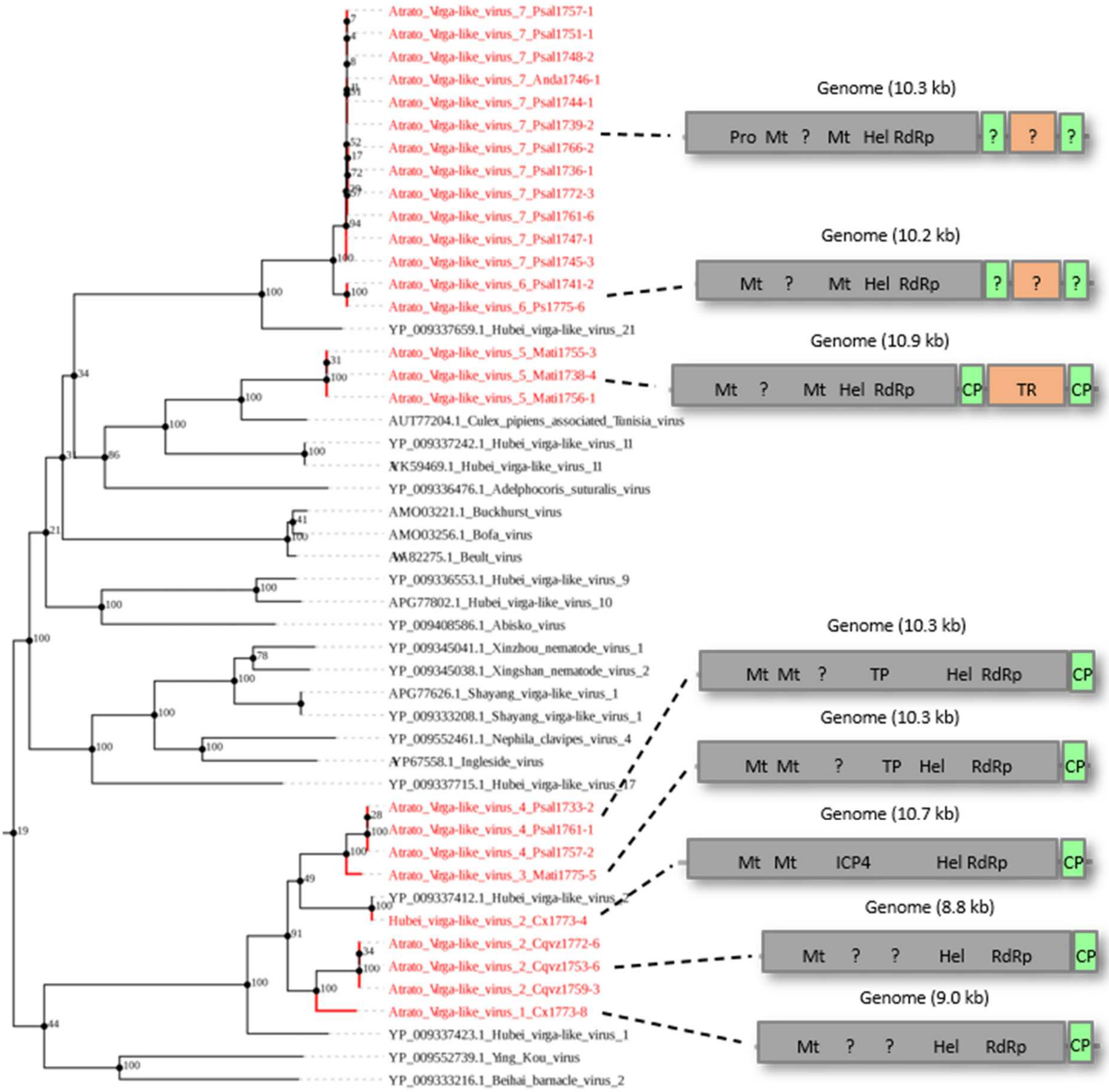


Figure 20: ML tree based on the AA sequence of the RdRp containing polyprotein of members of the family Virgaviridae. The names of the viruses discovered in this study are in red and their genome structures are shown on the right. See Figure 51 and Figure 52, appendix 11.6.1.3.2 for the fully labeled phylogenetic tree of the family Virgaviridae and Virga-like viruses.

---

[16] Atrato Virga-like virus: Referring to the **Atrato** river and the family **Virga**viridae

clades that incorporate several unassigned genera that might be incorporated into the family *Virgaviridae* or form yet unassigned families. Atrato Virga-like virus 1-4 cluster on the same glade with Hubei Virga-like virus 2 (HVLV2) sharing 52-56% AA identity on the RdRp coding ORF. AVlv 5 is on the same clade as *Culex pipiens* associated Tunisia virus sharing 41% AA sequence identity with the RdRp coding ORF, and AVlv 6 and 7 cluster with Hubei Virga-like virus 21 sharing 41% identity for the RdRp coding ORF (Table 12) Branching patterns and genome organizations (Figure 20) give evidence that the Virga-like viruses found in this study belong to three yet unassigned genera with the relatives just mentioned being the first discovered species.

6.2.2.4.2.3 Picornavirales

The order *Picornavirales* at present contains eight families, 98 genera, three subgenera, and 312 virus species with vertebrate, invertebrate, plant, and protist hosts. Their monopartite or bipartite linear positive-strand RNA genomes have a conserved sequence domain (Hel-Pro-Pol) in common (King 2011, Walker et al. 2020). A total of six new unsegmented virus genomes belonging to the order *Picornavirales* could be sequenced from the mosquito pools, of which four were assigned to the family *Iflaviridae*, one to the family *Polycipiviridae,* and one to the family *Marnaviridae* based on phylogeny of the polyprotein AA sequence (Figure 53, Figure 54).

6.2.2.4.2.3.1 Iflaviridae

The four new members of the family *Iflaviridae* have a non-segmented genome with a single ORF coding for a polyprotein (VPs-Hel-Pro-Pol) as characteristic of the family (Valles et al. 2017), (Figure 85). Isla virus, Chajerado virus, and Sabaleta virus[17] were detected in *Culex* mosquitos and are close relatives. Their closest known relatives based on the phylogeny of the polyprotein AA sequence are Bee Iflavirus 1 and Wuhan Insect Virus 13 (Figure 21). Atrato Picorna-like virus[18] was found in *Ma. titillans* mosquitoes and clusters on another clade with Hubei Picorna-like Virus 41. The family *Iflaviridae* was created in 2008 (Virus Taxonomy: 2008 Release EC 40, Istanbul, August 2008) and the only genus assigned until now is *Iflavirus*. Soon new genera will be assigned based on genome organization, branching pattern, and host range.

---

[17] **Isla**virus, **Chajerado**virus, **Sabaleta**virus: Referring to the villages **Isla**, **Chajeradó** & **Sabaleta** (Figure 4).

[18] Atrato Picorna-like virus: Referring to the **Atrato** River and the order **Picorna**virales (Figure 4).

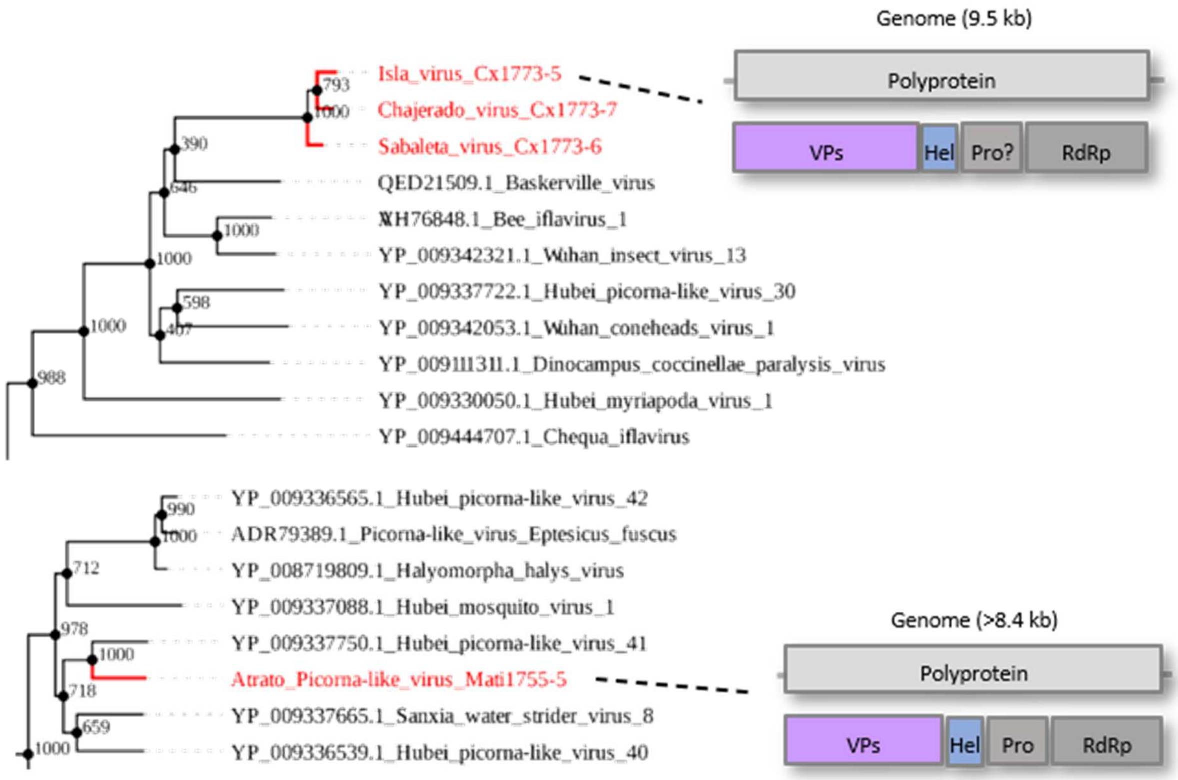


Figure 21: ML trees based on the AA sequence of the RdRp containing polyprotein of members of the family Iflaviridae. The names of the viruses discovered in this study are in red and their genome structures are shown on the right. See Figure 55, appendix 11.6.1.4.1 for the fully labeled phylogenetic tree of the family Iflaviridae.

6.2.2.4.2.3.2 *Polycipiviridae*

Members of the family *Polycipiviridae* conserve the Hel-Pro-Pol sequence domain typical for viruses of the order Picornavirales but have a polycistronic, non-segmented, linear, positive-sense RNA genome with at least five ORFs (Olendraite et al. 2019). One new member of the family *Polycipiviridae* could be

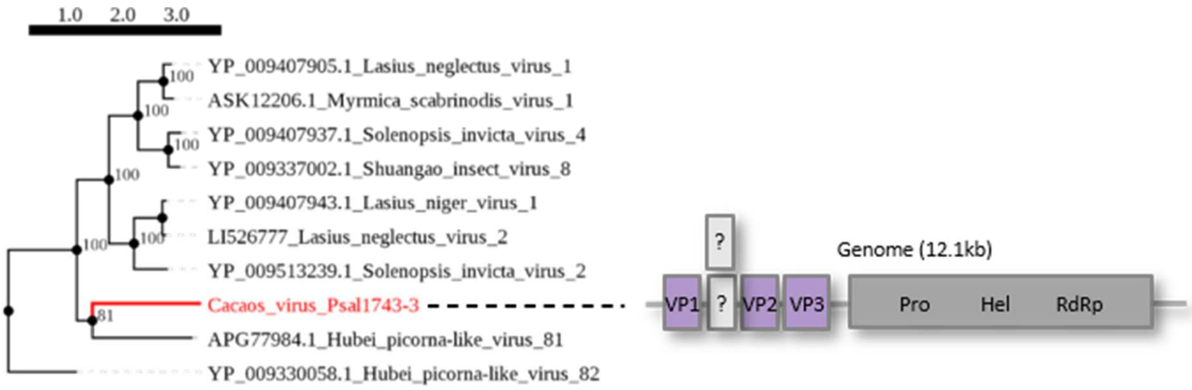


Figure 22: ML tree based on the AA sequence of the RdRp containing polyprotein of members of the family Polycipiviridae. The names of the viruses discovered in this study are in red and their genome structures are shown on the right. See Figure 56, appendix 11.6.1.4.2 for the fully labeled phylogenetic tree of the family Polycipiviridae.

detected in *Ps. albipes* and was called Cacaos virus[19]. Its genome structure is similar to viruses of the genus *Sopolyvirus* but its RdRp coding ORF shows only 31,2% similarity and 21% query cover with Solenopsis invicta virus 2 (AXL96895.1). Low AA sequence similarity and phylogenetic analysis based on the polyprotein AA sequence indicate, that Cacaos virus composes a species of a new genus we refer to as *Caosvirus*[20] (Figure 22).

6.2.2.4.2.3.3 Marnaviridae-like viruses

The members of the family *Marnaviridae* conserve the three-domain replication block (Hel-Pro-Pol) characteristic for the order *Picornavirales* but differ in genome organization as the nonstructural protein domains are located at the 3´ and the structural proteins at the 5´ end. The monopartite genome is approximately 8 to 10 kbs and codes for one or two ORFs (King 2011). One undescribed and unassigned virus species of the order *Picornavirales* was found in *Anopheles* and *Ps. albipes* mosquitoes and named Atrato Picorna-like virus 1[21] (Figure 23). It shares high AA sequence similarity with the CDSs of Zhejiang mosquito virus 1 (APG76656.1, AA 82%) and Yongsan picorna-like virus 2 (AXV43882.1, AA 90%), (Table 12). Traditionally, the *Marnaviruses* were associated with marine protists, but recently several insect-related Marna-like viruses have been described (Shi et al. 2016a). Phylogenetic analysis based on the AA sequence of the RdRp coding ORF shows, that these viruses share a common ancestor (Figure 53, Figure 54). Despite low AA similarity between the members of the two clades we propose to include these novel arthropod-derived viruses into the family *Marnaviridae*, as they are in the same clade and share the family typical genome structure. Furthermore, we propose the creation of two subfamilies that are supported by RdRp phylogeny and the source the viruses were derived from. The

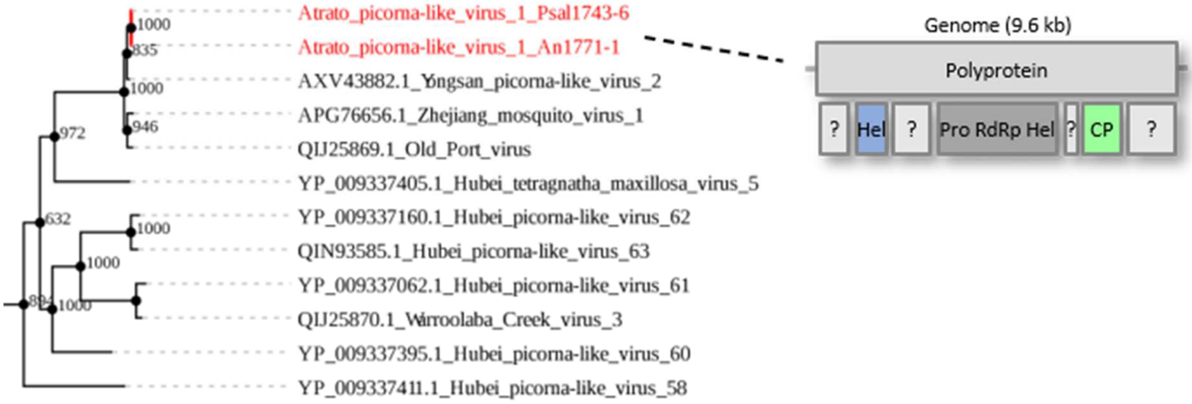


Figure 23: ML tree based on the AA sequence of the RdRp containing polyprotein of members of the family Marnavirirdae. The names of the viruses discovered in this study are in red and their genome structure is shown on the right. See Figure 57, appendix 11.6.1.4.3 for the fully labeled phylogenetic tree of the family Marnaviridae and Marna-like viruses.

---

[19] **Cacaos**virus: **Cacaos** is a settlement near the municipality of Mutatá (Figure 4).

[20] **Caos**virus (genus): Referring to the type species **Ca**ca**os** virus.

[21] **Atrato Picorna**-like virus 1: Referring to the **Atrato** River and the order **Picorna**virales.

classic *Marnaviridae* viruses detected in algae and diatoms might be assigned to the subfamily *Marnavirinae*[22] and the ones detected in arthropods to the subfamily *Athrovirinae*[23] (Figure 54).

6.2.2.4.2.4 *Tolivirales*

The order *Tolivirales* consists of three families, three subfamilies, 20 genera, and 128 accepted species that contain a monopartite or bipartite, linear, positive sense, ssRNA genome that codes for a conserved RdRp (Walker et al. 2020). A strain of Hubei mosquito virus 4, an unassigned formerly described Tombus-like virus was found in *Cx. nigripalpus*. All the viruses that cluster with Hubei mosquito virus 4 on a yet unassigned clade were derived from mosquitoes. Even though these viruses resemble the *Tombusviridae* genome structure, they show little concordance in the RdRp AA sequence with any assigned taxa and due to highly supported RdRp phylogeny and adaption to *Culicidae* hosts should conform to a new family we refer to as *Tolicuviridae*[24] (Figure 24).

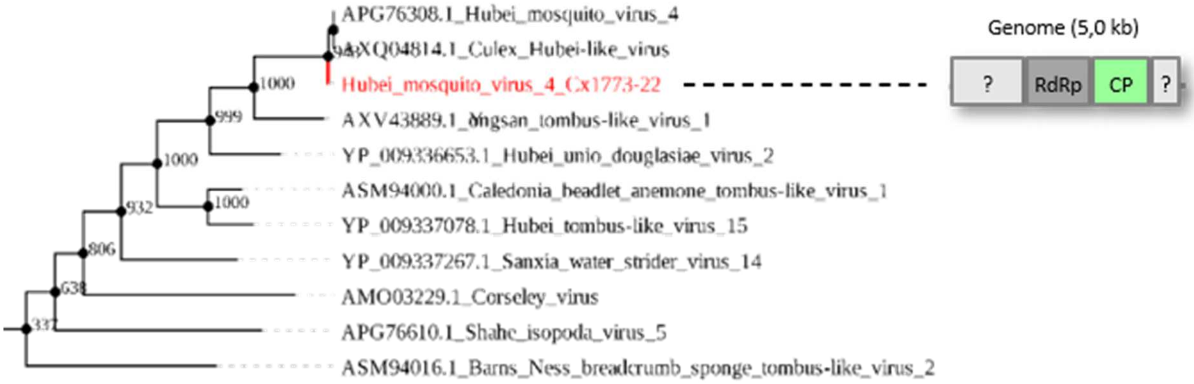


Figure 24: ML tree based on the AA sequence of the RdRp of members of the order Tolivirales.
The names of the viruses discovered in this study are in red and their genome structure is shown on the right. See Figure 58, Figure 59, and Figure 60, appendix 11.6.1.5 for the fully labeled phylogenetic trees of the orders Nadamuvirales and Tolivirales.

6.2.2.4.2.5 *Amarillovirales*

The order *Amarillovirales* includes one family, four genera, and 89 species (Walker et al. 2020). The family *Flaviviridae* is the only assigned family of the order. Typically *Flaviviruses* have a 9-13 Kb unipartite genome and many of the members of the family are arthropod-borne and a threat to human and veterinary health (Simmonds et al. 2017).

---

[22] ***Marna****virinae* (subfamily): Referring to the family **Marna**viridae, Marna Latin for sea.
[23] ***Athro****virinae* (subfamily): Referring to the **arthr**opods the viruses were derived from.
[24] ***Tolicu****viridae (family)*: From **To**mbusviridae- **li**ke **Cu**licidae affiliated viruses.

6.2.2.4.2.5.1 *Flaviviridae*

Three new viruses of the family *Flaviviridae* were found in the mosquitoes. Atrato Flavi-like virus 1[25] (AFlv1) was detected in a *Ma. titillans* and a *Culex* pool, Atrato Flavi-like virus 2 (AFlv2) in *Ps. albipes,* and Atrato Flavi-like virus 3 (AFlv3) in *Oc. serratus*. RdRp phylogeny shows, that AFlv1 shares a common ancestor with viruses of the genus *Flavivirus*, but it clusters on another branch with recently discovered and unassigned viruses that differ from typical *Flaviviruses* in genome length, genome structure, and host association. AFlv1 shares 26,5% percent AA sequence identity over 35% query cover with Lampyris noctiluca flavivirus 1. Due to the low AA similarity with other known viruses, phylogeny, and genome structure AFlv1 composes the first species of a new genus we refer to as *Atratovirus*[26]*.* AFlv2 and AFlv3 cluster with viruses from a recently discovered insect-specific glade of the genus Flavivirus (Figure 25).

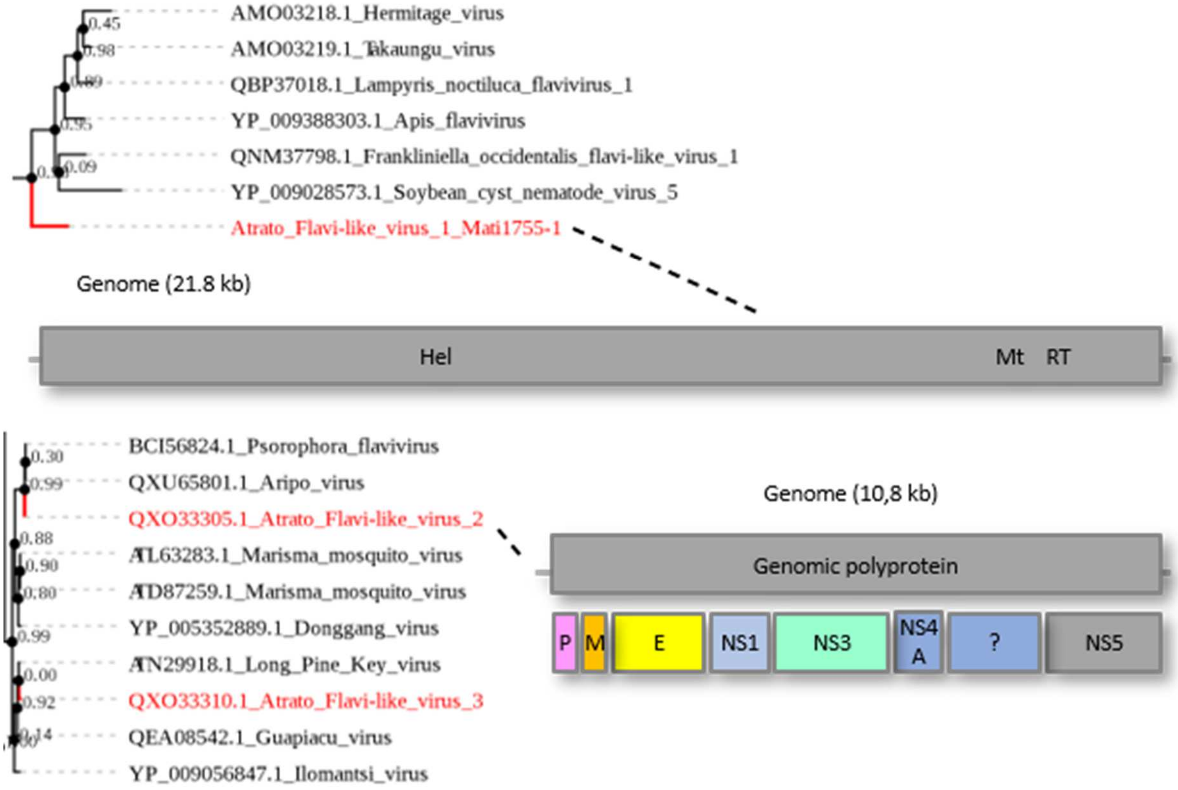


Figure 25: ML tree based on the AA sequence of the RdRp of members of the order Amarillovirales.
The names of the viruses discovered in this study are in red and their genome structure is shown. See Figure 61 and Figure 62, appendix 11.6.1.6 for the fully labeled phylogenetic trees of the order Amarillovirales.

---

[25] **Atrato Flavi**-like viurs 1: Referring to the **Atrato** River and the family **Flavi**viridae.

[26] **Atrato**virus (genus): From type species **Atrato** Flavi-like virus 1.

6.2.2.4.2.6 *Sobelivirales*

The order *Sobelivirales* is composed of three families, six genera, and 61 species (Walker et al. 2020). Six novel, unassigned viruses of the order *Sobelivirales* could be identified in the mosquito samples and were named Atrato Sobemo-like virus 1-6. Together with a variety of unassigned recently discovered viruses they form at least two main new clades that should give rise to several new genera and/or families (Figure 63, Figure 64).

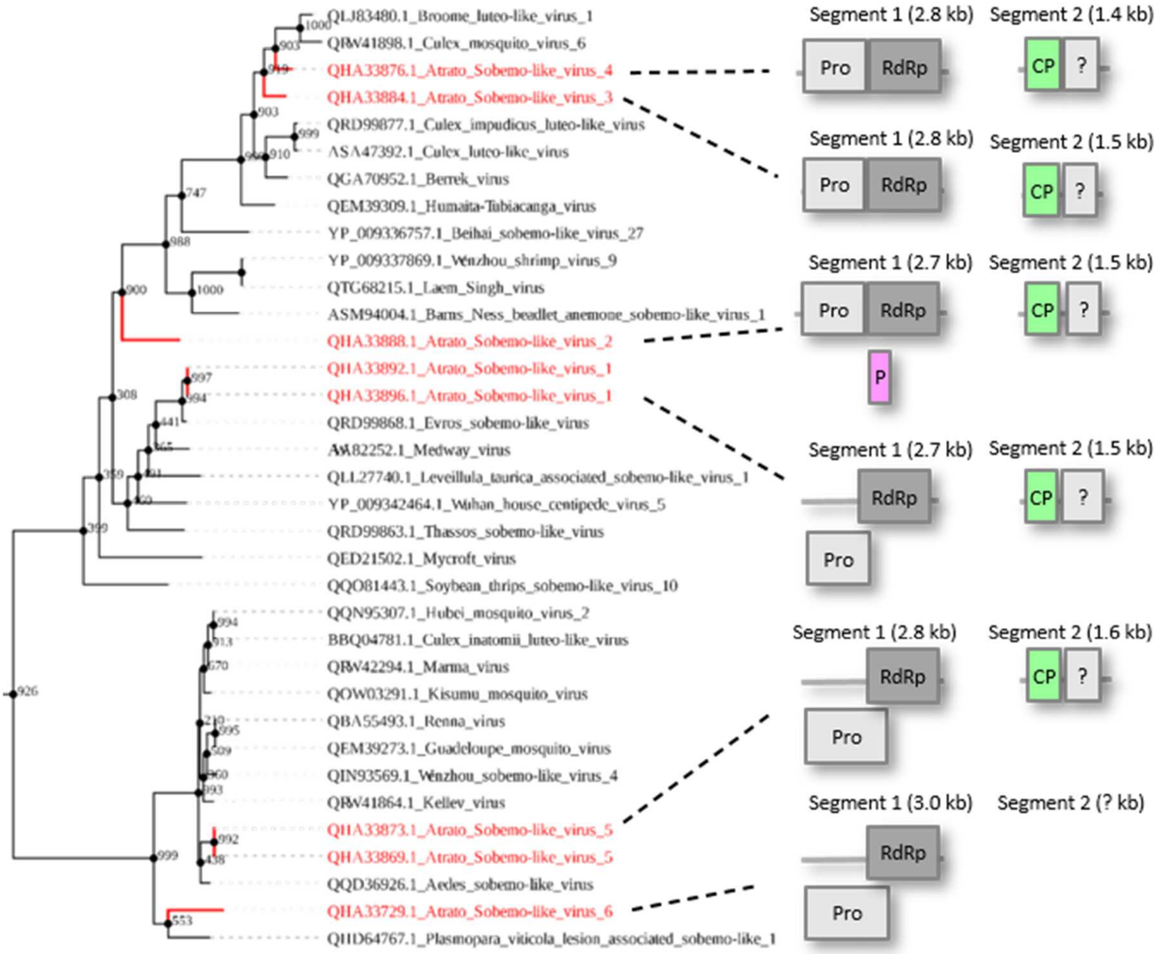


Figure 26: ML tree based on the AA sequence of the RdRp of members of the order Sobelivirales. The names of the viruses discovered in this study are in red and their genome structure is shown on the right. See Figure 63 and Figure 64, appendix 11.6.1.7 for the fully labeled phylogenetic trees of the order Amarillovirales.

6.2.2.4.3 Double-stranded RNA viruses

6.2.2.4.3.1 *Durnavirales*

The order *Durnavirales* comprises five families, ten genera, and 85 species (Walker et al. 2020). A total of six undescribed and unassigned Partiti-like viruses with a bipartite, monocistronic, linear genome coding an RdRp and a CP were spotted in the mosquitoes. They were named Atrato Partiti-like virus (APlv) 1-6. APlv 1-5 and are on an unassigned clade that consists of several subclades of recently discovered insect-associated viruses that might compose new genera of the family *Partitiviridae* (Figure 27). Blast search for APlv 6 resulted in low AA similarity with Hubei Partiti-like virus 20 (query cover: 71%/ identity: 27.0%) and RdRp phylogeny branching pattern showed that it forms a sister clade to the genus *Cryspovirus*. Due to low identity with other described viruses and RdRp phylogeny branching, we propose to establish a new genus in the family *Partitiviridae* we named *Atrapavirus*[27].

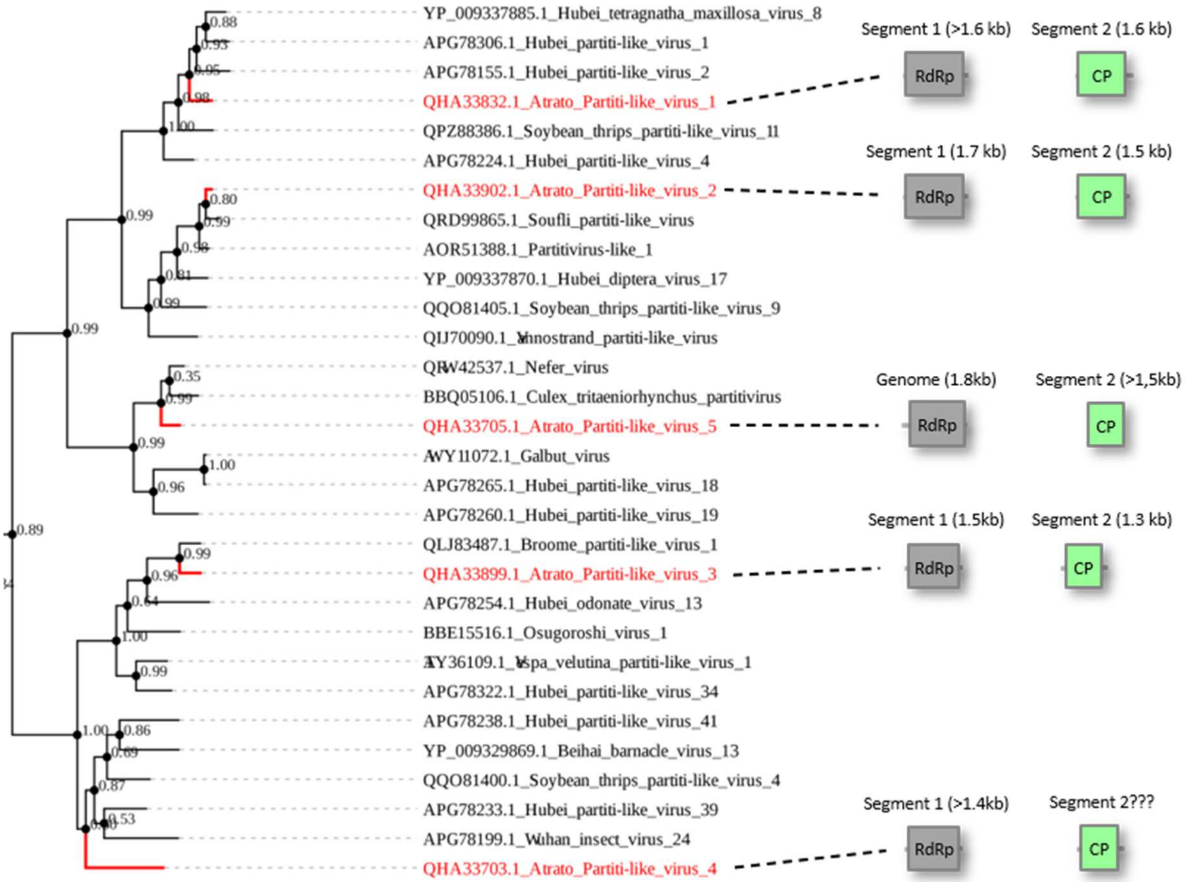


Figure 27: ML tree based on the AA sequence of the RdRp of members of the order Durnavirales.
The names of the viruses discovered in this study are in red and their genome structure is shown on the right. See Figure 65 and Figure 66, appendix 11.6.1.8 for the fully labeled phylogenetic trees of the order Durnavirales.

6.2.2.4.3.2 Ghabrivirales

The order of *Ghabrivirales* is composed of four families, nine genera, and 61 species (Walker et al. 2020). The five viruses found in this study were assigned to the families *Chrysoviridae* and *Totiviridae*.

6.2.2.4.3.2.1 Chrysoviridae

A novel member of the family *Chrysoviridae*, genus *Alphachrysovirus* (Figure 28) with a quadripartite, monocistronic, linear genome coding for an RdRp (3,5kb), Pro (3,3kb), Gly (3,0kb) and CP (3,1kb),

---

[27] ***Atrapa****virus* (genus): From type species **Atra**to **Pa**rtiti-like virus 6.

(Figure 90) was detected in a *Wyeomyia* pool and named Salado virus[28]. Its RdRp has 59% AA sequence identity with Hubei chryso-like virus 1 (*Cx. australicus*, ASA47348). Other close relatives are Shuangao chryso-like virus 1 (Cx. quinquefasciatus) and Hubei chryso-like virus 2 (Diptera, APG76028.1).

Figure 28: ML tree based on the AA sequence of the RdRp of members of the family Chrysoviridae. The name of the virus discovered in this study is in red and its genome structure is shown on the right. See Figure 67 and Figure 68, appendix 11.6.1.9 for the fully labeled phylogenetic trees of the order Ghabrivirales.

6.2.2.4.3.2.2 Totiviridae

Three undescribed and one recently discovered Toti-like viruses were detected in the mosquito samples. Murri virus was found in *Cq. venezuelensis,* Atrato virus in *Ma. titillans*, Murici virus in *Ps. funiculus*, *Cq. lynchi* and *Psorophora* mosquitoes, and Embera virus in *Wyeomyia* pools. Due to RdRp phylogeny, Murici virus and Embera virus cluster with recently described mosquito-derived viruses on a sister clade to the genus *Giardiavirus*. Branching pattern and low AA sequence similarity with assigned genera justify the creation of a new genus we refer to as *Totcuvirus*[29] (Figure 29). Embera

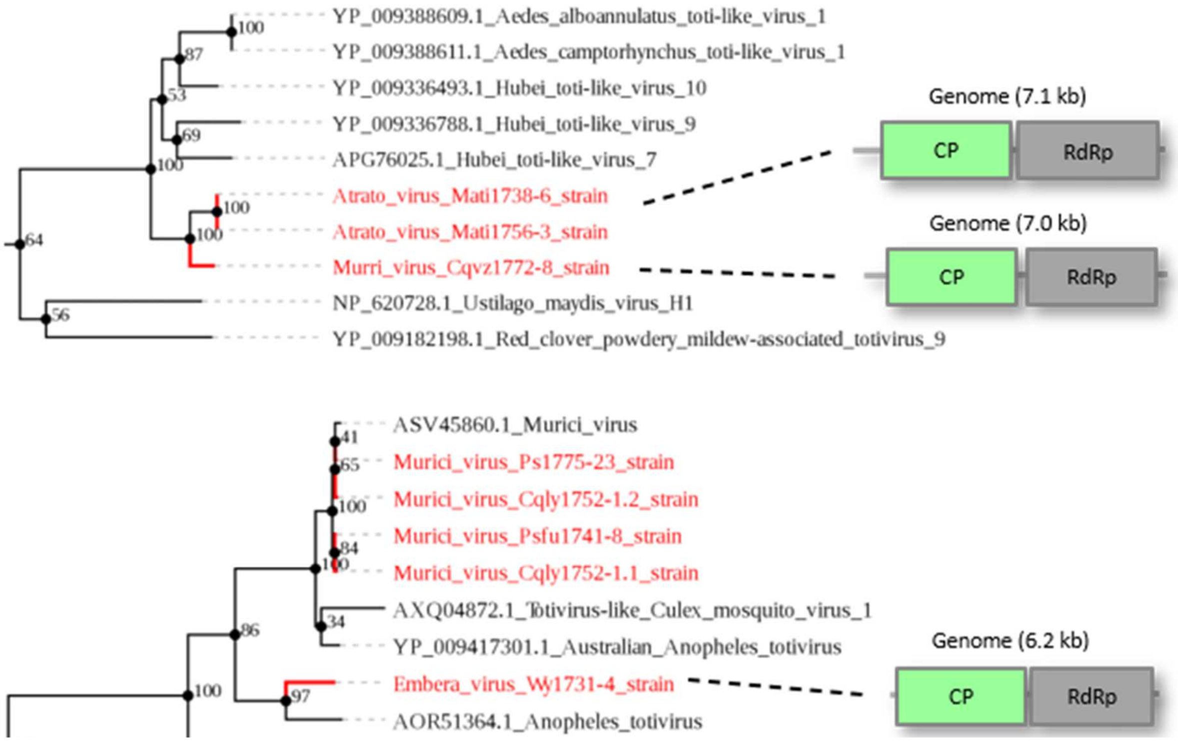


Figure 29: ML trees based on the AA sequence of the RdRp of members of the family Totiviridae. The names of the viruses discovered in this study are in red and their genome structure is shown on the right. See Figure 67 and Figure 68, appendix 11.6.1.9 for the fully labeled phylogenetic trees of the order Ghabrivirales.

---

[28] Referring to Salado a village in the research region.

[29] ***Totcu****virus* (genus): From **Tot**i-like **Cu**licidae derived viruses.

virus shares 58% RdRp AA sequence identity with Drama totivirus from *Oc. sticticus* (Fauver et al. 2016) and Pisingos virus 44% with Anopheles totivirus from *An. gambiae* (AOR51364). Together with other viruses recently described in mosquitoes, they form a deep rooting unassigned branch of the family *Totiviridae*. Atrato virus, Murri virus, and other recently described toti-like viruses give rise to a new clade at the root of the family *Chrysoviridae* but resemble the *Totiviridae* genome structure. The nearest known relative of Atrato virus and Murri virus is Schistocephalus solidus toti-like virus 2. Based on RdRp phylogeny this virus group should compose a new genus we tentatively call Murato virus[30] (Figure 29).

6.2.2.4.3.3 Reovirales

The order of *Reovirales* consists of one family, two subfamilies, 15 genera, and 97 species (Walker et al. 2020). A novel virus species of the order *Reovirales*, family *Reoviridae*, subfamily *Sedoreovirinae*, was detected in *Ps. albipes* mosquitoes and named Atrato Reo-like virus 1 (ARlv1)[31]. The AA sequence of the CDS of the RdRp coding segment of ARlv1 coincides 61% with the one of Aedes camptorhynchus reo-like virus (YP009389547.1). Even though these mosquito-derived unassigned viruses are in the same clade as the *Phytoreoviruses* (Figure 30), they only share 26% amino acid similarity with the RdRps of Rice gall dwarf virus (YP001111373.1) and Rice dwarf virus (BAA017074.1). Thus, according to the ICTV genus demarcation criteria for the family *Reoviridae* (King 2011), they should compose a new yet unassigned genus we refer to as *Relicuvirus*[32].

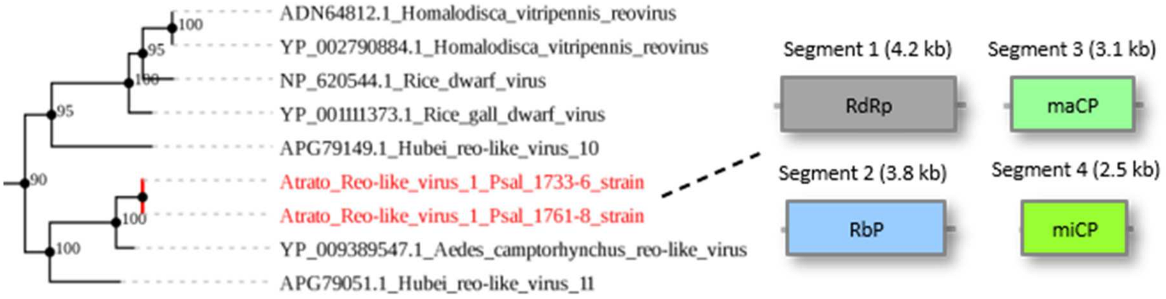


Figure 30: ML tree based on the AA sequence of the RdRp of members of the family Reoviridae.
The names of the viruses discovered in this study are in red and their genome structure is shown on the right. See Figure 69 and Figure 70, appendix 11.6.1.10 for the fully labeled phylogenetic trees of the order Reovirales.

---

[30] **Murato**virus (genus): From type species **Mu**rri virus and At**rato** virus.

[31] Referring to the Atrato river.

[32] **Relicu**virus (genus): From **Re**o-**li**ke **Cu**licidae derived virus.

6.2.2.4.4 Rep-encoding single-strand DNA viruses

6.2.2.4.4.1 *Piccovirales*

The order *Piccovirales* includes one family, three subfamilies, 26 genera, and 126 species (Walker et al. 2020). Two novel virus species of the order *Piccovirales*, family *Parvoviridae*, subfamily *Densovirinae* could be identified. Atrato Denso-like virus (ADlv) was found in *Ma. titillans* and Atrato Denso-like virus 1 (ADlv1) in *Ps. albipes*. ADlv and ADlv1 cluster on the same clade with Linvill Road virus and Haematobia irritans densovirus (not in figures) but the AA sequence on the nonstructural ORF 1 shows less than 30% amino acid sequence identity with any described virus of the family *Parvoviridae*, and therefore, due to the genus demarcation criteria of the ICTV for the subfamily *Densovirinae* give rise to a new genus we call *Adelivirus*[33] (Cotmore et al. 2019). ADlv and ADlv 1 have a monopartite, linear genome with approximately 5 kb genome length and 6 open reading frames. A nonstructural ORF, a structural ORF, and two VP-like protein-coding ORFs could be identified. The genome structures and phylogenetic relationships are shown in Figure 31.

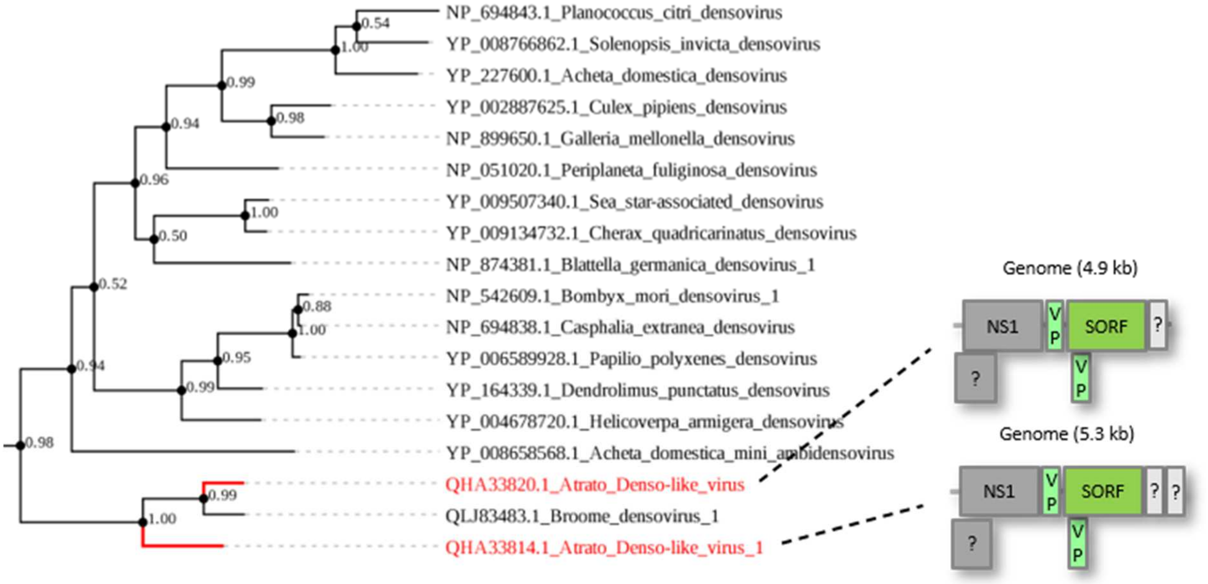


Figure 31: ML tree based on the AA sequence of the NS1 of members of the subfamily Densovirinae.
The names of the viruses discovered in this study are in red and their genome structure is shown on the right. See Figure 71 and Figure 72, appendix 11.6.1.11 for the fully labeled phylogenetic trees of the order Piccovirales.

---

[33] **Adeli**virus: from **A**trato **De**nso-**li**ke virus, type species of the genus

6.2.2.4.5 Reverse-transcribing viruses

6.2.2.4.5.1 Ortervirales

The order *Ortervirales* (formerly *Retrovirales*) contains five families, two subfamilies, 28 genera, and 238 species. Two undescribed viruses of the order *Ortervirales* were detected and named Atrato Retro-like virus (ARlv) and Chibugado virus. Chibugado virus was assigned to the family *Metaviridae* and ARlv to the family *Pseudoviridae* (Figure 76, Figure 77).

6.2.2.4.5.1.1 Pseudoviridae

The family *Pseudoviridae* by now includes three genera and 34 species. The genera *Hemivirus*, *Pseudovirus,* and *Sirevirus* have originally been assigned based on genome architecture and host range. These demarcation criteria are currently under revision as the genera *Pseudovirus* and *Hemivirus* turned out to be polyphyletic groups based on phylogenetic analysis of the AA sequence of the reverse transcriptase (RT), (Llorens et al. 2021). Atrato Retro-like virus has a monopartite, linear monocistronic genome of 4,3 kb coding for a Gag-Pol protein, and was found in *Ps. albipes* and *An. darlingi* and RT phylogeny based on the group-specific antigen polyprotein shows that it is in the same clade as other mosquito-associated viruses that have been described in *Culex* and *Aedes* recently (Figure 32). The branching pattern gives evidence for three main host-associated clades in the family *Pseudoviridae* which correspond to the kingdoms *Animalia*, *Plantae*, and *Fungi*. In this sense, we propose the creation of three subfamilies in the family *Pseudoviridae* named *Anipseuvirinae*[34], Plapseuvirinae[35], and Funpseuvirinae[36], and a phylogeny-based reassignment of the genera.

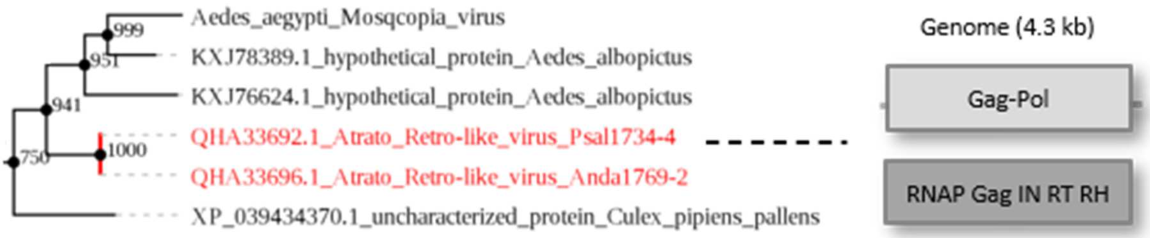


Figure 32: ML tree based on the AA sequence of the polymerase of members of the family Pseudoviridae.
The names of the viruses discovered in this study are in red and their genome structure is shown on the right. See Figure 76, Figure 77, and Figure 78, appendix 11.6.1.14 for the fully labeled phylogenetic trees of the order Ortervirales and the family Pseudoviridae.

---

[34] ***Anipseu****virinae* (subfamily): From kingdom ***Ani****malia* and family ***Pseu****doviridae.*
[35] ***Plapseu****virinae* (subfamily): From kingdom ***Pla****ntae* and family ***Pseu****doviridae.*
[36] ***Funpseu****virinae* (subfamily): From kingdom ***Fun****gi* and family ***Pseu****doviridae*.

6.2.2.4.5.1.2 *Metaviridae*

The family *Metaviridae* includes two genera and 31 species. Chibugado virus has a monopartite, monocistronic genome of 6,9 kb, with three ORFs coding for a GAG, Pro-RT-RH-IN polyprotein, and an ENV (Figure 94) and was assigned to the genus *Errantivirus* based on the phylogeny of the RT domain core (Figure 33, Figure 36).

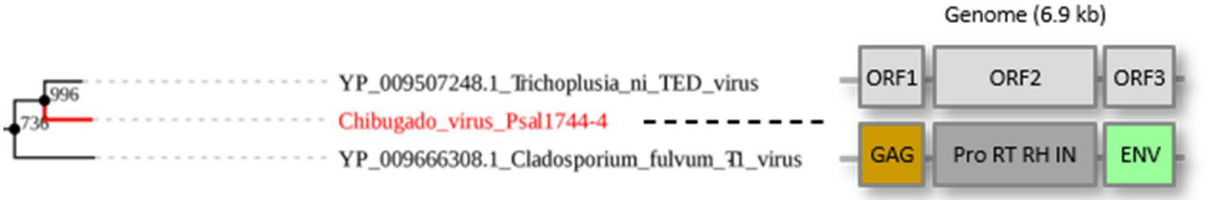


Figure 33: ML tree based on the AA sequence of the polymerase of members of the family Metaviridae.
The name of the virus discovered in this study is in red and its genome structure is shown on the right. See Figure 76, Figure 77 appendix 11.6.1.14 for the fully labeled phylogenetic trees of the order Ortervirales.

6.2.3 The mosquito virome

A total of 2002 mosquitos were pooled by species (genus) and geographic region in 189 pools of approximately 10 specimens. These pools were pooled again in 42 mosquito megapools and tested for viruses by NGS. The pools of the megapools that tested positive for a certain virus were then retested for this virus by species-specific (rt)PCRs (Förster 2019). Three quarters (74,6%) of the tested pools corresponded to four mosquito species: *Ps. albipes* (42,9%), *An. darlingi* (15,9%), *Ma titillans* (8,5%) and *Cq. venezuelensis* (6,3%).

Mosquito species	No. pools	Mosquito species	No. pools
Ps. albipes	83	*Cx. quinquefasciatus*	3
An. darlingi	30	*Ae. albopictus*	2
Ma. titillans	16	*Ps. ferox*	2
Cq. venezuelensis	12	*Cx. nigripalpus*	1
Oc. serratus	7	*An. spp.*	2
Cq. lynchi	7	*Cx. spp.*	4
Ps. funiculus	6	*Wy. spp.*	9
An. nuneztovari	4	*Ps. spp.*	1

Table 10: Mosquito pools tested for viruses by NGS and PCR
The number of pools tested for each mosquito species is shown. Pools corresponded to 8-10 specimens of the same species/genus.

At least 52 virus species could be identified in 12 mosquito species. Table 19 summarizes the prevalence with which each virus species was detected in each mosquito species (genus) and gives information on the taxonomical classification of the mosquito-associated viruses. Even though the sample size differed between the different mosquito species, certain trends could be observed. Most of the viruses (82.8%) detected were found in a specific mosquito species. The mosquito species that showed the highest associated virus diversity were *Cx. quinquefasciatus*, *Cq. venezuelensis*, and *Ma.*

titillans. More than half of the described viruses (53,9%) were derived from these three mosquito species. Nearly one-fifth (17,3%) of the viruses could be associated with *Culex* mosquitoes, beyond the fact that the *Culex* pools corresponded to only 4,2% of the pools tested. *Ma. titillans* corresponded to 8,5 % of the pools tested and 23,1% of the detected virus species and *Cq. venezuelensis* to 6,3% of the tested mosquito species and 13,5% of the viruses found.

Members of the order *Bunyavirales* were only found in *Culex* and *Ma. titillans* mosquitoes. Coredo virus was detected in 87,5% of the tested *Ma. titillans* pools and thus is the *Orthophasmavirus* with the highest prevalence throughout the mosquito populations. In contrast, members of the order *Jingchuvirales* were detected in the mosquito genera *Anopheles*, *Coquillettidiae*, *Psorophora,* and *Wyeomyia*, but were absent in *Culex* and *Mansonia*. Viruses of the order *Picornavirales* were exclusively detected in *An. darlingi*. Representatives of the order *Sobelivirales* were found in all tested genera except *Anopheles*. The order *Materellivirales* was present in all genera except *Wyeomyia* and *Ochlerotatus*. Hubei mosquito virus 4 was exclusively detected in *Cx. nigripalpus* and was the only *Tolivirales* member found. Atrato Flavi-like virus 1 was assigned to the order *Amarillovirales*, family *Flaviviridae,* and was found in a *Ma. titillans* and a *Culex* pool. Atrato Nege-like virus 1 was assigned to the genus *Negevirus*, a genus that has been proposed in 2016 (ICTV proposal 2016.011a-dP), but has never been accepted by the ICTV and thus corresponds to an unassigned +ssRNA virus.

The only viruses that could be detected in several mosquito species and/or genera were Atrato Picorna-like virus 1, Atrato Sobemo-like virus 1, Atrato Virga-like virus 4, Atrato Virga-like virus 7, Hubei Virga-like virus 2, Atrato Flavi-like virus 1, Atrato Partiti-like virus 2, Pisingos virus, and Atrato Retro-like virus. The virus species detected in the mosquito pools are listed in Table 11 according to the mosquito species they were found in.

Table 11: The virome of the most abundantly collected mosquito species.
Taxonomic classification of viruses detected in Oc. serratus, An. darling, An. nuneztovari, Cq. lynchi, Cq. venezuelensis, Cx. nigripalpus, Cx. quinquefasciatus, Ma. titillans, Ps. albipes, Ps. ferox, and Ps. funiculus (proposed taxa are in red).

Group	Order	Family	Genus	Virus species
Oc. serratus				
ssRNA(-)	*Mononegavirales*	*Rhabdoviridae*	*Atratorhabdovirus*	Atrato Rhabdo-like virus 2
ssRNA(-)	*Sobelivirales*	*Solemoviridae*	*Unassigned*	Atrato Sobemo-like virus 1
An. darlingi				
ssRNA(+)	*Tymovirales*	*Tymoviridae*	*Gendovirus*	Gengado virus
ssRNA(+)	*Tymovirales*	*Tymoviridae*	*Mudovirus*	Murindo virus
ssRNA(+)	*Martellivirales*	*Virgaviridae*	*Unassigned*	Atrato Virga-like virus 7
dsRNA	*Durnavirales*	*Partitiviridae*	*Unassigned*	Atrato Partiti-like virus 2
ssRNA(+)RT	*Ortervirales*	*Metaviridae*	*Hemivirus*	Atrato Retro-like virus
An. nuneztovari				
ssRNA(-)	*Jingchuvirales*	*Chuviridae*	*Atchuvirus*	Atrato Chu-like virus 2
ssRNA(+)	*Picornavirales*	*Marnaviridae*	*Unassigned*	Atrato Picorna-like virus 1
Cq. lynchi				

Group	**Order**	**Family**	**Genus**	**Virus species**
dsDNA	*Ghabrivirales*	*Totiviridae*	*Totcuvirus*	Pisingos virus
		Cq. venezuelensis		
ssRNA(-)	*Jingchuvirales*	*Chuviridae*	*Doliuvirus*	Atrato Chu-like virus 1
ssRNA(+)	*Sobelivirales*	*Solemoviridae*	*Unassigned*	Atrato Sobemo-like virus 3
ssRNA(+)	*Sobelivirales*	*Solemoviridae*	*Unassigned*	Atrato Sobemo-like virus 5
ssRNA(+)	*Martellivirales*	*Virgaviridae*	*Unassigned*	Atrato Virga-like virus 2
ssRNA(+)	*Martellivirales*	*Virgaviridae*	*Unassigned*	Atrato Virga-like virus 7
ssRNA(+)	*Unassigned*	*Unassigned*	*"Negevirus"*	Atrato Nege-like virus 1
dsRNA	*Ghabrivirales*	*Totiviridae*	*Muratovirus*	Murri virus
		Cx. nigripalpus		
ssRNA(+)	*Martellivirales*	*Virgaviridae*	*Unassigned*	Hubei Virga-like virus 2
ssRNA(+)	*Martellivirales*	*Tolicuviridae*	*Unassigned*	Hubei mosquito virus 4
		Cx. quinquefasciatus		
ssRNA(-)	*Bunyavirales*	*Phasmaviridae*	*Orthophasmavirus*	Culex Phasma-like virus
ssRNA(-)	*Bunyavirales*	*Phenuiviridae*	*Zheemosvirus*	Atrato Gouko-like virus 1
ssRNA(+)	*Martellivirales*	*Virgaviridae*	*Unassigned*	Hubei Virga-like virus 2
		Ma. titillans		
ssRNA(-)	*Bunyavirales*	*Phasmaviridae*	*Orthophasmavirus*	Coredo virus
ssRNA(-)	*Bunyavirales*	*Phasmaviridae*	*Feravirus*	Guagua virus
ssRNA(-)	*Bunyavirales*	*Phenuiviridae*	*Zheemosvirus*	Narangue virus
ssRNA(+)	*Picornavirales*	*Iflaviridae*	*Adelivirus*	Atrato Picorna-like virus
ssRNA(+)	*Sobelivirales*	*Solemoviridae*	*Unassigned*	Atrato Sobemo-like virus 1
ssRNA(+)	*Martellivirales*	*Virgaviridae*	*Unassigned*	Atrato Virga-like virus 3
ssRNA(+)	*Martellivirales*	*Virgaviridae*	*Unassigned*	Atrato Virga-like virus 5
ssRNA(+)	*Amarillovirales*	*Flaviviridae*	*Atratovirus*	Atrato Flavi-like virus 1
dsRNA	*Durnavirales*	*Partitiviridae*	*Unassigned*	Atrato Partiti-like virus 1
dsRNA	*Durnavirales*	*Partitiviridae*	*Unassigned*	Atrato Partiti-like virus 5
dsRNA	*Ghabrivirales*	*Totiviridae*	*Muratovirus*	Atrato virus
ssDNA	*Piccovirales*	*Parvoviridae*	*Adelivirus*	Atrato Denso-like virus
		Ps. albipes		
ssRNA(-)	*Jingchuvirales*	*Chuviridae*	*Obscuruvirus*	Atrato Chu-like virus 5
ssRNA(+)	*Picornavirales*	*Marnaviridae*	*Unassigned*	Atrato Picorna-like virus 1
ssRNA(+)	*Picornavirales*	*Polycipiviridae*	*Caosvirus*	Cacaos virus
ssRNA(+)	*Retrovirales*	*Metaviridae*	*Errantivirus*	Chibugado virus
ssRNA(+)	*Martellivirales*	*Virgaviridae*	*Unassigned*	Atrato Virga-like virus 4
ssRNA(+)	*Martellivirales*	*Virgaviridae*	*Unassigned*	Atrato Virga-like virus 7
dsRNA	*Durnavirales*	*Partitiviridae*	*Unassigned*	Atrato Partiti-like virus 3
dsRNA	*Durnavirales*	*Partitiviridae*	*Atrapavirus*	Atrato Partiti-like virus 6
dsRNA	*Reovirales*	*Reoviridae*	*Relicuvirus*	Atrato Reo-like virus 1
ssDNA	*Piccovirales*	*Parvoviridae*	*Adelivirus*	Atrato Denso-like virus 1
ssRNA(+)RT	*Ortervirales*	*Metaviridae*	*Relicuvirus*	Atrato Retro-like virus
		Ps. ferox		
ssRNA(+)	*Sobelivirales*	*Solemoviridae*	*Unassigned*	Atrato Sobemo-like virus 4
ssRNA(+)	*Martellivirales*	*Virgaviridae*	*Unassigned*	Atrato Virga-like virus 4
		Ps. funiculus		
ssRNA(+)	*Martellivirales*	*Virgaviridae*	*Unassigned*	Atrato Virga-like virus 4
ssRNA(+)	*Martellivirales*	*Virgaviridae*	*Unassigned*	Atrato Virga-like virus 6
dsRNA	*Ghabrivirales*	*Totiviridae*	*Totcuvirus*	Pisingos virus

6.3 Characterization of novel viruses found in human blood samples

An NGS-based virus search was promoted in blood samples on filter paper from patients that consulted a health centre with suspicion of infection with malaria in the Atrato River region in Colombia. A total of 46 blood samples were pooled in 6 pools and tested for viruses. At first, nucleic acids were extracted, virus particles were purified, and cDNA was synthesized. Then shotgun libraries were created, Illumina-sequenced and reads were de novo-assembled. A total of 12 viruses could be detected.

6.3.1 Viral polymerase coding segments identified in human blood samples by NCBI blastx

De novo-assembled contigs with nucleotide sequence lengths larger than 300 bps, that showed a sequencing coverage equal to or greater than one, were examined. Preselected contigs were aligned to the NCBI blastx database, Conserved Domain Database, and HHpred protein homology detection and structure prediction were used to identify virus-related genes and proteins. Then CDSs were predicted with NCBI ORF finder. Contigs that resulted in hits for viral polymerases are listed alphabetically by assigned name in Table 12.

Virus/ Pol coding segment	NCBI blastx Top Hit Description	Query cover	Per Ident	Hit Accession
Atrato circo-like virus 1	putative Rep protein [Circovirus-like genome DHCV-2]	29%	61,45%	YP_009259732.1
Atrato circo-like virus 2	replication-associated protein [Fringilla montifringilla Circoviridae sp.]	44%	100%	MW182726.1
Atrato circular strange virus 1	Rep [uncultured virus]	25%	91,91%	AUM61988.1
Atrato circular strange virus 2	replication-associated protein [Blackfly DNA Virus 12]	38%	57,63%	QCX35043.1
Atrato gemini-like virus 1	Rep [uncultured virus]	45%	54,75%	AUM61683.1
Atrato human blood virus	replication-associated protein [Fringilla montifringilla Circoviridae sp.]	44%	100%	QTE03314.1
Atrato Partiti-like virus 3	RdRp [Atrato Partiti-like virus 3]	99%	99,76%	QHA33899.1
Atrato Parvo-like virus 1	replication protein [Luscinia sibilans parvo-like hybrid virus]	33%	93,40%	QTE03982.1
Atrato Parvo-like virus 3	replication protein [Psittacidae aveparvovirus]	32%	93,20%	QTE03751.1
Atrato unclassified virus 1	replication-associated protein Rep [ssDNA virus sp.]	15%	63,31	QTP16102.1
Parvo-like hybrid virus UC2	Parvo-like hybrid virus UC2, partial genome	99%	99,76%	KF214646.1
Reagent-associated CRESS-like virus 7	Reagent-associated CRESS-like virus 7 isolate 13 replicase-like gene, partial sequence	96%	99,91%	MZ824231.1

Table 12: Viral polymerase coding segments identified by NCBI blastx in de novo assembled contigs from pooled human blood samples on filter paper.

The identified polymerase sequences were used to classify the viruses and thus get information on their genome structure comparing them to similar known viruses. By this, additional segments could be identified, and the coding proteins could be annotated by comparing them to similar viruses. Frequently, several viruses were present in one sample and the assignation of the segments was tricky.

6.3.2 Description of novel viruses from human blood on filter paper

Viruses were classified by phylogenetic analysis based on the AA sequence of the polymerase or replication-associated protein and are listed alphabetically by the order they were assigned (Table 13). Most of the viruses could not be classified to genus level, as there are not many known similar viruses in the NCBI GenBank for comparison and the ones available have not yet been assigned taxonomically. The human blood samples were from patients who consulted a medical centre with suspicion of malaria infection. Only pools were tested. Samples were not retested by PCR, and therefore the virus could not be associated with the patient and his clinical history. During blood sample acquisition, and sample preparation contamination might have occurred. For proper host identification, cell culture experiments might be promising. The prevalence with which each virus was found in the pools is shown in Table 14, and the genome structures in Figure 34.

gcom[1]	**Order**	**Family**	**Genus**	**Species**	**strain**	**Seqs[3]**	**depth**	**bps[2]**
ssDNA	Cirlivirales	Circoviridae	Unassigned	Atrato circo-like virus 1	Hosa1763-27	1	6	2559°
ssDNA	Cirlivirales	Circoviridae	Unassigned	Atrato circular strange virus 1	Hosa1777-17	1	3	3694°
ssDNA	Cirlivirales	Circoviridae	Unassigned	Atrato gemini-like virus	Hosa1778-39	1	1,5	3159°
ssDNA	Cirlivirales	Circoviridae	Unassigned	Atrato unclassified virus 1	Hosa1777-38	1	8	2575
ssDNA	Cirlivirales	Circoviridae	Unassigned	Reagent-associated CRESS-like virus 7	Hosa1763-128	1	6	1165°
ssDNA	Cremevirales	Unassigned	Atramanvirus	Atrato human blood virus	Hosa1774-108	1	74	2083°
dsRNA	Durnavirales	Partitiviridae	Unassigned	Atrato Partiti-like virus 3	Hosa1763-110	2	2-4	1243*, 1184*
ssDNA	Piccovirales	Parvoviridae	Unassigned	Atrato Parvo-like virus 1	Hosa-1774-42	1	9	3537
ssDNA	Piccovirales	Parvoviridae	Unassigned	Atrato Parvo-like virus 1	Hosa-1740-21	1	1	2336*
ssDNA	Piccovirales	Parvoviridae	Unassigned	Atrato Parvo-like virus 3	Hosa1778-23	1	2	3655
ssDNA	Piccovirales	Parvoviridae	Unassigned	Parvo-like hybrid virus UC2	Hosa1774-83	1	1	2494*
ssDNA	Piccovirales	Parvoviridae	Unassigned	Atrato Parvo-like virus 2	Hosa1774-69	1	1	2692*

Table 13: Short description of the viruses detected in human blood samples on filter paper
*Unassigned taxa or proposed taxa are in red. ([1] genome composition, [2] genome/ segment/ contig size in base pairs, [3] number of genome segments, * incomplete, ° circular).*

		NGS Pool					
		1762	1763	1774	1776	1777	1778
Virus species	**Atrato circular strange virus 1**	X	x	x	x	x	x
	Atrato human blood virus	X	x	x	x	x	x
	Atrato gemini-like virus 1		x	x		x	x
	Atrato Partiti-like virus 3		x				
	Atrato parvo-like virus 1	X	x	x	x	x	x
	Atrato parvo-like virus 3	X	x	x		x	x
	Atrato unclassified virus 1	X	x	x	x	x	x
	Parvo-like hybrid virus UC			x			
	Reagent-associated CRESS-like virus 7	X	x	x	x	x	
	Atrato parvo-like virus 2			x			

Table 14: Prevalence of viruses in NGS pools of human blood samples.

6.3.2.1 *Circlivirales*

In *Cressdnaviricota* phylogeny (Figure 74), Atrato Circo-like virus 1 (AClV1), Atrato circular strange virus 1, Atrato Gemini-like virus, Atrato unclassified virus 1, and Reagent-associated CRESS-like virus 7 shared a clade with members of the order *Ciclivirales*. *Circoviridae* phylogeny (Figure 75) including most of the accepted species shows, that these viruses cluster on a sister clade to the genera *Cyclovirus* and *Circovirus*. Atrato unclassified virus 1, Atrato circular strange virus 1, Atrato gemini-like virus, and Reagent-associated CRESS-like virus cluster together and their circular genome of 1,2 – 3,7 kb share the same genome structure with the *Circoviridae* characteristic ORI motive '(T/n)A(G/t)TATTAC' (Rosario et al. 2017) that is flanked by two ORFs in ambisense orientation coding for a Rep-Hel and a CP. Atrato unclassified virus 1 and Atrato circular strange virus 1 have a third ORF coding for a hypothetical protein with an unknown function in parallel with the Rep-Hel coding ORF. Atrato circo-like virus 1 has a 2,6 kb polycistronic, circular genome with three ORFs in unisense orientation that code for a Rep, a Hel, and a CP and two more ORFs with unknown functions in parallel to the Rep and Hel CDSs. We propose to include these viruses in the order *Circlivirales*, but due to evolutionary distance and branching pattern in RdRp phylogeny and differences in genome organization they might give rise to several new genera.

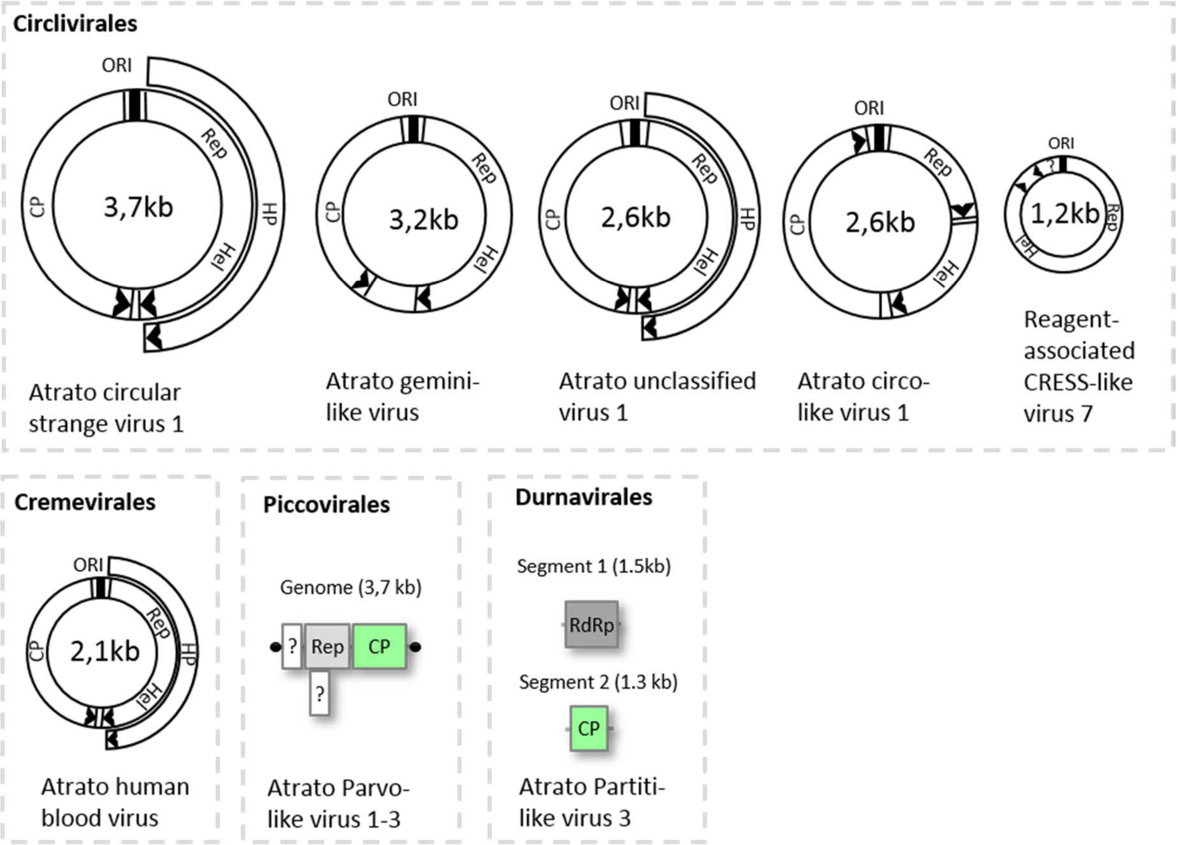


Figure 34: Genome structures of novel viruses of the orders Circlivirales, Cremevirales, Piccovirales, and Durnavirales derived from pools of human blood samples (Rep=replication-associated protein, CP=capsid protein, HP=hypothetical protein, black dots=palindromes/hairpins).

6.3.2.2 Cremevirales

Atrato human blood virus shares 100% AA identity for the Rep and the CP of a virus sequenced lately from an anal swab of bird species *Fringilla montifringilla* (QTE03314.1, QTE03315.1; Yang, S. and Zhang, W. 2021). Atrato human blood virus has a circular, covalently closed, ssDNA genome of 2,1 kb with an ORI and two ambisense orientated ORFs coding for a Rep-Hel and a CP protein and an additional ORF in parallel to the Rep-Hel coding ORF that codes for a hypothetical protein. In RdRp phylogeny (Figure 74) it shares a common ancestor with viruses from the order *Cremevirales*, but the evolutionary distance to the existing genera justifies the creation of a new genus (and probably family) we refer to as *Atramanvirus*[37], with Atrato human blood virus being the first described species.

6.3.2.3 Piccovirales

Piccovirales phylogeny based on the AA sequence of the nonstructural ORF 1 (NS1) shows that Atrato Parvo-like virus 1-3 and Parvo-like hybrid virus UC2 cluster together on a sister clade to the subfamily *Parvovirinae* in an unrooted maximum likelihood tree (Figure 73). Their linear, single-stranded DNA genomes have two main ORFs (Rep, CP) and several smaller ORFs with unknown functions coded on both stands and short terminal hairpin telomeres that are characteristic of *Parvoviridae* rolling hairpin replication (Cotmore et al. 2019). Evolutionary distance and low sequence similarity with *Parvovirinae* and *Densovirinae* members justify the creation of a new subfamily we refer to as *Parlivirinae*[38]. Atrato parvo-like viruses 1-3 showed sequence similarities to viruses from bird anal swabs (Table 12). The family *Parvoviridae* currently consists of 3 subfamilies. The members of the *Parvovirinae* infect non-human vertebrates and humans, the *Densovirinae* invertebrates, and the *Hamaparvovirinae* vertebrates and invertebrates. It is supposed, that the Parvo-like viruses described here infect humans as they were detected in human blood samples.

6.3.2.4 Durnavirales

Atrato Partiti-like virus 3 (APlv3) belongs to the family *Partitiviridae* and was also found in several mosquito samples (see Table 19, 11.5) and it is the only dsRNA virus detected in the blood sample pools. RdRp phylogeny and genome structure of APlv3 are discussed in chapter 6.2.2.4.3.1.

---

[37] ***Atraman****virus* (genus): Referring to the type species **Atra**to hu**man** blood virus.

[38] Subfamily **Parli**virinae: from **Par**vovirinae- **li**ke virus.

6.4 Other non-viral agent-related contigs from mosquito pools and blood samples

Even though samples were DNA/RNA digested before virus particle isolation and library construction, some contigs related to non-viral agents could be traced by NGS in the mosquito and blood sample pools. A full list of the non-viral agents' related contigs from the mosquito and blood samples is available in appendix 11.7. The most abundantly detected bacteria-related contigs from the mosquito pools belonged to the phylum *Proteobacteria*. Genera identified based on blastn search were *Wolbachia*, *Acinetobacter*, Rickettsia, Providencia, Rickettsiella, Arcobacter, Gilliamella, Frischella, Erwinia, Heliobacter, *Frischella*, *Providencia*, *Asaia*, *Variovorax*, *Serratia*, *Rhodoferax*, and *Acinetobacter*. Most of these bacteria are arthropod endosymbionts that live in the insect gut and are not known to be pathogenic in vertebrates. Some might be pathogenic to insects. Other bacteria that might cause disease in plants and insects and were detected in mosquito samples belong to the genus *Spiroplasma*.

Contigs related to *protozoan* parasites of the family *Trypanosomatidae*, genera *Trypanosoma*, *Blechomonas*, *Crithidia*, *Paratrypanosoma*, and *Trypanosoma* were also detected in the mosquito pools. *Trypanosoma brucei* causes sleeping sickness in humans and *Trypanosoma cruzi* Chagas disease. Most probably the *Trypanosoma* species detected in this study are novel and their host restriction is unclear. Trypanosoma sp. 1 showed 97% sequence identity with 18 s ribosomal gene of *Trypanosoma culicavium* transmitted by *Culex* mosquitoes to bird hosts. (Votýpka et al. 2012).

The human blood samples on filter paper also revealed contigs related to bacterial agents of the genera *Variovorax*, *Acinetobacter*, *Burkholderia*, *Arcobacter*, *Acinetobacter*, *Acidovorax*, *Diaphorobacter,* and *Xanthomonas, belonging to the phylum Proteobacteria*. *A. baumannii* is a frequent cause of hospital-acquired pneumonia, skin and wound infections, bacteremia, meningitis, and urinary tract infections (Kadri 2020). *Arcobacter* may cause bacteremia, endocarditis, peritonitis, gastroenteritis, and diarrhea in humans and is mainly transmitted through contaminated food or water (Ramees et al. 2017).

X. campestris is a bacterium that may cause black rot on vegetables but has also been reported as an opportunistic pathogen able to infect humans (Li et al. 1990). *Acidovorax oryza* is a serious rice pathogen but bloodstream infection in humans has been reported in a patient by Orsborne et al. 2014. Another pathogen detected and known to cause lung infection and cystic fibrosis in immunocompromised patients is *B. cepacian* (Lipowski et al. 2008). *Cruoricaptor ignavus* was once isolated from a blood culture from a man with bacteremia (Yassin et al. 2012). The protozoan parasite *Plasmodium vivax* responsible for malaria in humans was identified in a blood sample pool of 10 patients by the 28s ribosomal RNA gene.

7 Discussion

During this pilot study, mosquitos were collected in the Atrato River region in Northwest Colombia between August 2014 and December 2016. Mosquitoes were identified through morphology and molecular markers (COI, ITS2). More than 43 mosquito species could be identified. Most abundant mosquito species were screened for viruses in an (rt)PCR- and NGS-based virus search. More than 52 undescribed viruses were detected, and their genomes were sequenced and annotated. Then, they were taxonomically assigned through phylogenetic analyses based on the AA sequences of the polymerase CDS. The Atrato River valley harbours substantial biodiversity of mosquito-associated viruses, most of them being insect- and plant-specific. In addition, 46 human blood samples were NGS-sequenced and 12 novel viruses could be characterized.

7.1 The challenge of identifying mosquitoes

The geographical region of the Chocó located on the Colombian Pacific coast is one of the world´s biodiversity hotspots, as its formation process and geographical and climatical conditions have favoured speciation processes and evolution (Pérez-Escobar et al. 2019). The Atrato River Valley is part of the Chocó region and thus, it is not surprising that high mosquito biodiversity was discovered in the region.

Proper identification of *Culicidae* in this region was a tough challenge. Some species are easy to identify due to obvious morphological traits, the disponibility of barcoding regions, and the lack of species complexes of sibling species. Some mosquito species that we identified by morphology had not been barcoded before and we made available barcoding sequences for these species. For the first time, COI sequences of *Cq. lynchi*, *Cq. venezuelensis*, *Ma titillans*, *Ps. funiculus* and *Ps. albipes,* and ITS2 sequences of *An. neivai*, *Cq. lynchi*, *Cq. venezuelensis*, *Ma. indubitans*, *Ma. titillans*, *Oc. scapularis*, *Ps. albipes*, *Ps. funiculus*, *Wy. luteoventralis* and *Wy. pertinans* were uploaded to NCBI GenBank as references for molecular species identification.

Particularly, the subgenus *Melanoconion* of the genus *Culex* has been an object of study in the last years, as the subgenus is one of the largest subgenera with many groups and sibling species, many of them of public health interest (Torres-Gutierrez et al. 2016). The morphological identification of subgenus members is challenging as many species cannot be identified on the species level by the morphological traits of the female without pupal and larval stages, and not all the species have been described yet. In 2020, Sá et al. revised the *Atratus* group and presented a morphological key for the identification of all life stages of the group members, and described five new species. Phylogeny and molecular identification of the subgenus *Melanoconion* by barcoding regions, in particular the COI region, was investigated by Torres-Gutierrez et al. in 2018. Both studies concur with our results. In most cases, the COI is a suitable barcoding region for mosquito species discrimination. The real problem is that for many species COI sequences are not available, or the sequences available at NCBI GenBank have not been assigned properly to species level and thus lead to incongruent search results at NCBI GenBank.

Apart from *Cx. rabelloi* and *Ps. funiculus*, all the other mosquito species found in the present study had already been registered in the "Updated list of the mosquitoes of Colombia" (Rozo-Lopez and Mengual 2015). *Cx. rabelloi* was described by Forattini and Sallum for the first time in 1987 and was discovered in the municipality of Pariquera-Acú in Southern Brazil. Since 2018, COI sequences of four specimens of *Cx. rabelloi* that were collected at the same place are available at NCBI GenBank. The COI sequences of these specimens matched at 98,33% identity with a sequence of a *Culex* mosquito (KX779859.1) collected in this study in the village Gengadó located in the municipality of Vigía del Fuerte. Thus, this is the first report of *Cx. rabelloi* for Colombia.

Ps. funiculus is part of the *Psorophora confinnis* species complex and was first described in Colombia by Dyar in 1920, but later declared a synonym with *Ps. confinnis* (Dyar 1928). The same year the "Updated list of mosquitos of Colombia" was published, *Ps. funiculus* was resurrected from synonymy with *Ps. confinnis* based on isoenzyme and hybridization studies that confirmed that *Ps. confinnis* and *Ps. funiculus* were two different species. Due to Lanzaro et al. (2015), the specimens from Colombia correspond to *Ps. funiculus* and specimens from Argentina to *Ps. confinnis*. In this order of ideas, and accordance with our results, the COI sequence with accession number KM452781, uploaded to NCBI GenBank in 2014 (Hoyos-López 2018), and designated Psorophora (Grabhamia) sp., should be reassigned to *Ps. funiculus,* and the species should be included in the catalogue.

Many of the specimens of the genera *Wyeomia*, *Johnbelkinia,* and *Trichoprosopon* collected in the Atrato river valley could only be assigned to the genus level. The morphological keys available (Lane 1953, Zavortink 1979; Darsie 1985) were outdated and did not include new species described since then. For instance, in our study, we were able to distinguish six different *Trichoprosopon* species by their COI sequences, but we could not assign the barcodes to any species, as we were not able to identify the mosquitoes on species level by morphology, as our female specimens did not match to any species in the identification keys or could not be identified as males and larval stages were not available.

Genetic distance among marker regions may be used as a measure of the evolutionary divergence between mosquito species and populations and identify closely related species. We used the mean and maximum intraspecific K2P distance among ITS2 and COI sequences respectively, to estimate whether the barcoding sequences of the morphologically identified specimens were conspecific or allospecific. The typical mean intraspecific distance depends on the mosquito species, but generally speaking, a high discrepancy between the mean and maximal K2P distance is an indicator of the allospecifity among the specimens. For example, Marrelli et al. (2006) reviewed the taxonomy of Latin American Anophelines and observed intraspecific ITS2 sequence variations that ranged from 0.2% to 19% and ascribed the high K2P divergence to misidentification and/or sequencing errors. We calculated the K2P distance for all the mosquito species COI and ITS2 were sequenced. In general, intraspecific sequence divergence did not exceed 2-3%, except in the case of the ITS2 sequences of *Cq. venezuelensis* for which the calculated K2P distance was 8,8% (see Table 4 and Table 5). We interpreted the unexpectedly high value as an indicator of the presence of a sibling species that we misidentified and for which no ITS2 barcode has been uploaded yet.

An. nuneztovari is a species complex that includes at least three valid species: *An. nuneztovari*, *An. goeldii*, and *An. dunhami.* Female adults cannot be distinguished by morphology (Sallum et al. 2020). But for Colombia, only *An. nuneztovari* is registered (Rozo-Lopez and Mengual 2015) and Sierra et al. (2004) confirmed based on the homogeneity of the ITS2 region that *An. nuneztovari* specimens from Colombia are conspecific. In our research region the mean intraspecific K2P distances among the COI and ITS2 sequences of *An. nuneztovari* were low (≤0,1%), and COI phylogeny (Figure 39) confirmed that all the *An. nuneztovari* specimens collected by us were conspecific and correspond to *An. nuneztovari s.s.*. Barcoding and molecular identification do only work for mosquito species that have been identified properly by morphology before and for which sequences were uploaded to a gene bank. That is why there is a need for revised morphological keys and barcoding sequences available for each species.

7.2 Approaches to virus search

In this study, an (rt)PCR- and NGS-based virus search was conducted to identify mosquito-associated viruses that might be of human, veterinary, or crop health interest. The methods applied for sample collection, sample preparation, library preparation, PCR, NGS, and contig assembly shall be discussed in this retrospective. The focus of this project was the discovery of novel viruses, and the methods used for virus detection and characterization were derived from various publications and adjusted to the research objectives (see 4.4), having in mind the optimum sample collection, transportation, preservation, and cost and time efficiency.

Anthropophilic female mosquitoes and human blood samples from Indigenous and Afro-Colombian communities of the remote and tropical Atrato River valley were the basis of our research. Beyond the traps available for mosquito collection, human landing catch (HLC) was the method of choice, as the focus was on catching intact anthropophilic mosquitoes that might be involved in arbovirus transmission and pose a threat to human health. HLC is still the most efficient method for anthropophilic mosquito collection, as host-seeking anthropophilic females are attracted and relatively high yields are collected even in low mosquito densities (Lima et al. 2014, Kenea et al. 2017). Commonly used light traps or carbon dioxide traps have the disadvantage of attracting other insects than anthropophilic mosquitoes, the necessity of electricity or consumables, or damage of specimens by vents or removal (Huang et al. 2021, Bazin and Williams 2018). At the beginning of the fieldwork, the main objective was to identify the mosquito species in the region and preserve specimens for morphological and molecular identification. Desiccated mosquitoes may be preserved for many years at ambient temperature without degradation of specimens and their DNA, and transportation and storage are uncomplicated. After the identification of the mosquito species, additional specimens were collected for the systematic virus search in mosquitoes. This time, mosquitoes were collected and stored maintaining a cooling chain (-80°C or less) to preserve the integrity of the virus particles and prevent degradation, in particular of RNA viruses, which are extremely delicate (Burkhalter and Savage 2017), and thus facilitate full genome sequencing and virus cultivation in cell culture.

Sample preparation is a crucial step before NGS sequencing and depends on the study objectives. As the objective was not only the detection of viruses but also to sequence the whole virus genomes directly from the sample pools by NGS, the virus particles were purified and the nucleic acids of the host or other non-viral agents were digested before library preparation which employed the Nextera XT DNA Library Preparation Kit. Sequenced viral genomes were de novo assembled with Unicycler, an assembly pipeline developed for bacterial genome assembly that can assemble Illumina-only read sets, where it functions as a SPAdes optimizer using Bowtie2 and Pilon to polish the assembly (Wick et al. 2017). In the PubMed database, no publications could be found on virus assembly with Unicycler, but the Galaxy Training Network recently published a tutorial on how to assemble SARS-CoV-2 from human samples (Galaxy Training Network 2021). A crucial step for SARS-CoV-2 de novo genome assembly outlined in the tutorial is the subtraction of reads from the human reference genome by mapping the genome to a human reference genome. This post-NGS step is done to remove nucleic acids of the host genome before virus assembly and is comparable to the pre-NGS nucleic acids digestion step we applied for virus particle purification (Shi et al. 2015). The advantage of virus particle purification is that the proportion of virus reads to host reads and other contaminations is significantly increased. On the other hand, sequencing unpurified samples permits insight into the whole mosquito biome. Nevertheless, much more reads are necessary to sequence the full virus genomes as most of the generated reads will correspond to the host genome and other host-related agents or contaminations making the process more laborious and expensive. Despite the digestion of the unprotected nucleic acids, contigs related to non-viral agents could be identified in the mosquito pools, e.g., symbiotic bacteria or protozoans. Undoubtedly, NGS is a very effective, fast, and cost-effective way of searching for novel viruses and agents. In contrast, generic (rt)PCRs are very time-consuming, positive controls are required and only specific viruses or genera may be detected, whereas novel and unknown viruses stay undiscovered. Anyway, if a virus is detected by PCR, NGS is necessary for full genome acquisition. Nevertheless, PCRs are suitable for retesting the samples and confirming the NGS results (Förster 2020). To conclude, NGS is the most appropriate tool for exploring the great diversity of yet unknown viruses, whereas PCR is suitable for specific virus detection.

7.3 An insight into the virome of Colombian mosquitoes

Mosquitoes harbor a high diversity of viruses and arthropods are supposed to play a key role in RNA virus evolution, most probably due to their close interaction with plants, vertebrates, and invertebrates (Li et al. 2015). Metagenomic and metatranscriptomic approaches are commonly applied in virus search, discovery, and characterization and give insight into the virosphere, the genomic potential and activity of viruses, and their ancestry, evolution, and ecological dynamics (Shi et al. 2016a, Shi et al. 2018). Many of the most challenging human viral diseases like Dengue, Chikungunya, and Yellow fever are transmitted by mosquito vectors drawing researchers' attention to study the mosquito virome.

In a metagenomic approach, the viromes of the most representative mosquito species in the Atrato River region in Northwest Colombia were investigated. Mosquito species of the genera *Anopheles* (*An. darlingi*, *An. nuneztovari*), *Psorophora* (*Ps. albipes*, *Ps. ferox*, *Ps. funiculus*), *Coquillettidiae* (*Cq. lynchi*, *Cq. venezuelensis*), *Culex* (*Cx. nigripalpus*, *Cx. quinquefasciatus*), *Ochlerotatus* (*Oc. serratus*), and *Mansonia* (*Ma. titillans*) were screened for viruses. At least 52 viruses of 14 orders; ssRNA(-): *Mononegavirales*, *Jingchuvirales*, *Bunyavirales*; ssRNA(+): *Picornavirales*, *Tymovirales*, *Amarillovirales*, *Sobelivirales*, *Materellivirales*, *Tolivirales*; dsRNA: *Durnavirales*, *Reovirales*; ssRNA(RT): *Ortervirales* ssDNA: *Piccovirales*; dsDNA: *Ghabrivirales*) and one unassigned ssRNA(+) virus genus (*Negevirus*) could be identified, many of which showed similarities with recently discovered viruses in association with mosquitoes from all over the world.

In 2017, Shi et al. described 24 novel RNA viruses in *Culex* and *Ochlerotatus* mosquitoes from Western Australia in a metatranscriptomic approach. The species tested were *Oc. camptorhynchus*, *Oc. alboannulatus*, *Cx. globocoxitus*, *Cx. australicus* and *Cx. qinquefasciatus*. Many of the viruses described by Shi et al. show similarities with the ones found in the Atrato River region in Colombia. For example, Culex-Phasma-like virus (CPLV), a new (-)ssRNA virus of the order *Bunyavirales*, genus *Phasmaviridae* that showed high prevalence throughout the three *Culex* species just mentioned, was also detected in *Cx. quinquefasciatus* mosquitoes from the Atrato River region in Colombia. Surprisingly, the L and S segments of the CPLV strains from Australia, Colombia and Brazil show high AA sequence identity (>99%), and males are also affected by the virus (da Silva Ferreira et al. 2020). High virus prevalence in the distant *Culex* populations and high sequence identity between the different strains indicate, that CPLV most probably is an insect-specific virus restricted to the genus *Culex* and unlikely to cause severe disease in the insect host. Another virus found in Australian *Cx. globocoxitus* and *Cx. autralicus* is Culex luteo-like virus (CLLV). All near relatives of CLLV are mosquito-related and in RdRp phylogeny, they cluster with Atrato sobemo-like virus 3 and 4, but RdRp AA sequence identity is relatively low (65%) and ASLV 3 and 4 were derived from *Coquillettidiae* mosquitoes (see appendix 11.6.1.9 Figure 63 and Figure 64; and Table 11).

Chrysoviruses used to be associated with fungal hosts, but recently a clade of mosquito-associated *Chrysoviruses* has been described. Salado virus, a dsRNA virus of the family *Chrysoviridae* that was found in *Wyeomyia* mosquitoes in our study shows RdRP similarities to Shuangao Chryso-like virus 1 (59%, ASA47348.1) from *Cx. quinquefasciatus* and Hubei chryso-like virus 1 (59%, ASA47348) from *Cx. australicus*. Shi et al. (2017) used the abundance level of RNA in the library, prevalence, host association of close relatives, and co-appearance with other cellular microbes within the hosts as key attributes for host association and assume, that this clade within the family *Chrysoviridae* that is shared by the just mentioned viruses is specific to insects. Anyway, it cannot be ruled out that these viruses originated from bacterial, fungal, or protist hosts.

7.4 Virus phylogeny and evolution

During the last decade, NGS became affordable and made in-depth metagenomic sample analysis possible, and bring insight into the unimaginable biodiversity of viruses. Many mosquito-borne diseases are caused by viral agents (Lee et al. 2018). Thus, researchers focused NGS-sequencing of common vector species and related viruses and were confronted with a vast number of unknown viruses that revolutionized the understanding of virus taxonomy and evolution. Human pathogens and vertebrate viruses form only the tip of the iceberg of the mosquito virome which is characterized mainly by insect-specific and plant viruses (Moonen et al. 2023, Dietzgen et al. 2016).

Vertebrate RNA virus evolution has taken hundreds of millions of years and is closely related to the evolution of their hosts that began in the ocean. Our knowledge of virus diversity is still limited and thus conclusions on ancestral host groups might be erroneous (Shi et al. 2018). Nevertheless, Marklewitz et al. (2015) estimated the phylogenetic relationships of *Bunyaviruses* based on the polymerase protein and suggest, that the arbo-*bunyaviruses* have evolved from insect-specific viruses several times convergently, as deep branching viruses are typically associated with arthropod hosts. The novel *Bunyaviruses* we identified in mosquitoes belong to the families *Phenuiviridae* and *Phasmaviridae*. Atrato Gouko-like virus 1 and Narangue virus branch deep in the *Phenuiviridae* family with other recently discovered and yet unassigned viruses that were derived from mosquitoes. The family *Phenuiviridae* mainly consists of viruses related to invertebrate hosts. The only assigned genus in the family *Phenuiviridae* that shows plant tropism with a persistent transmission in invertebrates is the genus *Tenuiviridae*. Recently, new plant-infecting genera, tentatively named *Coguvirus* and *Rubovirus*, have been proposed (Navarro et al. 2018a; Navarro et al. 2018b,). Due to our analysis, the *Coguviruses* and the recently discovered and yet unassigned genus of mosquito-derived viruses we tentatively named *Zheemosvirus*, represent relatively deep branching genera in the family *Phenuiviridae*. In the case of the family *Phasmaviridae,* only invertebrate hosts have been discovered so far. Vertebrate and plant tropism might not have evolved, or plant and vertebrate viruses are rare in the family and have not been discovered yet.

Phylogenetic trees are used to make evolutionary and taxonomic inferences about organisms. Appropriate rooting is crucial as the root gives directionality to evolution and is the basis of molecular taxonomy. A widely used and popular rooting method is the outgroup method in which a divergent taxon (outgroup) is used to root the ingroup taxa. This means that you rely on prior knowledge of a phylogenetically close consensus outgroup for the sequence set you study. In particular for viral phylogenetic analysis where outgroups are often unknown and taxonomy is uncertain, midpoint rooting is applied to estimate evolutionary relationships (Kinene et al. 2016). In the present study, we tended to apply midrooted phylogenetical trees for estimating the evolutionary relationships of virus taxa, as many of the viruses discovered were novel. Moreover, taxonomical virus classification is currently undergoing major changes due to the discovery of a vast number of unknown viruses made possible by NGS-based approaches. In general, the midrooted maximum likelihood trees estimated based on the viral polymerase-related protein sequence in this study (see chapter 11.6.1) were similar to approaches from former studies (https://talk.ictvonline.org/ictv-reports/ictv_online_report).

7.5 Description of novel taxonomic groups

Our phylogenetical analyses led to the discovery of viruses that could not be assigned to any existing taxa and justify the creation of new virus taxa. Subsequently, the main characteristics of these new taxa and the distinguishing criteria for the assigned taxa shall be discussed.

7.5.1 Cacaos virus and the novel genus *Caosvirus*

The family *Polycipiviridae* is characterized by an unsegmented, linear, (-)ssRNA genome of 10- 12 kb, typically polycistronic with at least four ORFs. Currently, the three genera *Sopolycivirus*, *Hupolycivirus,* and *Chipolycivirus* are accepted by the ICTV. Demarcation criteria have not been established yet and the accepted genera were assigned based on the phylogenetic clustering of the AA sequence of the RdRp coding ORF5. All known family members have originated from an arthropod host (Olendraite et al. 2019). Host restriction could also be applied as a genus demarcation criterion, as the *Sopolycivirus* are delimited to ants (order *Hymenoptera*), the *Hupolycivirus* to the order *Odonata,* and the *Chipolycivirus* to spiders (order *Araneae*). Cacaos virus is the first member of the family *Polycipiviridae* that was found in mosquitoes (Order *Diptera*, family *Culicidae*). The 12 kb genome has 6 ORFs and the functions of 4 ORFs were identified by HHpred (VP1, VP2, VP3, RdRp, Figure 93, Figure 71), and it is the first described species of the novel genus *Caosvirus*.

7.5.2 Atrato Flavi-like virus 1 (AFlV1) and the novel genus *Atratovirus*

AFlV1 is a novel yet unassigned virus of the family *Flaviviridae* that shows similarities with the genus *Flavivirus*, but its genome length and structure are different from the typical *Flaviviruses*. In RdRp phylogeny, it clusters with other recently discovered Flavi-like viruses. Its genome was annotated with homology detection and structure prediction by HMM-HMM comparison and homologies to the NS5 (MT, RdRP, 5U0B), NS3 (Pro, 5WX1), Proxine (Pro, 6XB4), and an ENV like Gly (1SVB) could be identified (Söding 2005). These "long genome Flaviviruses (LGFs)" of 16 - 20kb genome length have been detected in a variety of hosts, e.g., plants, nematodes, and arthropods. Evolutionary relationships of the flavi-like superfamily have been studied by Paraskevopoulou et al. (2021). They believe LGFs to be ancestral to the *Pestivirus* and support their suggestion with a *Hepacivirus*-rooted maximum likelihood phylogenetic tree estimated based on the AA alignment of the RdRp region. In our midrooted approach, the LGFs grouped as a sister glade to the genus *Flavivirus* and might be ancestral to the *Flaviviruses* (Figure 62), but we did not include the *Jingmenvirus*, a novel segmented *Flavi*-like virus genus discovered recently (Shi et al. 2016b), as the genus has not been accepted by the ICTV yet. Thus, *Jingmenvirus* species are not listed in the ICTV master species list and the close relationship with the *Flaviviridae* family was not clear to us.

7.5.3 Murindo virus and Gengado virus and the novel genera *Mudovirus* and *Gendovirus*

Murindo virus and Gengado virus are novel viruses, and RdRp AA sequence phylogenetic analyses show that they root deep within the *Tymoviridae* family (Figure 48, Figure 49). Low AA sequence identity, branching pattern, host affiliation, and particular genome structures different from the accepted genera *Tymovirus*, *Marafivirus*, and *Maculavirus,* justify the creation of two new genera tentatively named *Mudovirus* and *Gendovirus*. Murindo virus has a 6 kb linear monopartite (+)ssRNA genome and resembles the typical *Tymovirales* genome structure (Mt-Pro-Hel-RdRp) coded in ORF1. An additional ORF coded for a CP. Gengado virus has the same gene order, but three protein-coding ORFs, ORF 1 (Mt-Pro), ORF2 (Hel-RdRp), and ORF3 (CP). The assigned genera in the family *Tymoviridae* have all been related to plant hosts, whereas the deep-rooting unassigned viruses like Murindo virus, Gengado virus, Guadeloupe Culex tymo-like virus (*Cx. quinquefasciatus*, MW434965), (Batson et al. 2021), Hubei macula like virus 1 and 2 (Odonata, NC_033254, NC_033260, (Shi et al. 2016a), and Bee macula-like virus 2 (Andrena Haemorrhoa, NC_040556) have been associated with arthropod hosts.

7.5.4 Atrato Partiti-like virus 6 (APlv6) and the novel genus *Atrapavirus*

APlv6 is a Parti-like virus that clusters in RdRp AA sequence phylogeny together with Cryptosporidium parvum virus 1 on a sister clade to the *Picobirnaviruses*, but RdRp AA sequence identity is very low (26%). We were not able to identify the second segment coding for the CP as common in the *Partitiviridae*. A second nested ORF was detected on the RdRp coding segment, but the putative protein could not be associated with any known protein by NBCI blast, CDD Search, or homology prediction. APlv6 was detected in a *Ps. albipes* pool that also contained other non-viral protozoan and bacterial sequences (Table 20) that might correspond to a possible host for APlv6.

7.5.5 Pisingos virus and Embera virus and the new genus *Totcuvirus*

So far, the family *Totiviridae* consists of five assigned genera: *Giardiavirus*, *Leishmaniavirus*, *Trichosomasvirus*, *Totivirus,* and *Victorivirus* (King 2011). The *Totivirus*, *Giardiavirus*, and *Victorivirus* are restricted to fungal hosts, the *Trichosomasvirus* (genus *Trichosomas*) and *Leishmaniavirus* (genus *Leishmania*) to *protozoans*. Embera virus has a genome of approximately 6,2kb with 2 coding ORFs (RdRp, CP) In RdRp phylogeny Embera virus and Pisingos virus cluster on two separate branches that form a sister clade of the *Giardiavirus*. The nearest known relatives of Embera virus are Drama totivirus (QRD99885) from *Oc. sticticus*, Ae. aegypti totivirus (Shi et al. 2019, QEM39153) and Anopheles totivirus from *An. gambiae* (Fauver et al. 2016, AOR51364).

Pisingos virus was detected in *Cq. venezuelensis*, *Cq. lynchi*, and *Ps. funiculus* and has a genome of about 7Kb that also codes for two proteins (RdRp, CP). It shares a common ancestor with Australian Anopheles totivirus (AATV), (Colmant et al. 2017, YP009417301), Culex inatomii totivirus (QTT60745), Culex modestus totivirus (QRD99875), Fitzroy Crossing toti-like virus 1 from *Cx. annulirostris* (Williams et al. 2020, QLJ83495), and Lindangsbacken virus from *Culex torrentium* (Pettersson et al. 2019, QGA70947). To preclude environmental contamination, Pettersson et al. established four criteria to decide about the host association of the mosquito-related viruses: (1) Total abundance of the virus in the library (>0,01%), (2) relation between host COXI and viral abundance, (3) presence of the virus in multiple libraries and (4) phylogenetic relationship to other viruses. If the virus meets at least two of the four criteria it is supposed to be associated with the mosquito host. In our case, the first two criteria cannot be taken into account, as the virus particles were purified by the digestion of the mosquito and other non-viral nucleic acids. Nevertheless, Pisingos virus meets the other two other criteria as it is present in multiple libraries and clusters with other mosquito-associated viruses in RdRp phylogeny. AATV was confirmed to replicate in its hosts *An. annulipes* and *An. hinesorum* by detection of virus-specific short interfering RNAs but could not be cultivated in *Ae. albopictus* C6/36 mosquito cells (Colmant et al. 2017). Deep branching of these mosquito-related viruses indicates, that the fungal-, and protozoan-related *Totiviridae* family might have evolved from arthropod viruses.

7.5.6 Murri virus and Atrato virus and the new genus *Muratovirus*

Murri virus and Atrato virus have a 7 kb dsRNA genome with 2 ORFs coding for an RdRp and a CP, as typical for members of the family *Totiviridae*, but the RdRp AA sequence phylogenetic tree shows that they, together with other recently described viruses, form a new yet unassigned genus tentatively called *Muratovirus*. No clear host restriction can be observed and the closest known relatives shared less than 47% AA sequence identity (Table 12). Schistocephalus solidus toti-like 2 virus was described in a fish parasiting tapeworm (Phylum: *Plathelminthes*, QJD26158) from Alaska (Hahn et al. 2020), Hubei toti-like virus 7 in *Dipteran* pools from China (Shi et al. 2016a, APG76025), Aedes camptorhynchus toti-like virus 1 (YP009388611) in mosquitoes in Australia (Shi et al. 2017), and a yet unnamed toti-like virus (OXV86394) in bird feces. These viruses cluster on a sister clade to the family *Chrysoviridae* and probably have fungal hosts that have not been identified yet.

7.5.7 Narangue and Atrato Gouko-like virus 1 and the novel genus *Zheemosvirus*

Narangue and Atrato Gouko-like virus 1 resemble the typical *Phenuiviridae* tripartite genome structure coding an L, M, and S segment and share a phylogenetic clade with Mothra virus detected in the moth *Cydia pomonella*. Thus, Narangue virus has been assigned to the genus *Mobuvirus* and renamed Narangue mobuvirus by the ICTV *Phenuiviridae* study group in 2020 (Marklewitz et al. 2020). The decision was taken without any justification and is not supported by our study. We agree, that Narangue virus and Atrato Gouko-like virus 1 are in the same clade as Mothra virus, but Narangue virus shares only 25% identity with the AA sequence of the CDS of the L-segment of Mothra virus and was not detected in moth but *Ma. titillans* mosquitoes. In addition, the M-segment of Mothra virus has two protein-coding ORFs meanwhile Narangue virus has only one, and the evolutionary distance estimated on the RdRp AA sequences is high (Figure 44). In our opinion, Narangue virus and Atrato Gouko-like virus 1 together with Zhee mosquito virus (KM817705) and Xinzhou mosquito virus (KM817701) detected in *An. sinensis* in China (Li et al. 2015), Salarivirus from the USA (KX924627), and Culex pseudovishnui bunya-like virus from Japan (Faizah et al. 2020 BBQ05095.1) should give rise to a new genus of mosquito associated viruses we refer to as *Zheemosvirus*[39].

---

[39] **Zheemos**virus (genus): From type species **Zhee mos**quito virus.

7.5.8 *Jingchuvirales* evolution and genome structures – novel genera *Obscuruvirus* and *Atchuvirus*

The members of the recently established Order *Jingchuvirales* show manifold genome organizations. Atrato Chu-like virus 1 was detected in *Cq. venezuelensis* and has a bipartite genome with two segments. The L-segment (7 kb) codes for an RdRp and the S-segment (4,1kb) for a Gly and an N. In RdRP phylogeny, it shares a branch with Chuvirus Mos8Chu0, genus *Doliuvirus* (also bipartite, KX924630) from *Culiseta minnesotae*, and thus was assigned to the genus. The sister genus *Culicidavirus* differs in genome composition. Culex mosquito viruses 4 and 5 were described in *Culex* mosquitoes (MK440640, MH188036, Pettersson et al. 2019, linear), Wuhan mosquito virus 8 in *Cx. tritaeniorhynchus* (Li et al. 2015, NC028265, circular) and Imjin River virus in *Cx. bitaeniorhynchus* (Hang et al. 2016, NC028482, linear), and all have a monopartite genome. The sister genus *Odonatavirus* represented by Hymenopteran chu-related virus OKIAV124 detected in *Nomada lathburiana* (Käfer et al. 2019, MW288189) also has a monopartite genome.

Atrato Chu-like virus 2 was described in *Anopheles* mosquitoes, has a bipartite genome with an L-segment (RdRp) and an S segment, and was assigned to the family *Chuviridae*, but could not be assigned to any existing genus. It shares a clade with Tacheng tick virus 4 (TTV4, YP009177716) detected in *Argas miniatus* (Li et al. 2015), (Figure 47), but the RdRp AA sequence identity between the two viruses is low (Table 12) and TTV4 is monopartite and circular and from a tick host. Thus, a new genus was proposed and tentatively called *Atchuvirus*[40].

Atrato Chu-like virus 5 (ATCLV5) was derived from *Ps. albipes* and has a linear, monopartite, tricistronic genome coding three proteins (Gly-N-RdRp, Figure 82), and it is the only known species of the recently proposed genus *Obscuruvirus*, family *Aliusviridae* (Di Paola et al. 2020). The genome of Atrato chu-like virus 4 is incomplete. Most probably its genome organization is similar to ATCLV5. Figure 35 shows the genome structure of representatives of the accepted *Jingchuvirales* genera and the novel viruses discovered in this study.

The known deep-rooting *Jingchuvirales* are characterized by a linear, monopartite genome with typically 3 coding ORFs (Gly-N-RdRp). In the deep branching *Chambyvirus* and *Myriadvirus* clades, a fourth ORF evolved coding for an additional Gly (Gly1-N-Gly2-N-RdRp). The genome size of the *Jingchuvirales* ranges from approximately 10 - 14 kb. The family *Aluiviridae* is characterized by a monopartite, linear genome of about 13-14 kb in size coding 3 ORFs (Gly-N-RdRp) The Gly coding ORF is nearly twice as long as in the sister family *Chuviridae*. The *Chuviridae* show variable genome

---

[40] **Atchu**virus: From the type species **At**rato **Chu**-like virus 2

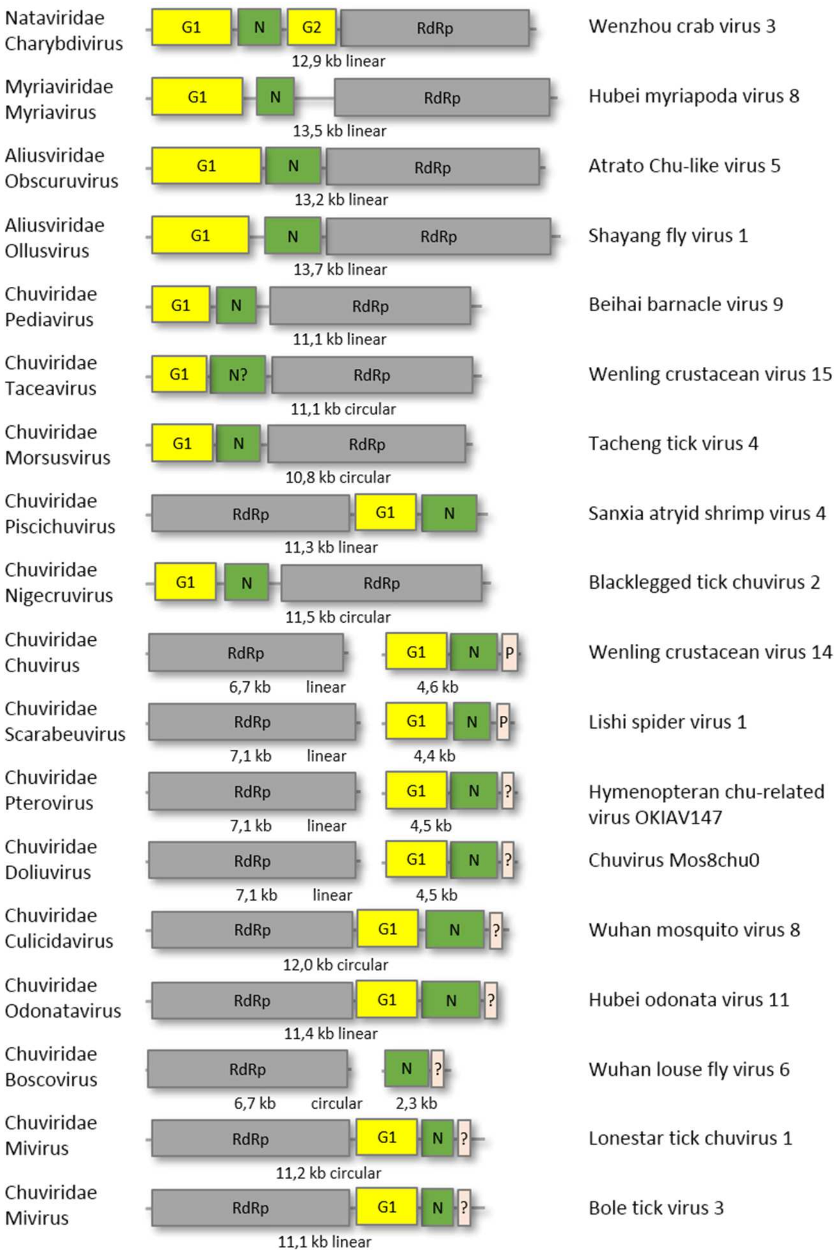


Figure 35: Jingchuvirales genome structures
The genome organizations of representatives of the accepted families and genera in the order Jingchuvirales are shown (N=nucleoprotein, G=glycoprotein, RdRp=RNA dependent RNA polymerase, P=Phosphoprotein, ?=unknown function).

organizations including linear, circular, monopartite, and bipartite genomes. In the genus *Nigecruvirus*, the linear, monopartite genome is arranged in a different way (RdRP-Gly-N). The most evolved known genera of the *Chuviridae* have a fourth ORF following the N CDS. The AA sequence coded by ORF4 shows homology to RNA binding Ps and might be an RdRp cofactor. In Wenling crustacean virus 4 (*Chuvirus*), HHPred for ORF 4 resulted in an 87% probability of being homologous to the oligomerization domain of parainfluenza virus P (6VAG). For Lishi Spider Virus 1 (*Scarabeuvirus*) ORF 4 showed a 79% probability of being homologous to the oligomerization domain of a P from Respiratory syncytial virus (6YP5). The genus *Boscovirus* consists of only two accepted members that are circular and bipartite, and the S-segment lacks a Gly.

7.5.9 Changes in *Ortervirales* taxonomy

Chibugado virus was assigned to the family *Metaviridae* by RdRp phylogeny (Figure 77). Recently the family *Metaviridae* was reviewed and now it consists of 2 genera and 31 accepted species. Chibugado virus was aligned to the *Metaviridae* sequence alignment available at the ICTV virus taxonomy profile (ICTV Virus Taxonomy Profile: *Metaviridae*, December 2021), and RdRp phylogeny showed that it belongs to the genus *Errantivirus* as it shares a clade with Drosophila melanogaster nomad virus (AF039416), Drosophila melanogaster HMS-Beagle virus (AF365402) and Ceratitis capitata yoyo virus (Zhou and Haymer 1997, U60529) and carries a third env-like gene considered to be a distinguishing feature of the *Errantivirus* (Figure 36).

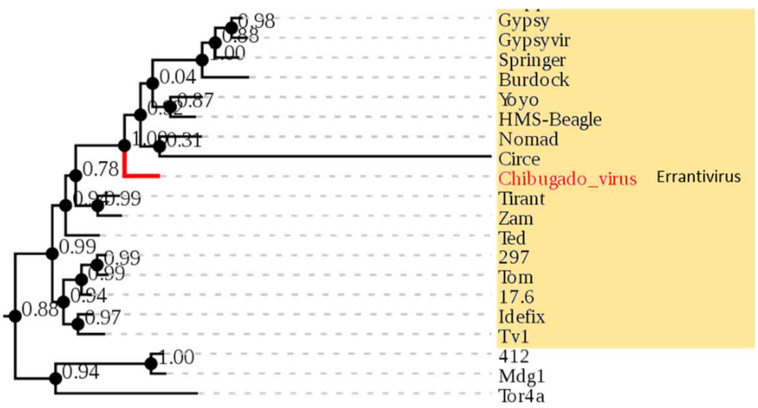


Figure 36: Phylogeny of the genus Errantivirus
Estimated based on the AA sequence of the CDS of the RT core domain. (Selected model of AA substitution: LG+G+F). The maximum likelihood tree including Errantivirus representatives from the ICTV master species list is displayed in a midrooted rectangular view. Nodes show SH-like branch support (aLRT). Novel species found in this study are in red.

Atrato Retro-like virus (ARLV) was assigned to the family *Pseudoviridae* (Figure 77). Recently, the family was reviewed. Thus, ARLV AA sequence was aligned to the *Pseudoviridae* sequence alignment available at the ICTV Taxonomy profile: *Pseudoviridae* (December 2021) and assigned to the genus *Hemivirus* by RdRp phylogeny (Figure 37). Nevertheless, the family *Pseudoviridae* needs further revision as the assigned genera are still polyphyletic. The closest known relatives of ARLV are Aedes aegypti mosqcopia virus (Tu and Hill 1999, AF134899) and Anopheles gambiae Mtanga virus (Ragsdale and Seiber 1999, AF387862) detected in mosquitoes.

Figure 37: Phylogeny of the genus Hemivirus Estimated based on the AA sequence of the CDS of the RT core domain. (Selected model of AA substitution: LG+G+F). The maximum likelihood tree including Hemivirus representatives from the ICTV master species list is displayed in a midrooted rectangular view. Nodes show SH-like branch support (aLRT). Novel species found in this study are in red. See Figure 78, appendix 11.6.1.14.1 for the fully labeled Pseudoviridae phylogenetic tree.

7.5.10 *Mononegavirales* and the novel genus *Atratorhabdovirus* in the family *Rhabdoviridae*

Atrato Rhabdo-like virus 3 (ARLV3) was found in *Oc. serratus* and Atrato Rhabdo-like virus 2 (ARLV2) in *Culex* mosquitoes and they were assigned to the family *Rhabdoviridae* by RdRp phylogeny (Figure 45, *Figure 46*), but could not be assigned on genus level. The closest known relatives of ARLV3 are Lasius neglectus virus 2 (MH477288, Kleanthous et al. 2019) detected in ants, Hymenopteran rhabdo-related virus 38 (MT153554), and Hymenopteran rhabdo-related virus 109 (MT153372, Käfer et al. 2019) from wasps. ARLV2 shares a clade with Shayang fly virus 3 (KM817636, Li et al. 2015) from *Chrysomya megacephala*, Wuhan house fly virus 2 (KM817649) from *Musca domestica,* and Hymenopteran rhabdo-related virus 24 (MW039260, Käfer et al. 2019) from *Heterodontonyx sp.*.

Recently the family *Rhabdoviridae* was reviewed (Walker et al. 2018) and now the family consists of 40 genera and 246 accepted species. ARLV2 and ARLV3 were aligned to the *Rhabdoviridae* sequence alignment available at the *Rhabdoviridae* ICTV Taxonomy profile, and ARLV3 could be assigned to the genus *Alphahymrhavirus* by RdRp phylogeny (Figure 38) and genome organization (N-P-M-G-L). ARLV2 AA sequence identity with accepted sister genera *Alphadrosrhavirus* and *Betahymrhavirus* is less than 50% and branch length and host association justify the creation of a new genus we tentatively called *Atratorhabdovirus*[41].

---

[41] ***Atratorhabdo**virus (genus): from first known species **Atrato rhabdo**-like virus 3.*

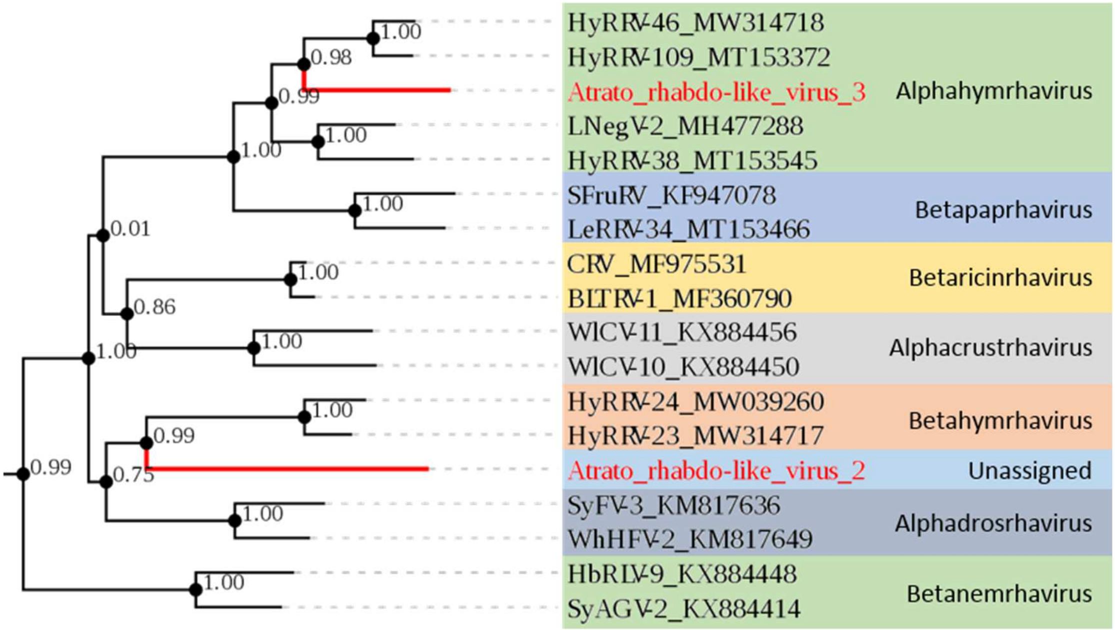


Figure 38: Phylogeny of the family Rhabdoviridae Estimated based on the AA sequence of the CDS of the RT core domain. (Selected model of AA substitution: LG+G+I+F). The maximum likelihood tree including representatives from the ICTV master species list (only part of the tree is shown) is displayed in a midrooted rectangular view. Nodes show SH-like branch support (aLRT). Novel species found in this study are in red.

7.5.11 Hubei mosquito virus 4 and the novel family *Tolicuviridae*

Hubei mosquito virus 4 (YP009330077, Shi et al. 2016a) was first described in mosquitoes in China, and in RdRp phylogeny, it clusters with Wenzhou tombus-like virus 11 detected in *Cx. tritaeniorhynchus* (YP009342051, Faizah et al. 2020) from Japan, Hammarskog tombus-like virus (QGA87328) detected in *Cq. richiardii*, Sweden, and Yongsan tombus-like virus 1 (YP0095532) detected in *St. albopicta* from South Korea in an unassigned sister clade to the order *Tolivirales* (Figure 59, Figure 60). The genome structure of these Toli-like viruses is similar to the one of the members of the *Tolivirales* and thus they should be included in the order, but we propose to assign these viruses to a new family tentatively named *Tolicuviridae*[42], based on the evolutionary distance and the fact that the classic known *Tolivirales* are restricted to plant hosts (apart from the *Alphacarmotetravirus*).

7.5.12 Atrato Reo-like virus 1 and the novel genus *Relicuvirus in the family Reoviridae*

Atrato Reo-like virus 1 (ARlv) was detected in two *Ps. albipes* pools. Eleven genome segments could be identified by comparing the contigs of two libraries that contained the same virus (Table 9), and the proteins coded on four segments could be identified (RdRp, RbP, maCP, miCP). The function of the proteins coded by the other segments is still unclear. In RdRp phylogeny ARlv is in a sister clade to the genus *Phytoreovirus* together with Aedes camptorhynchus reo-like virus (YP009389547, Shi et al. 2017)

---

[42] ***Toli**cu**viridae*** (family): From **Toli**-like **Cu**licidae derived arboviruses.

from Australia and Hubei reo-like virus 11 (APG79051, Shi et al. 2016a) detected in arthropods from China. These arthropod-derived viruses share low RdRp AA similarity with members of the genus *Phytoreovirus* (<26%) that were found in plant hosts and thus, in accordance with the ICTV demarcation criteria for the family *Reoviridae* (King 2011) a new genus tentatively called *Relicuvirus*[43] was proposed.

---

[43] ***Relicu**virus* (genus): From **Reo**-**li**ke **Cu**licidae derived viruses.

8 Conclusion and outlook

This pilot project aimed to identify the mosquito species present in the Atrato River valley in Northwest Colombia and get insight into the virome of the most abundant mosquito species, to identify novel viruses that might constitute a threat to human, animal, or crop health. Therefore, approx. 8000 adult mosquitoes were identified and approx. 2000 mosquitoes were screened for viruses in an NGS-based approach. In addition, six blood sample pools of 46 human finger-prick blood samples were screened for viruses.

In the Atrato River valley, more than 43 anthropophilic mosquito species of 14 genera could be identified by morphology and molecular markers (COI, ITS2). COI phylogeny gives evidence for at least 50 mosquito species (Figure 39), but only 40 could be identified at the species level (Table 17). About 80% of the collected specimens could be assigned to six predominant species: *Ma. titillans* (17,7%), *Ps. albipes* (16,5%), *An. darlingi* (15,8%), *An. nuneztovari* (10,9%), *Cq. venezuelensis* (8,9%), and *Cq. lynchi* (8,6%). Despite more than 100 years of investigation, the identification of mosquitoes is still a technical challenge. Especially the identification of members of the genera *Wyeomyia*, *Trichoprosopon*, *Johnbelkinia*, and *Culex*, subgenus *Melanoconion* caused problems and the specimens could only be identified partly, as morphological identification often requires all life stages, dichotomous keys are outdated or no barcoding regions are available, or because they might represent species that have not been described yet.

In Colombia studies on mosquito diversity mostly focus on the identification of species of genera of medical importance. Therefore, research is done mainly on *Anopheles* mosquitoes that are responsible for the transmission of malaria (Montoya-Lerma et al. 2011, Naranjo-Díaz et al. 2019; Altamiranda-Saavedra et al. 2017), or *Aedes* mosquitoes, particularly *Ae. aegypti* and *Ae. albopictus*, the main vectors of Dengue, Chikungunya, and Zika among other diseases (Calle-Tobón et al. 2022, Talbot et al. 2021). Moreover, researchers are interested in unraveling cryptic species complexes or groups (Sá et al. 2020, Motoki et al. 2021), but comprehensive inventories on mosquito species diversity are rare and frequently limited by sampling size due to the lack of financial resources for basic research. In 2018, Muñoz-Gamba et al. (2021) collected mosquitoes from the rainforest in the Sierra Nevada de Santa Marta, on the Northern coast of Colombia and identified seventeen species of seven genera, despite the low sample size of 123 mosquitoes. In another study carried out in the forests of the Magdalena Medio valley in 2017, Hoyos et al. (2021) collected 292 mosquitoes and were able to identify twenty species of eight genera. The species found in both studies coincided partly with the ones collected in our study and researchers faced the same problems we did with morphological classification and barcoding.

A more comprehensive species identification could be achieved by the recollection of all mosquito live stages and comparison with detailed morphological species descriptions as well as the extension of barcode marker regions databases. In this sense, this study contributed to the first COI barcodes of *Cq. lynchi*, *Cq. venezuelensis*, *Ma. indubitans*, *Ps. albipes,* and *Ps. funiculus,* and the first ITS2 barcodes for *An. neivai*, *Cq. lynchi*, *Cq. venezuelensis*, *Ma. indubitans*, *Ma. titillans*, *Oc. scapularis*, *Ps. albipes*, *Ps. funiculus*, *Wy. luteoventralis* and *Wy. pertinans*, which will facilitate molecular identification of the mentioned species in the future.

We characterized the virome of eleven mosquito species, nine of which have been associated with the transmission of diseases to animals or humans in former studies. *An. darlingi* and *An. nuneztovari* correspond to the main malaria vectors in Colombia (Montoya-Lerma et al. 2011). *Cq. venezuelensis* and *Oc. serratus* have been involved in Oropouche virus transmission in Brazil (Nunes et al. 2005, Mourããо et al. 2009). *Cx. quinquefasciatus*, *Cx. nigripalpu*s and *Ma. titillans* have been found infected with St. Louis encephalitis virus, Western equine encephalitis virus, and West Nile fever virus among other zoonotic diseases (Guo et al. 2016, Duguma et al. 2019, Beranek et al. 2018). *Ps. albipes* and *Ps. ferox* play a role in Venezuelan equine encephalitis virus and La Crosse virus transmission (Aragão et al. 2019). *Ps. funiculus* and *Cq. lynchi* have not yet been associated with viral diseases. Based on the phylogenetic analyses we assume, that the viruses found in this study are mainly insect or plant viruses and are not of immediate pathogenic potential for humans or livestock. Nevertheless, studying the mosquito virome is important, as a deeper understanding of virus evolution, genetic diversity, and host-virus interaction is gained. Moreover, insect-specific viruses might be used as a biocontrol agent against human health-relevant arboviruses or serve as vaccine candidates (Atoni et al. 2019).

Most of the viruses described in the mosquitoes could be ascribed to the (-)ssRNA viruses (96%), emphasizing the evolutionary success and importance of this group. Interestingly some mosquito species show a higher virus incidence and diversity than others. More than half (53,9%) of the described virus species were found in *Cx. quinquefasciatus*, *Ma. titillans* and *Cq. venezuelensis* mosquitoes. A trend via a mosquito species-specific virome could be observed. It is assumed that each mosquito species has its specific habitat and habits and thus bears a specific virome that has evolved along with the host and its environment. Most probably, the viruses detected in the mosquito pools correspond to insect or plant viruses, but further studies are necessary for confirmation (e.g. in vitro virus cultivation).

The genomes of 52 viruses derived from mosquito pools were sequenced completely or partly and annotated. Nearly all the coding proteins for the 52 viruses were identified. For the **(-)ssRNA viruses**, order *Bunyavirales*, three novel members of the family *Phasmaviridae*, Guagua virus, Coredo virus, and Culex Phasma-like virus, and two members of the family *Phenuiviridae*, Narangue virus, and Atrato

Gouko-like virus, were characterized. In the order *Mononegavirales*, two yet undescribed members of the family *Rhabdoviridae*, Atrato Rhabdo-like virus 2 and 3 were described, and the genus *Atratorhabdovirus was* established. Concerning the order *Jingchuvirales,* two members of the family *Chuviridae*, Atrato Chu-like virus 1 and 2, were described. Moreover, two members of the family *Aliusviridae*, Atrato Chu-like virus 2 and 3 were found.

Regarding the **(+)ssRNA viruses** in the order *Picornavirales*, members of the families *Iflaviridae*, *Marnaviridae* and *Polycipiviridae* could be sequenced and annotated. Isla virus, Sabaleta virus, Chajerado virus, and Atrato Picorna-like virus 1 belong to the family *Iflaviridae*. Atrato Picorna-like virus 1, belongs to the family *Marnaviridae*. In the family *Polycipiviridae*, Cacaos virus was described. In the order *Tymovirales,* Gengado virus, and Murindo virus, two members of the family *Tymoviridae* were described. The order *Amarillovirales* comprises the family *Flaviviridae,* of which three novel viruses were found. Atrato Flavi-like virus 2 and 3 were isolated in C6/36 cell culture and are insect-specific viruses sharing the *Flavivirus* characteristic genome. Atrato Flavi-like virus 1 on the other hand, has a much longer genome of 22 kb, and only three proteins (Hel, Mt, RT) could be annotated in the polyprotein, as the rest of the genome does not resemble any available viral proteins. Eight representatives of the order *Martellivirales*, family *Virgaviridae* were found. Concerning the order *Sobelivirales*, six representatives of the family *Solemoviridae*, Atrato Sobemo virus 1-6, were characterized. Atrato Nege-like virus 1, a novel (+)ssRNA virus of the unofficial genus *Negevirus* that has not been assigned to any order yet, has been described.

Concerning the **reverse transcribing ssRNA viruses**, Chibugado virus, a member of the family *Metaviridae*, and Atrato Retro-like virus of the family *Pseudoviridae* were discovered. Relating to the order *Ghabrivirales*, Salado virus, a member of the family *Chrysoviridae*, was characterized. Four more yet undescribed representatives of the family *Totiviridae* - Atrato virus, Embera virus, Murri virus, and Pisingos virus - were explored. Atrato Partiti-like virus 1-5 belong to the order *Durnavirales*, family *Partitiviridae*. In the order *Reovirales*, Atrato Reo-like virus 1 of the family *Reoviridae* was found, but only 4 monocistronic segments coding for an RdRp, an Rbp, a maCP, and a miCP could be annotated. In the order *Piccovirales* Atrato Denso-like virus and Atrato Denso-like virus 1, two ssDNA viruses of the family *Parvoviridae* were found.

Some of the viruses found in this study had been described before, but most were novel and many gave rise to new taxa. All the mosquito-related viruses could be classified phylogenetically based on the AA sequence of the polymerase protein, but only 14 species could be assigned to existing genera. For the viruses that could not be assigned, novel genera were proposed based on the phylogenies and taxa-specific criteria established by the ICTV. A total of thirteen new genera and one new family were proposed. In the family *Polycipiviridae*, Cacaosvirus gave rise to the novel genus *Caosvirus*, in the family

Flaviviridae, Atrato Flavi-like virus 1 to the genus *Atratovirus*, in the family *Tymoviridae*, Murindo virus to the genus *Mudovirus* and Gengado virus to the genus *Gendovirus*, in the family *Partitiviridae*, Atrato Partiti-like virus 6 to the genus *Atrapavirus*, in the family *Totiviridae*, Embera and Pisingos virus to the genus *Totcuvirus*, and Murri virus to the genus *Muratovirus*, in the family *Phenuiviridae*, Atrato Gouko-like virus 1 to the genus *Zheemosvirus*; in the family *Chuviridae*, Atrato Chu-like virus 2 to the genus *Atchuvirus*; in the family *Aliusviridae*, Atrato Chu-like virus 4 to the genus *Rachuvirus* and Atrato Chu-like virus 5 to the genus *Oscuruvirus*; in the family *Rhabdoviridae*, Atrato Rhabdo-like virus 2 to the genus *Atratorhabdovirus*; in the family *Reoviridae*, Atrato Reo-like virus 1 to the genus *Relicuvirus*. Hubei mosquito virus 4 gave rise to the new family *Tolicuviridae*.

To our knowledge, the present study is the first wide scale metagenomic study investigating the mosquito virome promoted in Colombia. Nevertheless, in recent years some novel viruses have been discovered in mosquitoes in Colombia through cell culture experiments. In 2017, Contreras et al. described Balsa virus, a novel *Rhabdovirus* that was isolated from *Cx. erraticus* collected in the municipality of San Bernardo del Viento on the Caribean coast. It shares a similar genome organization with Arboretum virus isolated from *Ae. fulvus* and Puerto Almendras virus isolated from *Ps. albigenu* in Puerto Almendras, Peru, and gave rise to the new genus *Almendravirus* within the family *Rhabdoviridae*.

From the same study site, Sinu virus, a novel member of the genus *Thogotovirus* within the family *Othomyxoviridae* (Contreras et al. 2017), and Chiqui virus, an unclassified member of the family *Reoviridae*, were isolated from a pool of unidentified mosquitoes (Contreras-Gutiérrez et al. 2018). The three viruses were isolated in C6/36 cells and their genomes were NGS-sequenced. They did not replicate in vertebrate cells and seem to be insect-specific viruses (ISVs) that do not pose a threat to humans or vertebrates. Nevertheless, investigating ISVs is of major importance, as they might have an impact on the transmission of pathogenic arboviruses and the fitness of their vectors and thus could be used as a control agent. In 2021, Patterson et al. investigated the effects of coinfection of mosquito cells with *Negeviruses* and *Alphaviruses* and showed that the replication of Venezuelan equine encephalitis virus and Chikungunya virus was inhibited by coinfection with *Negeviruses* inducing superinfection exclusion. In addition, chimers of public health-relevant viruses and ISVs could serve as vaccine candidates and contribute to the control of pathogen transmission (Patterson et al. 2020).

A total of twelve viruses could be detected in six blood sample pools of 46 patients with suspicion of malaria infection. Apart from Atrato Partiti-like virus 3, a dsRNA virus of the family *Partitiviridae*, all sequenced viruses belonged to the ssDNA viruses, and the majority were ascribed to the genera *Parvoviridae* (41,5%) and *Circoviridae* (41,5%). Atrato human blood virus was assigned to the order *Cremevirales.* Five more viruses of the order *Circlivirales* - Atrato circular strange virus 1, Atrato circular strange virus 1, Atrato Gemini-like virus, Reagent-associated CRESS-like virus 7, and Atrato Circo-like virus 1 - were described. Members of the families *Parvoviridae* and *Circoviridae* have been related to diseases in humans and non-human mammals and are transmitted horizontally by oral droplets or the fecal-oral route. Since the discovery of Human Parvovirus B19 in 1974, the causing agent of the slapped cheek syndrome, a rash-causing childhood disease, a variety of pathogens causing diseases in humans have been identified (Jianming Qiu 2017). Members of the family *Circoviridae* have also been detected in human and animal samples and might be associated with respiratory illness in humans (Tung Gia Phan 2014). Thus, it is quite possible that the *Parvoviruses* and *Circoviruses* found in the human blood samples in this study could be pathogenic to humans. Cell culture experiments in human cell lines should be the next step to confirm the presumption. Hundreds of blood samples from patients that saw a health center with suspicion of malaria infection were collected, but only 46 could be screened for viruses due to scarcity of resources and time limitations. Nevertheless, twelve viruses could be characterized in patients' samples, but further cell culture experiments are necessary to confirm their affiliation with human hosts and rule out contamination. From 2019 to 2022, Ciuoderis et al. (2022) investigated acute febrile illnesses in patients from four Colombian cities - Cali, Leticia, Villavicencio, Cucutá - and found evidence for malaria, dengue virus, and Oropouche virus infections in human serum in a PCR and NGS based study. Of the 2967 patients screened, 20,7% resulted positive for dengue virus. In addition, 68 Oropouche cases and ten coinfections with Oropouche virus and dengue virus could be confirmed. Colombia is endemic for dengue virus that is transmitted to humans by the vectors *Ae. aegypti* and *Ae. albopictus*. Since the introduction of *Ae. albopictus* to the USA in the early 1980s this invasive and highly adaptable species spread throughout the Americas and thus the cases of Dengue, Chikungunya, and Zika. In the Atrato River region, we could not confirm Dengue, Zika, or Chikungunya transmission, but we were able to identify 30 specimens of *Ae. albopictus*, 24 of which were collected in the municipality of Bellavista (Figure 4,Table 18). Oropouche virus is also endemic in Colombia and is mainly transmitted by midges of the species *Culicoides paraensis*, but *Ae. serratus* and *Cx. quinquefasciatus* might also be involved in transmission (Romero-Alvarez and Escobar 2018). Midges were not subject to our study and none of the mosquitoes and human blood samples tested here were infested with Oropouche virus. Apart from the viral agents, evidence was found for the presence of bacterial and protozoan organisms in mosquitoes and human blood samples that might be pathogenic to humans or insects.

In conclusion, the Atrato-River region in Northwest Colombia harbors a great diversity of mosquito species that harbor species-specific viromes and play a crucial role in virus transmission and evolution. Ongoing monitoring of the most abundantly collected mosquito species might lead to the discovery of unknown or emergent diseases that could pose a threat to human health, livestock, and crops.

9 References

Adams, Michael J.; Adkins, Scott; Bragard, Claude; Gilmer, David; Li, Dawei; MacFarlane, Stuart A. et al. (2017): ICTV Virus Taxonomy Profile: Virgaviridae. In *The Journal of general virology* 98 (8), pp. 1999–2000. DOI: 10.1099/jgv.0.000884.

Afgan, Enis; Baker, Dannon; Batut, Bérénice; van den Beek, Marius; Bouvier, Dave; Cech, Martin et al. (2018): The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2018 update. In *Nucleic acids research* 46 (W1), W537-W544. DOI: 10.1093/nar/gky379.

Aldhebiani, Amal Y. (2018): Species concept and speciation. In *Saudi journal of biological sciences* 25 (3), pp. 437–440. DOI: 10.1016/j.sjbs.2017.04.013.

Altamiranda-Saavedra, Mariano; Arboleda, Sair; Parra, Juan L.; Peterson, A. Townsend; Correa, Margarita M. (2017): Potential distribution of mosquito vector species in a primary malaria endemic region of Colombia. In *PloS one* 12 (6), e0179093. DOI: 10.1371/journal.pone.0179093.

Anisimova, Maria; Gascuel, Olivier (2006): Approximate likelihood-ratio test for branches: A fast, accurate, and powerful alternative. In *Systematic biology* 55 (4), pp. 539–552. DOI: 10.1080/10635150600755453.

Aragão, Andressa de Oliveira; Nunes Neto, Joaquim Pinto; Cruz, Ana Cecília Ribeiro; Casseb, Samir Mansour Moraes; Cardoso, Jedson Ferreira; da Silva, Sandro Patroca; Ishikawa, Edna Aoba Yassui (2019): Description and phylogeny of the mitochondrial genome of Sabethes chloropterus, Sabethes glaucodaemon and Sabethes belisarioi (Diptera: Culicidae). In *Genomics* 111 (4), pp. 607–611. DOI: 10.1016/j.ygeno.2018.03.016.

Ashfaq, Muhammad; Hebert, Paul D. N.; Mirza, Jawwad H.; Khan, Arif M.; Zafar, Yusuf; Mirza, M. Sajjad (2014): Analyzing mosquito (Diptera. Culicidae) diversity in Pakistan by DNA barcoding. In *PloS one* 9 (5), e97268. DOI: 10.1371/journal.pone.0097268.

Atoni, Evans; Zhao, Lu; Karungu, Samuel; Obanda, Vincent; Agwanda, Bernard; Xia, Han; Yuan, Zhiming (2019): The discovery and global distribution of novel mosquito-associated viruses in the last decade (2007-2017). In *Reviews in medical virology* 29 (6), e2079. DOI: 10.1002/rmv.2079.

Bahnck, Carolyn M.; Fonseca, Dina M. (2006): Rapid assay to identify the two genetic forms of Culex (Culex) pipiens L. (Diptera: Culicidae) and hybrid populations. In *The American journal of tropical medicine and hygiene* 75 (2), pp. 251–255.

Bangs, Michael J.; Pudiantari, Ratna; Gionar, Yoyo R. (2007): Persistence of Dengue Virus RNA in Dried *Aedes aegypti* (Diptera: Culicidae) Exposed to Natural Tropical Conditions. In *Journal of medical entomology* 44 (1), pp. 163–167. DOI: 10.1603/0022-2585(2007)44[163:podvri]2.0.co;2.

Batson, Joshua; Dudas, Gytis; Haas-Stapleton, Eric; Kistler, Amy L.; Li, Lucy M.; Logan, Phoenix et al. (2021): Single mosquito metatranscriptomics identifies vectors, emerging pathogens and reservoirs in one assay. In *eLife* 10. DOI: 10.7554/eLife.68353.

Bauhin, Caspar (1623): Pinax theatri botanici. Available online at https://www.biodiversitylibrary.org/bibliography/72114.

Bazin, Mathieu; Williams, Craig R. (2018): Mosquito traps for urban surveillance: collection efficacy and potential for use by citizen scientists. In *Journal of vector ecology: Journal of the Society for Vector Ecology* 43 (1), pp. 98–103. DOI: 10.1111/jvec.12288.

Beebe, Nigel W. (2018): DNA barcoding mosquitoes: advice for potential prospectors. In *Parasitology* 145 (5), pp. 622–633. DOI: 10.1017/S0031182018000343.

Beranek, M. D.; Gallardo, R.; Almirón, W. R.; Contigiani, M. S. (2018): First detection of Mansonia titillans (Diptera: Culicidae) infected with St. Louis encephalitis virus (Flaviviridae: Flavivirus) and Bunyamwera serogroup (Peribunyaviridae: Orthobunyavirus) in Argentina. In *Journal of vector ecology : journal of the Society for Vector Ecology* 43 (2), pp. 340–343. DOI: 10.1111/jvec.12320.

Berrocal, Luis; Peña, José; González, Marco; Mattar, Salim (2006): Virus del Oeste del Nilo: ecología y epidemiologia de un patógeno emergente en Colombia. In *Revista de salud publica (Bogota, Colombia)* 8 (2), pp. 218–228. DOI: 10.1590/s0124-00642006000200010.

Bragard, C.; Caciagli, P.; Lemaire, O.; Lopez-Moya, J. J.; MacFarlane, S.; Peters, D. et al. (2013): Status and prospects of plant virus control through interference with vector transmission. In *Annual review of phytopathology* 51, pp. 177–201. DOI: 10.1146/annurev-phyto-082712-102346.

Burkhalter, Kristen L.; Savage, Harry M. (2017): Detection of Zika Virus in Desiccated Mosquitoes by Real-Time Reverse Transcription PCR and Plaque Assay. In *Emerging Infectious Diseases* 23 (4), pp. 680–681. DOI: 10.3201/eid2304.161772.

Calle-Tobón, Arley; Pérez-Pérez, Juliana; Forero-Pineda, Nicolás; Chávez, Omar Triana; Rojas-Montoya, Winston; Rúa-Uribe, Guillermo; Gómez-Palacio, Andrés (2022): Local-scale virome depiction in Medellín, Colombia, supports significant differences between Aedes aegypti and Aedes albopictus. In *PloS one* 17 (7), e0263143. DOI: 10.1371/journal.pone.0263143.

Capella-Gutierrez, Salvador; Silla-Martinez, Jose M.; Gabaldon, Toni (2009): trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. In *Bioinformatics (Oxford, England)* 25 (15), pp. 1972–1973. DOI: 10.1093/bioinformatics/btp348.

Chew, Ching Hoong; Lim, Yvonne Ai Lian; Lee, Ping Chin; Mahmud, Rohela; Chua, Kek Heng (2012): Hexaplex PCR detection system for identification of five human Plasmodium species with an internal control. In *Journal of clinical microbiology* 50 (12), pp. 4012–4019. DOI: 10.1128/JCM.06454-11.

Ciuoderis, Karl A.; Berg, Michael G.; Perez, Lester J.; Hadji, Abbas; Perez-Restrepo, Laura S.; Aristizabal, Leidi Carvajal et al. (2022): Oropouche virus as an emerging cause of acute febrile illness in Colombia. In *Emerging microbes & infections* 11 (1), pp. 2645–2657. DOI: 10.1080/22221751.2022.2136536.

Coleman, Annette W. (2003): ITS2 is a double-edged tool for eukaryote evolutionary comparisons. In *Trends in Genetics* 19 (7), pp. 370–375. DOI: 10.1016/S0168-9525(03)00118-5.

Colmant, Agathe M. G.; Etebari, Kayvan; Webb, Cameron E.; Ritchie, Scott A.; Jansen, Cassie C.; van den Hurk, Andrew F. et al. (2017): Discovery of new orbiviruses and totivirus from Anopheles mosquitoes in Eastern Australia. In *Archives of virology* 162 (11), pp. 3529–3534. DOI: 10.1007/s00705-017-3515-x.

Contreras, Maria Angelica; Eastwood, Gillian; Guzman, Hilda; Popov, Vsevolod; Savit, Chelsea; Uribe, Sandra et al. (2017): Almendravirus: A Proposed New Genus of Rhabdoviruses Isolated from Mosquitoes in Tropical Regions of the Americas. In *The American journal of tropical medicine and hygiene* 96 (1), pp. 100–109. DOI: 10.4269/ajtmh.16-0403.

Contreras-Gutiérrez, María Angélica; Guzman, Hilda; Cardoso, Jedson F.; Popov, Vsevolod L.; Nunes, Marcio R. T.; Uribe, Sandra et al. (2018): Genome Sequence of Chiqui Virus, a Novel Reovirus Isolated from Mosquitoes Collected in Colombia. In *Microbiology resource announcements* 7 (12). DOI: 10.1128/MRA.00881-18.

Contreras-Gutiérrez, María Angélica; Nunes, Marcio R. T.; Guzman, Hilda; Uribe, Sandra; Suaza Vasco, Juan David; Cardoso, Jedson F. et al. (2017): Sinu virus, a novel and divergent orthomyxovirus related to members of the genus Thogotovirus isolated from mosquitoes in Colombia. In *Virology* 501, pp. 166–175. DOI: 10.1016/j.virol.2016.11.014.

Cotmore, Susan F.; Agbandje-McKenna, Mavis; Canuti, Marta; Chiorini, John A.; Eis-Hubinger, Anna-Maria; Hughes, Joseph et al. (2019): ICTV Virus Taxonomy Profile: Parvoviridae. In *The Journal of general virology* 100 (3), pp. 367–368. DOI: 10.1099/jgv.0.001212.

Cronquist, Arthur (1968): The evolution and classification of flowering plants. London: Nelson (Riverside studies in biology).

Da Silva Ferreira, Raquel; Da Toni Aquino Cruz, Lucinéia Claudia de; Souza, Vilma Juscineide de; da Silva Neves, Nilvanei Aparecido; Souza, Victor Costa de; Filho, Luciano Chaves Franco et al. (2020): Insect-specific viruses and arboviruses in adult male culicids from Midwestern Brazil. In *Infection, genetics and evolution : journal of molecular epidemiology and evolutionary genetics in infectious diseases* 85, p. 104561. DOI: 10.1016/j.meegid.2020.104561.

Darsie, Richard F. (1985): Mosquitoes of Argentina (Part 1). Part 1. Keys for Identification of Adult Females and Fourth Stage Larvae in Englisch and Spanish (Diptera, Culicidae). In *Mosquito Systematics* 17, pp. 153–253.

Darwin, C. R. (1859): On the origin of species by means of natural selection, or preservation of favoured races in the struggle for life. London.

Demari-Silva, Bruna; Foster, Peter G.; Oliveira, Tatiane M. P. de; Bergo, Eduardo S.; Sanabani, Sabri S.; Pessôa, Rodrigo; Sallum, Maria Anice M. (2015): Mitochondrial genomes and comparative analyses of Culex camposi, Culex coronator, Culex usquatus and Culex usquatissimus (Diptera:Culicidae), members of the coronator group. In *BMC genomics* 16, p. 831. DOI: 10.1186/s12864-015-1951-0.

Demari-Silva, Bruna; Suesdek, Lincoln; Sallum, Maria Anice Mureb; Marrelli, Mauro Toledo (2014): Wing geometry of Culex coronator (Diptera: Culicidae) from South and Southeast Brazil. In *Parasites & vectors* 7, p. 174. DOI: 10.1186/1756-3305-7-174.

Demari-Silva, Bruna; Vesgueiro, Fabiana Tavares; Sallum, Maria Anice Mureb; Marrelli, Mauro Toledo (2011): Taxonomic and phylogenetic relationships between species of the genus Culex (Diptera: culicidae) from Brazil inferred from the cytochrome c oxidase I mitochondrial gene. In *Journal of medical entomology* 48 (2), pp. 272–279. DOI: 10.1603/me09293.

Di Paola, N.; Dheilly, N. M.; Kuhn, J. H.; Junglen, S.; Paraskevopoulou, S.; Postler, T. S.; Shi, Mang (2020): Reorganize the order to include four new families, 18 new genera, and 22 new species (Jingchuvirales). International Committee on Taxonomy of Viruses.

Dietzgen, Ralf G.; Mann, Krin S.; Johnson, Karyn N. (2016): Plant Virus-Insect Vector Interactions: Current and Potential Future Research Directions. In *Viruses* 8 (11). DOI: 10.3390/v8110303.

Djadid, Navid D.; Gholizadeh, Saber; Tafsiri, Elham; Romi, Roberto; Gordeev, Mikhail; Zakeri, Sedigheh (2007): Molecular identification of Palearctic members of Anopheles maculipennis in northern Iran. In *Malaria journal* 6, p. 6. DOI: 10.1186/1475-2875-6-6.

Djikeng, Appolinaire; Spiro, David (2009): Advancing full length genome sequencing for human RNA viral pathogens. In *Future virology* 4 (1), pp. 47–53. DOI: 10.2217/17460794.4.1.47.

Donaldson, Eric F.; Haskew, Aimee N.; Gates, J. Edward; Huynh, Jeremy; Moore, Clea J.; Frieman, Matthew B. (2010): Metagenomic analysis of the viromes of three North American bat species: viral diversity among different bat species that share a common habitat. In *Journal of virology* 84 (24), pp. 13004–13018. DOI: 10.1128/JVI.01255-10.

Duguma, Dagne; Hall, Michael W.; Smartt, Chelsea T.; Debboun, Mustapha; Neufeld, Josh D. (2019): Microbiota variations in Culex nigripalpus disease vector mosquito of West Nile virus and Saint Louis Encephalitis from different geographic origins. In *PeerJ* 6, e6168. DOI: 10.7717/peerj.6168.

Dyar, H. G. (1920): The Grabhamia Group pf Psorophora. In *Insecutor Inscitiae Mensruus* 8, pp. 140–141.

Dyar, H. G. (1928): The mosquitoes of the Americas. Washington, DC (Pub. 387).

Efron, B. (1979): Bootstrap Methods: Another Look at the Jackknife. In *The Annals of Statistics* 7 (1), pp. 1–26. DOI: 10.1214/aos/1176344552.

Ellis, Brett R.; Wilcox, Bruce A. (2009): The ecological dimensions of vector-borne disease research and control. In *Cadernos de saude publica* 25 Suppl 1, S155-67. DOI: 10.1590/s0102-311x2009001300015.

Endoh, Daiji; Mizutani, Tetsuya; Kirisawa, Rikio; Maki, Yoshiyuki; Saito, Hidetoshi; Kon, Yasuhiro et al. (2005): Species-independent detection of RNA virus by representational difference analysis using non-ribosomal hexanucleotides for reverse transcription. In *Nucleic acids research* 33 (6), e65. DOI: 10.1093/nar/gni064.

Faizah, Astri Nur; Kobayashi, Daisuke; Isawa, Haruhiko; Amoa-Bosompem, Michael; Murota, Katsunori; Higa, Yukiko et al. (2020): Deciphering the Virome of Culex vishnui Subgroup Mosquitoes, the Major Vectors of Japanese Encephalitis, in Japan. In *Viruses* 12 (3). DOI: 10.3390/v12030264.

Fauver, Joseph R.; Grubaugh, Nathan D.; Krajacich, Benjamin J.; Weger-Lucarelli, James; Lakin, Steven M.; Fakoli, Lawrence S. et al. (2016): West African Anopheles gambiae mosquitoes harbor a taxonomically diverse virome including new insect-specific flaviviruses, mononegaviruses, and totiviruses. In *Virology* 498, pp. 288–299. DOI: 10.1016/j.virol.2016.07.031.

Folmer, O.; Black, M.; Hoeh, W.; Lutz, R.; Vrijenhoek, R. (1994): DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. In *Molecular marine biology and biotechnology* 3 (5), pp. 294–299.

Fontecha, Gustavo A.; Mendoza, Meisy; Banegas, Engels; Poorak, Mitra; Oliveira, Alexandre M. de; Mancero, Tamara et al. (2012): Comparison of molecular tests for the diagnosis of malaria in Honduras. In *Malaria journal* 11, p. 119. DOI: 10.1186/1475-2875-11-119.

Forattini, O. P. (1965): Entomologia médica: Editôra da Universidade de São Paulo (Entomologia médica). Available online at https://books.google.com.co/books?id=wFxZAAAAMAAJ.

Forattini, O. P.; Sallum, M. A. (1987): Studies on some species of Culex (Melanoconion), with the description of a new one from Southern Brazil (Diptera: Culicidae). In *Revista de saude publica* 21 (2), pp. 123–156. DOI: 10.1590/s0034-89101987000200006.

Forattini, Oswaldo Paulo (2002): Culicidologia médica. São Paulo: Edusp.

Forshey, Brett M.; Guevara, Carolina; Laguna-Torres, V. Alberto; Cespedes, Manuel; Vargas, Jorge; Gianella, Alberto et al. (2010): Arboviral etiologies of acute febrile illnesses in Western South America, 2000-2007. In *PLoS neglected tropical diseases* 4 (8), e787. DOI: 10.1371/journal.pntd.0000787.

Förster, Maria Sophie (2019): Molecular detection of novel mosquito-associated viruses in the Atrato River region in Colombia. Bachelor thesis. Technical University of Berlin, Berlin. Institute for Biotechnology.

Fuehrer, Hans-Peter; Fally, Markus A.; Habler, Verena E.; Starzengruber, Peter; Swoboda, Paul; Noedl, Harald (2011): Novel nested direct PCR technique for malaria diagnosis using filter paper samples. In *Journal of clinical microbiology* 49 (4), pp. 1628–1630. DOI: 10.1128/JCM.01792-10.

Galaxy Training Network (2021): Unicycler assembly of SARS-CoV-2 genome with preprocessing to remove human genome reads. With assistance of Cristóbal Gallardo. Available online at https://usegalaxy.org/training-material/topics/assembly/tutorials/assembly-with-preprocessing/slides-plain.html.

González Obando, Ranulfo; Carrejo Gironza, Nancy Soraya (2009): Introducción al estudio taxonómico de anopheles de Colombia. Claves y notas de distribución. 2. ed. Cali, Colombia: Programa Editorial Universidad del Valle (Colección Libros de Investigación).

Grubaugh, Nathan D.; McMenamy, Scott S.; Turell, Michael J.; Lee, John S. (2013): Multi-gene detection and identification of mosquito-borne RNA viruses using an oligonucleotide microarray. In *PLoS neglected tropical diseases* 7 (8), e2349. DOI: 10.1371/journal.pntd.0002349.

Grywna, Klaus; Kupfer, Bernd; Panning, Marcus; Drexler, Jan Felix; Emmerich, Petra; Drosten, Christian; Kümmerer, Beate M. (2010): Detection of all species of the genus Alphavirus by reverse transcription-PCR with diagnostic sensitivity. In *Journal of clinical microbiology* 48 (9), pp. 3386–3387. DOI: 10.1128/JCM.00317-10.

Gu, Wei; Miller, Steve; Chiu, Charles Y. (2019): Clinical Metagenomic Next-Generation Sequencing for Pathogen Detection. In *Annual review of pathology* 14, pp. 319–338. DOI: 10.1146/annurev-pathmechdis-012418-012751.

Guindon, Stephane; Dufayard, Jean-Francois; Lefort, Vincent; Anisimova, Maria; Hordijk, Wim; Gascuel, Olivier (2010): New algorithms and methods to estimate maximum-likelihood phylogenies:

assessing the performance of PhyML 3.0. In *Systematic biology* 59 (3), pp. 307–321. DOI: 10.1093/sysbio/syq010.

Guo, Xiao-Xia; Li, Chun-Xiao; Deng, Yong-Qiang; Xing, Dan; Liu, Qin-Mei; Wu, Qun et al. (2016): Culex pipiens quinquefasciatus: a potential vector to transmit Zika virus. In *Emerging microbes & infections* 5 (9), e102. DOI: 10.1038/emi.2016.102.

Hahn, Megan A.; Rosario, Karyna; Lucas, Pierrick; Dheilly, Nolwenn M. (2020): Characterization of viruses in a tapeworm: phylogenetic position, vertical transmission, and transmission to the parasitized host. In *The ISME journal* 14 (7), pp. 1755–1767. DOI: 10.1038/s41396-020-0642-2.

Hall, Roy A.; Blitvich, Bradley J.; Johansen, Cheryl A.; Blacksell, Stuart D. (2012): Advances in arbovirus surveillance, detection and diagnosis. In *Journal of biomedicine & biotechnology* 2012, p. 512969. DOI: 10.1155/2012/512969.

Hall, T. A. (1999): Bioedit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. In *Nucleic Acids Symposium Series* 41, pp. 95–98.

Hang, Jun; Klein, Terry A.; Kim, Heung-Chul; Yang, Yu; Jima, Dereje D.; Richardson, Jason H.; Jarman, Richard G. (2016): Genome Sequences of Five Arboviruses in Field-Captured Mosquitoes in a Unique Rural Environment of South Korea. In *Genome announcements* 4 (1). DOI: 10.1128/genomeA.01644-15.

Harbach, Ralph E. (2007): The Culicidae (Diptera): a review of taxonomy, classification and phylogeny *. DOI: 10.5281/zenodo.180118.

Harbach, Ralph E. (2012): Culex pipiens: species versus species complex taxonomic history and perspective. In *Journal of the American Mosquito Control Association* 28 (4 Suppl), pp. 10–23. DOI: 10.2987/8756-971X-28.4.10.

Harbach, Ralph E. (2013a): Mosquito Taxonomic Inventory. Subgenus Melanoconion Theobald, 1903. Available online at http://mosquito-taxonomic-inventory.info/simpletaxonomy/term/6182, checked on 6/3/2021.

Harbach, Ralph E. (2013b): Valid species. Mosquito Taxonomy Inventory. Available online at http://mosquito-taxonomic-inventory.info/valid-species-list, checked on 10/7/2020.

Hebert, Paul D. N.; Cywinska, Alina; Ball, Shelley L.; deWaard, Jeremy R. (2003a): Biological identifications through DNA barcodes. In *Proceedings. Biological sciences* 270 (1512), pp. 313–321. DOI: 10.1098/rspb.2002.2218.

Hebert, Paul D. N.; Penton, Erin H.; Burns, John M.; Janzen, Daniel H.; Hallwachs, Winnie (2004): Ten species in one. DNA barcoding reveals cryptic species in the neotropical skipper butterfly Astraptes fulgerator. In *Proceedings of the National Academy of Sciences of the United States of America* 101 (41), pp. 14812–14817. DOI: 10.1073/pnas.0406166101.

Hebert, Paul D. N.; Ratnasingham, Sujeevan; deWaard, Jeremy R. (2003b): Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. In *Proceedings. Biological sciences* 270 Suppl 1, S96-9. DOI: 10.1098/rsbl.2003.0025.

Hermanns, Kyra; Zirkel, Florian; Kopp, Anne; Marklewitz, Marco; Rwego, Innocent B.; Estrada, Alejandro et al. (2017): Discovery of a novel alphavirus related to Eilat virus. In *The Journal of general virology* 98 (1), pp. 43–49. DOI: 10.1099/jgv.0.000694.

Hordijk, Wim; Gascuel, Olivier (2005): Improving the efficiency of SPR moves in phylogenetic tree search methods based on maximum likelihood. In *Bioinformatics (Oxford, England)* 21 (24), pp. 4338–4347. DOI: 10.1093/bioinformatics/bti713.

Hoyos, Juliana; Carrasquilla, María Cristina; Leon, Cielo; Montgomery, Joel M.; Salyer, Stephanie J.; Komar, Nicholas; González, Camila (2021): Mosquito-vertebrate Interaction Networks in the Magdalena Medio Region of Colombia. In *Research Square*. DOI: 10.21203/rs.3.rs-540730/v1.

Hoyos-López, Richard (2018): Molecular Typing with COI - DNA Barcode of mosquitoes with medical importance from rural area of La Pintada, Antioquia, Colombia. Available online at https://www.researchgate.net/publication/323235286_Molecular_Typing_with_COI-DNA_Barcode_of_mosquitoes_with_medical_importance_from_rural_area_of_La_Pintada_Antioquia_Colombia.

Hoyos-López, Richard; Soto, Sandra Uribe; Rúa-Uribe, Guillermo; Gallego-Gómez, Juan Carlos (2015): Molecular identification of Saint Louis encephalitis virus genotype IV in Colombia. In *Memorias do Instituto Oswaldo Cruz* 110 (6), pp. 719–725. DOI: 10.1590/0074-02760280040.

Huang, Run; Song, Hongyun; Fang, Qian; Qian, Junping; Zhang, Yaodan; Jiang, Hong (2021): Laboratory and Greenhouse Performance of Five Commercial Light Traps for Capturing Mosquitoes in China. In *Journal of the American Mosquito Control Association* 37 (4), pp. 250–255. DOI: 10.2987/21-7012.

Huang, Yan-Jang S.; Higgs, Stephen; Vanlandingham, Dana L. (2019): Emergence and re-emergence of mosquito-borne arboviruses. In *Current opinion in virology* 34, pp. 104–109. DOI: 10.1016/j.coviro.2019.01.001.

Hulo, Chantal; Castro, Edouard de; Masson, Patrick; Bougueleret, Lydie; Bairoch, Amos; Xenarios, Ioannis; Le Mercier, Philippe (2011): ViralZone: a knowledge resource to understand virus diversity. In *Nucleic acids research* 39 (Database issue), D576-82. DOI: 10.1093/nar/gkq901.

ICTV (2018): The International Code of Virus Classification and Nomenclature. Available online at https://talk.ictvonline.org/information/w/ictv-information/383/ictv-code, checked on 10/7/2020.

ICTV (2019): ICTV Master Species List 2019 v2. With assistance of Markus Döring.

Junglen, Sandra (7/18/2016): Six (6) new species in one (1) new genus (Orthophasmavirus) to be included in the proposed family Phasmaviridae in the proposed order Bunyavirales. 2016.028a-dM.

Junglen, Sandra; Drosten, Christian (2013): Virus discovery and recent insights into virus diversity in arthropods. In *Current opinion in microbiology* 16 (4), pp. 507–513. DOI: 10.1016/j.mib.2013.06.005.

Junglen, Sandra; Marklewitz, Marco; Zirkel, Florian; Drosten, Christian (7/18/2016): One (1) new species in one new genus (Orthoferavirus), to be included in the proposed family Feraviridae in the proposed order Bunyavirales. 2016.021a-dM.

Kadri, Sameer S. (2020): Key Takeaways From the U.S. CDC's 2019 Antibiotic Resistance Threats Report for Frontline Providers. In *Critical care medicine* 48 (7), pp. 939–945. DOI: 10.1097/CCM.0000000000004371.

Käfer, Simon; Paraskevopoulou, Sofia; Zirkel, Florian; Wieseke, Nicolas; Donath, Alexander; Petersen, Malte et al. (2019): Re-assessing the diversity of negative strand RNA viruses in insects. In *PLoS pathogens* 15 (12), e1008224. DOI: 10.1371/journal.ppat.1008224.

Katoh, Kazutaka; Standley, Daron M. (2013): MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. In *Mol Biol Evol* 30 (4), pp. 772–780. DOI: 10.1093/molbev/mst010.

Kearse, Matthew; Moir, Richard; Wilson, Amy; Stones-Havas, Steven; Cheung, Matthew; Sturrock, Shane et al. (2012): Geneious Basic. An integrated and extendable desktop software platform for the organization and analysis of sequence data. In *Bioinformatics (Oxford, England)* 28 (12), pp. 1647–1649. DOI: 10.1093/bioinformatics/bts199.

Kenea, Oljira; Balkew, Meshesha; Tekie, Habte; Gebre-Michael, Teshome; Deressa, Wakgari; Loha, Eskindir et al. (2017): Comparison of two adult mosquito sampling methods with human landing catches in south-central Ethiopia. In *Malaria journal* 16 (1), p. 30. DOI: 10.1186/s12936-016-1668-9.

Kinene, T.; Wainaina, J.; Maina, S.; Boykin, L. M. (2016): Rooting Trees, Methods for. In *Encyclopedia of Evolutionary Biology*, pp. 489–493. DOI: 10.1016/B978-0-12-800049-6.00215-8.

King, Andrew (2011): Virus taxonomy. Ninth report of the International Committee on Taxonomy of Viruses. Oxford: Elsevier. Available online at http://www.sciencedirect.com/science/book/9780123846846.

Kleanthous, Eleni; Olendraite, Ingrida; Lukhovitskaya, Nina I.; Firth, Andrew E. (2019): Discovery of three RNA viruses using ant transcriptomic datasets. In *Archives of virology* 164 (2), pp. 643–647. DOI: 10.1007/s00705-018-4093-2.

Kohl, Claudia; Brinkmann, Annika; Dabrowski, Piotr W.; Radonić, Aleksandar; Nitsche, Andreas; Kurth, Andreas (2015): Protocol for metagenomic virus detection in clinical specimens. In *Emerging Infectious Diseases* 21 (1), pp. 48–57. DOI: 10.3201/eid2101.140766.

Koonin, Eugene V.; Dolja, Valerian V.; Krupovic, Mart; Varsani, Arvind; Wolf, Yuri I.; Yutin, Natalya et al. (2020): Global Organization and Proposed Megataxonomy of the Virus World. In *Microbiology and molecular biology reviews : MMBR* 84 (2). DOI: 10.1128/MMBR.00061-19.

Kotta-Loizou, Ioly; Castón, José R.; Coutts, Robert H. A.; Hillman, Bradley I.; Jiang, Daohong; Kim, Dae-Hyuk et al. (2020): ICTV Virus Taxonomy Profile: Chrysoviridae. In *The Journal of general virology* 101 (2), pp. 143–144. DOI: 10.1099/jgv.0.001383.

Kramer, L. D.; Wolfe, T. M.; Green, E. N.; Chiles, R. E.; Fallah, H.; Fang, Y.; Reisen, W. K. (2002): Detection of encephalitis viruses in mosquitoes (Diptera: Culicidae) and avian tissues. In *Journal of medical entomology* 39 (2), pp. 312–323. DOI: 10.1603/0022-2585-39.2.312.

Lambert, Amy J.; Lanciotti, Robert S. (2009): Consensus amplification and novel multiplex sequencing method for S segment species identification of 47 viruses of the Orthobunyavirus, Phlebovirus, and

Nairovirus genera of the family Bunyaviridae. In *Journal of clinical microbiology* 47 (8), pp. 2398–2404. DOI: 10.1128/JCM.00182-09.

Lane, John (1953): Neotropical Culicidae. Brazil: University of São Paulo (Vol I & II).

Lanzaro, Gregory C.; Collier, Travis C.; Lee, Yoosook (2015): Defining Genetic, Taxonomic, and Geographic Boundaries Among Species of the Psorophora confinnis (Diptera: Culicidae) Complex in North and South America. In *Journal of medical entomology* 52 (5), pp. 907–917. DOI: 10.1093/jme/tjv084.

Larsson, Anders (2014): AliView: a fast and lightweight alignment viewer and editor for large datasets. In *Bioinformatics* 30 (22), pp. 3276–3278. DOI: 10.1093/bioinformatics/btu531.

Laurito, Magdalena; Oliveira, Tatiane M. P. de; Almirón, Walter Ricardo; Sallum, Maria Anice Mureb (2013): COI barcode versus morphological identification of Culex (Culex) (Diptera: Culicidae) species: a case study using samples from Argentina and Brazil. In *Memorias do Instituto Oswaldo Cruz* 108 Suppl 1, pp. 110–122. DOI: 10.1590/0074-0276130457.

Laurito, Magdalena; Visintin, Andrés Mario; Almirón, Walter Ricardo (2008): Culex saltanensis morphological redescription of the immature and adult stages. In *Journal of the American Mosquito Control Association* 24 (2), pp. 203–210. DOI: 10.2987/5641.1.

Lee, Hobart; Halverson, Sara; Ezinwa, Ngozi (2018): Mosquito-Borne Diseases. In *Primary care* 45 (3), pp. 393–407. DOI: 10.1016/j.pop.2018.05.001.

Lee, Michael S. Y.; Palci, Alessandro (2015): Morphological Phylogenetics in the Genomic Age. In *Current biology : CB* 25 (19), R922-9. DOI: 10.1016/j.cub.2015.07.009.

Lefort, Vincent; Longueville, Jean-Emmanuel; Gascuel, Olivier (2017): SMS: Smart Model Selection in PhyML. In *Molecular biology and evolution* 34 (9), pp. 2422–2424. DOI: 10.1093/molbev/msx149.

Li, Ci-Xiu; Shi, Mang; Tian, Jun-Hua; Lin, Xian-Dan; Kang, Yan-Jun; Chen, Liang-Jun et al. (2015): Unprecedented genomic diversity of RNA viruses in arthropods reveals the ancestry of negative-sense RNA viruses. In *eLife* 4. DOI: 10.7554/eLife.05378.

Li, Z. X.; Bian, Z. S.; Zheng, H. P.; Yue, Y. S.; Yao, J. Y.; Gong, Y. P. et al. (1990): First isolation of Xanthomonas campestris from the blood of a Chinese woman. In *Chinese medical journal* 103 (5), pp. 435–439.

Lima, José Bento Pereira; Rosa-Freitas, Maria Goreti; Rodovalho, Cynara Melo; Santos, Fátima; Lourenço-de-Oliveira, Ricardo (2014): Is there an efficient trap or collection method for sampling Anopheles darlingi and other malaria vectors that can describe the essential parameters affecting transmission dynamics as effectively as human landing catches? - A Review. In *Memorias do Instituto Oswaldo Cruz* 109 (5), pp. 685–705. DOI: 10.1590/0074-0276140134.

Linnaeus, C. (Ed.) (1753): Species plantarum, exhibentes plantas rite cognitas, ad genera relatas, cum differentiis specificis, nominibus trivialibus, synonymis selectis, locis natalibus, secundum systema sexuale digestas. 2 volumes.

Lipowski, Dariusz; Rzadkiewicz, Ewa; Czekalska-Lachowicz, Ewa (2008): Burkholderia cepacia--nowy patogen wywołujacy zakazenia wśród pacjentów szpitalnych. In *Przeglad epidemiologiczny* 62 (1), pp. 7–17.

Llorens, Carlos; Soriano, Beatriz; Krupovic, Mart; Ictv, Report Consortium (2020): ICTV Virus Taxonomy Profile: Metaviridae. In *The Journal of general virology* 101 (11), pp. 1131–1132. DOI: 10.1099/jgv.0.001509.

Llorens, Carlos; Soriano, Beatriz; Krupovic, Mart; Ictv, Report Consortium (2021): ICTV Virus Taxonomy Profile: Pseudoviridae. In *The Journal of general virology* 102 (3). DOI: 10.1099/jgv.0.001563.

Madden, Tom (2003): The BLAST Sequence Analysis Tool: National Center for Biotechnology Information (US).

Madroñero, Johana; Corredor Rozo, Zayda Lorena; Escobar Pérez, Javier Antonio; Velandia Romero, Myriam Lucia (2019): Next generation sequencing and proteomics in plant virology: how is Colombia doing? In *Acta biol. Colomb.* 24 (3), pp. 423–438. DOI: 10.15446/abc.v24n3.79486.

Marchler-Bauer, Aron; Derbyshire, Myra K.; Gonzales, Noreen R.; Lu, Shennan; Chitsaz, Farideh; Geer, Lewis Y. et al. (2015): CDD: NCBI's conserved domain database. In *Nucleic acids research* 43 (Database issue), D222-6. DOI: 10.1093/nar/gku1221.

Marklewitz, M.; Paraskevopoulou, S.; Briese, T.; Charrel, R. N.; Choi, I-R.; Lamballerie, X. de et al. (2020): Create one new genus and 16 new species (Bunyavirales: Phenuiviridae). International Committee on Taxonomy of Viruses.

Marklewitz, Marco; Zirkel, Florian; Kurth, Andreas; Drosten, Christian; Junglen, Sandra (2015): Evolutionary and phenotypic analysis of live virus isolates suggests arthropod origin of a pathogenic RNA virus family. In *Proceedings of the National Academy of Sciences of the United States of America* 112 (24), pp. 7536–7541. DOI: 10.1073/pnas.1502036112.

Marrelli, Mauro Toledo; Sallum, Maria Anice Mureb; Marinotti, Osvaldo (2006): The second internal transcribed spacer of nuclear ribosomal DNA as a tool for Latin American anopheline taxonomy - a critical review. In *Memorias do Instituto Oswaldo Cruz* 101 (8), pp. 817–832. DOI: 10.1590/s0074-02762006000800002.

Michaud, V.; Gil, P.; Kwiatek, O.; Prome, S.; Dixon, L.; Romero, L. et al. (2007): Long-term storage at tropical temperature of dried-blood filter papers for detection and genotyping of RNA and DNA viruses by direct PCR. In *Journal of virological methods* 146 (1-2), pp. 257–265. DOI: 10.1016/j.jviromet.2007.07.006.

Miranda, Jorge; Mattar, Salim; Gonzalez, Marco; Hoyos-López, Richard; Aleman, Ader; Aponte, Jose (2019): First report of Culex flavivirus infection from Culex coronator (Diptera: Culicidae), Colombia. In *Virology journal* 16 (1), p. 1. DOI: 10.1186/s12985-018-1108-2.

Montoya-Lerma, James; Solarte, Yezid A.; Giraldo-Calderón, Gloria Isabel; Quiñones, Martha L.; Ruiz-López, Freddy; Wilkerson, Richard C.; González, Ranulfo (2011): Malaria vector species in Colombia: a review. In *Mem. Inst. Oswaldo Cruz* 106 Suppl 1, pp. 223–238. DOI: 10.1590/S0074-02762011000900028.

Moonen, Jurgen P.; Schinkel, Michelle; van der Most, Tom; Miesen, Pascal; van Rij, Ronald P. (2023): Composition and global distribution of the mosquito virome - A comprehensive database of insect-specific viruses. In *One health (Amsterdam, Netherlands)* 16, p. 100490. DOI: 10.1016/j.onehlt.2023.100490.

Mora-Salamanca, Andrés Felipe; Porras-Ramírez, Alexandra; La Hoz Restrepo, Fernando Pío de (2020): Estimating the burden of arboviral diseases in Colombia between 2013 and 2016. In *International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases* 97, pp. 81–89. DOI: 10.1016/j.ijid.2020.05.051.

Motoki, Maysa T.; Linton, Yvonne-Marie; Conn, Jan E.; Ruiz-Lopez, Fredy; Wilkerson, Richard C. (2021): Phylogenetic Network of Mitochondrial COI Gene Sequences Distinguishes 10 Taxa Within the Neotropical Albitarsis Group (Diptera: Culicidae), Confirming the Separate Species Status of Anopheles albitarsis H (Diptera: Culicidae) and Revealing a Novel Lineage, Anopheles albitarsis J. In *Journal of medical entomology* 58 (2), pp. 599–607. DOI: 10.1093/jme/tjaa211.

Mourãão, Maria Paula G.; Bastos, Michelle S.; Gimaqu, João Boscoe L.; Mota, Bruno Rafaelle; Souza, Giselle S.; Grimmer, Gustavo Henrique N. et al. (2009): Oropouche fever outbreak, Manaus, Brazil, 2007-2008. In *Emerging Infectious Diseases* 15 (12), pp. 2063–2064. DOI: 10.3201/eid1512.090917.

Muñoz-Gamba, Andrew S.; Laiton-Donato, Katherine; Perdomo-Balaguera, Erick; Castro, Lyda R.; Usme-Ciro, José A.; Parra-Henao, Gabriel (2021): Molecular characterization of mosquitoes (Diptera: Culicidae) from the Colombian rainforest. In *Revista do Instituto de Medicina Tropical de Sao Paulo* 63, e24. DOI: 10.1590/S1678-9946202163024.

Naranjo-Díaz, Nelson; Altamiranda-Saavedra, Mariano; Correa, Margarita M. (2019): Anopheles species composition and entomological parameters in malaria endemic localities of North West Colombia. In *Acta tropica* 190, pp. 13–21. DOI: 10.1016/j.actatropica.2018.10.011.

Navarro, Beatriz; Minutolo, Maria; Stradis, Angelo de; Palmisano, Francesco; Alioto, Daniela; Di Serio, Francesco (2018a): The first phlebo-like virus infecting plants: a case study on the adaptation of negative-stranded RNA viruses to new hosts. In *Molecular plant pathology* 19 (5), pp. 1075–1089. DOI: 10.1111/mpp.12587.

Navarro, Beatriz; Zicca, Stefania; Minutolo, Maria; Saponari, Maria; Alioto, Daniela; Di Serio, Francesco (2018b): A Negative-Stranded RNA Virus Infecting Citrus Trees: The Second Member of a New Genus Within the Order Bunyavirales. In *Front. Microbiol.* 9, Article 2340. DOI: 10.3389/fmicb.2018.02340.

Navarro, Juan-Carlos; Medina, Gladys; Vasquez, Clovis; Coffey, Lark L.; Wang, Eryu; Suárez, Alexander et al. (2005): Postepizootic persistence of Venezuelan equine encephalitis virus, Venezuela. In *Emerging Infectious Diseases* 11 (12), pp. 1907–1915. DOI: 10.3201/eid1112.050533.

Nunes, Márcio Roberto Teixeira; Martins, Lívia Carício; Rodrigues, Sueli Guerreiro; Chiang, Jannifer Oliveira; Da Azevedo, Raimunda do Socorro Silva; Da Rosa, Amelia P. A. Travassos; Da Vasconcelos, Pedro Fernando Costa (2005): Oropouche virus isolation, southeast Brazil. In *Emerging Infectious Diseases* 11 (10), pp. 1610–1613. DOI: 10.3201/eid1110.050464.

Olendraite, Ingrida; Brown, Katherine; Valles, Steven M.; Firth, Andrew E.; Chen, Yanping; Guérin, Diego M. A. et al. (2019): ICTV Virus Taxonomy Profile: Polycipiviridae. In *The Journal of general virology* 100 (4), pp. 554–555. DOI: 10.1099/jgv.0.001241.

Orsborne, Christopher; Hardy, Alison; Isalska, Barbara; Williams, Simon G.; Muldoon, Eavan G. (2014): Acidovorax oryzae catheter-associated bloodstream infection. In *Journal of clinical microbiology* 52 (12), pp. 4421–4424. DOI: 10.1128/JCM.00657-14.

Padilla, Julio César; Lizarazo, Fredy Eberto; Murillo, Olga Lucía; Mendigaña, Fernando Antonio; Pachón, Edwin; Vera, Mauricio Javier (2017): Epidemiología de las principales enfermedades transmitidas por vectores en Colombia, 1990-2016. In *Biomedica : revista del Instituto Nacional de Salud* 37 (0), pp. 27–40. DOI: 10.7705/biomedica.v37i0.3769.

Paraskevopoulou, Sofia; Käfer, Simon; Zirkel, Florian; Donath, Alexander; Petersen, Malte; Liu, Shanlin et al. (2021): Viromics of extant insect orders unveil the evolution of the flavi-like superfamily. In *Virus Evolution* 7 (1), Article veab030. DOI: 10.1093/ve/veab030.

Patel, Pranav; Landt, Olfert; Kaiser, Marco; Faye, Oumar; Koppe, Tanja; Lass, Ulrich et al. (2013): Development of one-step quantitative reverse transcription PCR for the rapid detection of flaviviruses. In *Virology journal* 10, p. 58. DOI: 10.1186/1743-422X-10-58.

Patterson, Edward I.; Kautz, Tiffany F.; Contreras-Gutierrez, Maria A.; Guzman, Hilda; Tesh, Robert B.; Hughes, Grant L.; Forrester, Naomi L. (2021): Negeviruses Reduce Replication of Alphaviruses during Coinfection. In *Journal of virology* 95 (14), e0043321. DOI: 10.1128/JVI.00433-21.

Patterson, Edward I.; Villinger, Jandouwe; Muthoni, Joseph N.; Dobel-Ober, Lucien; Hughes, Grant L. (2020): Exploiting insect-specific viruses as a novel strategy to control vector-borne disease. In *Current opinion in insect science* 39, pp. 50–56. DOI: 10.1016/j.cois.2020.02.005.

Pérez-Escobar, Oscar Alejandro; Lucas, Eve; Jaramillo, Carlos; Monro, Alexandre; Morris, Sarah K.; Bogarín, Diego et al. (2019): The Origin and Diversification of the Hyperdiverse Flora in the Chocó Biogeographic Region. In *Frontiers in Plant Science* 10, p. 1328. DOI: 10.3389/fpls.2019.01328.

Pettersson, John H-O; Shi, Mang; Eden, John-Sebastian; Holmes, Edward C.; Hesson, Jenny C. (2019): Meta-Transcriptomic Comparison of the RNA Viromes of the Mosquito Vectors Culex pipiens and Culex torrentium in Northern Europe. In *Viruses* 11 (11). DOI: 10.3390/v11111033.

Powers, A. M.; Roehrig, John T. (2011): Alphaviruses. In *Methods in molecular biology (Clifton, N.J.)* 665, pp. 17–38. DOI: 10.1007/978-1-60761-817-1_2.

Prado, Irina; Rosario, Delfina; Bernardo, Lídice; Alvarez, Mayling; Rodríguez, Rosmari; Vázquez, Susana; Guzmán, María G. (2005): PCR detection of dengue virus using dried whole blood spotted on filter paper. In *Journal of virological methods* 125 (1), pp. 75–81. DOI: 10.1016/j.jviromet.2005.01.001.

Ragsdale, Nancy N.; Seiber, James N. (Eds.) (1999): Pesticides: managing risks and optimizing benefits. With assistance of Nancy N. Ragsdale. Washington, DC: [Verlag nicht ermittelbar] (ACS Symposium Series, 734).

Ramees, Thadiyam Puram; Dhama, Kuldeep; Karthik, Kumaragurubaran; Rathore, Ramswaroop Singh; Kumar, Ashok; Saminathan, Mani et al. (2017): Arcobacter: an emerging food-borne zoonotic pathogen, its public health concerns and advances in diagnosis and control - a comprehensive review. In *The veterinary quarterly* 37 (1), pp. 136–161. DOI: 10.1080/01652176.2017.1323355.

Romero-Alvarez, Daniel; Escobar, Luis E. (2018): Oropouche fever, an emergent disease from the Americas. In *Microbes and infection* 20 (3), pp. 135–146. DOI: 10.1016/j.micinf.2017.11.013.

Rosario, Karyna; Breitbart, Mya; Harrach, Balázs; Segalés, Joaquim; Delwart, Eric; Biagini, Philippe; Varsani, Arvind (2017): Revisiting the taxonomy of the family Circoviridae: establishment of the genus Cyclovirus and removal of the genus Gyrovirus. In *Archives of virology* 162 (5), pp. 1447–1463. DOI: 10.1007/s00705-017-3247-y.

Roundy, Christopher M.; Azar, Sasha R.; Rossi, Shannan L.; Weaver, Scott C.; Vasilakis, Nikos (2017): Insect-Specific Viruses: A Historical Overview and Recent Developments. In *Advances in virus research* 98, pp. 119–146. DOI: 10.1016/bs.aivir.2016.10.001.

Rozo-Lopez, Paula; Mengual, Ximo (2015): Updated list of the mosquitoes of Colombia (Diptera: Culicidae). In *Biodiversity data journal* (3), e4567. DOI: 10.3897/BDJ.3.e4567.

Ruiz-Lopez, Freddy; Wilkerson, Richard C.; Conn, Jan E.; McKeon, Sascha N.; Levin, David M.; Quiñones, Martha L. et al. (2012): DNA barcoding reveals both known and novel taxa in the Albitarsis Group (Anopheles: Nyssorhynchus) of Neotropical malaria vectors. In *Parasites & vectors* 5, p. 44. DOI: 10.1186/1756-3305-5-44.

Sá, Ivy Luizi Rodrigues de; Hutchings, Rosa Sá Gomes; Hutchings, Roger William; Sallum, Maria Anice Mureb (2020): Revision of the Atratus Group of Culex (Melanoconion) (Diptera: Culicidae). In *Parasites & vectors* 13 (1), p. 269. DOI: 10.1186/s13071-020-3982-x.

Sallum, M. A.; Forattini, O. P. (1996): Revision of the Spissipes Section of Culex (Melanoconion) (Diptera:Culicidae). In *Journal of the American Mosquito Control Association* 12 (3 Pt 2), pp. 517–600.

Sallum, Maria Anice Mureb; Obando, Ranulfo González; Carrejo, Nancy; Wilkerson, Richard C. (2020): Identification keys to the Anopheles mosquitoes of South America (Diptera: Culicidae). I. Introduction. In *Parasites & vectors* 13 (1), p. 583. DOI: 10.1186/s13071-020-04298-6.

Shi, Chenyan; Beller, Leen; Deboutte, Ward; Yinda, Kwe Claude; Delang, Leen; Vega-Rúa, Anubis et al. (2019): Stable distinct core eukaryotic viromes in different mosquito species from Guadeloupe, using single mosquito viral metagenomics. In *Microbiome* 7 (1), p. 121. DOI: 10.1186/s40168-019-0734-2.

Shi, Chenyan; Liu, Yi; Hu, Xiaomin; Xiong, Jinfeng; Zhang, Bo; Yuan, Zhiming (2015): A metagenomic survey of viral abundance and diversity in mosquitoes from Hubei province. In *PloS one* 10 (6), e0129845. DOI: 10.1371/journal.pone.0129845.

Shi, Mang; Lin, Xian-Dan; Chen, Xiao; Tian, Jun-Hua; Chen, Liang-Jun; Li, Kun et al. (2018): The evolutionary history of vertebrate RNA viruses. In *Nature* 556 (7700), pp. 197–202. DOI: 10.1038/s41586-018-0012-7.

Shi, Mang; Lin, Xian-Dan; Tian, Jun-Hua; Chen, Liang-Jun; Chen, Xiao; Li, Ci-Xiu et al. (2016a): Redefining the invertebrate RNA virosphere. In *Nature* 540 (7634), pp. 539–543. DOI: 10.1038/nature20167.

Shi, Mang; Lin, Xian-Dan; Vasilakis, Nikos; Tian, Jun-Hua; Li, Ci-Xiu; Chen, Liang-Jun et al. (2016b): Divergent Viruses Discovered in Arthropods and Vertebrates Revise the Evolutionary History of the Flaviviridae and Related Viruses. In *Journal of virology* 90 (2), pp. 659–669. DOI: 10.1128/JVI.02036-15.

Shi, Mang; Neville, Peter; Nicholson, Jay; Eden, John-Sebastian; Imrie, Allison; Holmes, Edward C. (2017): High-Resolution Metatranscriptomics Reveals the Ecological Dynamics of Mosquito-Associated RNA Viruses in Western Australia. In *Journal of virology* 91 (17). DOI: 10.1128/JVI.00680-17.

Sierra, Diana M.; Velez, Ivan Dario; Linton, Yvonne-Marie (2004): Malaria vector anopheles (Nyssorhynchus) nuneztovari comprises one genetic species in colombia based on homogeneity of nuclear ITS2 rDNA. In *Journal of medical entomology* 41 (3), pp. 302–307. DOI: 10.1603/0022-2585-41.3.302.

Simmonds, Peter; Becher, Paul; Bukh, Jens; Gould, Ernest A.; Meyers, Gregor; Monath, Tom et al. (2017): ICTV Virus Taxonomy Profile: Flaviviridae. In *The Journal of general virology* 98 (1), pp. 2–3. DOI: 10.1099/jgv.0.000672.

Sirivanakarn, S. (1982): A review of the systematics and a proposed scheme of internal classification of the New World subgenus Melanoconion of Culex (Diptera, Culicidae. In *Mosquito Systematics* 14 (4), pp. 265–333.

Smith, Julie L.; Fonseca, Dina M. (2004): Rapid assays for identification of members of the Culex (Culex) pipiens complex, their hybrids, and other sibling species (Diptera: culicidae). In *The American journal of tropical medicine and hygiene* 70 (4), pp. 339–345.

Söding, Johannes (2005): Protein homology detection by HMM-HMM comparison. In *Bioinformatics* 21 (7), pp. 951–960. DOI: 10.1093/bioinformatics/bti125.

Stang, Alexander; Korn, Klaus; Wildner, Oliver; Uberla, Klaus (2005): Characterization of virus isolates by particle-associated nucleic acid PCR. In *Journal of clinical microbiology* 43 (2), pp. 716–720. DOI: 10.1128/JCM.43.2.716-720.2005.

Talbot, Benoit; Sander, Beate; Cevallos, Varsovia; González, Camila; Benítez, Denisse; Carissimo, Claudio et al. (2021): Determinants of Aedes mosquito density as an indicator of arbovirus transmission risk in three sites affected by co-circulation of globally spreading arboviruses in Colombia, Ecuador and Argentina. In *Parasites & vectors* 14 (1), p. 482. DOI: 10.1186/s13071-021-04984-z.

Torres-Gutierrez, Carolina; Bergo, Eduardo Sterlino; Emerson, Kevin J.; Oliveira, Tatiane M. P. de; Greni, Susan; Sallum, Maria Anice Mureb (2016): Mitochondrial COI gene as a tool in the taxonomy of mosquitoes Culex subgenus Melanoconion. In *Acta tropica* 164, pp. 137–149. DOI: 10.1016/j.actatropica.2016.09.007.

Tu, Z.; Hill, J. J. (1999): MosquI, a novel family of mosquito retrotransposons distantly related to the Drosophila I factors, may consist of elements of more than one origin. In *Mol Biol Evol* 16 (12), pp. 1675–1686. DOI: 10.1093/oxfordjournals.molbev.a026081.

Unlu, Isik; Kramer, Wayne L.; Roy, Alma F.; Foil, Lane D. (2010): Detection of West Nile virus RNA in mosquitoes and identification of mosquito blood meals collected at alligator farms in Louisiana. In *Journal of medical entomology* 47 (4), pp. 625–633. DOI: 10.1603/me09087.

Valles, S. M.; Chen, Y.; Firth, A. E.; Guérin, D. M. A.; Hashimoto, Y.; Herrero, S. et al. (2017): ICTV Virus Taxonomy Profile: Iflaviridae. In *The Journal of general virology* 98 (4), pp. 527–528. DOI: 10.1099/jgv.0.000757.

Vázquez, Ana; Sánchez-Seco, María-Paz; Palacios, Gustavo; Molero, Francisca; Reyes, Noelia; Ruiz, Santiago et al. (2012): Novel flaviviruses detected in different species of mosquitoes in Spain. In *Vector borne and zoonotic diseases (Larchmont, N.Y.)* 12 (3), pp. 223–229. DOI: 10.1089/vbz.2011.0687.

Vesgueiro, Fabiana Tavares; Demari-Silva, Bruna; Malafronte, Rosely dos Santos; Sallum, Maria Anice Mureb; Marrelli, Mauro Toledo (2011): Intragenomic variation in the second internal transcribed spacer of the ribosomal DNA of species of the genera Culex and Lutzia (Diptera: Culicidae). In *Memorias do Instituto Oswaldo Cruz* 106 (1), pp. 1–8. DOI: 10.1590/s0074-02762011000100001.

Villesen P. (2007): FaBox: an online toolbox for fasta sequences. In *Mol Ecol Notes* 7 (6), pp. 965–968. DOI: 10.1111/j.1471-8286.2007.01821.x.

Villinger, Jandouwe; Mbaya, Martin K.; Ouso, Daniel; Kipanga, Purity N.; Lutomiah, Joel; Masiga, Daniel K. (2017): Arbovirus and insect-specific virus discovery in Kenya by novel six genera multiplex high-resolution melting analysis. In *Molecular ecology resources* 17 (3), pp. 466–480. DOI: 10.1111/1755-0998.12584.

Votýpka, Jan; Szabová, Jana; Rádrová, Jana; Zídková, Lenka; Svobodová, Milena (2012): Trypanosoma culicavium sp. nov., an avian trypanosome transmitted by Culex mosquitoes. In *International journal of systematic and evolutionary microbiology* 62 (Pt 3), pp. 745–754. DOI: 10.1099/ijs.0.032110-0.

Walker, Peter J.; Blasdell, Kim R.; Calisher, Charles H.; Dietzgen, Ralf G.; Kondo, Hideki; Kurath, Gael et al. (2018): ICTV Virus Taxonomy Profile: Rhabdoviridae. In *The Journal of general virology* 99 (4), pp. 447–448. DOI: 10.1099/jgv.0.001020.

Walker, Peter J.; Siddell, Stuart G.; Lefkowitz, Elliot J.; Mushegian, Arcady R.; Adriaenssens, Evelien M.; Dempsey, Donald M. et al. (2020): Changes to virus taxonomy and the Statutes ratified by the International Committee on Taxonomy of Viruses (2020). In *Archives of virology* 165 (11), pp. 2737–2748. DOI: 10.1007/s00705-020-04752-x.

Weaver, Scott C.; Reisen, William K. (2010): Present and future arboviral threats. In *Antiviral research* 85 (2), pp. 328–345. DOI: 10.1016/j.antiviral.2009.10.008.

Wick, Ryan R.; Judd, Louise M.; Gorrie, Claire L.; Holt, Kathryn E. (2017): Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. DOI: 10.1101/096412.

Will, Kipling W.; Mishler, Brent D.; Wheeler, Quentin D. (2005): The perils of DNA barcoding and the need for integrative taxonomy. In *Systematic biology* 54 (5), pp. 844–851. DOI: 10.1080/10635150500354878.

Williams, Simon H.; Levy, Avram; Yates, Rachel A.; Somaweera, Nilusha; Neville, Peter J.; Nicholson, Jay et al. (2020): The Diversity and Distribution of Viruses Associated with Culex annulirostris Mosquitoes from the Kimberley Region of Western Australia. In *Viruses* 12 (7). DOI: 10.3390/v12070717.

Yassin, A. F.; Inglis, T. J. J.; Hupfer, H.; Siering, C.; Schumann, P.; Busse, H-J; Aravena-Román, M. (2012): Cruoricaptor ignavus gen. nov., sp. nov., a novel bacterium of the family Flavobacteriaceae isolated from blood culture of a man with bacteraemia. In *Systematic and applied microbiology* 35 (7), pp. 421–426. DOI: 10.1016/j.syapm.2012.08.003.

Zavortink, Thomas J. (1979): Mosquito studies (Diptera, Culicidae). XXXV. The new sabethine genus Johnbelkinia and a preliminary reclassification of the composite genus Trichoprosopon. In *Contributions of the American Entomological Institute* 17 (1).

Zhou, Q.; Haymer, D. S. (1997): Molecular structure of yoyo, a gypsy-like retrotransposon from the mediterranean fruit fly, Ceratitis capitata. In *Genetica* 101 (3), pp. 167–178. DOI: 10.1023/a:1018347425870.

Zittra, Carina; Flechl, Eva; Kothmayer, Michael; Vitecek, Simon; Rossiter, Heidemarie; Zechmeister, Thomas; Fuehrer, Hans-Peter (2016): Ecological characterization and molecular differentiation of Culex pipiens complex taxa and Culex torrentium in eastern Austria. In *Parasites & vectors* 9, p. 197. DOI: 10.1186/s13071-016-1495-4.

10 Curriculum vitae

David Joachim Schiemann

BIOLOGIST, AGILE PROJECT MANAGER, PHD CANDIDATE

PROFILE

Biologist (BSc.), with a Master's degree (MSc.) in biology, and doctoral candidate (Dr. rer. nat.) at Johannes Gutenberg University in Mainz, Germany, with a broad knowledge of molecular biology, microbiology, epidemiology, entomology, and virology, with research experience in the area of vector-borne diseases, lab-work and fieldwork, with ethnic communities in Colombia, national and international contacts with renowned research facilities, excellent English and Spanish skills, and the capacity to manage projects and work in interdisciplinary work groups.

CONTACT

Hinter der Kirche 39
55129 Mainz. Germany
daschiemann@gmail.com
+49 (0)162 8670072

PERSONAL DATA

Date of birth: 30.04.1981
Place of birth: Wolfach
Nationality: German
Martial status: Single

COMPETENCES

Project management
Mosquito identification
Virus search
Cellculture
Phylogeny
PCR
NGS data analysis
Field work

SKILLSET

Communication
Adaptability
Spirit of discovery
Problem solving
Creativity
Team work

STUDIES

Agile Project Manager level advanced (TÜV certified)

Habmann Group GbR, Stuttgart, Germany | **Jul. 2021 - Nov. 2021**

- Leadership, Conflict Management, Employee Management.
- Business Development and Project Management.
- Scrum Master - Leader

Certificate: 04.11.2021

Doctorate in Natural Sciences (Dr. rer. nat.)

Johannes Gutenberg University, Mainz, Germany | **Feb. 2014 - now**

- Thesis: Mosquito species diversity and mosquito-associated viruses from the Atrato River valley in Colombia.
- Sponsored by the Robert Koch Institute, the German Academic Exchange Service, and the Hoechst Foundation.

Diplom studies in Biology

Johannes Gutenberg University, Mainz, Germany | **Abr. 2003 - Jun. 2010**

- Thesis: Identification of the Anopheles species present in Tierradentro, Córdoba, Colombia, and determination of their natural infection with Plasmodium.
- Sponsored by the German Academic Exchange Service.

Diplom: 21.06.2010

PROFESSIONAL EXPERIENCE

Secondary school teacher

Erich-Kästner-Realschule plus, Wörrstadt | **Sep. 2022 - Sep. 2023**

Biology and chemistry teacher for 7th to 9th grade

Research Associate
Laboratory of Molecular Biology of Virus

National University of Colombia - Faculty of Medicine, Bogotá| **Jan. 2018 - Dez 2020**

- Viral genome assembly and annotation.
- Mosquito and virus phylogeny.
- Next Generation Sequencing data analysis.

LANGUAGES

German (native language)
English (fluently)
Spanish (fluently)

IT-EXPERTISE

Microsoft Office
Google Workspace
Genious
Bioedit
Figtree
Trimal
PhyML

PUBLICATIONS

Schiemann DJ, Pinzón ML, Hankeln T. Anthropophilic Anopheles species composition and malaria in Tierradentro, Córdoba, Colombia. Mem Inst Oswaldo Cruz. 2014;109(3):384–387. doi:10.1590/0074-0276130483

MEMBERSHIPS

National Research Platform for Zoonoses, Institute of Virology Westphalian Wilhelms University Münster

PROFESSIONAL EXPERIENCE

Research Associate
Centre of Biological Threats and Special Pathogens

Robert Koch Institute - Ministry of Health, Berlín, Germany | **Oct. 2016 - Dic. 2017**

- Development and implementation of molecular tests for the detection of viruses in mosquitoes and blood samples.
- Isolation of novel viruses.
- Metagenomic analysis of mosquito viromes.

Research Associate
Laboratory of Molecular Biology of Virus

National University of Colombia - Faculty of Medicine, Bogotá | **Feb. 2014 - Sep. 2016**

- Morphological and molecular identification of mosquitoes.
- Mosquito phylogeny.

Research Associate
Laboratory of Malaria

University of Antioquia - Medellín, Colombia | **Sep. 2012 - Mar. 2013**

- Genotyping of Plasmodium vivax.
- Evaluation of antimalarial drugs from medicinal plants.

German Teacher
Multilingual programme

University of Antioquia - Medellín, Colombia | **Feb. 2013 - May. 2013**

German courses level I, II and V, 240 hours.

Research Associate
Laboratory of Parasitology

University Hospital of Heidelberg - Germany | **Jul. 2010 - Sep. 2010**

- Drug resistance in Plasmodium falciparum.

Research Associate
Laboratory of Entomology

National University of Colombia - Faculty of Medicine, Bogotá | **Abr. 2009 - Jun. 2010**

- Morphological and molecular identification of Anopheles mosquitoes.
- Identification of malaria vectors in Colombia.

Mainz, April 2023

11 Appendix

11.1 Mosquito barcoding regions

11.1.1 COI sequences of mosquitoes from the Atrato River valley

Table 15: NCBI blastn hits for the COI sequences from mosquito species found in this study

ID	Accession Seq.	NCBI blastn Hit Description/ species	Q. cov.	Per. Id.	Accession Hit
An. darlingi					
Mr 136	MN997596	Anopheles darlingi isolate Northern mitochondrion,	100%	99,0%	GQ918272.1
Mr 152	MN997604	Anopheles darlingi isolate MPC06-6 cytochrome c	100%	99,0%	HM022406.1
Mr 173	MN997588	Anopheles darlingi voucher AC20_21_100	100%	99,0%	JF923693.1
Mr 181	MN997585	Anopheles darlingi voucher AC20_21_100	100%	99,0%	JF923693.1
Mu 110	MN997584	Anopheles darlingi voucher AC20_21_100	100%	99,0%	JF923693.1
Mu 317	MN997587	Anopheles darlingi voucher AC20_21_100	100%	99,0%	JF923693.1
Mu 334	MN997595	Anopheles darlingi isolate Northern mitochondrion,	100%	99,0%	GQ918272.1
Na 652	MN997592	Anopheles darlingi voucher AC20_21_100	100%	99,0%	JF923693.1
Na 788	MN997603	Anopheles darlingi cytochrome c oxidase subunit I	100%	99,7%	MF598461.1
Ve 820	MN997602	Anopheles darlingi isolate MPC06-6 cytochrome c	99%	99,0%	HM022406.1
Vi 2157	MN997599	Anopheles darlingi isolate MPC06-6 cytochrome c	100%	99,0%	HM022406.1
Am 164	MN997591	Anopheles darlingi voucher AC20_21_100	100%	99,0%	JF923693.1
Am 258	MN997597	Anopheles darlingi isolate MPC06-6 cytochrome c	100%	99,0%	HM022406.1
Mr 30	MN997586	Anopheles darlingi voucher AC20_21_100	100%	99,0%	JF923693.1
R 269	MN997600	Anopheles darlingi isolate MPC06-6 cytochrome c	100%	99,0%	HM022406.1
R 336	MN997593	Anopheles darlingi voucher AC20_21_100	100%	99,0%	JF923693.1
R 743	MN997590	Anopheles darlingi isolate MPC06-6 cytochrome c	100%	99,0%	HM022406.1
Vi 8	MN997589	Anopheles darlingi isolate MPC06-6 cytochrome c	99%	99,0%	HM022406.1
R 771	MN997601	Anopheles darlingi isolate MPC06-6 cytochrome c	100%	99,0%	HM022406.1
R 266	MN997598	Anopheles darlingi clone MS4_7 mitochondrion,	100%	99,1%	MF381650.1
An. neivai					
Mr 164	MN997605	Anopheles neivai isolate 1V50V210H10E1	92%	99,0%	KU900811.1
An. nuneztovari					
Ca 2	MN997554	Anopheles nuneztovari haplotype 28 cytochrome	100%	100,0%	KU865556.1
Ca 27	MN997568	Anopheles nuneztovari haplotype 28 cytochrome	100%	99,0%	KU865556.1
Ca 37	MN997576	Anopheles nuneztovari haplotype 28 cytochrome	100%	99,0%	KU865556.1
Ca 47	MN997583	Anopheles nuneztovari haplotype 28 cytochrome	100%	99,0%	KU865556.1
Ca 55	MN997548	Anopheles nuneztovari haplotype 28 cytochrome	100%	100,0%	KU865556.1
Ca 65	MN997555	Anopheles nuneztovari haplotype 28 cytochrome	100%	100,0%	KU865556.1
Ca 72	MN997556	Anopheles nuneztovari haplotype 28 cytochrome	100%	100,0%	KU865556.1
Ca 79	MN997577	Anopheles nuneztovari haplotype 28 cytochrome	100%	99,0%	KU865556.1
Ca 85	MN997578	Anopheles nuneztovari haplotype 28 cytochrome	100%	99,0%	KU865556.1
Ca 93	MN997582	Anopheles nuneztovari haplotype 28 cytochrome	100%	99,0%	KU865556.1
Mr 144	MN997544	Anopheles nuneztovari haplotype 28 cytochrome	100%	100,0%	KU865556.1
Mu 208	MN997540	Anopheles nuneztovari haplotype 28 cytochrome	100%	100,0%	KU865556.1
Mu 222	MN997550	Anopheles nuneztovari haplotype 28 cytochrome	100%	100,0%	KU865556.1
Mu 230	MN997581	Anopheles nuneztovari haplotype 28 cytochrome	100%	99,0%	KU865556.1
Mu 240	MN997572	Anopheles nuneztovari haplotype 28 cytochrome	100%	99,0%	KU865556.1
Mu 247	MN997542	Anopheles nuneztovari haplotype 28 cytochrome	100%	100,0%	KU865556.1
Mu 258	MN997571	Anopheles nuneztovari haplotype 28 cytochrome	100%	99,0%	KU865556.1
Mu 283	MN997569	Anopheles nuneztovari haplotype 28 cytochrome	100%	99,0%	KU865556.1
Mu 310	MN997580	Anopheles nuneztovari haplotype 28 cytochrome	100%	99,0%	KU865556.1
Mu 60	MN997545	Anopheles nuneztovari haplotype 28 cytochrome	100%	100,0%	KU865556.1
Mu 72	MN997557	Anopheles nuneztovari haplotype 28 cytochrome	100%	100,0%	KU865556.1
Mu 98	MN997549	Anopheles nuneztovari haplotype 28 cytochrome	100%	100,0%	KU865556.1
Na 688	MN997546	Anopheles nuneztovari haplotype 28 cytochrome	100%	100,0%	KU865556.1
R 315	MN997570	Anopheles nuneztovari haplotype 28 cytochrome	100%	99,0%	KU865556.1
R 357	MN997575	Anopheles nuneztovari haplotype 28 cytochrome	93%	99,0%	KU865556.1
R 364	MN997537	Anopheles nuneztovari haplotype 28 cytochrome	95%	100,0%	KU865556.1
R 371	MN997559	Anopheles nuneztovari haplotype 28 cytochrome	94%	100,0%	KU865556.1
R 523	MN997562	Anopheles nuneztovari haplotype 28 cytochrome	93%	100,0%	KU865556.1

ID	Accession Seq.	NCBI blastn Hit Description/ species	Q. cov.	Per. Id.	Accession Hit
Ve 113	MN997560	Anopheles nuneztovari haplotype 28 cytochrome	93%	100,0%	KU865556.1
Vi 324	MN997558	Anopheles nuneztovari haplotype 28 cytochrome	100%	100,0%	KU865556.1
Vi 532	MN997539	Anopheles nuneztovari haplotype 28 cytochrome	100%	100,0%	KU865556.1
Vi 575	MN997547	Anopheles nuneztovari haplotype 28 cytochrome	100%	100,0%	KU865556.1
Vi 801	MN997565	Anopheles nuneztovari haplotype 28 cytochrome	100%	99,0%	KU865556.1
Am 266	MN997566	Anopheles nuneztovari haplotype 28 cytochrome	100%	99,0%	KU865556.1
Am 297	MN997567	Anopheles nuneztovari haplotype 28 cytochrome	100%	99,0%	KU865556.1
Am 367	MN997573	Anopheles nuneztovari haplotype 28 cytochrome	100%	99,0%	KU865556.1
Ca 111	MN997574	Anopheles nuneztovari haplotype 28 cytochrome	100%	99,0%	KU865556.1
Ca 119	MN997541	Anopheles nuneztovari haplotype 28 cytochrome	100%	100,0%	KU865556.1
Ca 128	MN997552	Anopheles nuneztovari haplotype 28 cytochrome	100%	100,0%	KU865556.1
Ca 174	MN997538	Anopheles nuneztovari haplotype 28 cytochrome	100%	100,0%	KU865556.1
Ca 190	MN997536	Anopheles nuneztovari haplotype 28 cytochrome	100%	100,0%	KU865556.1
Ca 196	MN997563	Anopheles nuneztovari haplotype 28 cytochrome	100%	99,0%	KU865556.1
Ca 207	MN997564	Anopheles nuneztovari haplotype 28 cytochrome	100%	99,0%	KU865556.1
R 380	MN997561	Anopheles nuneztovari haplotype 28 cytochrome	94%	100,0%	KU865556.1
Ve 106	MN997551	Anopheles nuneztovari haplotype 28 cytochrome	100%	100,0%	KU865556.1
Ve 108	MN997553	Anopheles nuneztovari haplotype 28 cytochrome	100%	100,0%	KU865556.1
Am 163	MN997579	Anopheles nuneztovari haplotype 28 cytochrome	96%	99,0%	KU865556.1
An. pseudopunctipennis					
R 324	MN997612	Anopheles pseudopunctipennis isolate MFO01-14	99%	99,0%	HM022407.1
An. punctimacula					
Cu 209	MN997528	Anopheles punctimacula isolate GUA8501	100%	99,0%	KC354818.1
R 256	MN997529	Anopheles punctimacula isolate GUA8501	99%	99,0%	KC354818.1
R 365	MN997535	Anopheles punctimacula isolate SS1-12 cytochrome	97%	99,0%	HQ622626.1
R 826	MN997534	Anopheles punctimacula isolate GUA8501	100%	99,0%	KC354818.1
Ve 297	MN997533	Anopheles punctimacula isolate GUA8501	99%	99,0%	KC354818.1
Vi 677	MN997531	Anopheles punctimacula isolate GUA8501	100%	99,0%	KC354818.1
Mu 88	MN997532	Anopheles punctimacula isolate GUA8501	100%	99,0%	KC354818.1
Am 415	MN997530	Anopheles punctimacula isolate GUA8501	99%	99,0%	KC354818.1
Cq. lynchi					
Ve 579	MN997675	Culex cinctellus mitochondrial COI gene for	99%	87,0%	AB738190.1
		Coquillettidia ochracea isolate 31-1 cytochrome	99%	87,0%	KT358437.1
Mr 421	MN997683	Coquillettidia ochracea isolate 31-1 cytochrome	100%	87,0%	KT358437.1
		Culex cinctellus mitochondrial COI gene for	99%	87,0%	AB738190.1
Ve 485	MN997684	Culex cinctellus mitochondrial COI gene for	93%	87,0%	AB738190.1
Ve 707	MN997677	Culex cinctellus mitochondrial COI gene for	92%	87,0%	AB738190.1
Ve 392	MN997678	Culex cinctellus mitochondrial COI gene for	94%	87,0%	AB738190.1
Ve 624	MN997681	Culex cinctellus mitochondrial COI gene for	93%	87,0%	AB738190.1
Mr 214	MN997676	Coquillettidia ochracea isolate 31-1 cytochrome	100%	87,0%	KT358437.1
		Culex cinctellus mitochondrial COI gene for	99%	87,0%	AB738190.1
Ve 636	MN997679	Culex cinctellus mitochondrial COI gene for	99%	87,0%	AB738190.1
Ve 754	MN997685	Culex cinctellus mitochondrial COI gene for	94%	88,0%	AB738190.1
Ve 515	MN997680	Culex cinctellus mitochondrial COI gene for	96%	87,0%	AB738190.1
Ve 487	MN997682	Culex cinctellus mitochondrial COI gene for	99%	87,0%	AB738190.1
Cq. venezuelensis					
Na 806	MN997705	Coquillettidia nigricans isolate BAP8 cytochrome	85%	97,0%	KM452754.1
Na 628	MN997706	Coquillettidia nigricans isolate BAP8 cytochrome	85%	97,0%	KM452754.1
Ve 302	MN997701	Coquillettidia nigricans isolate BAP8 cytochrome	81%	97,0%	KM452754.1
Ca 52	MN997707	Coquillettidia nigricans isolate BAP8 cytochrome	85%	97,0%	KM452754.1
Cu 103	MN997686	Coquillettidia nigricans isolate BAP8 cytochrome	85%	97,0%	KM452754.1
Ve 304	MN997704	Coquillettidia nigricans isolate BAP8 cytochrome	85%	97,0%	KM452754.1
R 454	MN997702	Coquillettidia nigricans isolate BAP8 cytochrome	80%	97,0%	KM452754.1
Ve 268	MN997691	Coquillettidia nigricans isolate BAP8 cytochrome	85%	97,0%	KM452754.1
Ca 146	MN997692	Coquillettidia nigricans isolate BAP8 cytochrome	85%	97,0%	KM452754.1
Am 312	MN997696	Coquillettidia nigricans isolate BAP8 cytochrome	85%	97,0%	KM452754.1
Ve 249	MN997697	Coquillettidia nigricans isolate BAP8 cytochrome	84%	97,0%	KM452754.1
R 83	MN997689	Coquillettidia nigricans isolate BAP8 cytochrome	81%	97,0%	KM452754.1
Ve 259	MN997688	Coquillettidia nigricans isolate BAP8 cytochrome	85%	97,0%	KM452754.1

ID	Accession Seq.	NCBI blastn Hit Description/ species	Q. cov.	Per. Id.	Accession Hit
R 227	MN997694	Coquillettidia nigricans isolate BAP8 cytochrome	82%	97,0%	KM452754.1
R 13	MN997693	Coquillettidia nigricans isolate BAP8 cytochrome	83%	97,0%	KM452754.1
Ve 276	MN997695	Coquillettidia nigricans isolate BAP8 cytochrome	82%	97,0%	KM452754.1
R 224	MN997700	Coquillettidia nigricans isolate BAP7 cytochrome	80%	97,0%	KM452753.1
R 969	MN997687	Coquillettidia nigricans isolate BAP8 cytochrome	81%	97,0%	KM452754.1
R 22	MN997699	Coquillettidia nigricans isolate BAP8 cytochrome	80%	97,0%	KM452754.1
R 91	MN997690	Coquillettidia nigricans isolate BAP8 cytochrome	81%	97,0%	KM452754.1
R 81	MN997698	Coquillettidia nigricans isolate BAP8 cytochrome	83%	97,0%	KM452754.1
Ve 256	MN997703	Coquillettidia nigricans isolate BAP8 cytochrome	82%	97,0%	KM452754.1
Cx. coronator					
Be 18	MN997483	Culex saltanensis voucher F010 cytochrome c	99%	98,0%	KF919232.1
		Culex surinamensis voucher CDC3_1 cytochrome c	99%	99,0%	KF919233.1
		Culex maxi mitochondrial partial COI gene for	100%	99,0%	HE599225.
		Culex coronator isolate RS10_109 mitochondrion,	100%	99,0%	MF040162.1
		Culex usquatissimus isolate RO25_19 mitochondrion,	100%	99,0%	MF040163.1
Mu 275	MN997482	Culex maxi mitochondrial partial COI gene for	100%	99,0%	HE599225.1
		Culex coronator isolate RS10_109 mitochondrion,	100%	99,0%	MF040162.1
		Culex usquatissimus isolate RO25_19 mitochondrion,	100%	99,0%	MF040163.1
Cx. sp. 10					
Ve 300	MN997437	Culex (Melanoconion) nr. gnomatos gnomat_AC	94%	98,0%	KX779886.1
Cx. nigripalpus					
Ve 157	MN997478	Culex conspirator voucher ZFMK:PR069-Colombia	98%	99,0%	KM593054.1
		Culex nigripalpus voucher ZFMK:PR007-Colombia	96%	99,0%	KM592992.1
		Culex declarator voucher ZFMK:PR032-Colombia COI	98%	99,0%	KM593017.1
		Culex lactator voucher ZFMK:PR012-Colombia COI	98%	98,0%	KM592997.1
		Culex bidens isolate Cx_bidens_Cba_15_14	93%	99,0%	KY581215.1
		Culex interfor isolate Cx_interfor_LR_15_23	93%	99,0%	KY581272.1
		Culex mollis voucher MB10225 cytochrome oxidase	93%	99,0%	MF172288.1
Mr 165	MN997474	Culex declarator voucher ZFMK:PR061-Colombia COI	100%	99,0%	KM593046.1
		Culex interfor isolate Cx_interfor_LR_15_23	99%	99,0%	KY581272.1
		Culex conspirator voucher ZFMK:PR069-Colombia	100%	99,0%	KM593054.1
		Culex nigripalpus voucher ZFMK:PR007-Colombia	100%	100,0%	KM592992.1
		Culex mollis voucher MB10225 cytochrome oxidase	100%	100,0%	MF172288.1
Ve 150	MN997476	Culex nigripalpus voucher ZFMK:PR007-Colombia	100%	99,0%	KM592992.1
		Culex mollis voucher MB10225 cytochrome oxidase	100%	99,0%	MF172288.1
Mu 206	MN997481	Culex interfor isolate Cx_interfor_LR_15_23	99%	99,0%	KY581272.1
		Culex declarator voucher ZFMK:PR070-Colombia COI	100%	99,0%	KM593055.1
		Culex bidens isolate Cx_bidens_Cba_15_14	99%	99,0%	KY581215.1
		Culex conspirator voucher ZFMK:PR069-Colombia	100%	99,0%	KM593054.1
		Culex nigripalpus voucher ZFMK:PR007-Colombia	100%	99,0%	KM592992.1
		Culex mollis voucher MB10225 cytochrome oxidase	100%	99,0%	MF172288.1
Ve 404	MN997480	Culex mollis voucher MB10225 cytochrome oxidase	100%	99,0%	MF172288.1
		Culex nigripalpus voucher PR cytochrome c oxidase	99%	99,0%	KF919231.1
		Culex conspirator voucher ZFMK:PR069-Colombia	98%	99,0%	KM593054.1
		Culex declarator voucher ZFMK:PR032-Colombia COI	98%	99,0%	KM593017.1
Mu 330	MN997472	Culex conspirator voucher ZFMK:PR069-Colombia	100%	99,0%	KM593054.1
		Culex nigripalpus voucher ZFMK:PR007-Colombia	100%	99,0%	KM592992.1
		Culex mollis voucher MB10225 cytochrome oxidase	100%	99,0%	MF172288.1
Am 347	MN997470	Culex mollis voucher MB10225 cytochrome oxidase	100%	99,9%	MF172288.1
		Culex nigripalpus voucher ZFMK:PR007-Colombia	100%	99,0%	KM592992.1
Ve 19	MN997473	Culex mollis voucher MB10225 cytochrome oxidase	100%	100,0%	MF172288.1
		Culex nigripalpus voucher ZFMK:PR007-Colombia	100%	100,0%	KM592992.1
Ve 447	MN997475	Culex nigripalpus voucher ZFMK:PR007-Colombia	100%	100,0%	KM592992.1
		Culex mollis voucher MB10225 cytochrome oxidase	100%	100,0%	MF172288.1
Ve 892	MN997477	Culex mollis voucher MB10225 cytochrome oxidase	100%	99,0%	MF172288.1
		Culex nigripalpus voucher ZFMK:PR007-Colombia	100%	99,0%	KM592992.1
Ve 399	MN997479	Culex mollis voucher MB10225 cytochrome oxidase	100%	100,0%	MF172288.1
		Culex nigripalpus voucher ZFMK:PR007-Colombia	100%	100,0%	KM592992.1
Ve 156	MN997471	Culex mollis voucher MB10225 cytochrome oxidase	100%	99,0%	MF172288.1

ID	Accession Seq.	NCBI blastn Hit Description/ species	Q. cov.	Per. Id.	Accession Hit
		Culex nigripalpus voucher ZFMK:PR007-Colombia	100%	99,0%	KM592992.1
Cx. quinquefasciatus					
Mu 23	MN997457	Culex pipiens mitochondrial partial COI gene for	100%	99,0%	HG793437.1
Am 413	MN997460	Culex pipiens isolate stin.cpip_contig781 cytochrome	100%	100,0%	KP293425.1
Mu 118	MN997459	Culex pipiens isolate stin.cpip_contig781 cytochrome	100%	100,0%	KP293425.1
Mu 38	MN997467	Culex pipiens mitochondrial partial COI gene for	100%	100,0%	HF562662.1
Am 394	MN997462	Culex pipiens isolate stin.cpip_contig781 cytochrome	100%	100,0%	KP293425.1
Vi 1066	MN997463	Culex pipiens isolate stin.cpip_contig781 cytochrome	100%	100,0%	KP293425.1
Mu 9	MN997464	Culex pipiens isolate stin.cpip_contig781 cytochrome	100%	100,0%	KP293425.1
Am 417	MN997465	Culex pipiens isolate stin.cpip_contig781 cytochrome	100%	100,0%	KP293425.1
Am 411	MN997466	Culex pipiens isolate stin.cpip_contig781 cytochrome	100%	100,0%	KP293425.1
Am 375	MN997468	Culex quinquefasciatus from USA mitochondrion,	99%	99,0%	HQ724617.1
Am 385	MN997458	Culex pipiens isolate stin.cpip_contig781 cytochrome	100%	100,0%	KP293425.1
Am 454	MN997461	Culex pipiens isolate stin.cpip_contig781 cytochrome	100%	100,0%	KP293425.1
Am 462	MN997469	Culex pipiens mitochondrial partial COI gene for	100%	99,0%	HG793467.1
Cx. sp. 2					
Vi 1383	MN997450	Culex (Melanoconion) nr. portesi AC312_8	100%	99,0%	KX779889.1
Cx. educator					
Am 253	MN997411	Culex (Melanoconion) nr. theobaldi AC127_1	100%	99,0%	KX779891.1
Am 256	MN997416	Culex educator voucher ZFMK:PR057-Colombia COI	100%	99,0%	KM593042.1
		Culex (Melanoconion) nr. theobaldi AC127_1	100%	99,0%	KX779891.1
Mu 45	MN997413	Culex educator voucher ZFMK:PR057-Colombia COI	100%	99,0%	KM593042.1
		Culex (Melanoconion) nr. theobaldi AC127_1	100%	99,0%	KX779891.1
Bo 48	MN997412	Culex educator voucher ZFMK:PR057-Colombia COI	100%	99,0%	KM593042.1
		Culex (Melanoconion) nr. theobaldi AC127_1	100%	99,0%	KX779891.1
R 58	MN997415	Culex (Melanoconion) nr. theobaldi AC127_1	100%	99,0%	KX779891.1
R 38	MN997414	Culex educator voucher ZFMK:PR057-Colombia COI	100%	99,0%	KM593042.1
		Culex (Melanoconion) nr. theobaldi AC127_1	100%	99,0%	KX779891.1
Cx. rabelloi					
Ve 866	MN997527	Culex rabelloi voucher PA1_2 cytochrome c oxidase	100%	98.33%	KX779859.1
Cx. sp. 8					
Ve 400	MN997443	Culex (Melanoconion) nr. vomerifer AC322_1	93%	98,0%	KX779888.1
Ve 897	MN997445	Culex (Melanoconion) nr. vomerifer AC322_1	93%	98,0%	KX779888.1
Ve 891	MN997444	Culex (Melanoconion) nr. vomerifer AC322_1	100%	98,0%	KX779888.1
Ve 893	MN997446	Culex (Melanoconion) nr. vomerifer AC322_1	92%	98,0%	KX779888.1
Ve 983	MN997447	Culex (Melanoconion) nr. vomerifer AC322_1	100%	98,0%	KX779888.1
Ve 815	MN997448	Culex (Melanoconion) nr. vomerifer AC322_1	92%	98,0%	KX779888.1
Ve 908	MN997449	Culex (Melanoconion) nr. vomerifer AC322_1	100%	98,0%	KX779888.1
Cx.sp. 2					
Ve 743	MN997434	Culex (Melanoconion) nr. pedroi AC153_1	100%	98,0%	KX779887.1
Cx. sp. 1					
Ve 405	MN997455	Culex spissipes voucher spis_2 cytochrome c oxidase	93%	96,0%	KX779869.1
Ve 407	MN997410	Culex spissipes voucher spis_2 cytochrome c oxidase	96%	96,2%	KX779869.1
Am 260	MN997452	Culex spissipes voucher spis_2 cytochrome c oxidase	100%	96,0%	KX779869.1
Ve 408	MN997453	Culex spissipes voucher spis_2 cytochrome c oxidase	92%	96,0%	KX779869.1
R 522	MN997451	Culex spissipes voucher spis_2 cytochrome c oxidase	93%	96,0%	KX779869.1
Am 313	MN997455	Culex spissipes voucher spis_2 cytochrome c oxidase	93%	96,0%	KX779869.1
Ve 656	MN997456	Culex spissipes voucher spis_2 cytochrome c oxidase	100%	95,0%	KX779869.1
Cx. sp .3					
Ve 433	MN997426	Culex intrincatus voucher PM1_4 cytochrome c	92%	96,0%	KX779831.1
		Culex conspirator voucher ZFMK:PR065-Colombia	98%	95,0%	KM593050.1
Ve 421	MN997425	Culex conspirator voucher ZFMK:PR065-Colombia	98%	94,0%	KM593050.1
		Culex idottus voucher SP71_117 cytochrome c	100%	95,0%	KX779819.1
		Culex eastor voucher 1B cytochrome c oxidase	100%	95,0%	KX779803.1
		Culex intrincatus voucher PM1_4 cytochrome c	93%	96,0%	KX779831
Ve 425	MN997428	Culex intrincatus voucher PM1_4 cytochrome c	100%	96,0%	KX779831.1
Ve 886	MN997708	Culex intrincatus voucher PM1_4 cytochrome c	100%	96,0%	KX779831.1
Ve 431	MN997420	Culex +G292:M293conspirator voucher	98%	95,0%	KM593050.1
		Culex intrincatus voucher PM1_4 cytochrome c	93%	96,0%	KX779831.1

ID	Accession Seq.	NCBI blastn Hit Description/ species	Q. cov.	Per. Id.	Accession Hit
Ve 446	MN997422	Culex intrincatus voucher PM1_4 cytochrome c	100%	96,0%	KX779831.1
Ve 434	MN997423	Culex conspirator voucher ZFMK:PR065-Colombia	98%	94,0%	KM593050.1
		Culex intrincatus voucher PM1_4 cytochrome c	92%	96,0%	KX779831.1
		Culex idottus voucher SP71_117 cytochrome c	92%	95,0%	KX779819.1
Ve 452	MN997424	Culex intrincatus voucher PM1_4 cytochrome c	92%	96,0%	KX779831.1
		Culex conspirator voucher ZFMK:PR065-Colombia	98%	95,0%	KM593050.1
Ve 894	MN997418	Culex intrincatus voucher PM1_4 cytochrome c	100%	96,0%	KX779831.1
		Culex conspirator voucher ZFMK:PR065-Colombia	98%	95,0%	KM593050.1
		Culex intrincatus voucher PM1_4 cytochrome c	93%	96,0%	KX779831.1
		Culex idottus voucher SP71_117 cytochrome c	93%	95,0%	KX779819.1
Ve 438	MN997421	Culex intrincatus voucher PM1_4 cytochrome c	96%	96,0%	KX779831.1
Ve 445	MN997417	Culex conspirator voucher ZFMK:PR065-Colombia	98%	95,0%	KM593050.1
		Culex intrincatus voucher PM1_4 cytochrome c	92%	96,0%	KX779831.1
Ve 863	MN997419	Culex atratus isolate A03 cytochrome c oxidase	99%	95,3%	MH376749.1
		Culex intrincatus voucher PM1_4 cytochrome c	93%	96,0%	KX779831.1
Ve 888	MN997427	Culex idottus voucher SP71_117 cytochrome c	99%	96,0%	KX779819.1
		Culex intrincatus voucher PM1_4 cytochrome c	99%	96,0%	KX779831.1
		Culex conspirator voucher ZFMK:PR065-Colombia	98%	95,0%	KM593050.1
		Culex intrincatus voucher PM1_4 cytochrome c	92%	96,0%	KX779831.1
		Culex idottus voucher SP71_117 cytochrome c	92%	96,0%	KX779819.1
Cx. sp. 4					
Ve 804	MN997430	Culex conspirator voucher ZFMK:PR065-Colombia	98%	96,0%	KM593050.1
		Culex eastor voucher 1B cytochrome c oxidase	93%	96,0%	KX779803.1
		Culex intrincatus voucher PA1_5 cytochrome c	93%	95,0%	KX779828.1
Ve 803	MN997432	Culex intrincatus voucher PA1_5 cytochrome c	100%	95,0%	KX779828.1
		Culex eastor voucher 1B cytochrome c oxidase	100%	96,0%	KX779803.1
		Culex conspirator voucher ZFMK:PR068-Colombia	100%	96,0%	KM593053.1
		Culex eastor voucher 1B cytochrome c oxidase	100%	96,0%	KX779803.1
Ve 830	MN997433	Culex conspirator voucher ZFMK:PR068-Colombia	98%	95,0%	KM593053.1
		Culex eastor voucher 1B cytochrome c oxidase	93%	96,0%	KX779803.1
		Culex intrincatus voucher PA1_5 cytochrome c	93%	95,0%	KX779828.1
Ve 529	MN997431	Culex intrincatus voucher PA1_5 cytochrome c	100%	95,0%	KX779828.1
		Culex eastor voucher 1B cytochrome c oxidase	100%	96,0%	KX779803.1
		Culex conspirator voucher ZFMK:PR068-Colombia	98%	96,0%	KM593053.1
Ve 531	MN997429	Culex conspirator voucher ZFMK:PR065-Colombia	100%	96,0%	KM593050.1
		Culex intrincatus voucher PM1_1x cytochrome c	100%	96,0%	KX779826.1
		Culex eastor voucher 1B cytochrome c oxidase	100%	96,0%	KX779803.1
Cx. sp. 5					
Ve 409	MN997439	Culex (Melanoconion) nr. pedroi ped1 cytochrome c	94%	95,0%	KX779846.1
		Culex (Melanoconion) nr. gnomatos gnomat_AC	94%	93,0%	KX779886.1
Ve 406	MN997440	Culex (Melanoconion) nr. gnomatos gnomat_AC	100%	93,0%	KX779886.1
		Culex (Melanoconion) nr. pedroi ped1 cytochrome c	92%	95,0%	KX779846.1
R 996	MN997438	Culex (Melanoconion) nr. pedroi ped1 cytochrome c	100%	95,0%	KX779846.1
Cx.sp. 6					
Ve 717	MN997435	Culex (Melanoconion) nr. pedroi ped1 cytochrome c	100%	93,0%	KX779846.1
		Culex (Melanoconion) nr. gnomatos gnomat_AC	100%	96,0%	KX779886.1
Ve 853	MN997436	Culex (Melanoconion) nr. gnomatos gnomat_AC	93%	96,0%	KX779886.1
Cx. sp. 7					
Ve 848	MN997441	Culex pedroi isolate Cped11 cytochrome c oxidase	99%	93,0%	KX379626.1
		Culex crybda voucher AC316_9 cytochrome c	99%	93,0%	KX779884.1
		Culex ribeirensis voucher IG20_13 cytochrome c	100%	93,0%	KX779863.1
Ve 887	MN997442	Culex pedroi isolate Cped11 cytochrome c oxidase	99%	93,0%	KX379626.
		Culex crybda voucher AC316_9 cytochrome c	99%	93,0%	KX779884.1
		Culex ribeirensis voucher IG20_13 cytochrome c	94%	93,0%	KX779863.1
		Culex crybda voucher AC316_9 cytochrome c	94%	93,0%	KX779884.1
		Culex pedroi isolate Cped11 cytochrome c oxidase	94%	93,0%	KX379626.
Hg. lucifer					
Ro 299	MN997668	Haemagogus lucifer isolate CDCLB3-2_1 cytochrome	68%	99,0%	KT766506.1
Li. durhamii					

ID	Accession Seq.	NCBI blastn Hit Description/ species	Q. cov.	Per. Id.	Accession Hit
R 292	MN997613	Limatus durhamii voucher MB10129 cytochrome	100%	99.85%	MF172331.1
Ma. indubitans					
Mu 145	MN997669	Culex crybda voucher AC316_9 cytochrome c	99%	90,0%	KX779884.1
		Ochlerotatus aurifer voucher NEONTculicid2038	100%	91,0%	JX259522.1
		Mansonia titillans isolate CCSBVCH_Ma.titi5	100%	90,1%	KY859902.1
Mu 164	MN997670	Culex crybda voucher AC307_12 cytochrome c	99%	90,0%	KX779881.1
		Ochlerotatus aurifer voucher NEONTculicid2038	100%	91,0%	JX259522.1
Mu 329	MN997672	Ochlerotatus aurifer voucher NEONTculicid2038	100%	91,0%	JX259522.1
Mu 155	MN997671	Culex bitaeniorhynchus voucher 20974_CxbitB09	100%	90,0%	KU380407.1
		Ochlerotatus aurifer voucher NEONTculicid2038	100%	91,0%	JX259522.1
Ma. titillans					
R 968	MN997666	Haemagogus lucifer voucher ZFMK:PR051-Colombia	96%	89,0%	KM593036.1
		Culex (Melanoconion) nr. vomerifer AC322_1	99%	88,0%	KX779888.1
		Wyeomyia splendida voucher MB10652 cytochrome	100%	89,0%	MF172500.1
Am 355	MN997667	Wyeomyia splendida voucher MB10652 cytochrome	100%	89,0%	MF172500.1
		Haemagogus lucifer voucher ZFMK:PR051-Colombia	91%	89,0%	KM593036.1
Na 755	MN997665	Wyeomyia splendida voucher MB10652 cytochrome	100%	88,0%	MF172500.1
Ma. sp. 1					
Ca 130	MN997674	Mansonia titillans isolate CCSBVCH_Ma.titi5	100%	99,4%	KY859902.1
Ma. sp. 2					
Mu 148	MN997673	Mansonia titillans isolate CCSBVCH_Ma.titi5	100%	88,6%	KY859902.1
		Culex ribeirensis voucher IG20_13 cytochrome c	99%	88,4%	KX779863.1
Oc. angustivittatus					
Am 17	MN997504	Ochlerotatus angustivittatus voucher ZFMK:PR076-	100%	99,0%	KM593061.1
Oc. scapularis					
Ve 211	not available	Ochlerotatus scapularis voucher ST10038	95%	99,0%	MF172266.1
Ve 282	MN997484	Ochlerotatus scapularis voucher ST10288	92%	99,0%	MF172267.1
Oc. serratus					
R 433	MN997486	Ochlerotatus atlanticus voucher NEONTculicid2130	100%	97,0%	JX259519.1
		Ochlerotatus pertinax cytochrome oxidase subunit I	99%	98,0%	MG242509.1
		Ochlerotatus serratus voucher ST10048 cytochrome	100%	98,0%	MF172270.1
Am 233	MN997500	Ochlerotatus serratus voucher ST10048 cytochrome	100%	98,0%	MF172270.1
Ve 606	MN997499	Ochlerotatus serratus voucher ST10046 cytochrome	100%	98,0%	MF172269.1
		Ochlerotatus pertinax cytochrome oxidase subunit I	94%	99,0%	MG242509.1
		Ochlerotatus serratus voucher ST10046 cytochrome	94%	98,0%	MF172269.1
		Ochlerotatus atlanticus voucher NEONTculicid2130	94%	96,0%	JX259519.1
Ve 668	MN997498	Ochlerotatus serratus voucher ST10048 cytochrome	100%	98,0%	MF172270.1
		Ochlerotatus atlanticus voucher NEONTculicid2130	100%	97,0%	JX259519.1
		Ochlerotatus pertinax cytochrome oxidase subunit I	92%	98,0%	MG242509.1
Ve 660	MN997492	Ochlerotatus serratus voucher ST10048 cytochrome	93%	98,0%	MF172270.1
Ve 486	MN997489	Ochlerotatus serratus voucher ST10048 cytochrome	100%	98,0%	MF172270.1
Bo 119	MN997485	Ochlerotatus pertinax cytochrome oxidase subunit I	99%	98,0%	MG242509.1
		Ochlerotatus serratus voucher ST10048 cytochrome	99%	98,0%	MF172270.1
		Ochlerotatus atlanticus voucher NEONTculicid2130	99%	97,0%	JX259519.1
Ve 420	MN997488	Ochlerotatus serratus voucher ST10048 cytochrome	94%	98,0%	MF172270.1
		Ochlerotatus atlanticus voucher NEONTculicid2130	94%	97,0%	JX259519.1
		Ochlerotatus pertinax cytochrome oxidase subunit I	94%	98,0%	MG242509.1
Vi 1179	MN997491	Ochlerotatus serratus voucher ST10046 cytochrome	100%	98,0%	MF172269.1
R 607	MN997487	Ochlerotatus pertinax cytochrome oxidase subunit I	99%	98,0%	MG242509.1
		Ochlerotatus atlanticus voucher NEONTculicid2130	100%	97,0%	JX259519.1
		Ochlerotatus serratus voucher ST10048 cytochrome	100%	98,0%	MF172270.1
R 439	MN997490	Ochlerotatus serratus voucher ST10046 cytochrome	93%	98,0%	MF172269.1
Ve 201	MN997497	Ochlerotatus atlanticus voucher NEONTculicid2130	96%	97,0%	JX259519.1
		Ochlerotatus pertinax cytochrome oxidase subunit I	96%	98,0%	MG242509.1
		Ochlerotatus serratus voucher ST10048 cytochrome	96%	99,0%	MF172270.1
R 511	MN997495	Ochlerotatus serratus voucher ST10048 cytochrome	93%	99,0%	MF172270.1
Ve 165	MN997496	Ochlerotatus serratus voucher ST10048 cytochrome	100%	99,0%	MF172270.1
Ve 784	MN997493	Ochlerotatus serratus voucher ST10048 cytochrome	93%	99,0%	MF172270.1
Ve 530	MN997494	Ochlerotatus serratus voucher ST10048 cytochrome	100%	99,0%	MF172270.1

ID	Accession Seq.	NCBI blastn Hit Description/ species	Q. cov.	Per. Id.	Accession Hit
Oc. sp. 1					
Ve 797	MN997502	Ochlerotatus pertinax cytochrome oxidase subunit I	94%	95,0%	MG242509.1
		Ochlerotatus serratus voucher ST10046 cytochrome	94%	94,0%	MF172269.1
		Ochlerotatus atlanticus voucher NEONTculicid2130	94%	94,0%	JX259519.1
Ve 514	MN997503	Ochlerotatus serratus voucher ST10286 cytochrome	99%	95,0%	MF172268.1
		Ochlerotatus pertinax cytochrome oxidase subunit I	99%	95,0%	MG242509.1
		Ochlerotatus atlanticus voucher NEONTculicid2131	92%	95,0%	JX259518.1
Oc. sp. 2					
Vi 1316	MN997501	Ochlerotatus atlanticus cytochrome oxidase subunit	99%	95,3%	MG242469.1
		Ochlerotatus pertinax cytochrome oxidase subunit I	99%	95,0%	MG242509.1
		Ochlerotatus serratus voucher ST10046 cytochrome	99%	95,0%	MF172269.1
Ps. albipes					
Ve 264	MN997523	Psorophora ferox voucher ZFMK:PR077-Colombia	99%	94,0%	KM593062.1
Ve 13	MN997522	Psorophora ferox voucher ZFMK:PR077-Colombia	95%	94,0%	KM593062.1
Be 24	MN997526	Psorophora ferox voucher ST10041 cytochrome	100%	94,0%	MF172349.1
Vi 944	MN997521	Psorophora ferox voucher ST10041 cytochrome	100%	95,0%	MF172349.1
Be 3	MN997520	Psorophora ferox voucher ST10049 cytochrome	100%	95,0%	MF172347.1
Am 31	MN997525	Psorophora ferox voucher ZFMK:PR052-Colombia	97%	94,0%	KM593037.1
Am 557	MN997524	Psorophora ferox voucher ST10049 cytochrome	100%	95,0%	MF172347.1
Ps. cingulata					
Mu 280	MN997513	Psorophora cingulata voucher ZFMK:PR004-	100%	99,0%	KM592989.1
Ps. colombiae					
Mu 75	MN997514	Psorophora columbiae cytochrome oxidase subunit I	99%	99,0%	MG242534.1
Ps. ferox					
Mr 200	MN997517	Psorophora ferox voucher ZFMK:PR077-Colombia	100%	99,0%	KM593062.1
Vi 1882	MN997515	Psorophora ferox voucher ZFMK:PR077-Colombia	100%	99,0%	KM593062.1
Ve 197	MN997516	Psorophora ferox voucher ZFMK:PR077-Colombia	96%	99,0%	KM593062.1
Am 61	MN997519	Psorophora ferox voucher ST10293 cytochrome	100%	99,0%	MF172348.1
Vi 166	MN997518	Psorophora ferox voucher ST10049 cytochrome	100%	99,0%	MF172347.1
Ps. funiculus					
Ve 648	MN997507	Psorophora (Grabhamia) sp. RHL-2015 isolate	82%	99,0%	KM452781.1
		Psorophora cingulata voucher ZFMK:PR004-	98%	94,0%	KM592989.1
Vi 1251	MN997511	Psorophora (Grabhamia) sp. RHL-2015 isolate	85%	99,0%	KM452781.1
Ve 661	MN997505	Psorophora (Grabhamia) sp. RHL-2015 isolate	85%	98,0%	KM452781.1
		Psorophora cingulata voucher ZFMK:PR004-	98%	94,0%	KM592989.1
Vi 991	MN997506	Psorophora (Grabhamia) sp. RHL-2015 isolate	84%	98,0%	KM452781.1
		Psorophora cingulata voucher ZFMK:PR004-	99%	95,0%	KM592989.1
Ve 623	MN997510	Psorophora (Grabhamia) sp. RHL-2015 isolate	83%	98,0%	KM452781.1
		Psorophora cingulata voucher ZFMK:PR004-	99%	94,0%	KM592989.1
Ve 649	MN997512	Psorophora (Grabhamia) sp. RHL-2015 isolate	82%	98,0%	KM452782.1
Ve 758	MN997508	Psorophora cingulata voucher ZFMK:PR004-	98%	95,0%	KM592989.1
		Psorophora (Grabhamia) sp. RHL-2015 isolate	82%	98,0%	KM452782.1
Ve 756	MN997509	Psorophora cingulata voucher ZFMK:PR004-	98%	95,0%	KM592989.1
		Psorophora (Grabhamia) sp. RHL-2015 isolate	80%	98,0%	KM452782.1
St. albopicta (Ae. albopictus)					
Am 274	MN997608	Aedes albopictus haplotype 38 cytochrome oxidase	100%	100,0%	MF148286.1
Ve 483	MN997609	Aedes albopictus haplotype H34 cytochrome c	100%	99,0%	KC690929.1
Am 348	MN997610	Aedes albopictus haplotype 38 cytochrome oxidase	100%	100,0%	MF148286.1
Am 213	MN997611	Aedes albopictus haplotype H34 cytochrome c	100%	99,9%	KC690929.1
Am 346	MN997606	Aedes albopictus haplotype 44 cytochrome oxidase	100%	99,0%	MF148292.1
Am 435	MN997607	Aedes albopictus haplotype 44 cytochrome oxidase	100%	99,0%	MF148292.1
Tr. digitatum					
Ve 580	MN997658	Trichoprosopon digitatum voucher MB10002	93%	100,0%	MF172408.1
Tr. sp. 1					
Ve 642	MN997654	Trichoprosopon digitatum voucher CCDB-10764-A10	100%	93,0%	KF671033.1
		Trichoprosopon compressum voucher ST10251	93%	94,0%	MF172405.1
Ve 582	MN997656	Trichoprosopon digitatum voucher CCDB-10764-A10	100%	93,0%	KF671033.1
		Trichoprosopon compressum voucher ST10251	95%	94,0%	MF172405.1
Ve 545	MN997655	Trichoprosopon digitatum voucher CCDB-10764-A10	96%	93,0%	KF671033.1

ID	Accession Seq.	NCBI blastn Hit Description/ species	Q. cov.	Per. Id.	Accession Hit
		Shannoniana schedocyclia voucher ST10241	96%	93,0%	MF172384.1
		Trichoprosopon compressum voucher ST10251	96%	94,0%	MF172405.1
Ve 497	MN997657	Trichoprosopon digitatum voucher CCDB-10764-A10	95%	93,0%	KF671033.1
		Trichoprosopon compressum voucher ST10251	95%	94,0%	MF172405.1
Tr. Sp. 2					
Ve 535	MN997653	Trichoprosopon digitatum voucher MB10002	92%	94,5%	MF172408.1
Tr. sp. 3					
R 118	MN997663	Wyeomyia lamellata voucher MB10209 cytochrome	100%	95,0%	MF172448.1
		Wyeomyia complosa voucher MB10297 cytochrome	100%	91,0%	MF172438.1
Tr. sp. 4					
Ve 504	MN997662	Wyeomyia complosa voucher MB10297 cytochrome	100%	91,0%	MF172438.1
		Wyeomyia surinamensis voucher MB10482	93%	93,0%	MF172514.1
Tr. sp. 5					
R 432	MN997659	Wyeomyia aporonoma voucher MB10691	92%	91,0%	MF172423.1
		Wyeomyia complosa voucher MB10447 cytochrome	92%	92,0%	MF172439.1
Wy. aporonoma					
Ve 760	MN997648	Wyeomyia aporonoma voucher MB10691	100%	97,0%	MF172423.1
R 391	MN997650	Wyeomyia aporonoma voucher MB10691	93%	97,0%	MF172423.1
Ve 507	MN997651	Wyeomyia aporonoma voucher MB10691	93%	97,0%	MF172423.1
Wy. luteoventralis					
Mu 338	MN997661	Wyeomyia luteoventralis voucher ZFMK:PR055-	100%	100,0%	KM593040.1
Mu 339	MN997660	Wyeomyia luteoventralis voucher ZFMK:PR055-	98%	99,7%	KM593040.1
Wy. melanocephala					
R 156	MN997664	*Wyeomyia melanocephala voucher MB10264*	93%	97,0%	MF172463.1
Wy. pertinans					
R 216	MN997617	Wyeomyia pertinans voucher MB10503 cytochrome	100%	98,0%	MF172479.1
Ve 757.1	MN997616	Wyeomyia pertinans voucher MB10503 cytochrome	100%	98,0%	MF172479.1
Ve 673	MN997614	Wyeomyia pertinans voucher MB10503 cytochrome	95%	98,0%	MF172479.1
R 201	MN997620	Wyeomyia pertinans voucher MB10503 cytochrome	100%	98,0%	MF172479.1
R 113	MN997621	Wyeomyia pertinans voucher MB10503 cytochrome	100%	98,0%	MF172479.1
Ve 769	MN997622	Wyeomyia pertinans voucher MB10503 cytochrome	100%	98,0%	MF172479.1
Ve 762	MN997623	Wyeomyia pertinans voucher MB10503 cytochrome	100%	98,0%	MF172479.1
Ve 778	MN997624	Wyeomyia pertinans voucher MB10503 cytochrome	94%	98,0%	MF172479.1
Ve 788	MN997625	Wyeomyia pertinans voucher MB10503 cytochrome	95%	98,0%	MF172479.1
Ve 763	MN997636	Wyeomyia pertinans voucher MB10503 cytochrome	92%	98,0%	MF172479.1
R 295	MN997648	Wyeomyia pertinans voucher MB10503 cytochrome	100%	98,0%	MF172479.1
R 291	MN997619	Wyeomyia pertinans voucher MB10503 cytochrome	100%	98,0%	MF172479.1
Vi 1204	MN997626	Wyeomyia pertinans voucher MB10503 cytochrome	100%	98,0%	MF172479.1
Ve 783	MN997618	Wyeomyia pertinans voucher MB10503 cytochrome	93%	98,0%	MF172479.1
R 422	MN997615	Wyeomyia pertinans voucher MB10503 cytochrome	100%	98,0%	MF172479.1
R 438	MN997632	Wyeomyia pertinans voucher MB10503 cytochrome	93%	98,0%	MF172479.1
R 198	MN997633	Wyeomyia pertinans voucher MB10503 cytochrome	92%	98,0%	MF172479.1
Ve 772	MN997629	Wyeomyia pertinans voucher MB10503 cytochrome	94%	99,0%	MF172479.1
R 420	MN997627	Wyeomyia pertinans voucher MB10503 cytochrome	100%	99,0%	MF172479.1
R 150	MN997628	Wyeomyia pertinans voucher MB10503 cytochrome	100%	99,0%	MF172479.1
Ve 753	MN997634	Wyeomyia pertinans voucher MB10503 cytochrome	100%	98,0%	MF172479.1
Ve 759	MN997635	Wyeomyia pertinans voucher MB10503 cytochrome	95%	98,0%	MF172479.1
Ve 802	MN997630	Wyeomyia pertinans voucher MB10503 cytochrome	93%	98,0%	MF172479.1
Mu 341	MN997631	Wyeomyia pertinans voucher MB10503 cytochrome	100%	99,0%	MF172479.1
Ve 785	MN997643	Wyeomyia pertinans voucher MB10503 cytochrome	93%	98,0%	MF172479.1
Ve 502	MN997642	Wyeomyia pertinans voucher MB10503 cytochrome	100%	98,0%	MF172479.1
R 158	MN997641	Wyeomyia pertinans voucher MB10503 cytochrome	100%	98,0%	MF172479.1
R 782	MN997644	Wyeomyia pertinans voucher MB10503 cytochrome	100%	98,0%	MF172479.1
R 192	MN997647	Wyeomyia pertinans voucher MB10503 cytochrome	93%	98,0%	MF172479.1
Ve 381	MN997637	Wyeomyia pertinans voucher MB10503 cytochrome	92%	98,0%	MF172479.1
Ve 465	MN997640	Wyeomyia pertinans voucher MB10503 cytochrome	94%	98,0%	MF172479.1
R 633	MN997638	Wyeomyia pertinans voucher MB10503 cytochrome	100%	98,0%	MF172479.1
Ve 776	MN997639	Wyeomyia pertinans voucher MB10503 cytochrome	100%	98,0%	MF172479.1
Ve 500	MN997645	Wyeomyia pertinans voucher MB10503 cytochrome	93%	98,0%	MF172479.1

ID	Accession Seq.	NCBI blastn Hit Description/ species	Q. cov.	Per. Id.	Accession Hit
Ve 508	MN997646	Wyeomyia pertinans voucher MB10503 cytochrome	100%	98,0%	MF172479.1
		Wy. sp. 1			
R 169	MN997652	Wyeomyia aporonoma voucher MB10691	99%	92,0%	MF172423.1
		Wyeomyia arthrostigma voucher MB10347	99%	91,0%	MF172432.1
		Wyeomyia aphobema voucher CCDB-10764-B10	99%	91,0%	KF671035.1

11.1.2 ITS2 sequences of mosquitoes from the Atrato River valley

Table 16: NCBI blastn hits for ITS2 sequences from mosquito species found in this study

ID	Accession Seq.	NCBI blastn Hit Description/ species	Q. cov.	Per. Id.	Accession Hit
		An. apicimacula			
Mu 1	MT151997	Anopheles apicimacula 5.8S ribosomal RNA gene, partial	100%	99,0%	KF436935.1
		An. darlingi			
Na 652	MT151916	Anopheles darlingi 5.8S ribosomal RNA gene, partial	100%	100,0%	KF436940.1
Na 647	MT151917	Anopheles darlingi 5.8S ribosomal RNA gene, partial	100%	100,0%	KF436940.1
Na 600	MT151918	Anopheles darlingi 5.8S ribosomal RNA gene, partial	100%	100,0%	KF436940.1
Mr 181	MT151919	Anopheles darlingi 5.8S ribosomal RNA gene, partial	100%	100,0%	KF436940.1
Na 788	MT151920	Anopheles darlingi 5.8S ribosomal RNA gene, partial	99%	99,8%	KF436940.1
Vi 2157	MT151943	Anopheles darlingi 5.8S ribosomal RNA gene, partial	100%	99,6%	KF436940.1
Na 573	MT151921	Anopheles darlingi 5.8S ribosomal RNA gene, partial	100%	100,0%	KF436940.1
		An. neivai			
Mr 164	MT151946	Anopheles homunculus isolate E-10225 clone 1 5.8S	100%	81,0%	FJ176952.1
		An. nuneztovari			
Ca 201	MT151922	Anopheles nuneztovari 5.8S ribosomal RNA gene, partial	100%	99,4%	KF436936.1
Mu 98	MT151923	Anopheles nuneztovari 5.8S ribosomal RNA gene, partial	100%	99,8%	KF436936.1
Vi 575	MT151924	Anopheles nuneztovari 5.8S ribosomal RNA gene, partial	100%	100,0%	KF436936.1
Vi 324	MT151925	Anopheles nuneztovari 5.8S ribosomal RNA gene, partial	100%	100,0%	KF436936.1
Mr 144	MT151926	Anopheles nuneztovari 5.8S ribosomal RNA gene, partial	100%	100,0%	KF436936.1
Ca 207	MT151927	Anopheles nuneztovari 5.8S ribosomal RNA gene, partial	100%	100,0%	KF436936.1
Ca 128	MT151928	Anopheles nuneztovari 5.8S ribosomal RNA gene, partial	100%	100,0%	KF436936.1
Ca 79	MT151929	Anopheles nuneztovari 5.8S ribosomal RNA gene, partial	100%	100,0%	KF436936.1
Ca 65	MT151930	Anopheles nuneztovari 5.8S ribosomal RNA gene, partial	100%	100,0%	KF436936.1
Ca 37	MT151931	Anopheles nuneztovari 5.8S ribosomal RNA gene, partial	100%	100,0%	KF436936.1
Ca 2	MT151932	Anopheles nuneztovari 5.8S ribosomal RNA gene, partial	100%	100,0%	KF436936.1
Ca 111	MT151933	Anopheles nuneztovari 5.8S ribosomal RNA gene, partial	100%	100,0%	KF436936.1
Ca 279	MT151934	Anopheles nuneztovari 5.8S ribosomal RNA gene, partial	100%	100,0%	KF436936.1
Vi 532	MT151935	Anopheles nuneztovari 5.8S ribosomal RNA gene, partial	100%	99,8%	KF436936.1
Na 688	MT151936	Anopheles nuneztovari 5.8S ribosomal RNA gene, partial	100%	99,8%	KF436936.1
Mu 72	MT151937	Anopheles nuneztovari 5.8S ribosomal RNA gene, partial	100%	99,8%	KF436936.1
Ca 174	MT151938	Anopheles nuneztovari 5.8S ribosomal RNA gene, partial	100%	99,8%	KF436936.1
Vi 801	MT151939	Anopheles nuneztovari 5.8S ribosomal RNA gene, partial	100%	100,0%	KF436936.1
Ca 119	MT151940	Anopheles nuneztovari 5.8S ribosomal RNA gene, partial	100%	100,0%	KF436936.1
Ca 85	MT151941	Anopheles nuneztovari 5.8S ribosomal RNA gene, partial	100%	100,0%	KF436936.1
Ca 72	MT151944	Anopheles nuneztovari 5.8S ribosomal RNA gene, partial	100%	100,0%	KF436936.1
Ca 93	MT151947	Anopheles nuneztovari 5.8S ribosomal RNA gene, partial	100%	100,0%	KF436936.
Ca 190	MT151948	Anopheles nuneztovari 5.8S ribosomal RNA gene, partial	100%	100,0%	KF436936.
		An. pseudopunctipennis			
Mu 295	MT151998	Anopheles pseudopunctipennis 5.8S rRNA gene, complete	100%	98,1%	U49735.1
		An. punctimacula			
Vi 677	MT151952	Anopheles punctimacula 5.8S ribosomal RNA gene and	100%	99,8%	KF436941.1
Mr 88	MT151953	Anopheles punctimacula 5.8S ribosomal RNA gene and	100%	99,5%	KF436941.1
Cu 209	MT151954	Anopheles punctimacula 5.8S ribosomal RNA gene and	100%	99,5%	KF436941.1
		Cq. lynchi			
Mr 214	MT151942	Culex atratus B Williams & Savage (2009) voucher FL2007-	36%	89,3%	HQ317357.1
Mr-44	MT151950	Culex atratus B Williams & Savage (2009) voucher FL2007-	33%	85,9%	HQ317357.1
		Aedes palpalae partial 5.8S rRNA gene, ITS2 and partial	39%	83,0%	FM211130.1

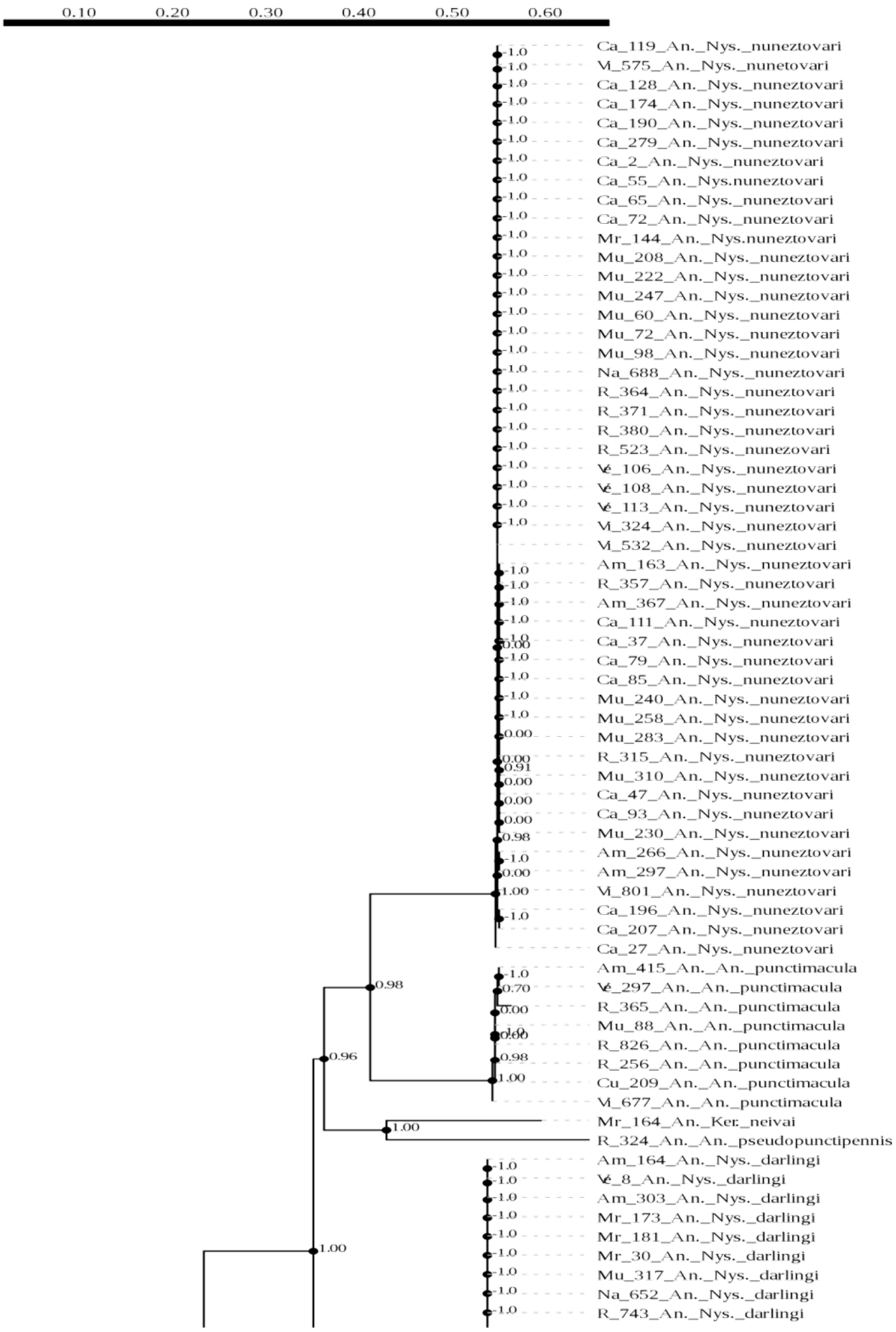
ID	Accession Seq.	NCBI blastn Hit Description/ species	Q. cov.	Per. Id.	Accession Hit
		Aedes belleci partial 5.8S rRNA gene, ITS2 and partial 28S	39%	83,0%	FM211125.1
		Aedes nigropterum partial 5.8S rRNA gene, ITS2 and	39%	83,0%	FM211127.1
		Aedes circumluteolus partial 5.8S rRNA gene, ITS2 and	39%	83,0%	FM211131.1
Cq. venezuelensis					
Vi 2149	MT151955	Tripteroides atripes voucher VAITC4313 5.8S ribosomal	50%	93,6%	KU495708.1
		Culex nigripalpus clone FJNGITS5 18S ribosomal RNA	44%	91,4%	AF521663.1
Cu 103	MT151956	Tripteroides atripes voucher VAITC4313 5.8S ribosomal	45%	91,5%	KU495708.1
		Culex mulrennani voucher FL2006-27-02 clone 2 18S	45%	91,6%	HQ317374.1
Ca 99	MT151966	Tripteroides atripes voucher VAITC4313 5.8S ribosomal	43%	92,1%	KU495708.1
Na 628	MT151978	Aedes japonicus clone Aejap_CH_ZHc2_2_2 5.8S	36%	97,3%	KF471621.1
		Tripteroides atripes voucher VAITC4313 5.8S ribosomal	42%	90,2%	KU495708.1
Na 806	MT151980	Aedes japonicus clone Aejap_CH_ZHc2_2_2 5.8S	35%	96,3%	KF471621.1
Cx. nigripalpus					
Ca 117	MT151951	Culex nigripalpus clone NFL1J32 putative 5.8S rRNA gene,	100%	99,5%	U33024.1
Cx. pipiens					
Mu 9	MT151945	Culex pipiens clone QBZ3447J putative 18S ribosomal RNA	100%	100,0%	U22124.1
Mr 18	MT151987	Culex pipiens clone 2 5.8S ribosomal RNA gene, partial	100%	99,6%	KU743933.1
Cx. sp. 2					
Vi 385	MT151972	Culex pilosus voucher FL2006-06-01 clone 2 18S	76%	89,2%	HQ317382.1
		Culex nr. pedroi PERU-02 5.8S ribosomal RNA gene,	93%	83,3%	AY633732.1
		Culex sacchettae from Brazil 5.8S ribosomal RNA gene,	71%	88,7%	AY633735.1
		Culex portesi 5.8S ribosomal RNA gene, partial sequence;	82%	84,1%	AY633733.1
Vi1383	MT151985	Culex sacchettae from Brazil 5.8S ribosomal RNA gene,	82%	89,5%	AY633735.1
Cx. sp.1					
Na 591	MT151964	Culex abominator voucher TX2003-27-03 clone 2 18S	69%	85,6%	HQ317348.1
		Culiseta inconspicua voucher VAITC4904 5.8S ribosomal	69%	83,9%	KU495655.1
		Culex peccator voucher LA2003-30-02 clone 2 18S	69%	83,9%	HQ317378.1
Ma. indubitans					
Mu 155	MT151967	Ochlerotatus sagax voucher VAITC4305C clone 2 5.8S	61%	84,6%	KX866215.1
		Ochlerotatus vittiger voucher VAITC4301C 5.8S ribosomal	61%	84,1%	KU495700.1
Mu 145	MT151981	Ochlerotatus sagax voucher VAITC4305C clone 2 5.8S	63%	83,2%	KX866215.1
Ma. titillans					
Ca 43	MT151949	Ochlerotatus sagax voucher VAITC4305C clone 2 5.8S	45%	96,5%	KX866215.1
		Culiseta inconspicua voucher VAITC4904 5.8S ribosomal	44%	97,1%	KU495655.1
		Ochlerotatus vittiger voucher VAITC4301D clone 3 5.8S	45%	96,5%	KX866233.1
		Ochlerotatus camptorhynchus voucher VAITC4330 clone 2	44%	97,1%	KX866190.1
		Ochlerotatus theobaldi voucher VAITC4302D clone 4 5.8S	46%	96,5%	KX866227.1
Be 10	MT151959	Ochlerotatus sagax voucher VAITC4305C clone 2 5.8S	42%	95,5%	KX866215.1
Vi 59	MT151960	Ochlerotatus sagax voucher VAITC4305C clone 2 5.8S	42%	95,5%	KX866215.1
		Culiseta inconspicua voucher VAITC4904 5.8S ribosomal	41%	96,3%	KU495655.1
Mr 95	MT151961	Ochlerotatus sagax voucher VAITC4305C clone 2 5.8S	39%	94,9%	KX866215.1
Mr 61	MT151962	Ochlerotatus sagax voucher VAITC4305C clone 2 5.8S	39%	94,9%	KX866215.1
Mr 3	MT151965	Ochlerotatus sagax voucher VAITC4305C clone 2 5.8S	42%	95,1%	KX866215.1
Na 755	MT151982	Toxorhynchites ambionensis 5.8S ribosomal RNA gene,	58%	86,8%	U48377.1
		Culex nr. pedroi PERU-02 5.8S ribosomal RNA gene,	58%	86,1%	AY633732.1
Mr 34	MT151992	Borichinda cavernicola isolate R1 5.8S ribosomal RNA	34%	81,6%	EF370409.1
		Aedes aegypti isolate LAB2 5.8S ribosomal RNA gene,	31%	81,2%	MH142327.1
Mr 154	MT151993	Aedes metallicus voucher metal01 5.8S ribosomal RNA	23%	90,2%	MG232628.1
		Aedes metallicus isolate 14702-AemetF05 18S ribosomal	23%	90,2%	KU056494.1
Mr 9	MT151994	Aedes metallicus voucher metal01 5.8S ribosomal RNA	24%	90,2%	MG232628.1
		Aedes mascarensis voucher masca01 5.8S ribosomal RNA	26%	87,3%	MG232627.1
Ma. sp. 1					
Mu 148	MT151999	Aedes albopictus clone S20 5.8S ribosomal RNA gene,	73%	81,9%	MN062748.1
		Culex nr. pedroi MIR-01 5.8S ribosomal RNA gene, partial	64%	84,7%	AY633726.1
Oc. scapularis					
Vi 1316	MT151957	Aedes mallochi voucher VAITC4303 5.8S ribosomal RNA	100%	87,3%	KU495686.1
		Ochlerotatus sagax voucher VAITC4305D clone 1 5.8S	82%	93,8%	KX866219.1
		Ochlerotatus tormentor voucher NC-T-9 5.8S ribosomal	92%	90,1%	KF307744.1
Vi 754	MT151958	Aedes mallochi voucher VAITC4303 5.8S ribosomal RNA	100%	87,3%	KU495686.1

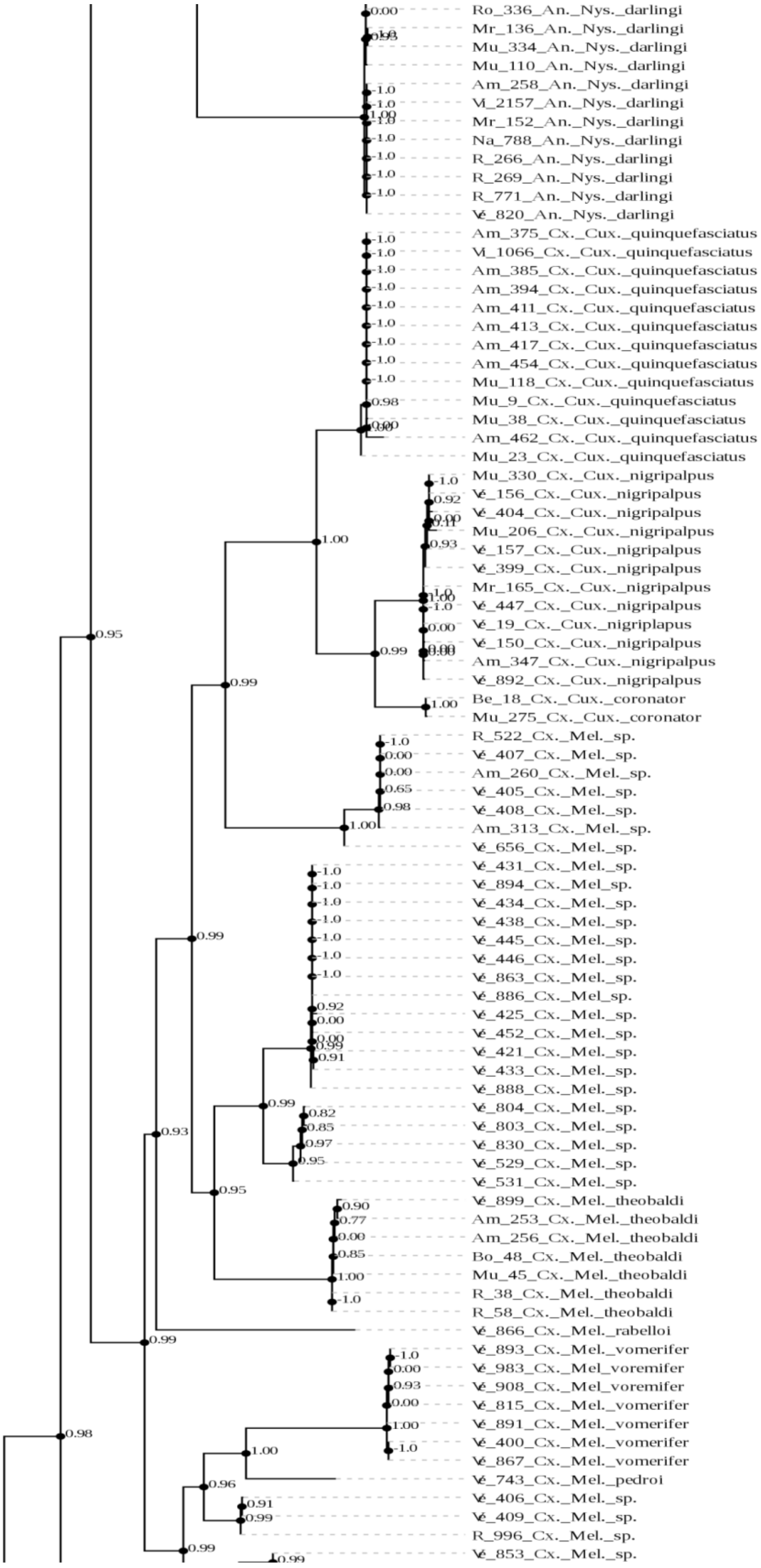
ID	Accession Seq.	NCBI blastn Hit Description/ species	Q. cov.	Per. Id.	Accession Hit
Vi 880	MT151970	Ochlerotatus tormentor voucher NC-T-9 5.8S ribosomal	95%	92,6%	KF307744.1
Vi 1576	MT151973	Ochlerotatus tormentor voucher NC-T-9 5.8S ribosomal	98%	92,5%	KF307744.1
		Ochlerotatus atlanticus voucher NC-A-15 5.8S ribosomal	97%	90,8%	KF307743.1
Oc. serratus					
Vi 1173	MT151963	Ochlerotatus atlanticus voucher NC-A-15 5.8S ribosomal	80%	96,7%	KF307743.1
		Ochlerotatus tormentor voucher NC-T-9 5.8S ribosomal	91%	90,7%	KF307744.1
Bo 69	MT151969	Ochlerotatus atlanticus voucher NC-A-15 5.8S ribosomal	88%	96,7%	KF307743.1
		Ochlerotatus tormentor voucher NC-T-9 5.8S ribosomal	87%	97,0%	KF307744.1
Bo 119	MT151977	Ochlerotatus atlanticus voucher NC-A-15 5.8S ribosomal	96%	96,4%	KF307743.1
		Ochlerotatus tormentor voucher NC-T-9 5.8S ribosomal	94%	96,6%	KF307744.1
Vi 899	MT151989	Ochlerotatus atlanticus voucher NC-A-15 5.8S ribosomal	96%	99,6%	KF307743.1
		Ochlerotatus tormentor voucher NC-T-9 5.8S ribosomal	95%	98,7%	KF307744.1
Ps. albipes					
Vi 944	MT151968	Ochlerotatus camptorhynchus voucher VAITC4330 clone 2	77%	95,6%	KX866190.1
		Ochlerotatus sagax voucher VAITC4305C clone 2 5.8S	100%	85,4%	KX866215.1
Be 5	MT151971	Ochlerotatus camptorhynchus voucher VAITC4330 clone 2	76%	95,3%	KX866190.1
Vi 868	MT151974	Ochlerotatus camptorhynchus voucher VAITC4330 clone 2	84%	95,6%	KX866190.1
		Ochlerotatus sagax voucher VAITC4305A clone 4 5.8S	92%	88,0%	KX866210.1
Be 15	MT151975	Ochlerotatus camptorhynchus voucher VAITC4330 clone 2	84%	95,6%	KX866190.1
		Ochlerotatus sagax voucher VAITC4305D clone 1 5.8S	92%	88,3%	KX866219.1
Be 22		Ochlerotatus camptorhynchus voucher VAITC4330 clone 2	82%	94,9%	KX866190.1
Be 8	MT151983	Ochlerotatus camptorhynchus voucher VAITC4330 clone 2	81%	93,8%	KX866190.1
Be 4	MT151984	Ochlerotatus camptorhynchus voucher VAITC4330 clone 2	85%	94,5%	KX866190.1
Vi 755	MT151986	Ochlerotatus camptorhynchus voucher VAITC4330 clone 2	86%	95,0%	KX866190.1
Be 3	MT151991	Ochlerotatus camptorhynchus voucher VAITC4330 clone 2	98%	95,1%	KX866190.1
Be 14	MT151990	Psorophora ferox voucher ferox01 5.8S ribosomal RNA	100%	82,0%	MG232643.1
Be 3	MT151991	Ochlerotatus camptorhynchus voucher VAITC4330 clone 2	98%	95,1%	KX866190.1
Be 19	MT151995	Ochlerotatus camptorhynchus voucher VAITC4330 clone 2	100%	95,3%	KX866190.1
Ps. ferox					
Vi 1882	MT151979	Psorophora ferox 5.8S ribosomal RNA (5.8S rRNA) gene, 3'	91%	98,6%	M95129.1
Bo 144	MT151988	Psorophora ferox voucher ferox01 5.8S ribosomal RNA	99%	98,4%	MG232643.1
Ps. funiculus					
Vi 991	MT152001	Ochlerotatus sagax voucher VAITC4305D clone 1 5.8S	56%	87,3%	KX866219.1
		Ochlerotatus vittiger voucher VAITC4301A 5.8S ribosomal	57%	86,5%	KU495699.1
Wy. luteoventralis					
Mu 338	MT151976	Aedes sp. isolate BS07 5.8S ribosomal RNA gene, partial	54%	89,6%	MF623851.1
		Aedes aegypti clone Kass_1.3 5.8S ribosomal RNA gene,	54%	89,9%	MF142280.1
Wy. pertinans					
Mu 341	MT151996	Culicidae sp. JBB11-2_Tr 5.8S ribosomal RNA gene, partial	25%	91,5%	KY117397.1
		Culiseta inconspicua voucher VAITC4904 5.8S ribosomal	25%	89,1%	KU495655.1
		Aedes albopictus isolate Aa26 internal transcribed spacer	25%	87,0%	KY703654.1
Wy. sp. 1					
Mu 340	MT152000	Aedes flavopictus clone 14_4 18S ribosomal RNA gene,	51%	88,4%	AF353531.1
		Aedes metallicus isolate 14702-AemetD09 18S ribosomal	51%	87,4%	KU056497.1

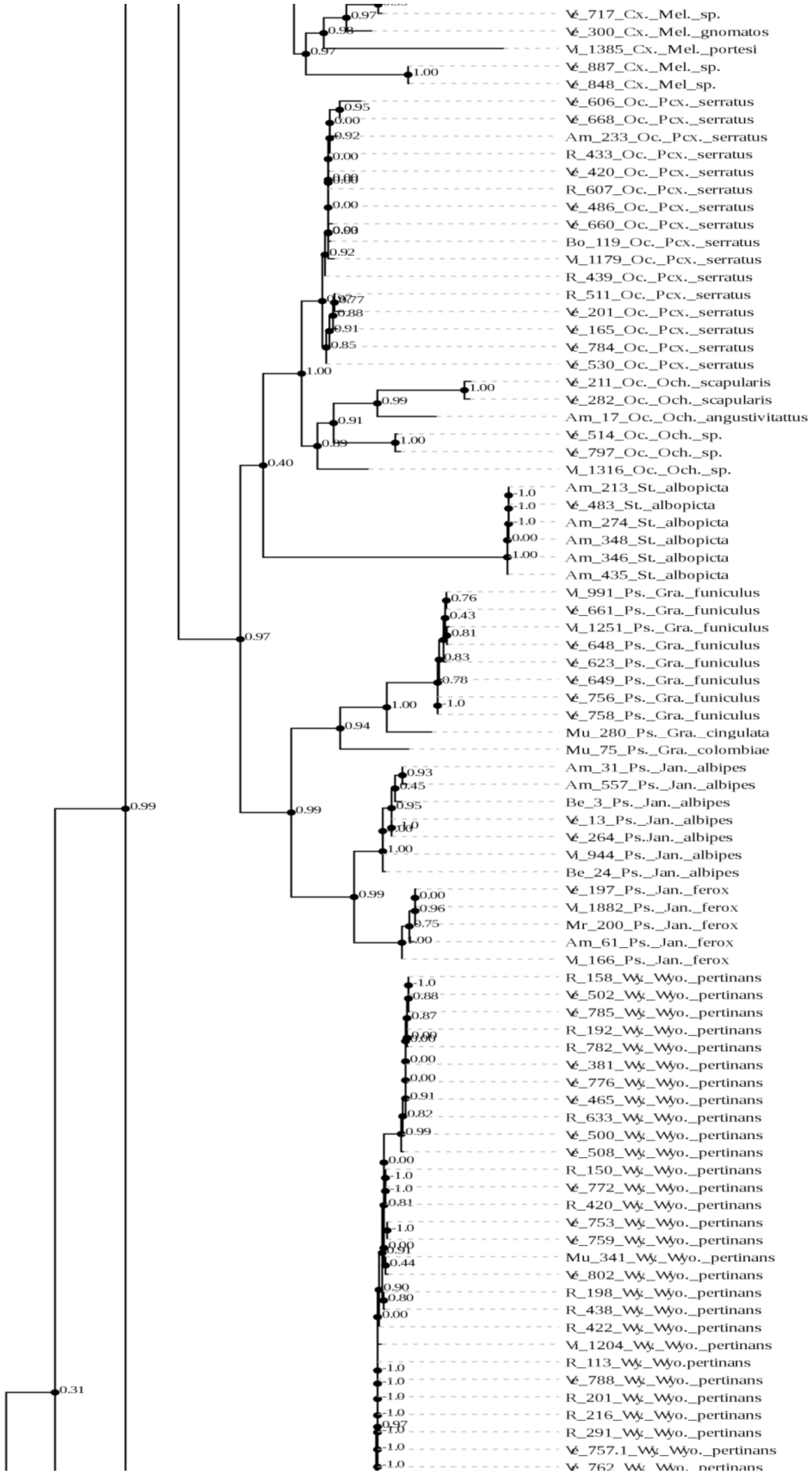
11.2 Mosquito phylogenies

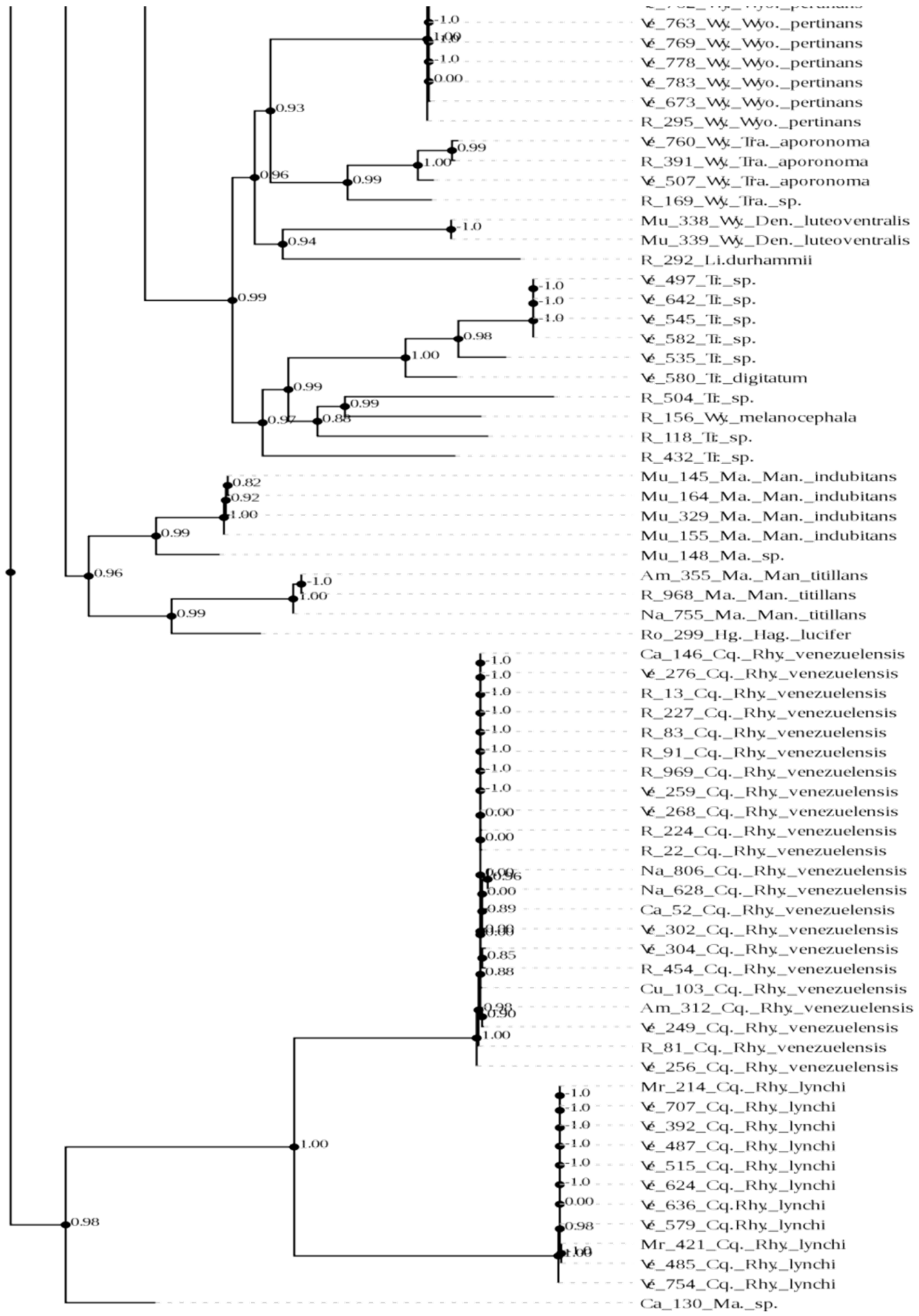
11.2.1 Phylogenetic tree based on the COI sequences of the mosquitoes from this study

Figure 39: Mosquito phylogeny estimated based on the nucleotide sequence of the COI gene (selected model GTR+G+I). The midrooted maximum likelihood tree of mosquitoes found in this study is displayed in a rectangular view. Nodes show aLRT SH-like support values and branch length is shown in the upper left corner.




Ro_336_An._Nys._darlingi
Mr_136_An._Nys._darlingi
Mu_334_An._Nys._darlingi
Mu_110_An._Nys._darlingi
Am_258_An._Nys._darlingi
Vi_2157_An._Nys._darlingi
Mr_152_An._Nys._darlingi
Na_788_An._Nys._darlingi
R_266_An._Nys._darlingi
R_269_An._Nys._darlingi
R_771_An._Nys._darlingi
Vé_820_An._Nys._darlingi
Am_375_Cx._Cux._quinquefasciatus
Vi_1066_Cx._Cux._quinquefasciatus
Am_385_Cx._Cux._quinquefasciatus
Am_394_Cx._Cux._quinquefasciatus
Am_411_Cx._Cux._quinquefasciatus
Am_413_Cx._Cux._quinquefasciatus
Am_417_Cx._Cux._quinquefasciatus
Am_454_Cx._Cux._quinquefasciatus
Mu_118_Cx._Cux._quinquefasciatus
Mu_9_Cx._Cux._quinquefasciatus
Mu_38_Cx._Cux._quinquefasciatus
Am_462_Cx._Cux._quinquefasciatus
Mu_23_Cx._Cux._quinquefasciatus
Mu_330_Cx._Cux._nigripalpus
Vé_156_Cx._Cux._nigripalpus
Vé_404_Cx._Cux._nigripalpus
Mu_206_Cx._Cux._nigripalpus
Vé_157_Cx._Cux._nigripalpus
Vé_399_Cx._Cux._nigripalpus
Mr_165_Cx._Cux._nigripalpus
Vé_447_Cx._Cux._nigripalpus
Vé_19_Cx._Cux._nigriplapus
Vé_150_Cx._Cux._nigripalpus
Am_347_Cx._Cux._nigripalpus
Vé_892_Cx._Cux._nigripalpus
Be_18_Cx._Cux._coronator
Mu_275_Cx._Cux._coronator
R_522_Cx._Mel._sp.
Vé_407_Cx._Mel._sp.
Am_260_Cx._Mel._sp.
Vé_405_Cx._Mel._sp.
Vé_408_Cx._Mel._sp.
Am_313_Cx._Mel._sp.
Vé_656_Cx._Mel._sp.
Vé_431_Cx._Mel._sp.
Vé_894_Cx._Mel_sp.
Vé_434_Cx._Mel._sp.
Vé_438_Cx._Mel._sp.
Vé_445_Cx._Mel._sp.
Vé_446_Cx._Mel._sp.
Vé_863_Cx._Mel._sp.
Vé_886_Cx._Mel_sp.
Vé_425_Cx._Mel._sp.
Vé_452_Cx._Mel._sp.
Vé_421_Cx._Mel._sp.
Vé_433_Cx._Mel._sp.
Vé_888_Cx._Mel._sp.
Vé_804_Cx._Mel._sp.
Vé_803_Cx._Mel._sp.
Vé_830_Cx._Mel._sp.
Vé_529_Cx._Mel._sp.
Vé_531_Cx._Mel._sp.
Vé_899_Cx._Mel._theobaldi
Am_253_Cx._Mel._theobaldi
Am_256_Cx._Mel._theobaldi
Bo_48_Cx._Mel._theobaldi
Mu_45_Cx._Mel._theobaldi
R_38_Cx._Mel._theobaldi
R_58_Cx._Mel._theobaldi
Vé_866_Cx._Mel._rabelloi
Vé_893_Cx._Mel._vomerifer
Vé_983_Cx._Mel_voremifer
Vé_908_Cx._Mel_voremifer
Vé_815_Cx._Mel._vomerifer
Vé_891_Cx._Mel._vomerifer
Vé_400_Cx._Mel._vomerifer
Vé_867_Cx._Mel._vomerifer
Vé_743_Cx._Mel._pedroi
Vé_406_Cx._Mel._sp.
Vé_409_Cx._Mel._sp.
R_996_Cx._Mel._sp.
Vé_853_Cx._Mel._sp.
0.95
0.98
1.00
0.99
0.99
1.00
0.99
0.93
0.99
0.95
0.99
0.99
0.96
1.00


Vé_717_Cx._Mel._sp.
Vé_300_Cx._Mel._gnomatos
Vi_1385_Cx._Mel._portesi
Vé_887_Cx._Mel._sp.
Vé_848_Cx._Mel_sp.
Vé_606_Oc._Pcx._serratus
Vé_668_Oc._Pcx._serratus
Am_233_Oc._Pcx._serratus
R_433_Oc._Pcx._serratus
Vé_420_Oc._Pcx._serratus
R_607_Oc._Pcx._serratus
Vé_486_Oc._Pcx._serratus
Vé_660_Oc._Pcx._serratus
Bo_119_Oc._Pcx._serratus
Vi_1179_Oc._Pcx._serratus
R_439_Oc._Pcx._serratus
R_511_Oc._Pcx._serratus
Vé_201_Oc._Pcx._serratus
Vé_165_Oc._Pcx._serratus
Vé_784_Oc._Pcx._serratus
Vé_530_Oc._Pcx._serratus
Vé_211_Oc._Och._scapularis
Vé_282_Oc._Och._scapularis
Am_17_Oc._Och._angustivitattus
Vé_514_Oc._Och._sp.
Vé_797_Oc._Och._sp.
Vi_1316_Oc._Och._sp.
Am_213_St._albopicta
Vé_483_St._albopicta
Am_274_St._albopicta
Am_348_St._albopicta
Am_346_St._albopicta
Am_435_St._albopicta
Vi_991_Ps._Gra._funiculus
Vé_661_Ps._Gra._funiculus
Vi_1251_Ps._Gra._funiculus
Vé_648_Ps._Gra._funiculus
Vé_623_Ps._Gra._funiculus
Vé_649_Ps._Gra._funiculus
Vé_756_Ps._Gra._funiculus
Vé_758_Ps._Gra._funiculus
Mu_280_Ps._Gra._cingulata
Mu_75_Ps._Gra._colombiae
Am_31_Ps._Jan._albipes
Am_557_Ps._Jan._albipes
Be_3_Ps._Jan._albipes
Vé_13_Ps._Jan._albipes
Vé_264_Ps.Jan._albipes
Vi_944_Ps._Jan._albipes
Be_24_Ps._Jan._albipes
Vé_197_Ps._Jan._ferox
Vi_1882_Ps._Jan._ferox
Mr_200_Ps._Jan._ferox
Am_61_Ps._Jan._ferox
Vi_166_Ps._Jan._ferox
R_158_Wy._Wyo._pertinans
Vé_502_Wy._Wyo._pertinans
Vé_785_Wy._Wyo._pertinans
R_192_Wy._Wyo._pertinans
R_782_Wy._Wyo._pertinans
Vé_381_Wy._Wyo._pertinans
Vé_776_Wy._Wyo._pertinans
Vé_465_Wy._Wyo._pertinans
R_633_Wy._Wyo._pertinans
Vé_500_Wy._Wyo._pertinans
Vé_508_Wy._Wyo._pertinans
R_150_Wy._Wyo._pertinans
Vé_772_Wy._Wyo._pertinans
R_420_Wy._Wyo._pertinans
Vé_753_Wy._Wyo._pertinans
Vé_759_Wy._Wyo._pertinans
Mu_341_Wy._Wyo._pertinans
Vé_802_Wy._Wyo._pertinans
R_198_Wy._Wyo._pertinans
R_438_Wy._Wyo._pertinans
R_422_Wy._Wyo._pertinans
Vi_1204_Wy._Wyo._pertinans
R_113_Wy._Wyo.pertinans
Vé_788_Wy._Wyo._pertinans
R_201_Wy._Wyo._pertinans
R_216_Wy._Wyo._pertinans
R_291_Wy._Wyo._pertinans
Vé_757.1_Wy._Wyo._pertinans
Vé_762_Wy._Wyo._pertinans

Ve_763_Wy._Wyo._pertinans
Ve_769_Wy._Wyo._pertinans
Ve_778_Wy._Wyo._pertinans
Ve_783_Wy._Wyo._pertinans
Ve_673_Wy._Wyo._pertinans
R_295_Wy._Wyo._pertinans
Ve_760_Wy._Tra._aporonoma
R_391_Wy._Tra._aporonoma
Ve_507_Wy._Tra._aporonoma
R_169_Wy._Tra._sp.
Mu_338_Wy._Den._luteoventralis
Mu_339_Wy._Den._luteoventralis
R_292_Li.durhammii
Ve_497_Tr._sp.
Ve_642_Tr._sp.
Ve_545_Tr._sp.
Ve_582_Tr._sp.
Ve_535_Tr._sp.
Ve_580_Tr._digitatum
R_504_Tr._sp.
R_156_Wy._melanocephala
R_118_Tr._sp.
R_432_Tr._sp.
Mu_145_Ma._Man._indubitans
Mu_164_Ma._Man._indubitans
Mu_329_Ma._Man._indubitans
Mu_155_Ma._Man._indubitans
Mu_148_Ma._sp.
Am_355_Ma._Man_titillans
R_968_Ma._Man._titillans
Na_755_Ma._Man._titillans
Ro_299_Hg._Hag._lucifer
Ca_146_Cq._Rhy._venezuelensis
Ve_276_Cq._Rhy._venezuelensis
R_13_Cq._Rhy._venezuelensis
R_227_Cq._Rhy._venezuelensis
R_83_Cq._Rhy._venezuelensis
R_91_Cq._Rhy._venezuelensis
R_969_Cq._Rhy._venezuelensis
Ve_259_Cq._Rhy._venezuelensis
Ve_268_Cq._Rhy._venezuelensis
R_224_Cq._Rhy._venezuelensis
R_22_Cq._Rhy._venezuelensis
Na_806_Cq._Rhy._venezuelensis
Na_628_Cq._Rhy._venezuelensis
Ca_52_Cq._Rhy._venezuelensis
Ve_302_Cq._Rhy._venezuelensis
Ve_304_Cq._Rhy._venezuelensis
R_454_Cq._Rhy._venezuelensis
Cu_103_Cq._Rhy._venezuelensis
Am_312_Cq._Rhy._venezuelensis
Ve_249_Cq._Rhy._venezuelensis
R_81_Cq._Rhy._venezuelensis
Ve_256_Cq._Rhy._venezuelensis
Mr_214_Cq._Rhy._lynchi
Ve_707_Cq._Rhy._lynchi
Ve_392_Cq._Rhy._lynchi
Ve_487_Cq._Rhy._lynchi
Ve_515_Cq._Rhy._lynchi
Ve_624_Cq._Rhy._lynchi
Ve_636_Cq.Rhy._lynchi
Ve_579_Cq.Rhy._lynchi
Mr_421_Cq._Rhy._lynchi
Ve_485_Cq._Rhy._lynchi
Ve_754_Cq._Rhy._lynchi
Ca_130_Ma._sp.
1.0
1.00
1.0
1.0
0.00
0.93
0.99
1.00
0.96
0.99
1.0
0.94
0.99
1.0
1.0
1.0
0.98
1.00
0.99
0.99
0.97
0.88
0.82
0.92
1.00
0.99
0.96
1.0
1.00
0.99
1.0
1.0
1.0
1.0
1.0
1.0
1.0
1.0
0.00
0.00
0.00
0.96
0.00
0.89
0.00
0.00
0.85
0.88
0.98
0.90
1.00
1.0
1.0
1.0
1.0
1.0
1.0
0.00
0.98
1.0
1.00
1.00
0.98

11.3 Mosquito species identified in the Atrato River valley

Table 17: Mosquito species identified and identification methods applied.

Species	**Morphology**	**COI**	**ITS2**
Ad. squamipennis	✓	✗	✗
An. apicimacula	✓	✗	✓
An. calderoni	✓	✗	✗
An. darlingi	✓	✓	✓
An. malefactor	✓	✗	✗
An. neivai	✓	✓	✗
An. nuneztovari	✓	✓	✓
An. pseudopunctipennis	✓	✓	✓
An. punctimacula	✓	✓	✓
An. shannoni	✓	✗	✗
An. triannulatus	✓	✗	✗
Cq. albicosta	✓	✗	✗
Cq. lynchi	✓	✓	✗
Cq. venezuelensis	✓	✓	✗
Cx. coronator	✗	✓	✗
Cx. nigripalpus	✓	✓	✓
Cx. quinquefasciatus	✓	✗	✓
Cx. rabelloi	✗	✓	✗
Cx. educator	✗	✓	✗
Hg. regalis	✓	✗	✗
Hg.lucifer	✓	✓	✗
Jb. longipes	✓	✗	✗
Li. durhammii	✓	✓	✗
Ma. indubitans	✓	✗	✗
Ma. titillans	✓	✗	✗
Oc. angustivittatus	✓	✓	✗
Oc. scapularis	✓	✓	✗
Oc. serratus	✓	✗	✗
Ps. albipes	✓	✗	✗
Ps. cingulata	✓	✓	✗
Ps. colombiae	✓	✓	✗
Ps. ferox	✓	✓	✓
Ps. funiculus	✓	✗	✗
Sa. tridentatus	✓	✗	✗
St. albopicta	✓	✓	✗
Tr. digitatum	✓	✓	✗
Wy. aporonoma	✗	✓	✗
Wy. luteoventralis	✗	✓	✗
Wy. melanocephala	✗	✓	✗
Wy. pertinans	✗	✓	✗

11.4 Mosquito species abundance in the Atrato River vallley

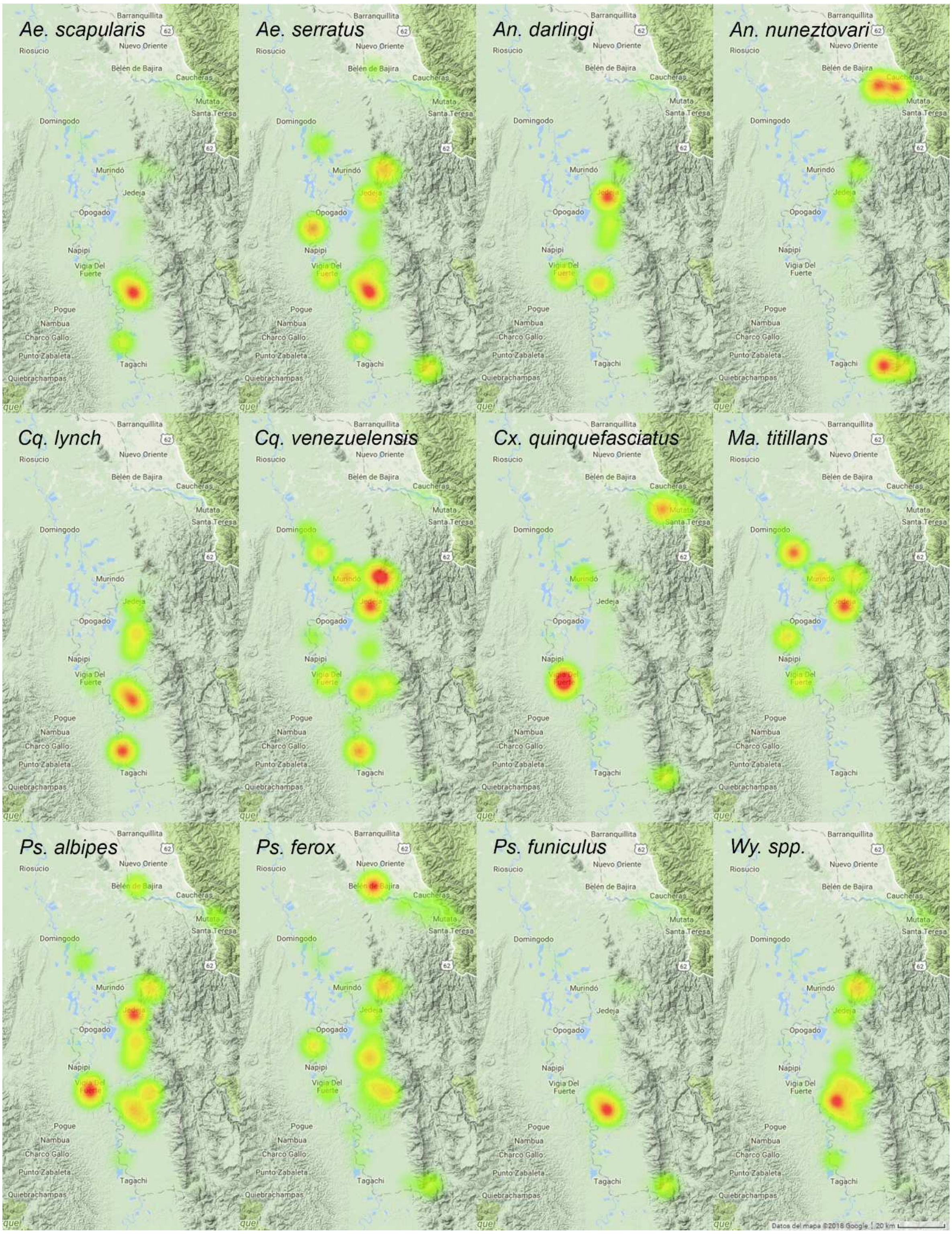


Figure 40: Relative abundance of the main mosquito species by locality (red=abundant, yellow=less abundant, green=rare, no color=not found/not tested). Heatmaps were created with google fusion tables. Unfortunately, google fusion tables was closed down by Google in 2020 and therefore dynamic maps are no longer available online.

Table 18: Mosquito species collected in each location of the research region. The total number of identified specimens is shown. Species in black were identified by morphology and COI blastn and represent the total amount of collected specimens. Species in red were identified by COI blastn only and do not represent the total amount of specimens collected, as these species could not be identified based on morphological characters and not all specimens that could not be identified were COI sequenced.

No.	Location	*Ad. squamipennis*	*An. darlingi*	*An. neivai*	*An. nuneztovari*	*An. pseudopunctipennis*	*An. punctimacula*	*Cq. lynchi*	*Cq. venezuelenis*	*Cx. sp. 8*	*Cx. coronator*	*Cx. sp. 10*	*Cx. nigripalpus*	*Cx. sp. 2*	*Cx. quinquefasciatus*	*Cx. rabelloi*	*Cx. educator*	*Hg. lucifer*	*Li. durhammii*	*Ma. indubitans*	*Ma. titillans*	*Oc. angustivittatus*	*Oc. scapularis*	*Oc. serratus*	*Ps. albipes*	*Ps. cingulata*	*Ps. colombiae*	*Ps. ferox*	*Ps. funiculus*	*St. albopicta*	*Tr. digitatum*	*Wy. aporonoma*	*Wy. luteoventralis*	*Wy. melanocephala*	*Wy. pertinans*
1	Belén de Bajirá										1		2										1	4	53			19							
2	La Secreta		18		127	1	1				3		5		1					5									2						
3	Los Pisingos		7		85										2							1							3						
4	Urada		13		183		5		4												2								1						
5	Caucheras						1																												
6	Caña Verales				6						1																								
7	Chontadural				1										45		1																		
8	Mutatá				1										11																				
9	Sabaleta		1		1								14											1	26				1				2		
	Los Cacaos		2		13								2							19	1			1	92			2	1						
	Rancho Alegre		4		44		1								3											1	1								
10	Carmen del Darién								16				1								33		1		10										
11	Vigía del Curvaradó	2					1		44						1						322			9	27										
12	Murindo		2		1		1		74				3		12		2				194	1			4										
13	Murindo Viejo		11		1				74												99														
14	Guagua		44		33	2	2	1	91									1	2		64	1	1	30	55	3		12				1		1	10
15	Isla		1		2	1			6												2			6	51			1							
16	Coredó		2	1	1								2											1				1							
17	Ñarangue		467		42		4	40	109						2						361			27	207			5							2
18	Chajeradó		103		16			90	2			1	2				2				4		2	4	96			3							
19	Chibugadó		65		4			45	14												8			7	55			12							
20	Puerto Antioquia							1	11		1			1							141			40	4			7							
21	Vigía del Fuerte		7				2	9	33		1	1			57						67	2	2	1	96					2					
22	Bellavista		210					11	11				1		44						67			20	161			2		24					
23	Playa Murrí				1				5												1	26													
24	Jarapetó																					21		11				6	1						
25	Turriquitado Llano		3		1			2	32						1						9				110			10							
26	Gengadó		250		2			108	75	6			9		1	1	1				17		13	27	39			1	26	1		1			11
27	Partadó							160	3	1			12		1							1	46	52	118			1	73	2	21	1			5
28	Salado							4	1													1	2		77										1
29	Buchadó		2						9						3						1														
30	San Antonio de Padua							196	85		1											1	11	15	13			1		1					
31	Vidrí		28		211		1						1																						
32	Vegaez		2		84	1		10							14							1	1	19	1			3	17						1
33	Isleta																																		
	Palmiros																				2														

11.5 Virus prevalence in mosquito species

Virus Species					Mosquito species													
Group	**Order**	**Family**	**Genus**	**Species**	*Oc. serratus*	*An. darlingi*	*An. nuneztovari*	*Cq. lynchi*	*Cq. venezuelensis*	*Cx. nigripalpus*	*Cx. quinquefasciatus*	*Cx. spp.*	*Ma. titillans*	*Ps. albipes*	*Ps. ferox*	*Ps. funiculus*	*Ps. spp.*	*Wy. spp.*
ssRNA(-)	Bunyavirales	Phasmaviridae	Orthophasmavirus	Culex Phasma-like virus							2/3	1/4						
				Coredo virus									7/8					
			Feravirus	Guagua virus									1/8					
		Phenuiviridae	Unassigned	Atrato Gouko-like virus 1							2/3	1/4						
			Unassigned	Narangue virus									1/8					
	Jingchuvirales	Chuviridae	Unassigned	Atrato Chu-like virus 1					1/3									
				Atrato Chu-like virus 2			1/4											
				Atrato Chu like virus 4														?
				Atrato Chu-like virus 5										1/83				
	Mononegavirales	Rhabdoviridae	Unassigned	Atrato Rhabdo-like virus 2	2/7													
				Atrato Rhabdo-like virus 3								?						
ssRNA(+)	Picornavirales	Iflaviridae	Unassigned	Atrato Picorna-like virus									1/8					
				Chajerado virus								1/4						
				Isla virus								1/4						
				Sabaleta virus								?						
		Unassigned	Unassigned	Atrato Picorna-like virus 1			1/4							1/83				
		Polycipiviridae	Unassigned	Cacaos virus										?				
	Tymovirales	Tymoviridae	Unassigned	Gengado virus		?												
			Unassigned	Murindó virus		1/30												
	Sobelivirales	Solemoviridae	Unassigned	Atrato Sobemo-like virus 1	1/7								1/8					
				Atrato Sobemo-like virus 2								1/4						
				Atrato Sobemo-like virus 3					1/6									
				Atrato Sobemo-like virus 4											1/2			
				Atrato Sobemo-like virus 5					1/3									

Virus Species					Mosquito species													
Group	**Order**	**Family**	**Genus**	**Species**	*Oc. serratus*	*An. darlingi*	*An. nuneztovari*	*Cq. lynchi*	*Cq. venezuelensis*	*Cx. nigripalpus*	*Cx. quinquefasciatus*	*Cx. spp.*	*Ma. titillans*	*Ps. albipes*	*Ps. ferox*	*Ps. funiculus*	*Ps. spp.*	*Wy. spp.*
				Atrato Sobemo-like virus 6														1/9
	Martellivirales	Unassigned	Unassigned	Atrato Virga-like virus 1								1/4						
				Atrato Virga-like virus 2					5/6									
				Atrato Virga-like virus 3									1/8					
				Atrato Virga-like virus 4										5/83	1/2	6/6	1/1	
				Atrato Virga-like virus 5									1/2					
				Atrato Virga-like virus 6												1/6		
				Atrato Virga-like virus 7		3/30			1/3					1/2				
				Hubei Virga-like virus 2						1/1	1/3	1/4						
	Tolivirales	Unassigned	Unassigned	Hubei mosquito virus 4						1/1								
	Amarillovirales	Flaviviridae	Unassigned	Atrato Flavi-like virus 1								?	1/8					
	Unassigned	Unassigned	Negevirus	Atrato Nege-like virus 1					1/12									
dsRNA	Ghabrivirales	Chrysoviridae	Unassigned	Salado virus														1/9
	Durnavirales	Partitiviridae	Unassigned	Atrato Partiti-like virus 1									3/8					
				Atrato Partiti-like virus 2		?												
				Atrato Partiti-like virus 3								1/4		1/83				
				Atrato Partiti-like virus 4														
				Atrato Partiti-like virus 5									1/8					
				Atrato Partiti-like virus 6										2/83				
	Reovirales	Reoviridae	Unassigned	Atrato Reo-like virus 1										1/83				
	Ghabrivirales	Totivirirdae	Unassigned	Atrato virus									?					
				Embera virus														1/9
				Murri virus					1/3									
				Pisingos virus				1/7								?	?	
ssDNA	Piccovirales	Parvoviridae	Unassigned	Atrato Denso-like virus 1										3/83				

Virus Species					Mosquito species													
Group	**Order**	**Family**	**Genus**	**Species**	*Oc. serratus*	*An. darlingi*	*An. nuneztovari*	*Cq. lynchi*	*Cq. venezuelensis*	*Cx. nigripalpus*	*Cx. quinquefasciatus*	*Cx. spp.*	*Ma. titillans*	*Ps. albipes*	*Ps. ferox*	*Ps. funiculus*	*Ps. spp.*	*Wy. spp.*
				Atrato Denso-like virus									1/8					
ssRNA(+)RT	Ortervirales	Unassigned	Unassigned	Atrato Retro-like virus		?								?				
	Ortervirales	Metaviridae	Unassigned	Chibugado virus										4/83				

Table 19: Virus prevalence in mosquito species
The proportion of the mosquito pools (10-20 specimens) tested positive for a certain virus species by (rt)PCR is specified (background: red=high prevalence, yellow=medium prevalence, green=low prevalence, ?=prevalence unknown). Virus species in red have been detected by NGS but have not been confirmed by PCR.

11.6 Virus phylogenies and genome structures

11.6.1 Phylogenetic trees

11.6.1.1 Bunyavirales phylogeny

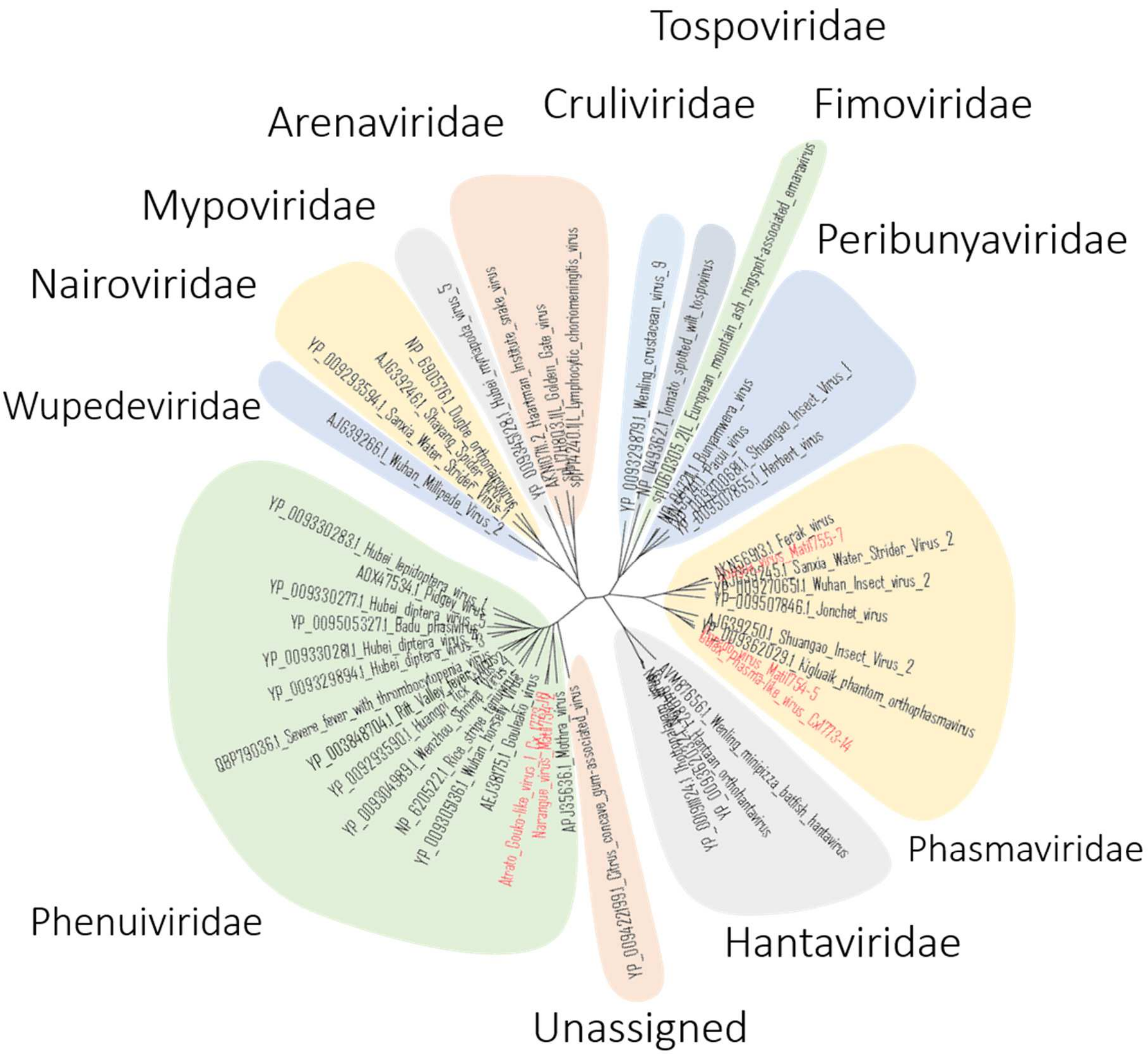


Figure 41: Bunyavirales phylogeny estimated based on the AA sequence of the CDS of the L-segment (selected model of AA substitution: LG +G+I+F). An unrooted maximum likelihood tree including all major families and genera type species is displayed in a radial view. Bootstrap values and branch length are shown in Figure 42. Novel species found in this study are in red.

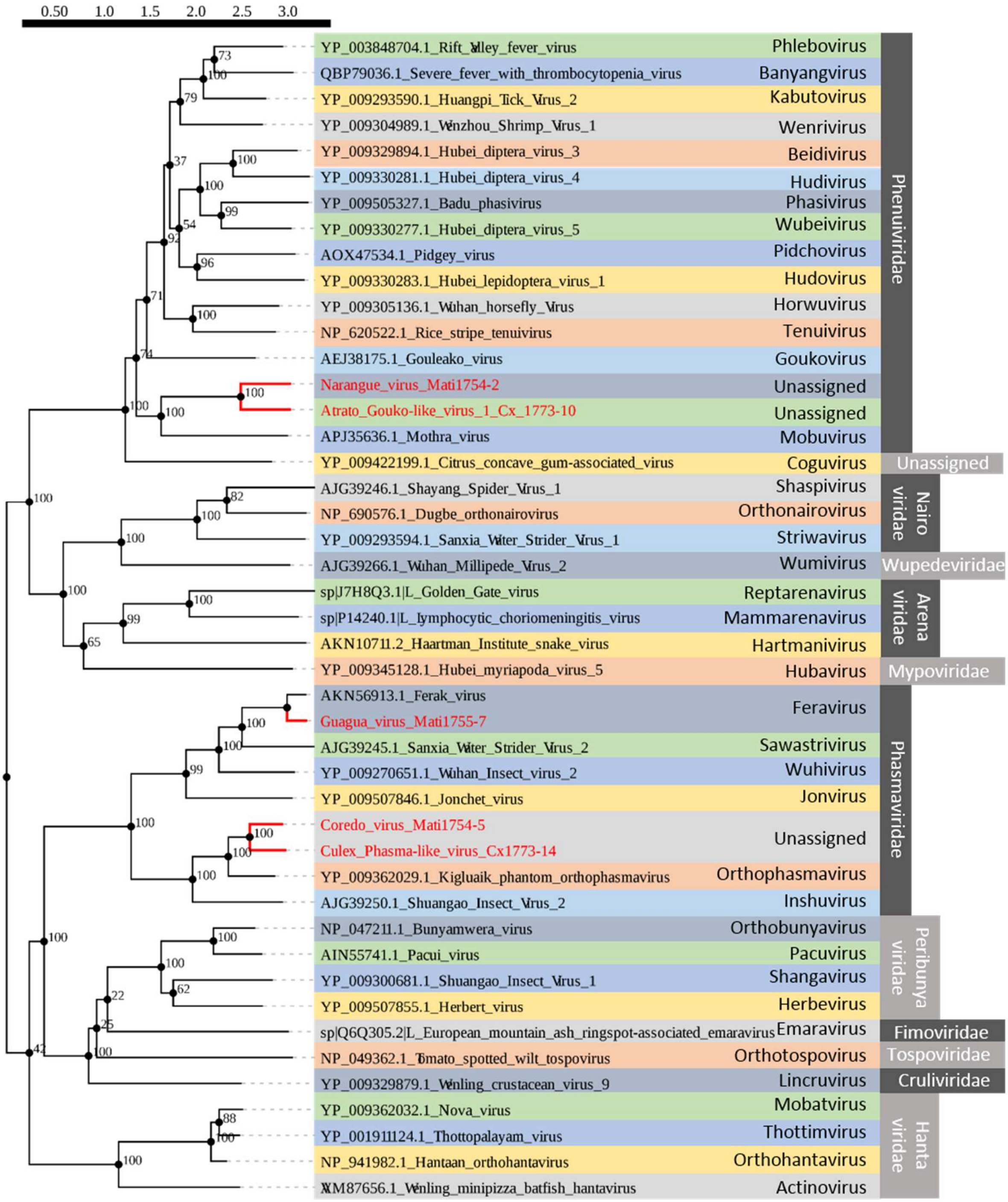


Figure 42: Bunyavirales phylogeny estimated based on the AA sequence of the CDS of the L-segment (selected model of AA substitution: LG +G+I+F). A midrooted maximum likelihood tree including all major families and genera type species is displayed in a rectangular view. Nodes show bootstrap values (bootstrap=100) and branch length is shown in the upper left corner. Novel species found in this study are in red.

11.6.1.1.1 Phasmaviridae phylogeny

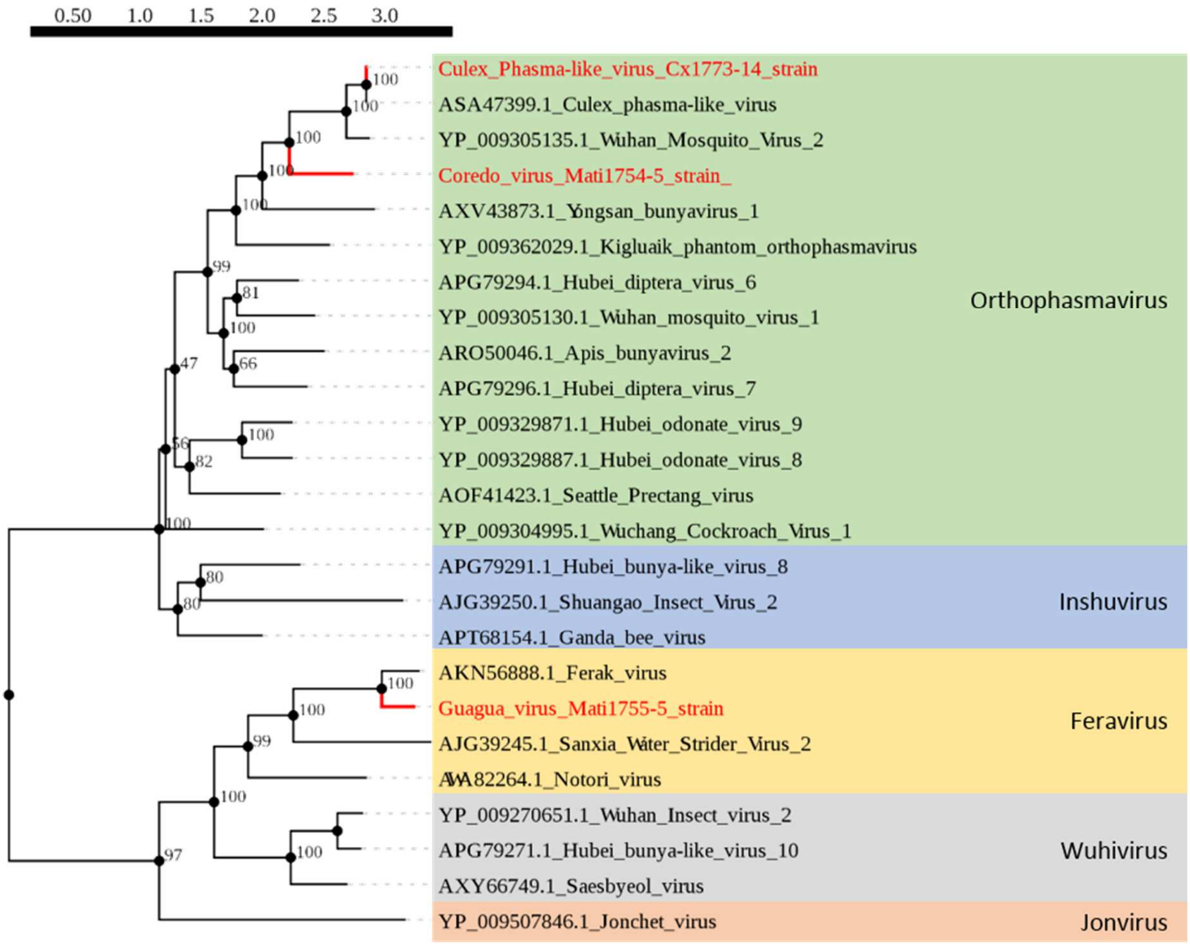


Figure 43: Phasmaviridae phylogeny estimated based on the AA sequence of the CDS of the L-segment (selected model of AA substitution: LG +G+I+F). A midrooted maximum likelihood tree including genera representatives and novel species is displayed in a rectangular view. Nodes show bootstrap values (bootstrap=100) and branch length is shown in the upper left corner. Novel species found in this study are in red.

11.6.1.1.2 Phenuiviridae phylogeny

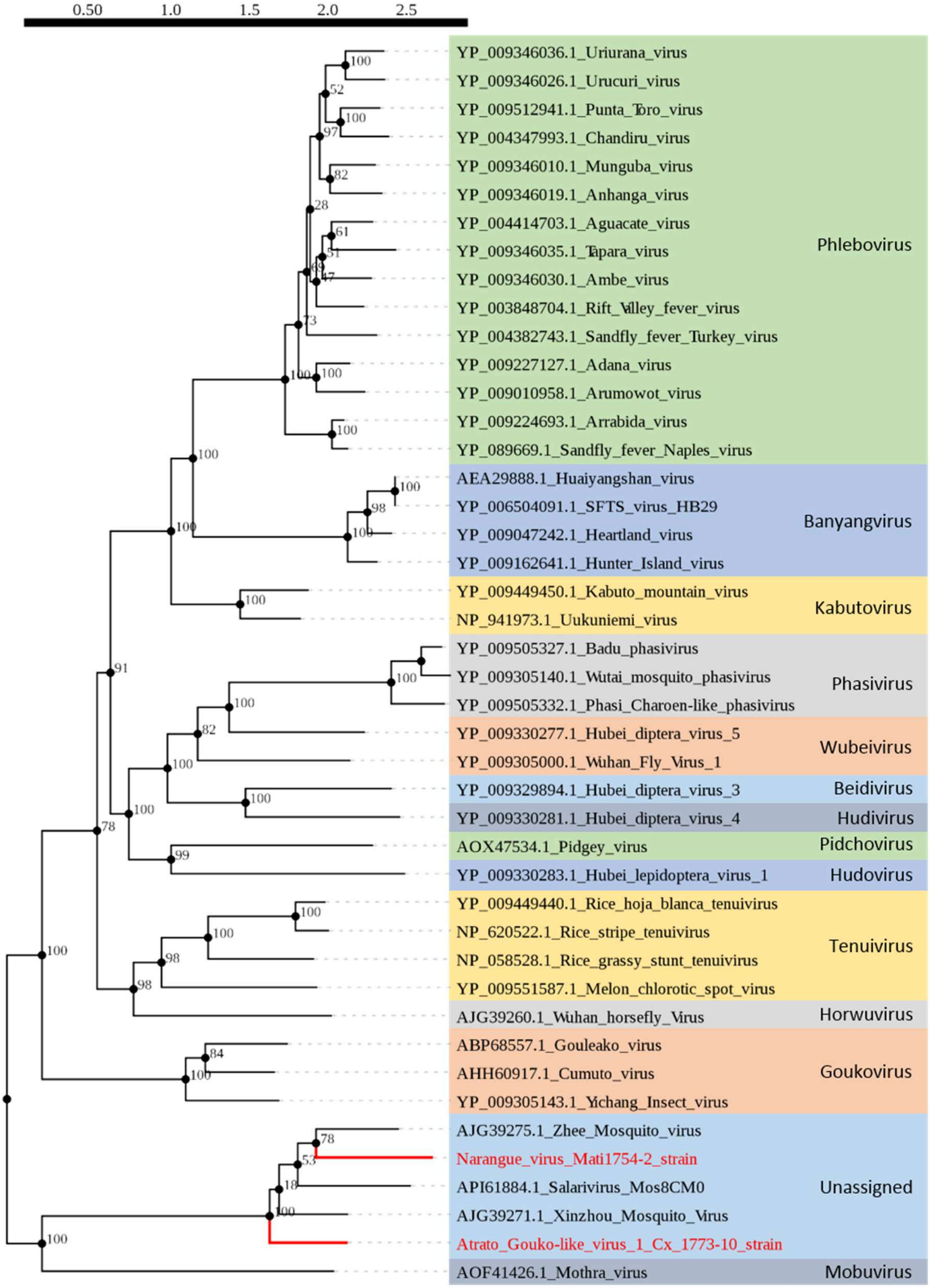


Figure 44: Phenuiviridae phylogeny estimated based on the AA sequence of the CDS of the L-segment (selected model of AA substitution: LG +G+I+F). A midrooted maximum likelihood tree including genera representatives and novel species is displayed in a rectangular view. Nodes show bootstrap values (bootstrap=100) and branch length is shown in the upper left corner. Novel species found in this study are in red.

11.6.1.2 Mononegavirales and Jingchuvirales phylogeny

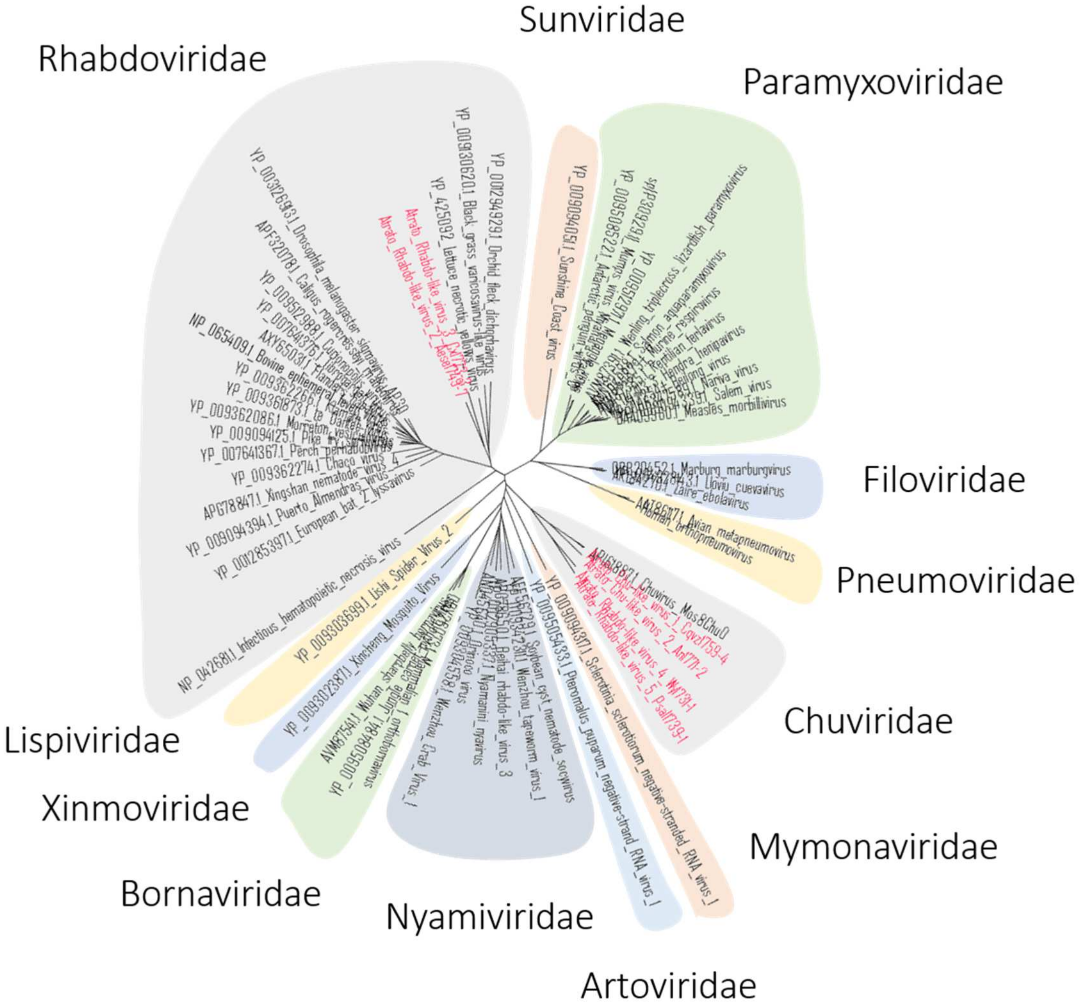


Figure 45: Mononegavirales and Jingchuvirales phylogeny estimated based on the AA sequence of the CDS of the L-segment (selected model of AA substitution: LG +G+I+F). An unrooted maximum likelihood tree including all major families and genera type species is displayed in a radial view. Bootstrap values and branch length are shown in Figure 46*. Novel species found in this study are in red.*

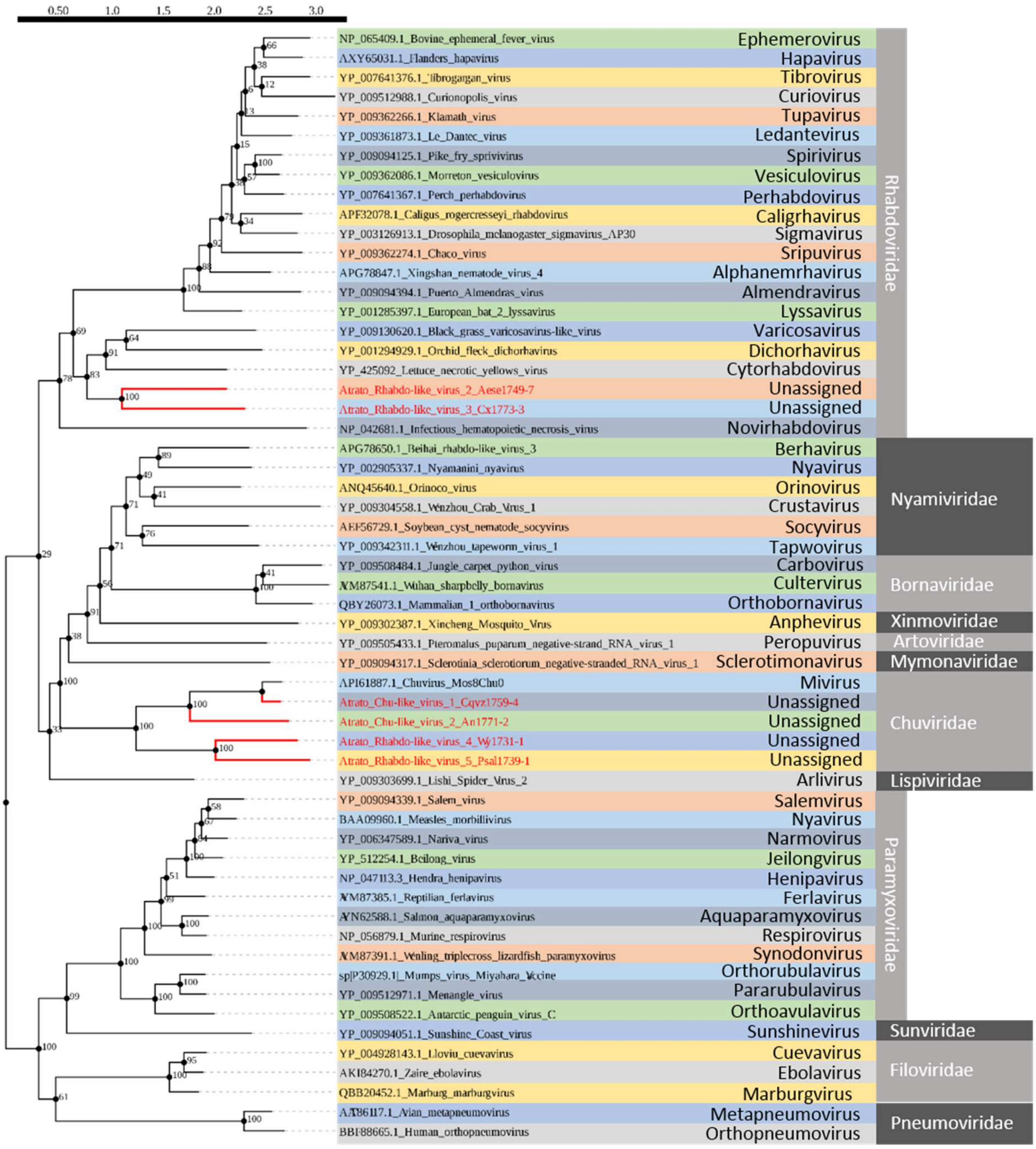


Figure 46: Mononegavirales and Jingchuvirales phylogeny estimated based on the AA sequence of the CDS of the L-segment (selected model of AA substitution: LG +G+I+F). A midrooted maximum likelihood tree including all major families and genera type species is displayed in a rectangular view. Nodes show bootstrap values (bootstrap=100) and branch length is shown in the upper left corner. Novel species found in this study are in red. The recently established Order Jingchuvirales, family Chuviridae, due to midrooted RdRp phylogeny are in the same clade as the families Nyamiviridae, Bornaviridae, Xinmoviridae, Artoviridae, Mymonaviridae, and Lispiviridae. Even though the new order Jingchuvirales has been accepted by the ICTV in 2018, the phylogenetic relationship between the family Chuviridae and the order Mononegavirales is still being discussed. More information is in the text.

11.6.1.2.1 Jingchuvirales phylogeny

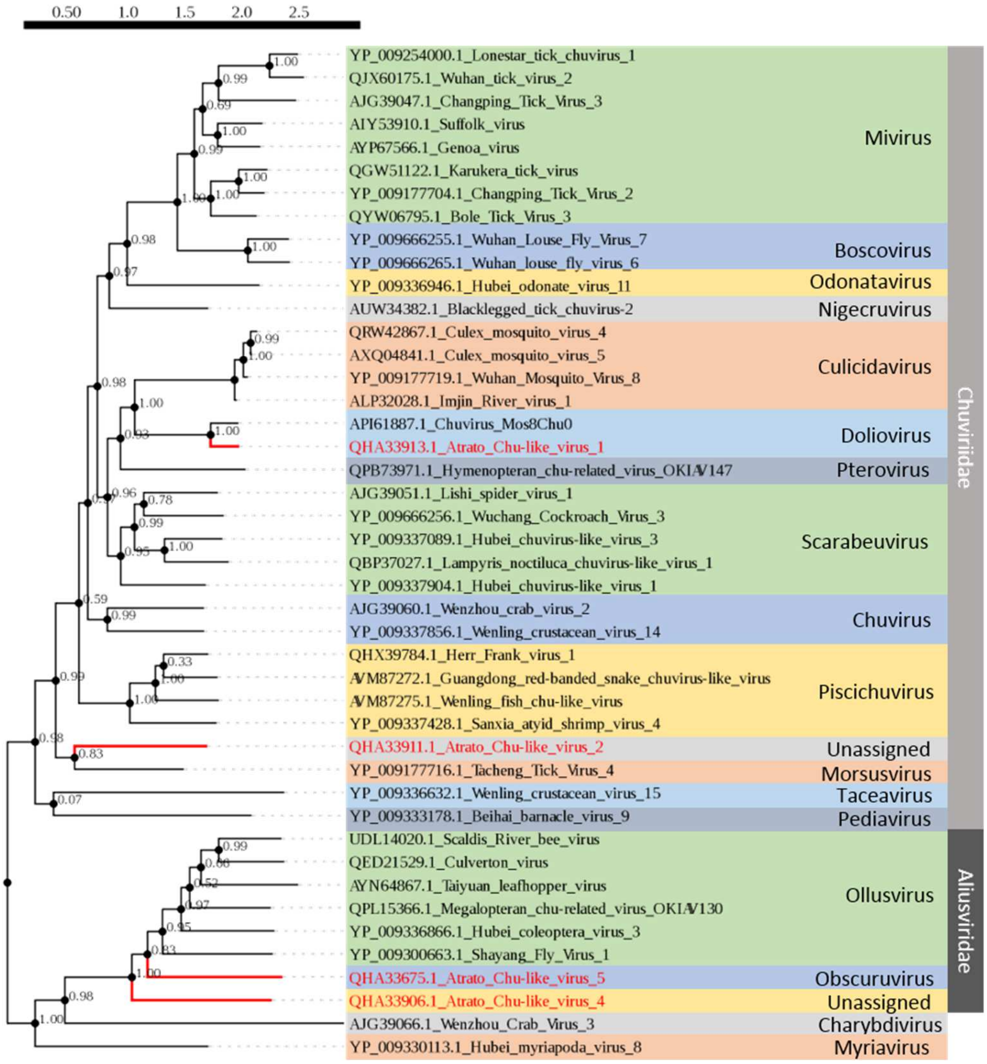


Figure 47: Jingchuvirales phylogeny estimated based on the AA sequence of the CDS of the L-segment or the RdRp coding segment (selected model of AA substitution: LG +G+I+F). A midrooted maximum likelihood tree including Jingchuvirales species from the ICTV master species list 2020, is displayed in a rectangular view. Nodes show bootstrap values (bootstrap=1000) and branch length is shown in the upper left corner. Novel species found in this study are in red.

11.6.1.3 Hepevirales, Martellivirales, and Tymovirales phylogeny

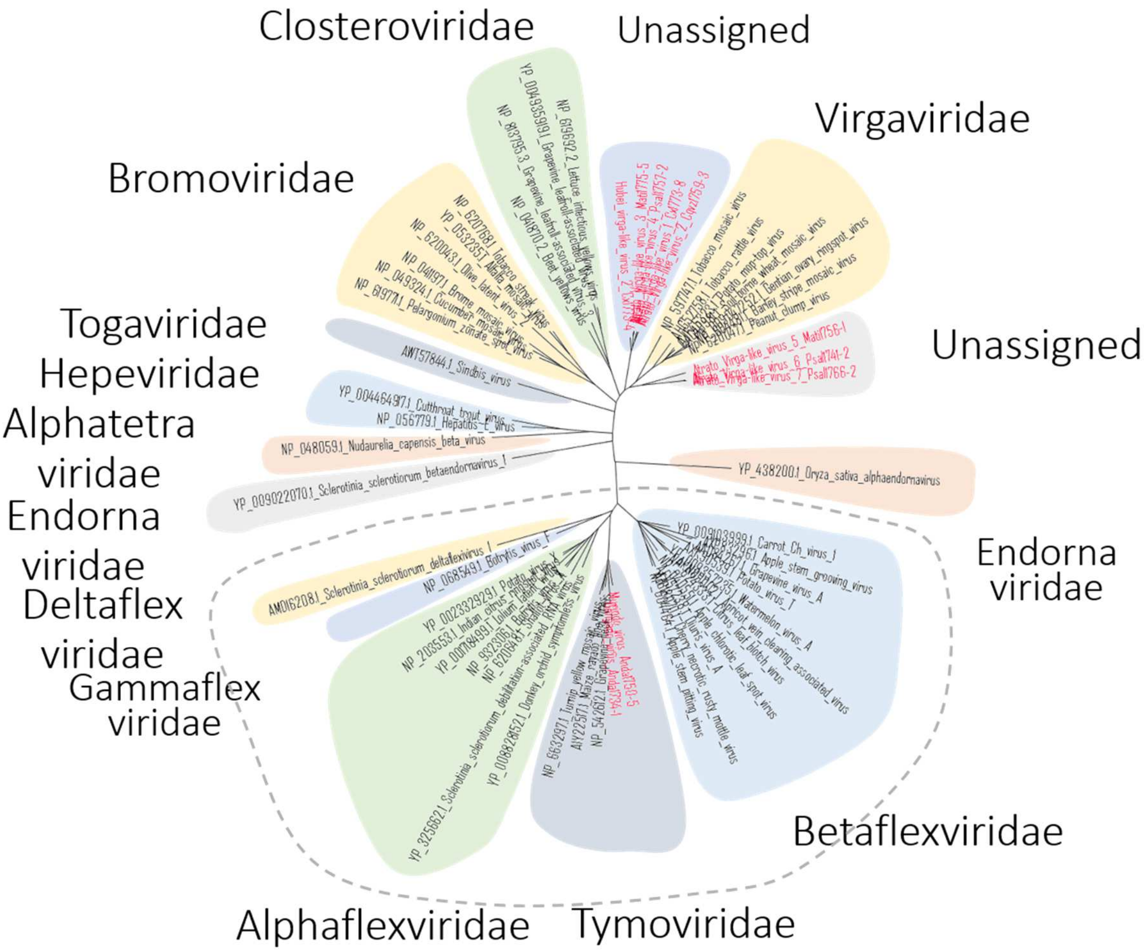


Figure 48: Phylogeny of the Hepeviridae & Virgaviridae clade estimated based on the AA sequence of the CDS of the RdRp containing ORF (selected model of AA substitution: LG +G+I+F). An unrooted maximum likelihood tree including genera type species for the families Virgaviridae, Endornaviridae, Betaflexviridae, Tymoviridae, Alphaflexviridae, Gammaflexviridae, Deltaflexviridae, Endornaviridae, Alphatetraviridae, Hepeviridae, Togaviridae, Bromoviridae and Closteroviridae is displayed in a radial view. The order Tymovirales is marked with a grey dashed line. Bootstrap values and branch length are shown in Figure 49.. Novel species found in this study are in red.

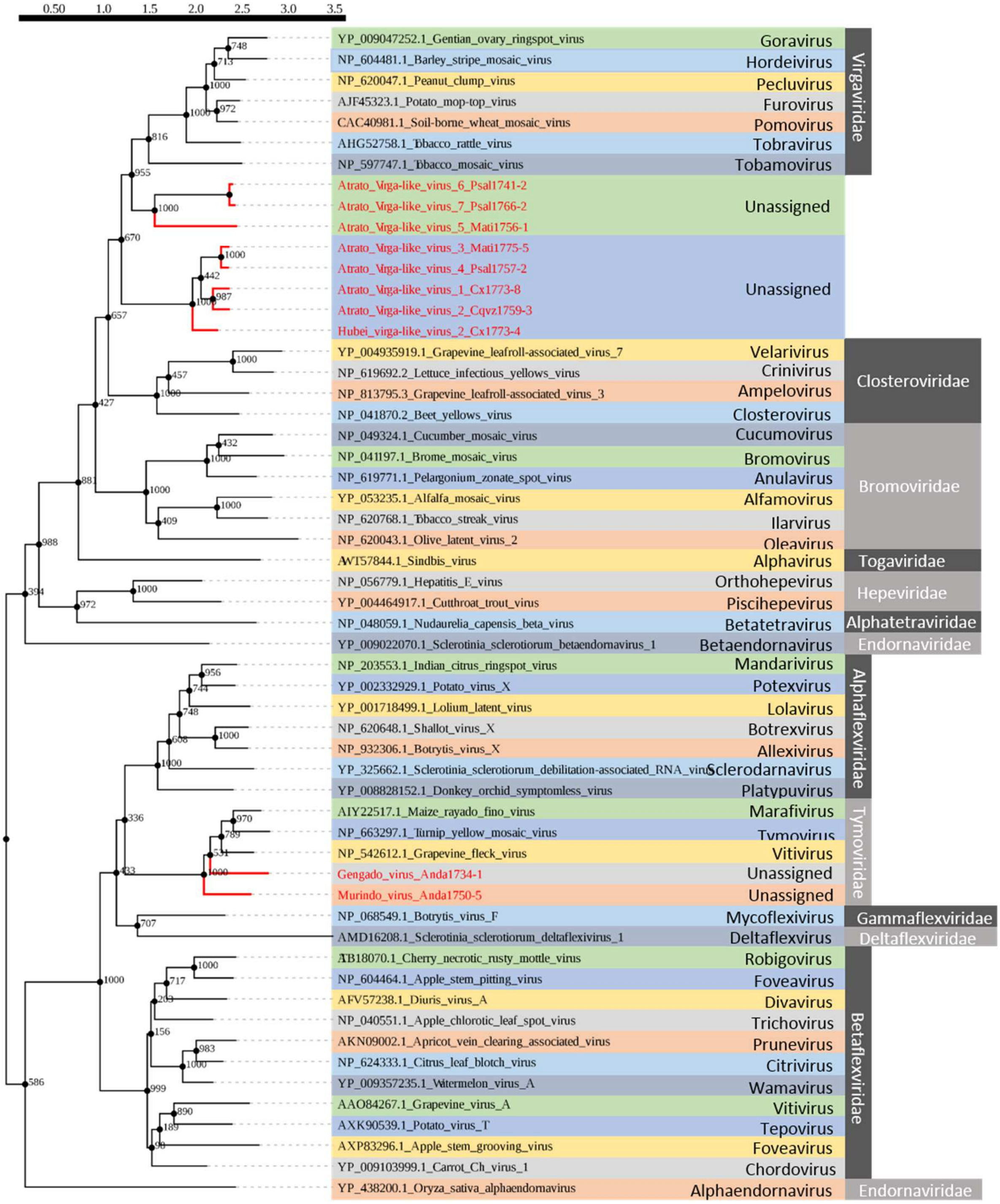


Figure 49: Phylogeny of the Hepeviridae and Virgaviridae clade estimated based on the AA sequence of the CDS of the RdRp containing ORF (selected model of AA substitution: LG +G+I+F). A midrooted maximum likelihood tree including Virgaviridae, Bromoviridae, Closteroviridae, Togaviridae, Endornaviridae, Tymovirales, Hepeviridae, Alphatetraviridae genera type species is displayed in a rectangular view. Nodes show bootstrap values (bootstrap=1000) and branch length is shown in the upper left corner. Novel species found in this study are in red.

11.6.1.3.1 Tymoviridae phylogeny

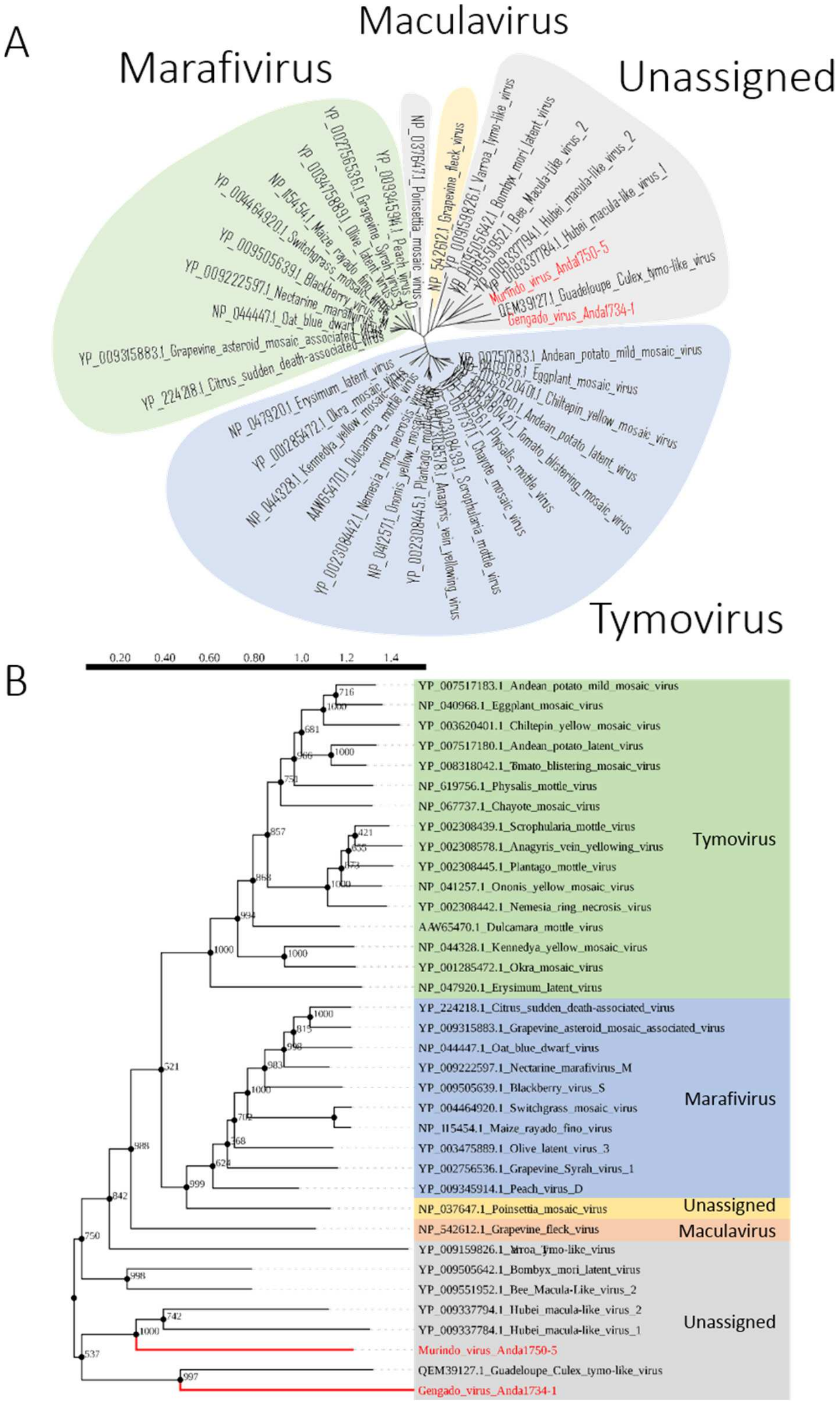


Figure 50: Phylogeny of the family Tymoviridae estimated based on the AA sequence of the CDS of the RdRp containing ORF (selected model of AA substitution RtREV +G+I+F). A maximum likelihood tree including Tymoviridae genera representatives from the ICTV Master Species list and unassigned Tymo-like species is displayed in (A) in an unrooted radial view and (B) a midrooted rectangular view. Nodes show bootstrap values (bootstrap=1000) and branch length is shown in the upper left corner. Novel species found in this study are in red.

11.6.1.3.2 Virgaviridae phylogeny

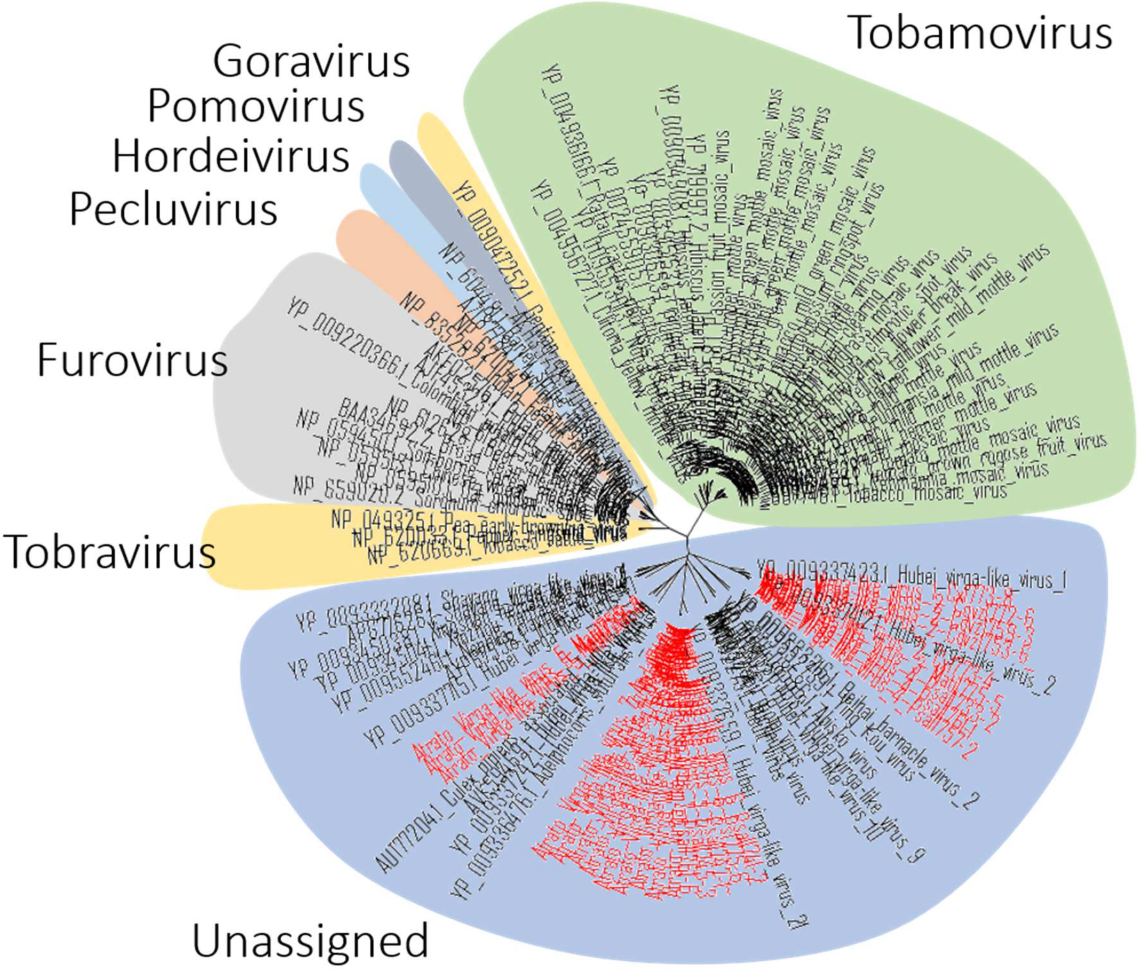


Figure 51: Phylogeny of the family Virgaviridae and Virga-like viruses based on the AA sequence of the CDS of the RdRp containing ORF (selected model of AA substitution: LG +G+I+F). An unrooted maximum likelihood tree including Virgaviridae genera representatives from the ICTV Master Species list and novel viruses is displayed in a radial view. Bootstrap values and branch length are shown in Figure 52. Novel species found in this study are in red.

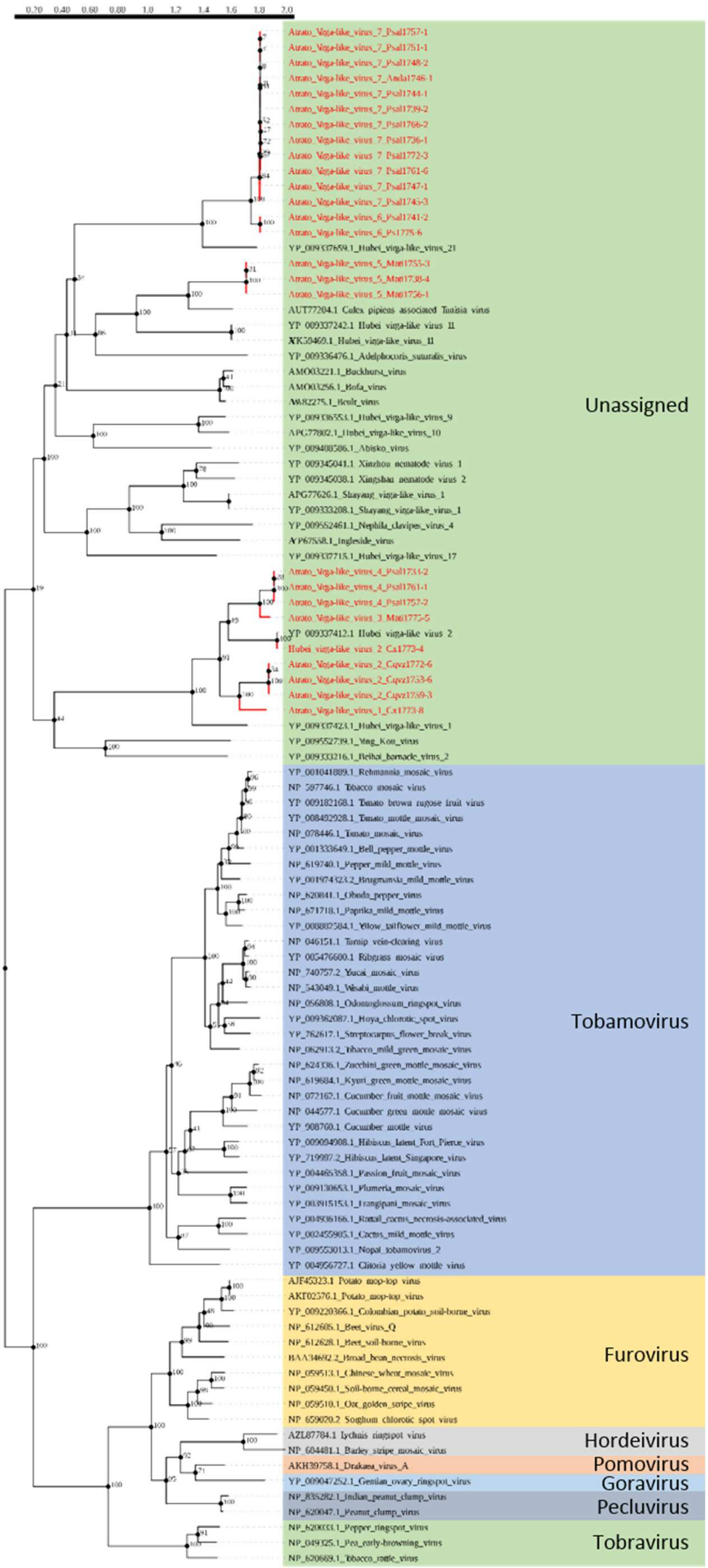


Figure 52: Phylogeny of the family Virgaviridae and Virga-like viruses based on the AA sequence of the CDS of the RdRp containing ORF (selected model of AA substitution: LG +G+I+F). A midrooted maximum likelihood tree including Virgaviridae genera representatives from the ICTV Master Species list and novel viruses is displayed in a rectangular view. Nodes show bootstrap values (bootstrap=100) and branch length is shown in the upper left corner. Novel species found in this study are in red.

11.6.1.4 Picornavirales phylogeny

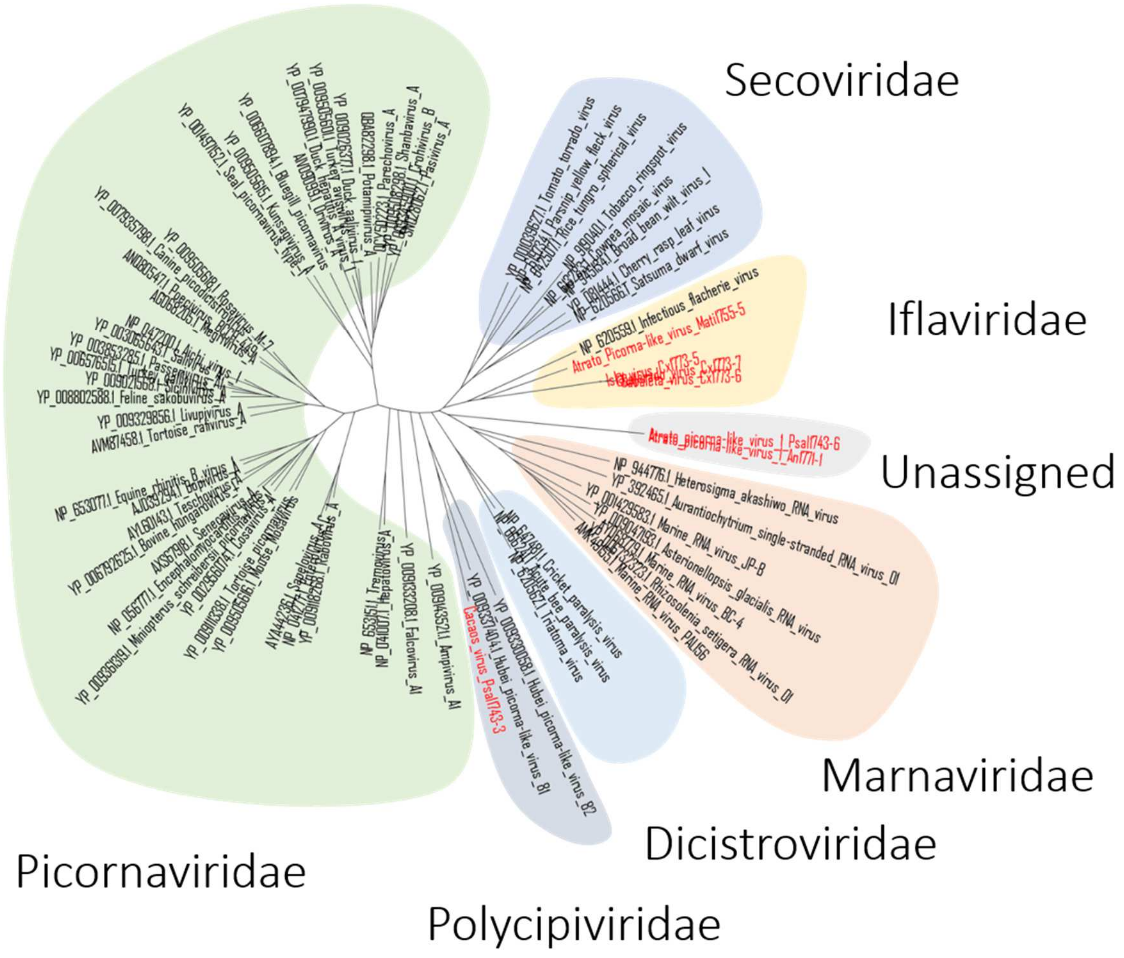


Figure 53: Picornavirales phylogeny estimated based on the AA sequence of the CDS of the RdRp containing ORF (selected model of AA substitution: LG +G+I+F). An unrooted maximum likelihood tree including all major families and genera type species is displayed in a radial view. Bootstrap values and branch length are shown in Figure 54. Novel species found in this study are in red.

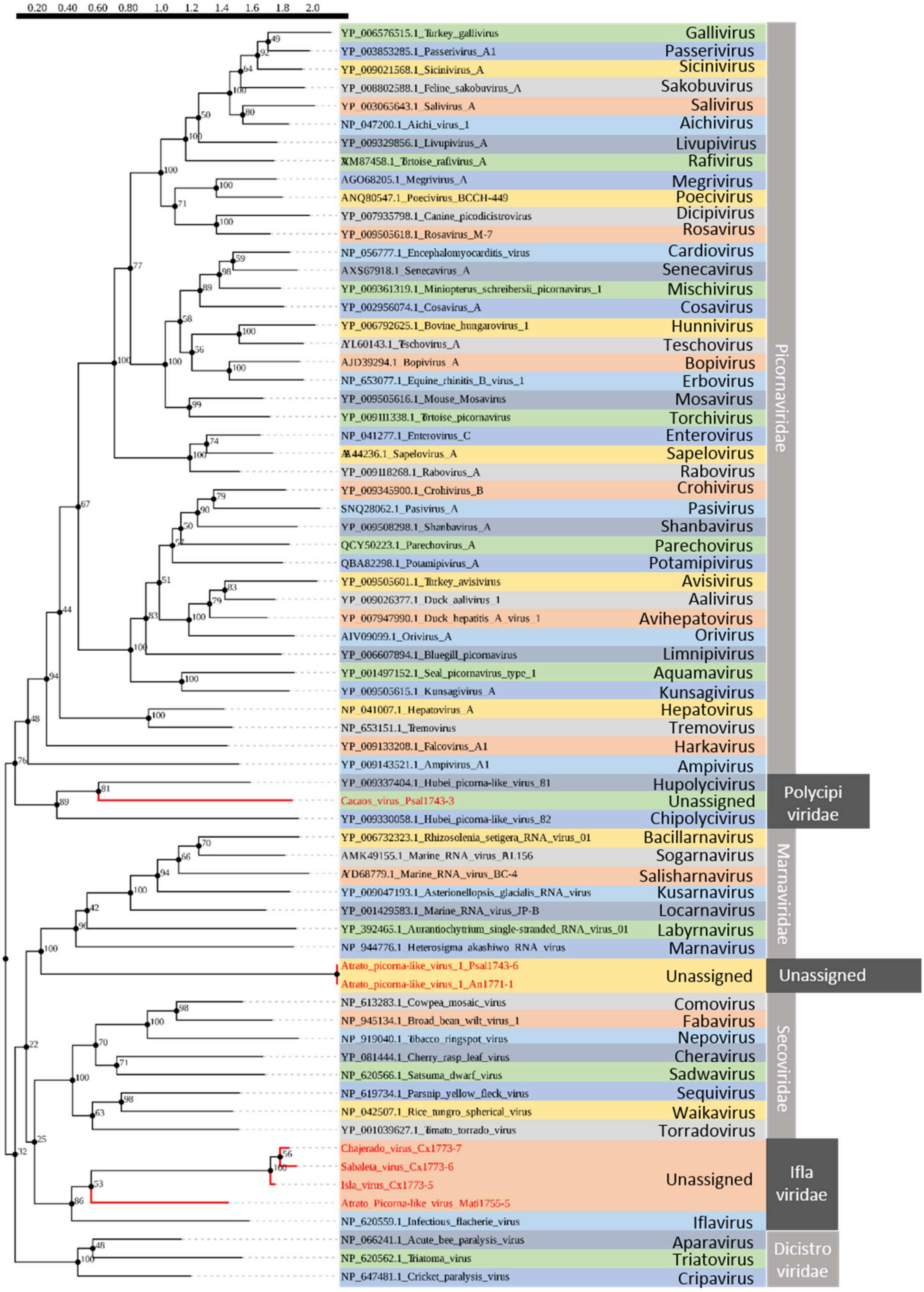


Figure 54: Picornavirales phylogeny estimated based on the AA sequence of the CDS of the RdRp containing ORF (selected model of AA substitution: LG +G+I+F). A midrooted maximum likelihood tree including all major families and genera type species is displayed in a rectangular view. Nodes show bootstrap values (bootstrap=100) and branch length is shown in the upper left corner. Novel species found in this study are in red.

11.6.1.4.1 Iflavirididae phylogeny

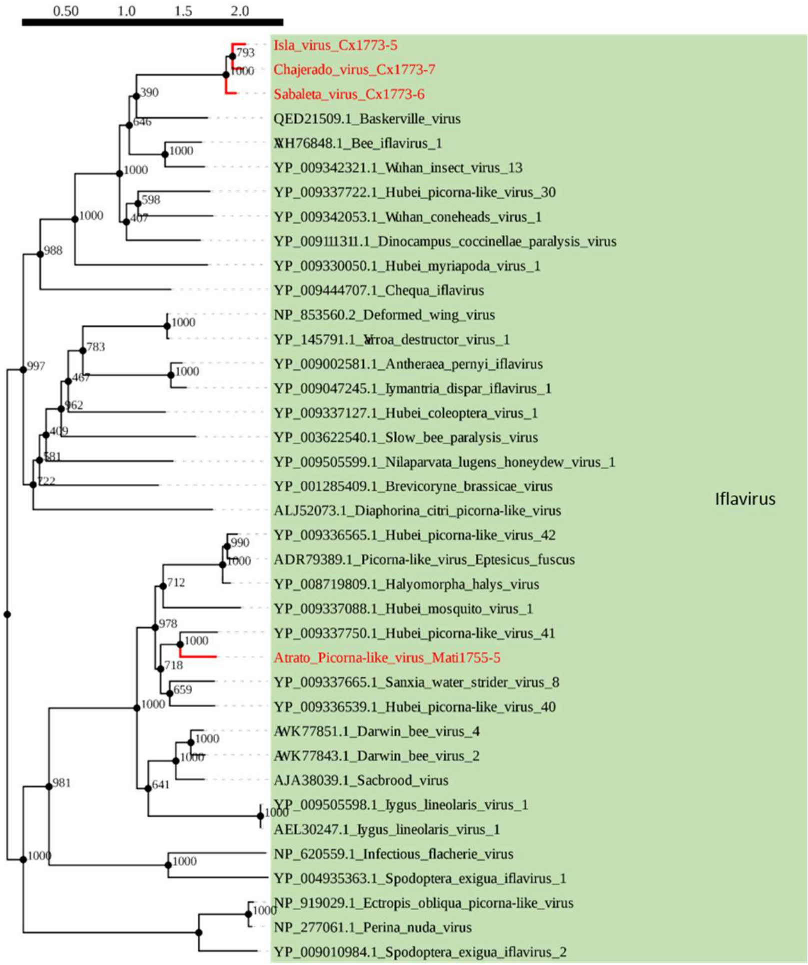


Figure 55: Iflaviridae phylogeny estimated based on the AA sequence of the CDS of the RdRp containing polyprotein (selected model of AA substitution: LG +G+I+F). A midrooted maximum likelihood tree including Iflavirus representatives from the ICTV master species list and Ifla-like viruses is displayed in a rectangular view. Nodes show bootstrap values (bootstrap=1000) and branch length is shown in the upper left corner. Novel species found in this study are in red.

11.6.1.4.2 Polycipiviridae phylogeny

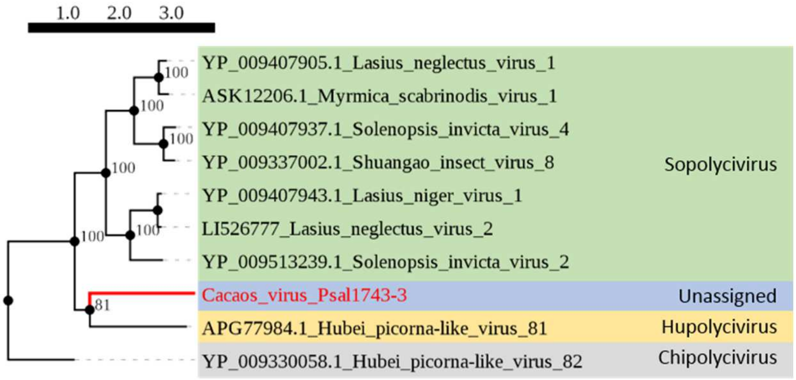


Figure 56: Polycipiviridae phylogeny estimated based on the AA sequence of the CDS of ORF5 (Pro-Hel-RdRp), (selected model of AA substitution: LG +G+I+F). A midrooted maximum likelihood tree including all major families and described species is displayed in a rectangular view. Nodes show bootstrap values (bootstrap=100) and branch length is shown in the upper left corner. Novel species found in this study are in red.

11.6.1.4.3 Marnaviridae phylogeny

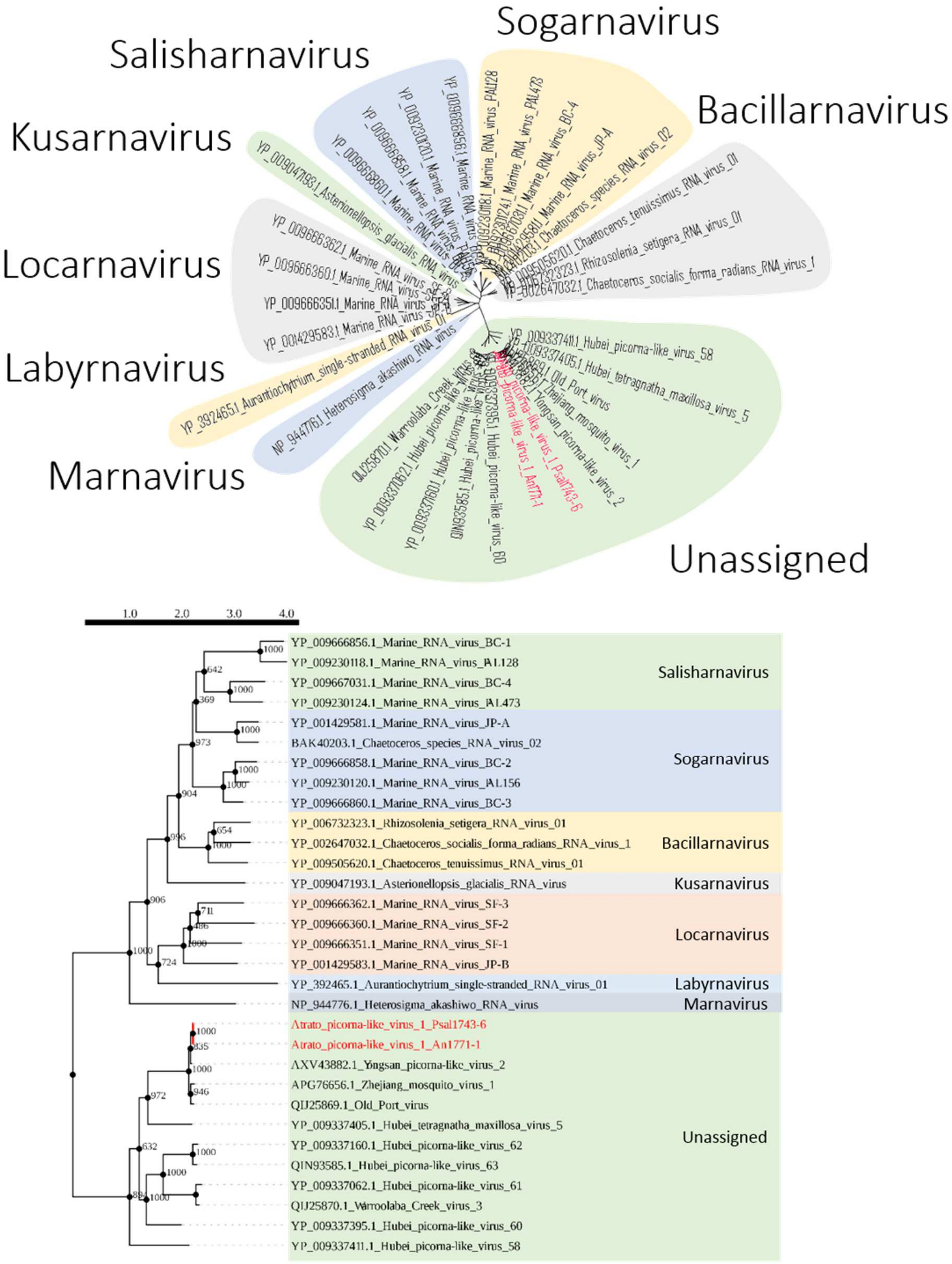


Figure 57: Phylogeny of the family Marnaviridae estimated based on the AA sequence of the CDS of the RdRp coding ORF (selected model of AA substitution LG +G+I+F). A maximum likelihood tree including Marnaviridae genera representatives from the ICTV Master Species list and unassigned Marna-like species is displayed in (A) an unrooted radial view and (B) a midrooted rectangular view. Nodes show bootstrap values (bootstrap=1000) and branch length is shown in the upper left corner. Novel species found in this study are in red.

11.6.1.5 Nodamuvirales and Tolivirales phylogeny

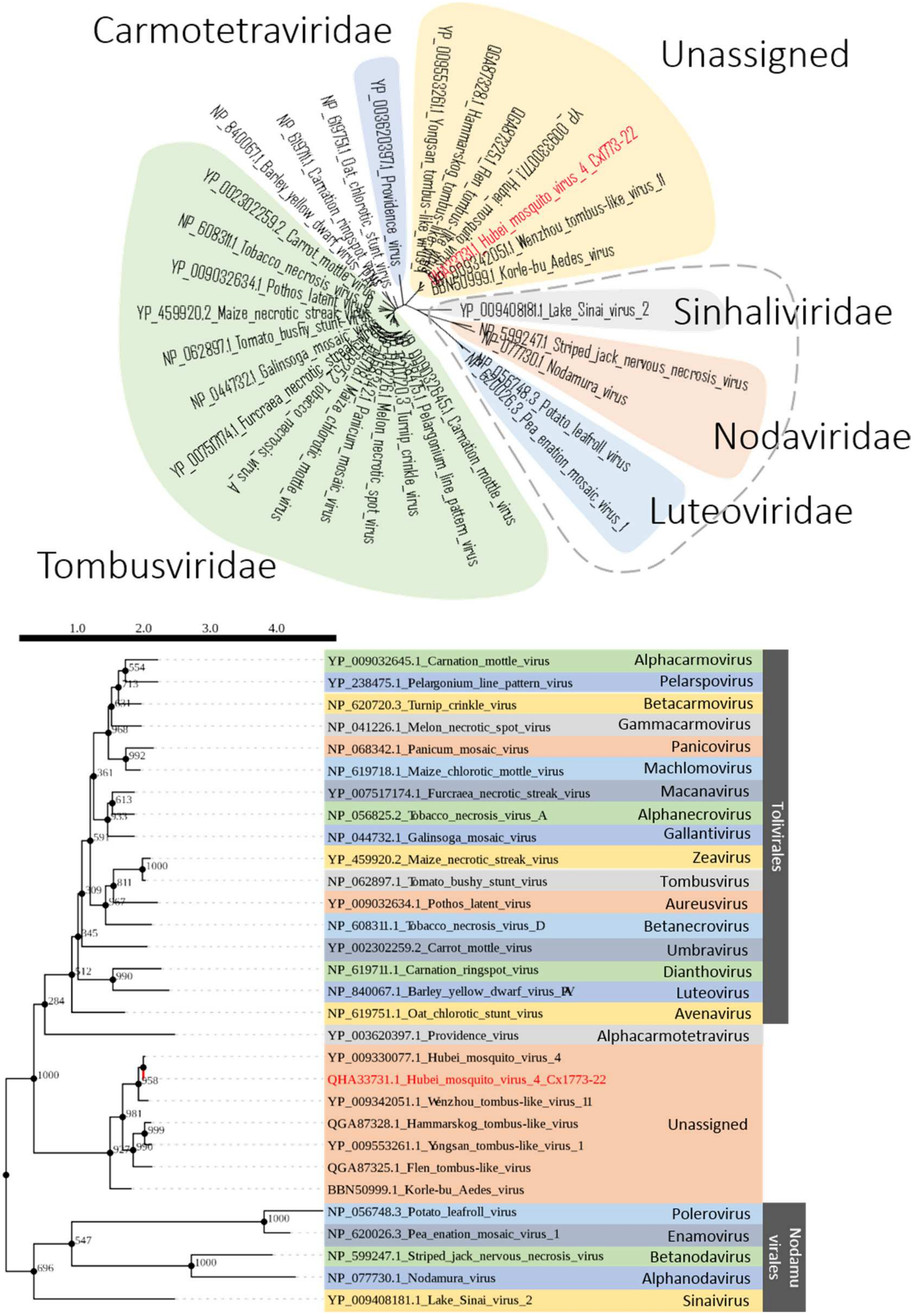


Figure 58: Phylogeny of the orders Tolivirales and Nodamuvirales estimated based on the AA sequence of the CDS of the RdRp containing ORF (selected model of AA substitution: LG +G+I+F). A maximum likelihood tree including genera type species and unassigned Tombus-like viruses is displayed in (A) an unrooted radial view and (B) a midrooted rectangular view. Nodes show bootstrap values (bootstrap=1000) and branch length is shown in the upper left corner. Viruses found in this study are in red.

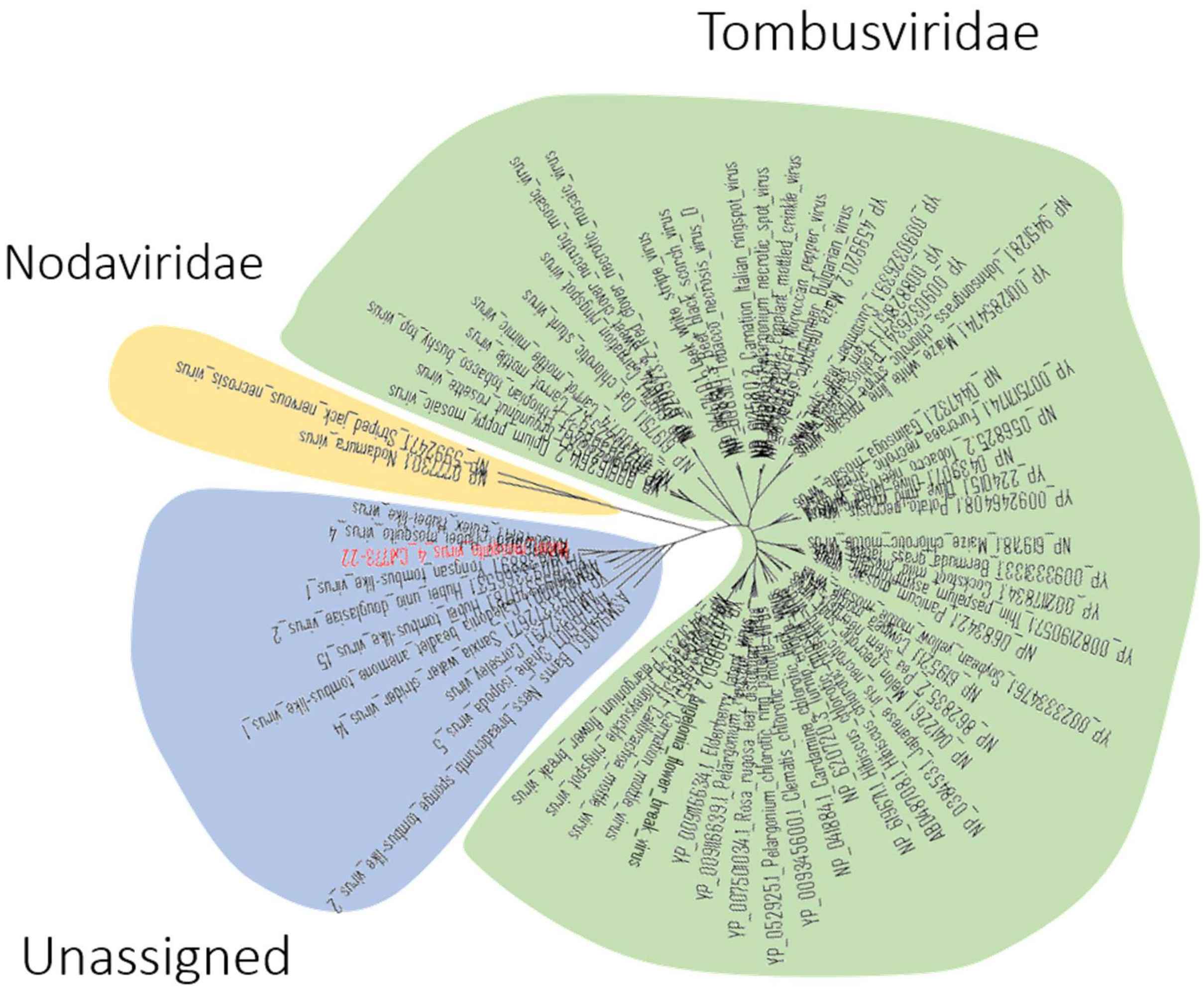


Figure 59: Phylogeny of the Tombus-Noda clade and Tombus-like viruses estimated based on the AA sequence of the CDS of the RdRp containing ORF (selected model of AA substitution: LG +G+I+F). An unrooted maximum likelihood tree including Tobusviridae genera representatives from the ICTV Master Species list, Nodaviridae genera type species and novel viruses is displayed in a radial view. Bootstrap values and branch length are shown in Figure 60. Novel species found in this study are in red. Delfuerte virus is a synonym for Hubei mosquito virus 4.

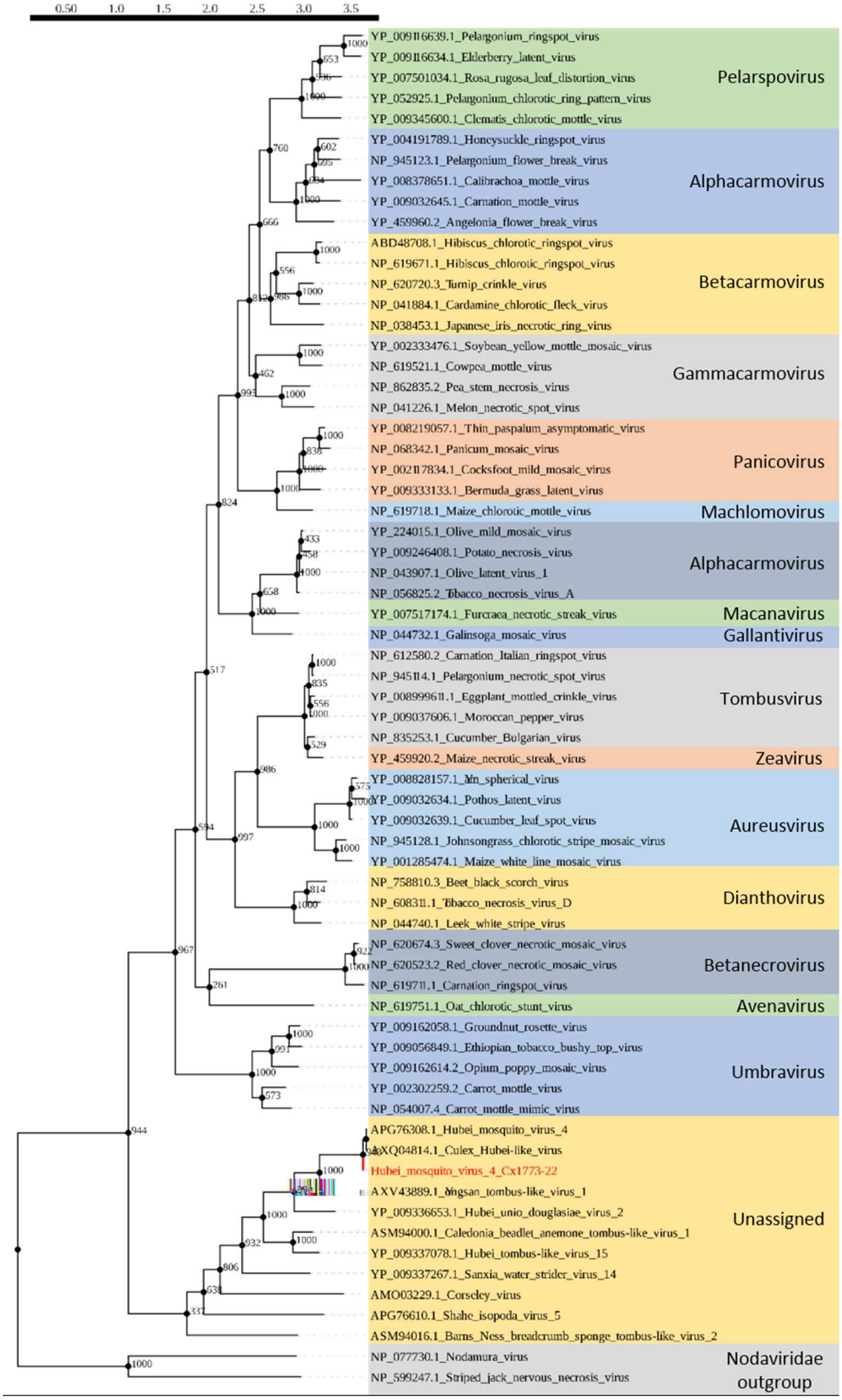


Figure 60: Phylogeny of the Tombus-Noda clade estimated based on the AA sequence of the CDS of the RdRp containing ORF (selected model of AA substitution: LG +G+I+F). A Midrooted maximum likelihood tree including genera representatives from the ICTV Master Species list, Nodaviridae genera type species and novel viruses is displayed in a rectangular view. Nodes show bootstrap values (bootstrap=1000) and branch length is shown in the upper left corner. Novel species found in this study are in red. Delfuerte virus is a synonym for Hubei mosquito virus 4.

11.6.1.6 *Amarillovirales phylogeny*

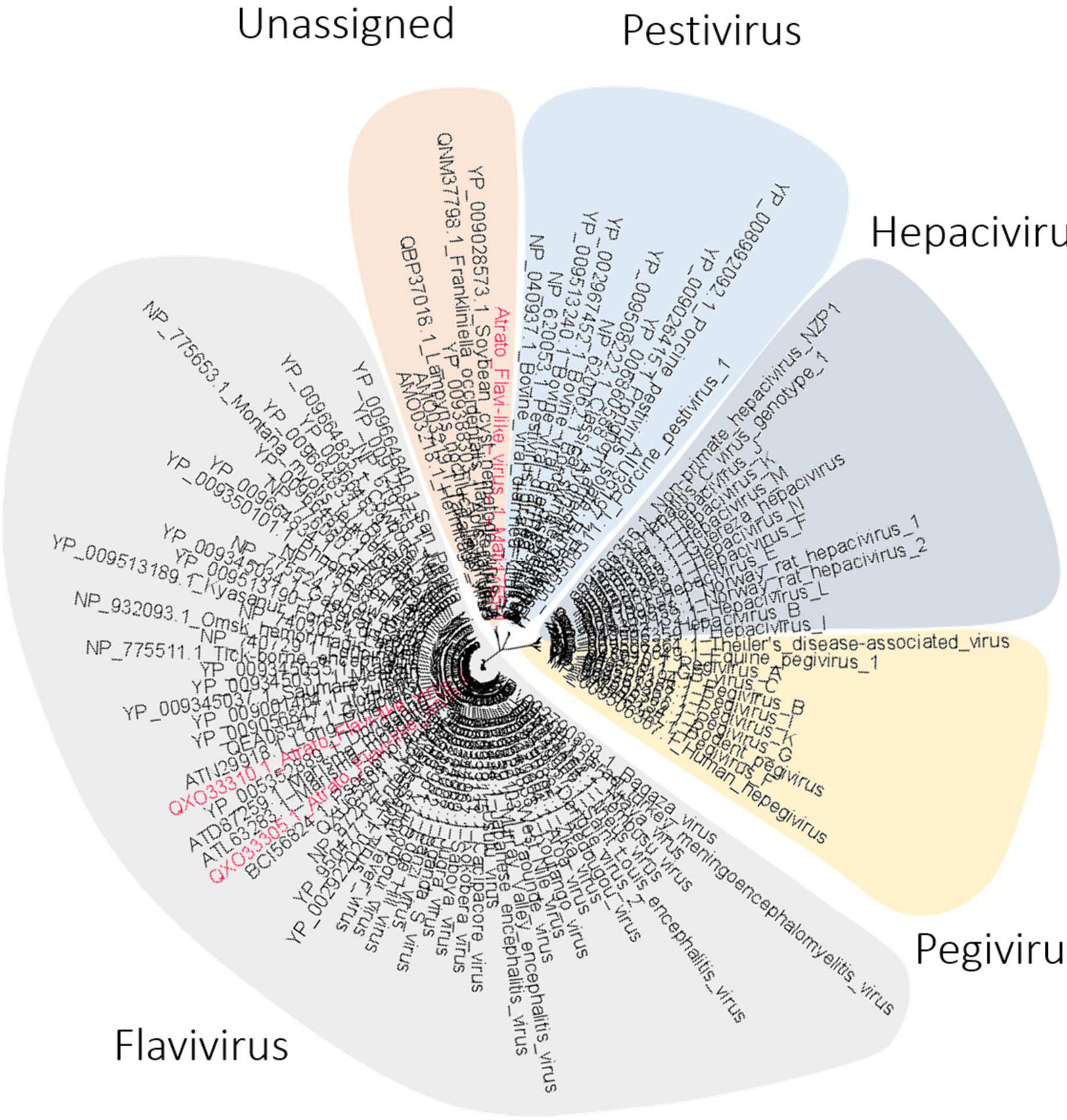


Figure 61: Phylogeny of the order Amarillovirales estimated based on the AA sequence of the CDS of the RdRp (selected model of AA substitution: LG +G+I+F). A maximum likelihood tree including Flavivirus, Pestivirus, Pegivirus, and Hepacivirus species from the ICTV master species list, and unassigned Flavi-like species is displayed in an unrooted radial view. Bootstrap values (bootstrap=1000) and branch length are shown in Figure 62. Novel species found in this study are in red.

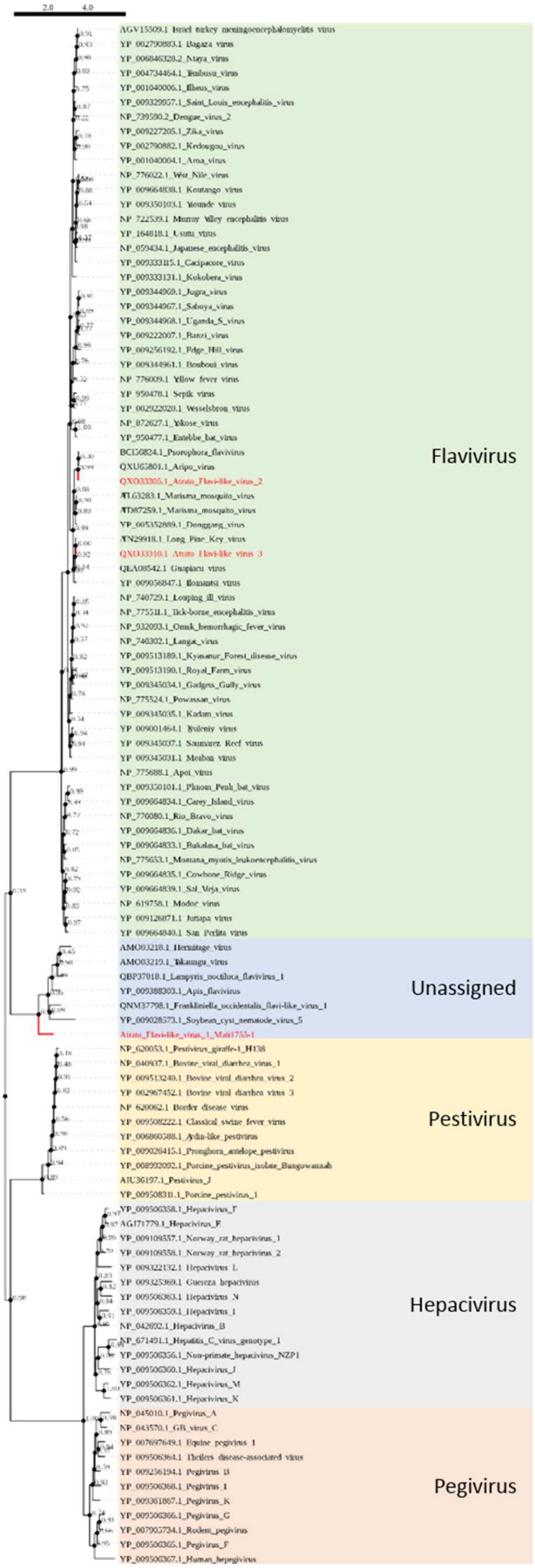


Figure 62: Phylogeny of the order Amarillovirales estimated based on the AA sequence of the CDS of the RdRp (selected model of AA substitution: LG +G+I+F). A maximum likelihood tree including Flavivirus, Pestivirus, Pegivirus, and Hepacivirus species from the ICTV master species list and unassigned Flavi-like species is displayed in a midrooted rectangular view. Nodes show bootstrap values (bootstrap=1000) and branch length is shown in the upper left corner. Novel species found in this study are in red.

11.6.1.7 Sobelivirales phylogeny

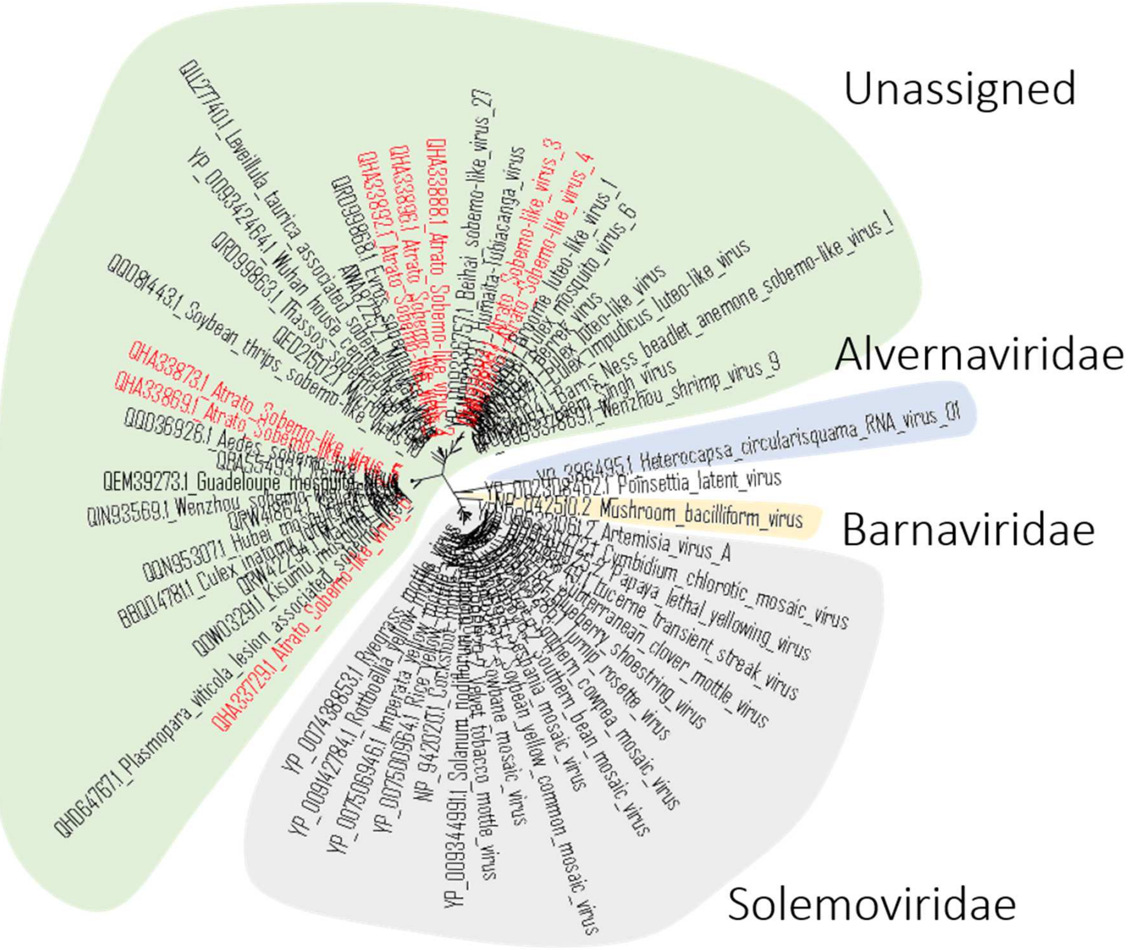


Figure 63: Phylogeny of the order Sobelivirales estimated based on the AA sequence of the CDS of the RdRp (selected model of AA substitution: RtREV +G+I+F). A maximum likelihood tree including species from the ICTV master species list and unassigned viruses is displayed in an unrooted radial view. Support values and branch length are shown in Figure 64. Novel species found in this study are in red.

Figure 64: Phylogeny of the order Sobelivirales estimated based on the AA sequence of the CDS of the RdRp (selected model of AA substitution: RtREV +G+I+F). A maximum likelihood tree including species from the ICTV master species list and unassigned viruses is displayed in an unrooted, rectangular view. Nodes show aLRT SH-like support values and branch length is shown in the upper left corner. Novel species found in this study are in red.

11.6.1.8 Durnavirales phylogeny

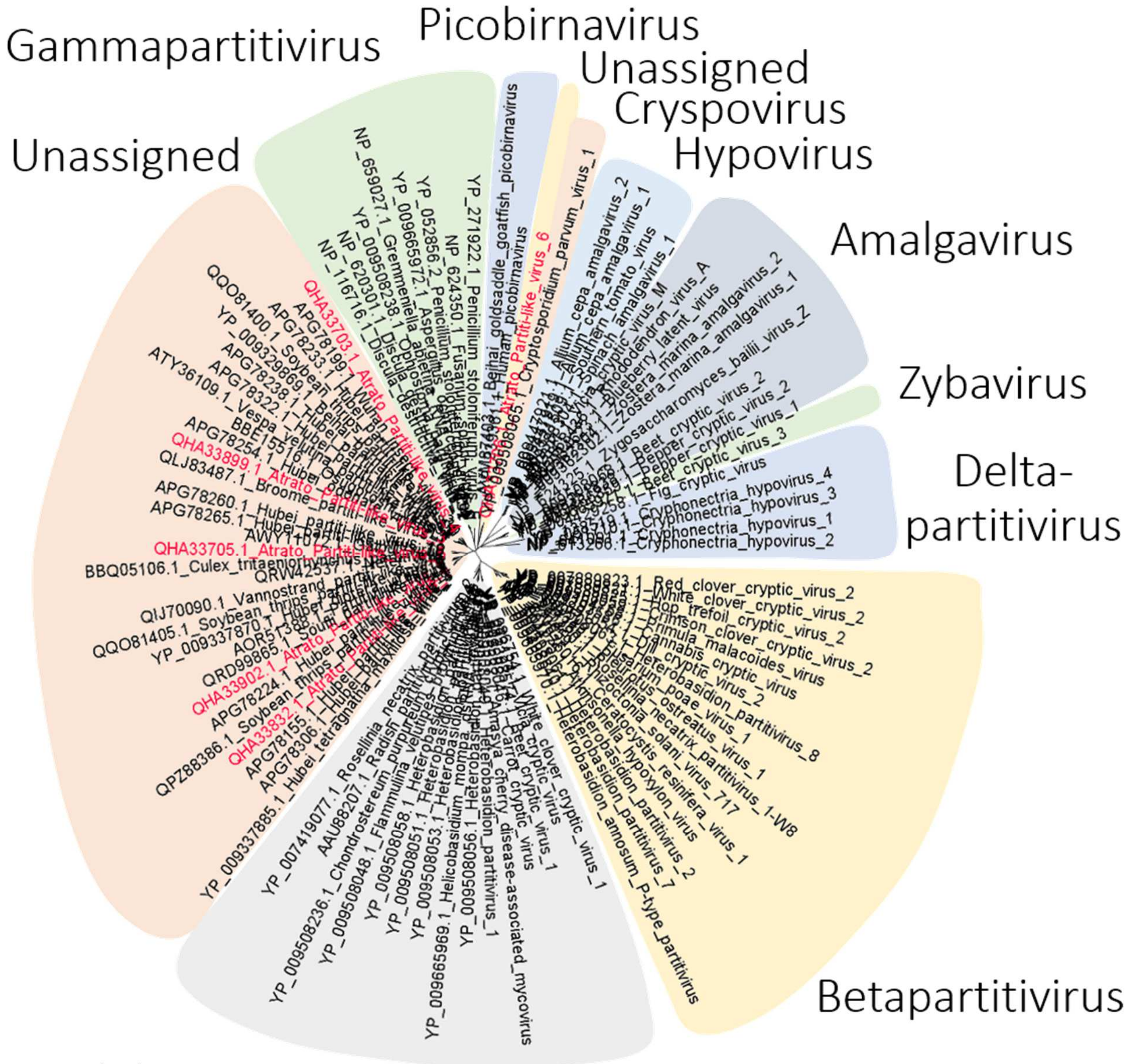


Figure 65: Phylogeny of the order Durnavirales estimated based on the AA sequence of the CDS of the RdRp (selected model of AA substitution: LG +G+I+F). A maximum likelihood tree of Partitiviridae, Hypoviridae, Amalgaviridae, and Picornaviridae species from the ICTV master species list and Partiti-like unassigned viruses is displayed in an unrooted radial view. Bootstrap values (bootstrap=1000) and branch length are shown in Figure 66. Novel species found in this study are in red.

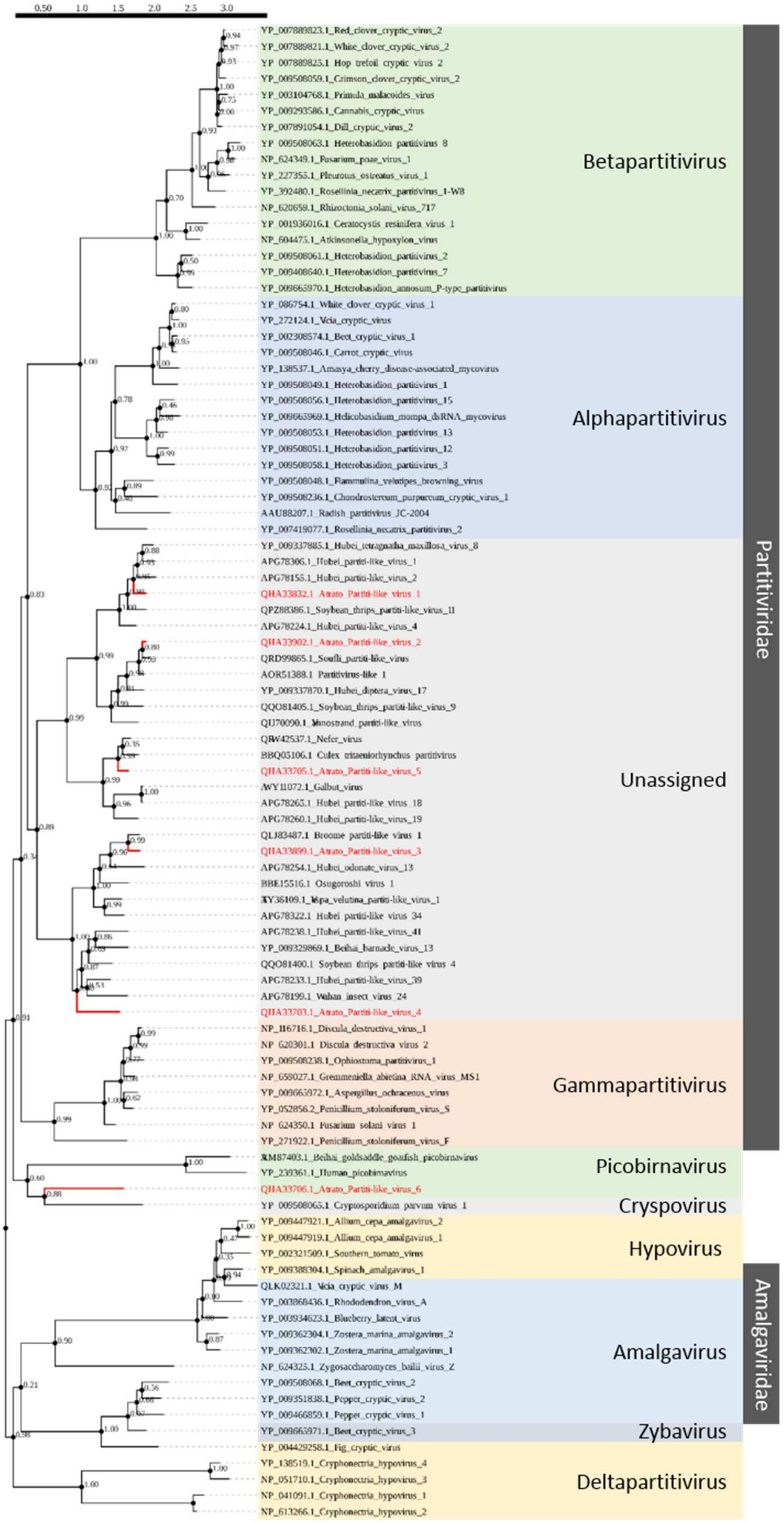


Figure 66: Phylogeny of the order Durnavirales estimated based on the AA sequence of the CDS of the RdRp (selected model of AA substitution: LG +G+I+F). A maximum likelihood tree of Partitiviridae, Hypoviridae, Amalgaviridae, and Picornaviridae species from the ICTV master species list and Partiti-like unassigned viruses is displayed in a midrooted rectangular view. Nodes show bootstrap values (bootstrap=1000) and branch length are shown in the upper left corner. Novel species found in this study are in red.

11.6.1.9 *Ghabrivirales phylogeny*

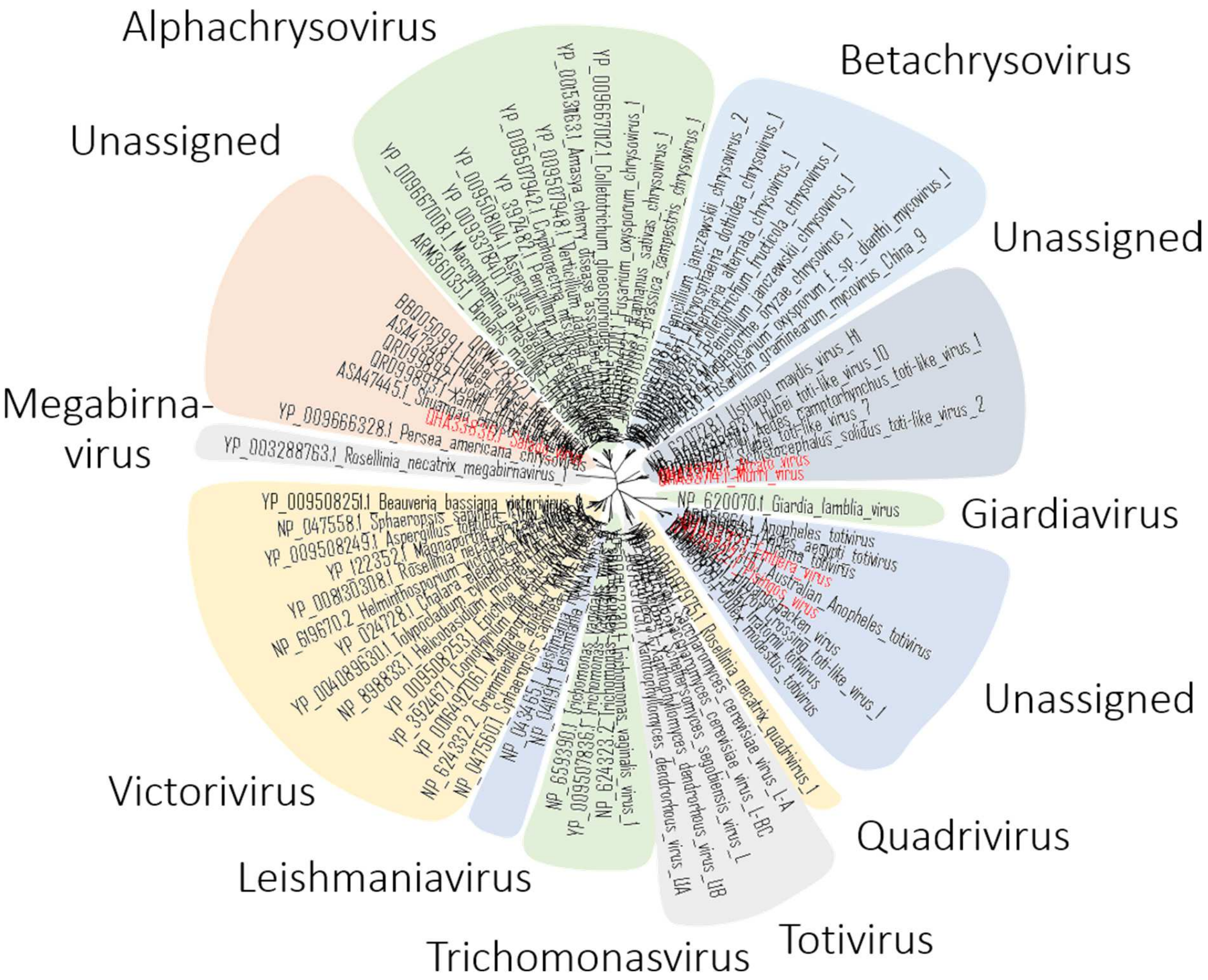


Figure 67: Phylogeny of the order Ghabrivirales estimated based on the AA sequence of the CDS of the RdRp containing ORF (selected model of AA substitution: LG +G+I+F). An unrooted maximum likelihood tree including genera representatives from the ICTV Master Species list and yet unassigned viruses is displayed in a radial view. Bootstrap values and branch length are shown in Figure 68. Novel species found in this study are in red.

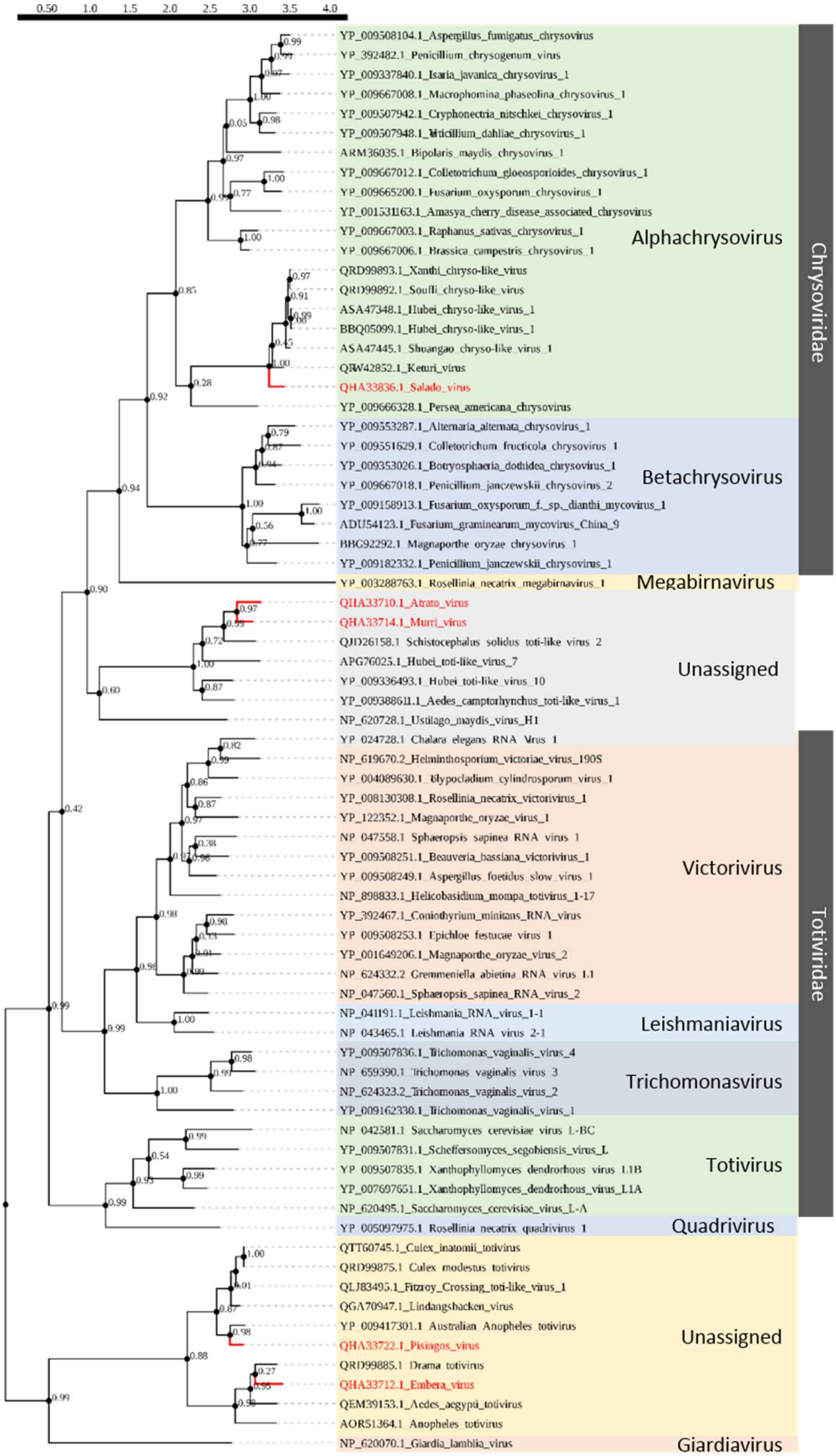


Figure 68: Phylogeny of order Ghabrivirales estimated based on the AA sequence of the CDS of the RdRp containing ORF (selected model of AA substitution: LG +G+I+F). An unrooted maximum likelihood tree including genera representatives from the ICTV Master Species list and unassigned viruses is displayed in a rectangular view. Nodes show bootstrap values (bootstrap=1000) and branch length is shown in the upper left corner. Novel species found in this study are in red.

11.6.1.10 Reovirales phylogeny

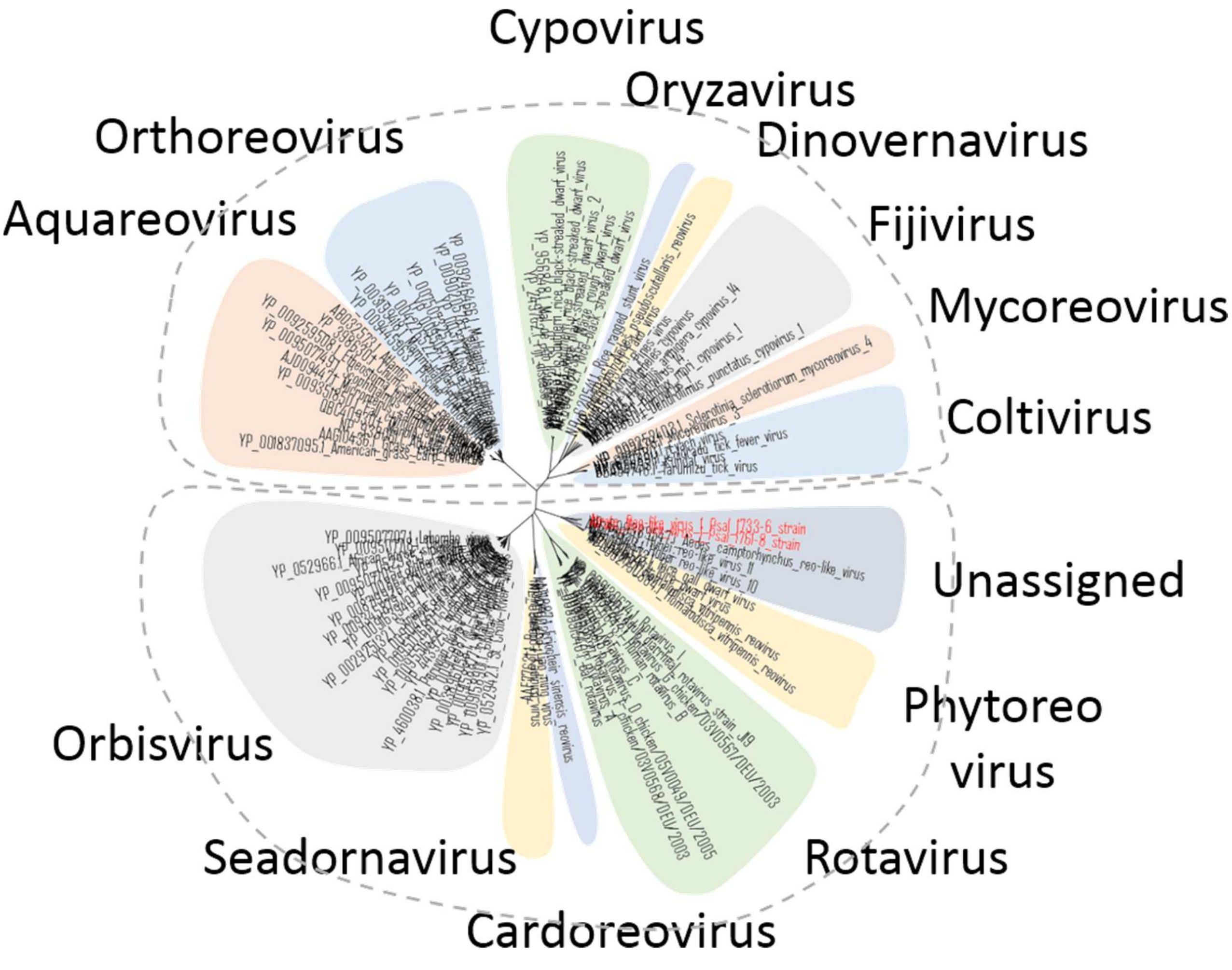


Figure 69: Phylogeny of the order Reovirales estimated based on the AA sequence of the CDS of the RdRp coding segment (selected model of AA substitution: RtREV+G+I+F). An unrooted maximum likelihood tree including genera representatives from the ICTV Master Species list and novel viruses is displayed in a radial view. Bootstrap values and branch length are shown in Figure 70. Novel species found in this study are in red.

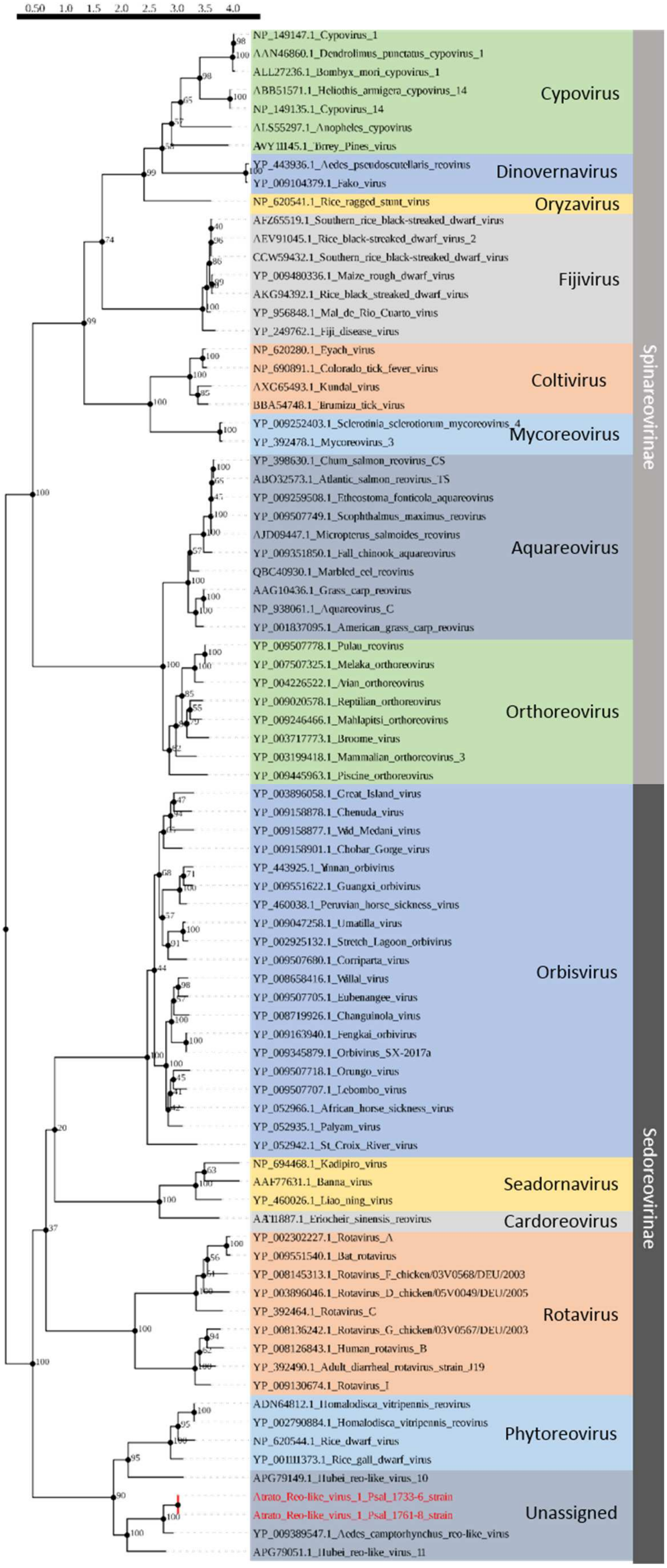


Figure 70: Phylogeny of the order Reovirales estimated based on the AA sequence of the CDS of the RdRp coding segment (selected model of AA substitution: RtREV+G+I+F). A midrooted maximum likelihood tree including genera representatives from the ICTV Master Species list and novel viruses is displayed in a rectangular view. Nodes show bootstrap values (bootstrap=100) and branch length is shown in the upper left corner. Novel species found in this study are in red.

11.6.1.11 Piccovirales phylogeny

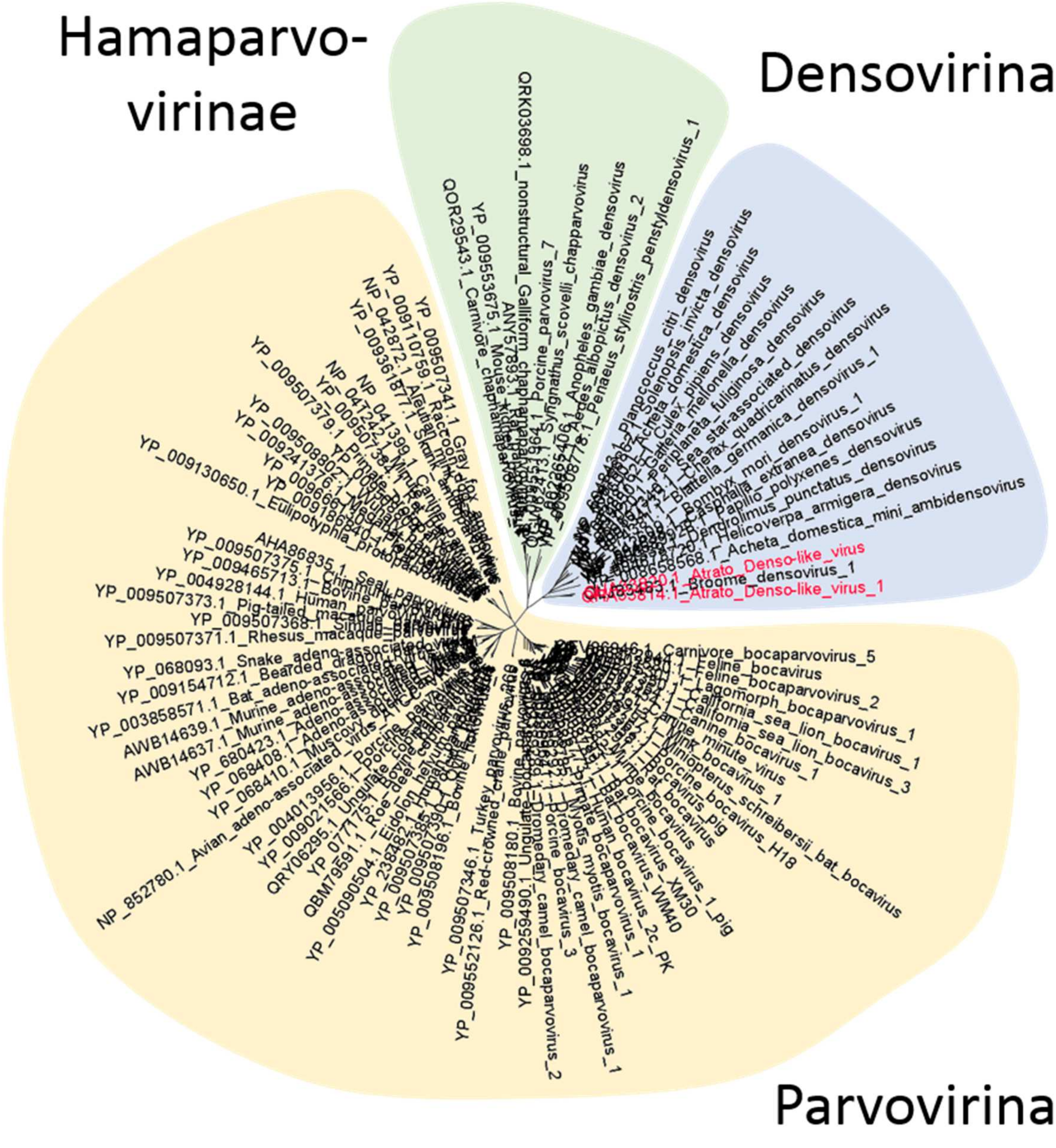


Figure 71: Phylogeny of the order Piccovirales estimated based on the AA sequence of the CDS of the nonstructural ORF (NS1), (selected model of AA substitution: LG+G+I+F). An unrooted maximum likelihood tree including genera representatives from the ICTV Master Species list and novel viruses is displayed in a radial view. Bootstrap values and branch length are shown in Figure 72. Novel species found in this study are in red.

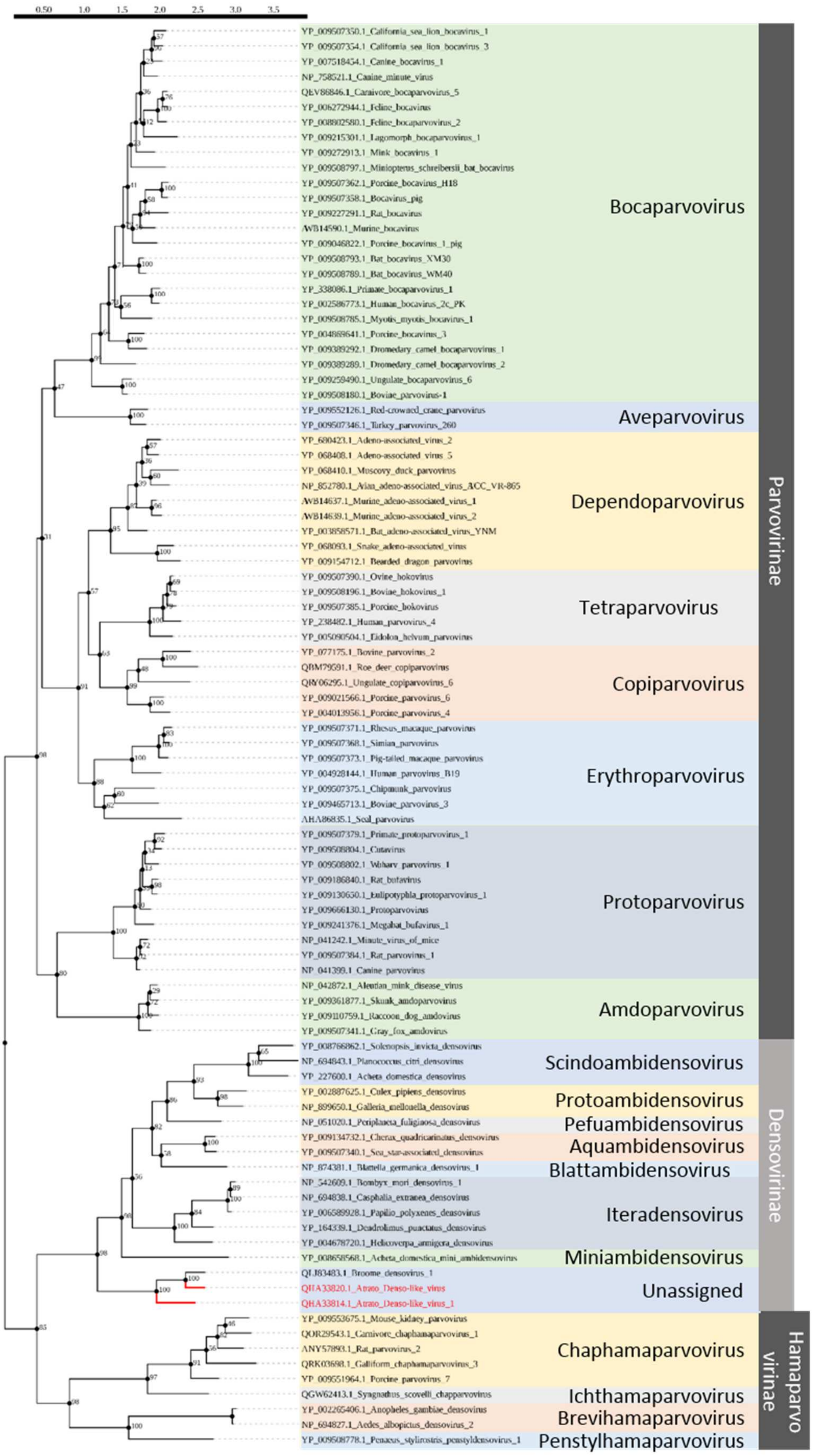


Figure 72: Phylogeny of the order Piccovirales estimated based on the AA sequence of the CDS of the non-structural ORF (NS1), (selected model of AA substitution: LG+G+I+F). A midrooted maximum likelihood tree including genera representatives from the ICTV Master Species list and unassigned viruses is displayed in a rectangular view. Nodes show bootstrap values (bootstrap=100) and branch length is shown in the upper left corner. Novel species found in this study are in red.

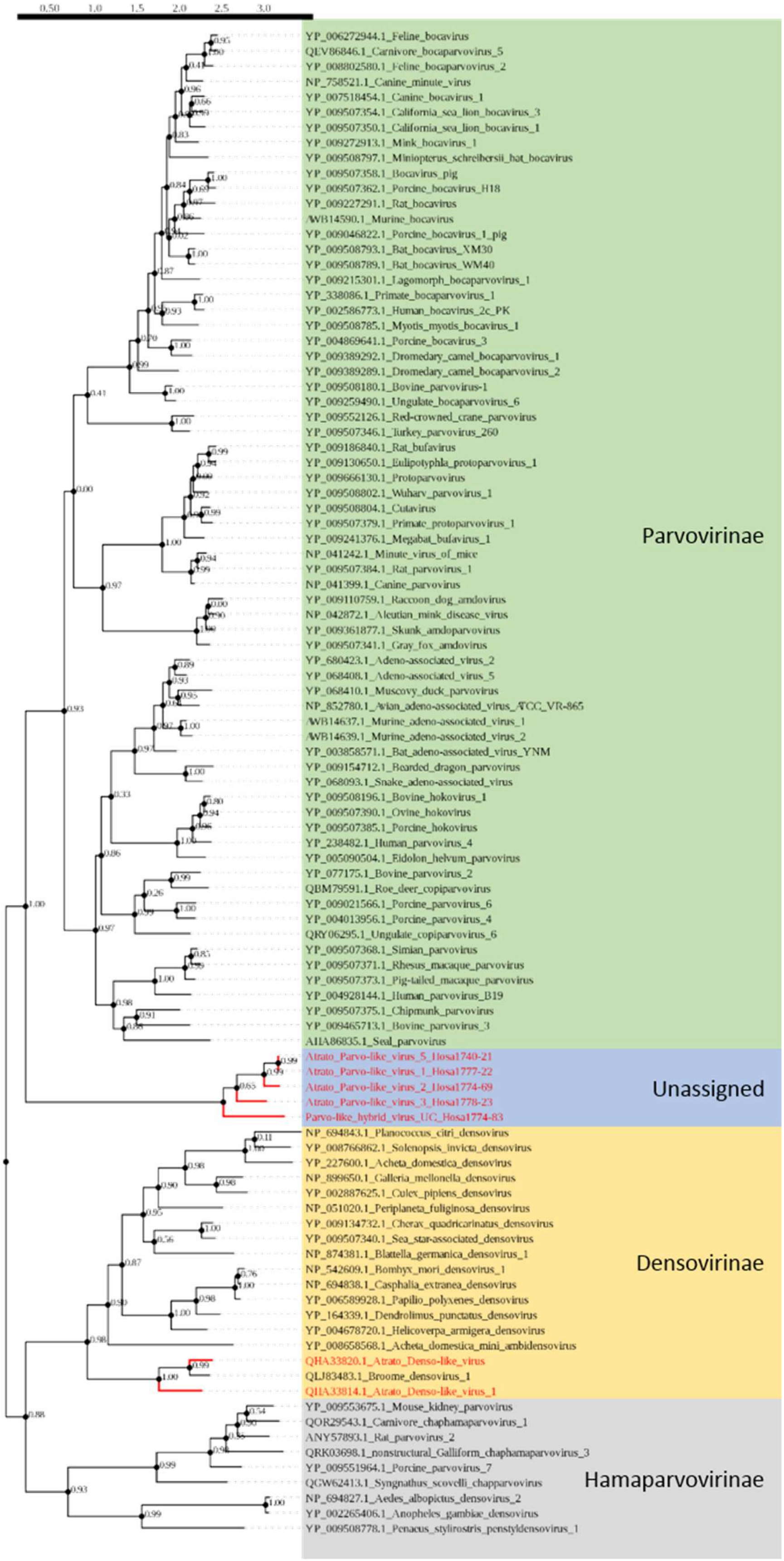


Figure 73: Phylogeny of the order Piccovirales estimated based on the AA sequence of the CDS of the non-structural ORF (NS1), (selected model of AA substitution: LG+G+I+F). A midrooted maximum likelihood tree including genera representatives from the ICTV Master Species list and unassigned viruses is displayed in a rectangular view. Nodes show aLRT SH-like support values and branch length is shown in the upper left corner. Novel species found in this study are in red. Figure 53 is similar to Figure 52 but includes novel viruses from blood samples.

11.6.1.12 Cressdanviricota phylogeny

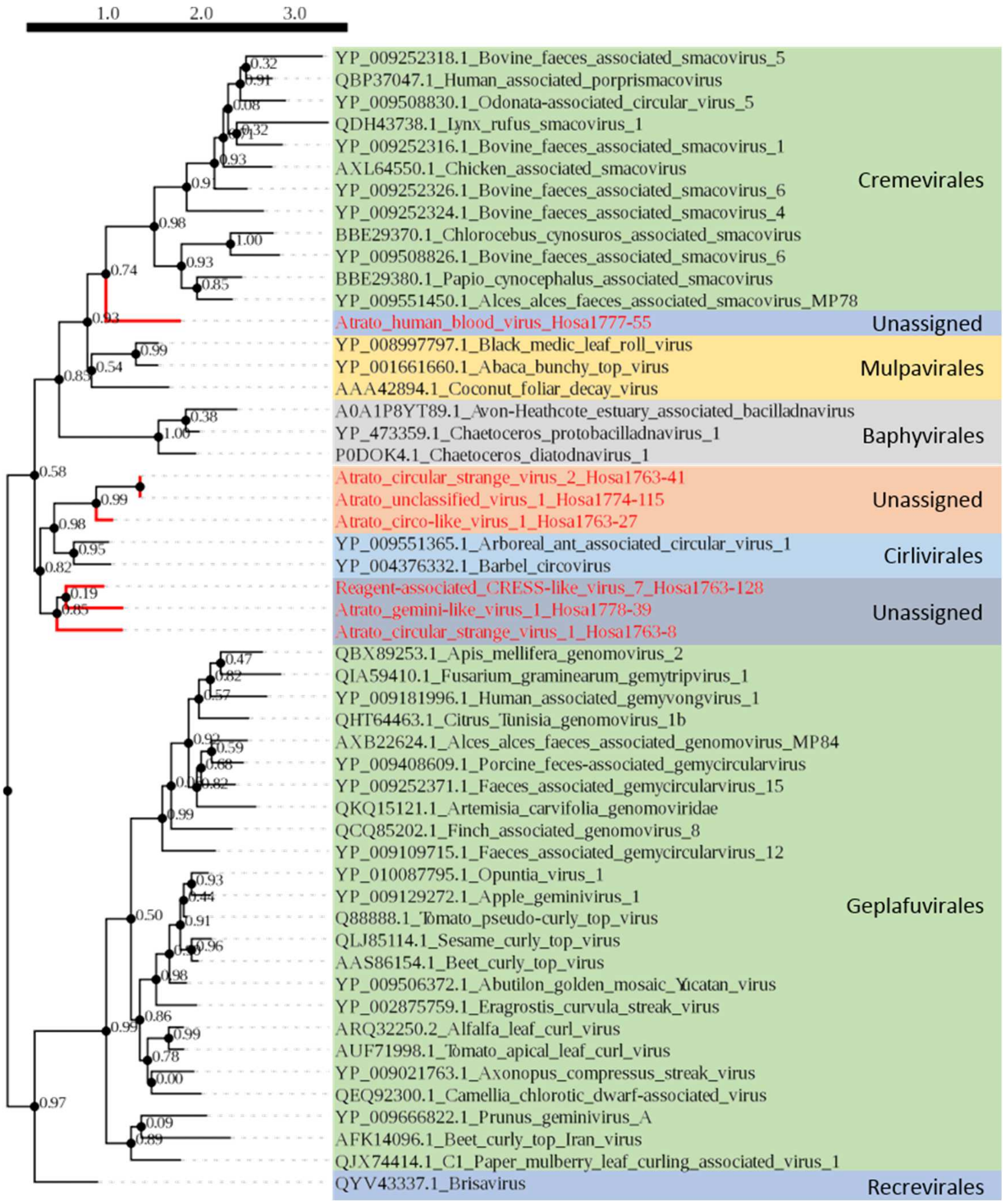


Figure 74: Phylogeny of the phylum Cressdnaviricota estimated based on the AA sequence of the CDS of the replication protein (selected model of AA substitution: LG+G+I). A maximum likelihood tree including genera representatives from the ICTV Master Species list and unassigned novel viruses is displayed in a midrooted, rectangular view. Nodes show aLRT SH-like support values and branch length is shown in the upper left corner. Novel species found in this study are in red.

11.6.1.13 Ciclivirales phylogeny

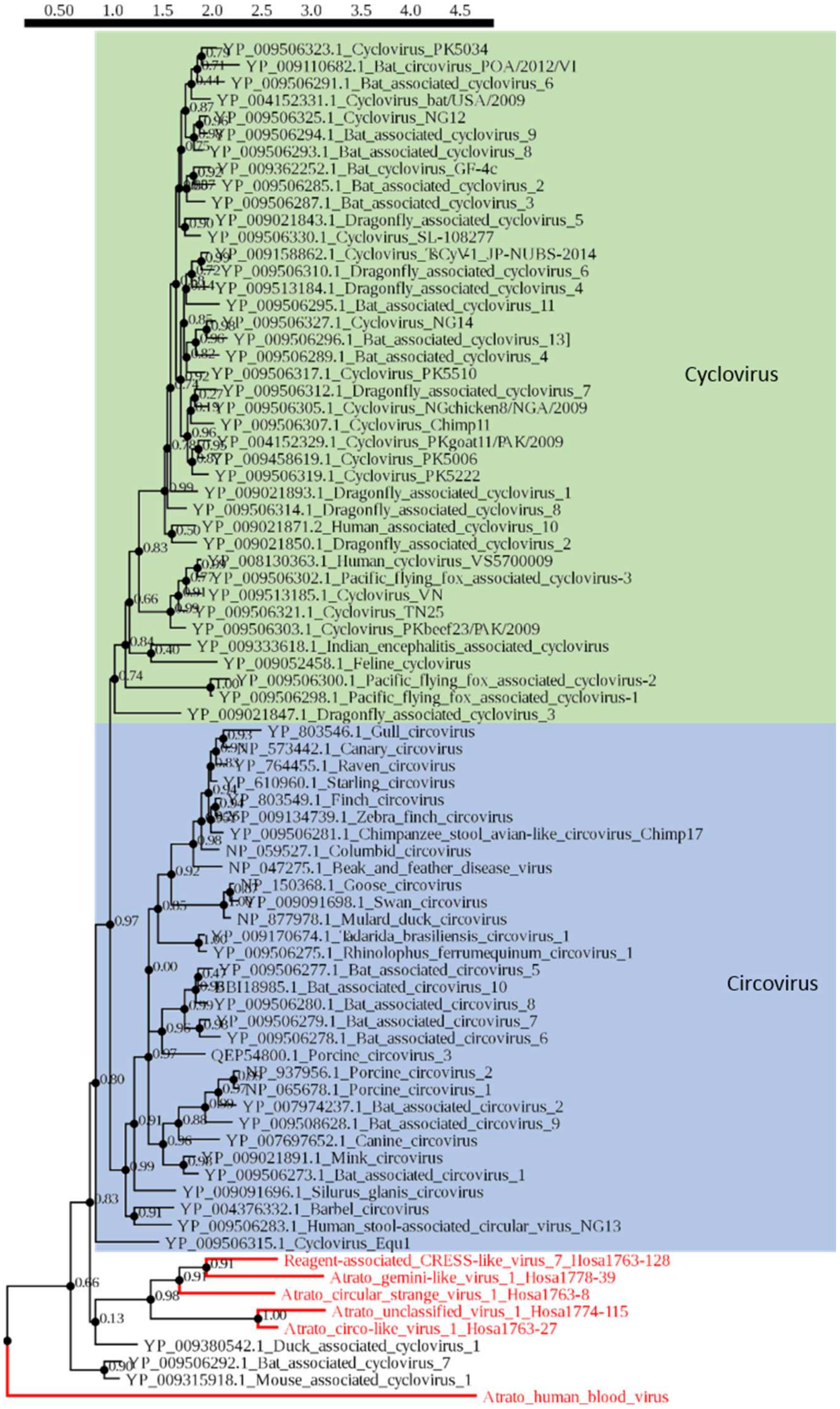


Figure 75: Phylogeny of the order Circlivirales estimated based on the AA sequence of the CDS of the replication protein (selected model of AA substitution: LG+G+I). A maximum likelihood tree including representatives from the ICTV master species list and unassigned Circoviridae-like viruses is displayed in a rooted, rectangular view (outgroup: Atrato human blood virus.) Nodes show aLRT SH-like support values and branch length is shown in the upper left corner. Novel species found in this study are in red.

11.6.1.14 Ortervirales phylogeny

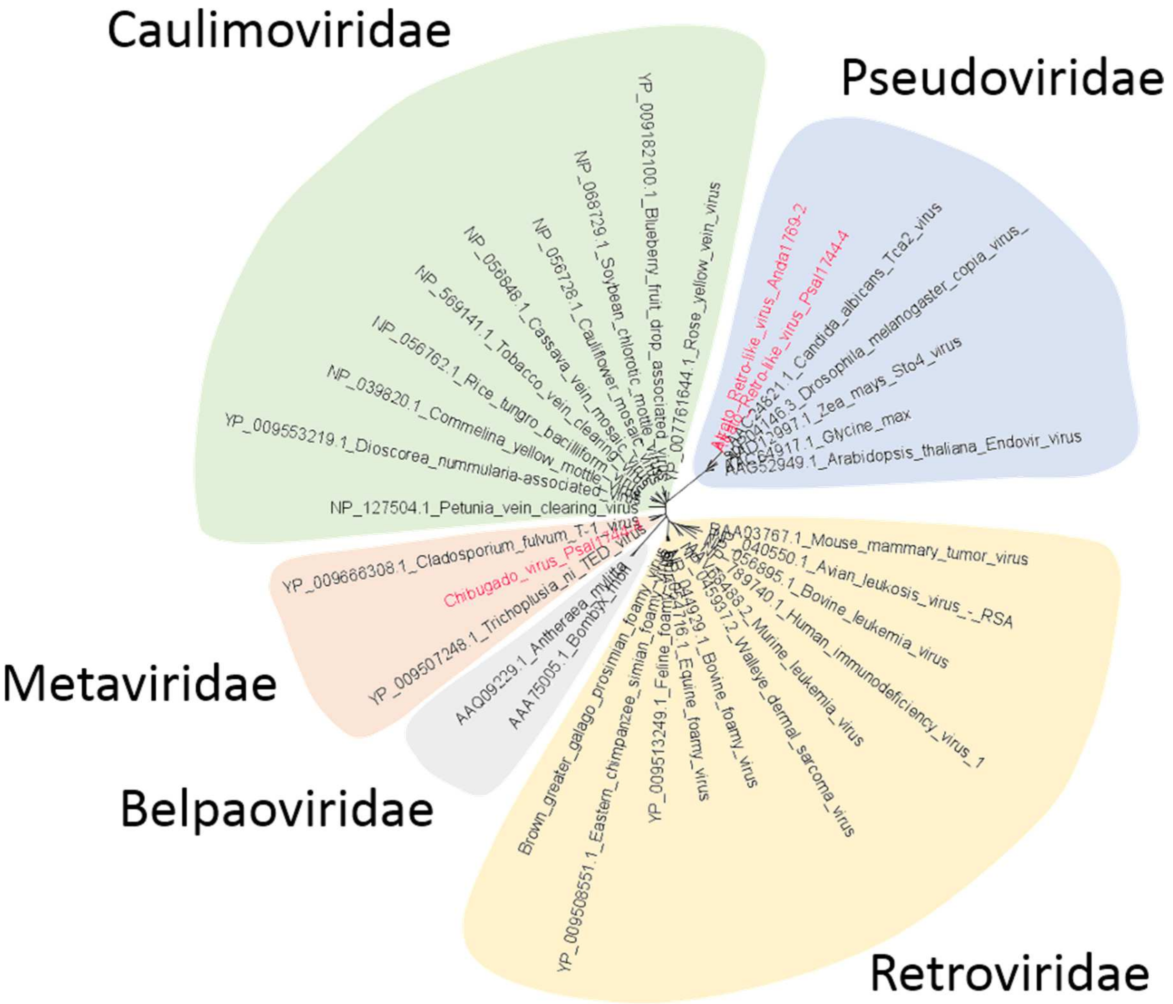


Figure 76: Phylogeny of the order Ortervirales estimated based on the AA sequence of the CDS of the polymerase gene (selected model of AA substitution: RtREV +G+I+F). An unrooted maximum likelihood tree including genera type species and unassigned viruses is displayed in a radial view. Bootstrap values and branch length are shown in Figure 77. Novel species found in this study are in red.

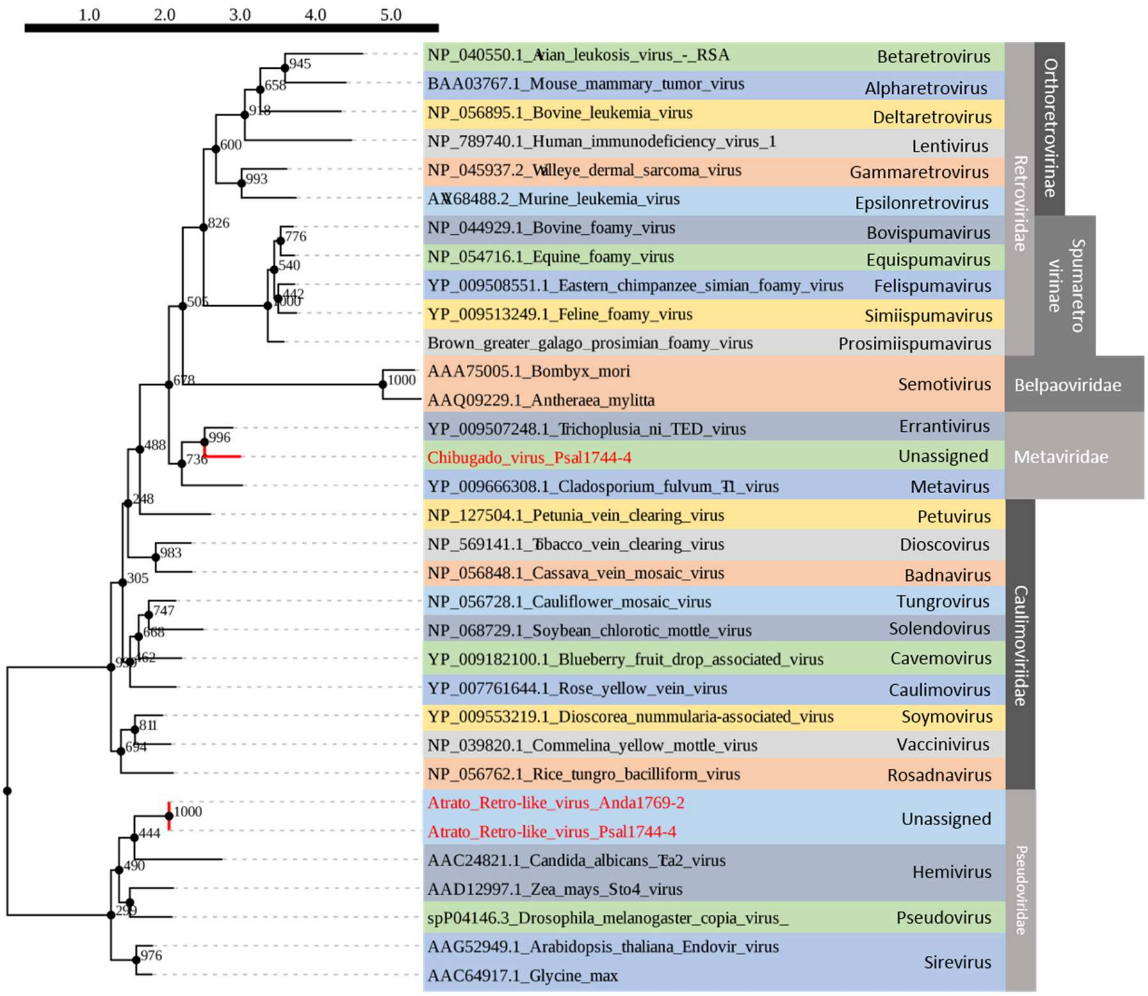


Figure 77: Phylogeny of the order Ortervirales estimated based on the AA sequence of the CDS of the polymerase gene (selected model of AA substitution: RtREV +G+I+F). A maximum likelihood tree of genera type species and unassigned viruses is displayed in a midrooted rectangular view. Nodes show bootstrap values (bootstrap=1000) and branch length is shown in the upper left corner. Novel species found in this study are in red.

11.6.1.14.1 Pseudoviridae

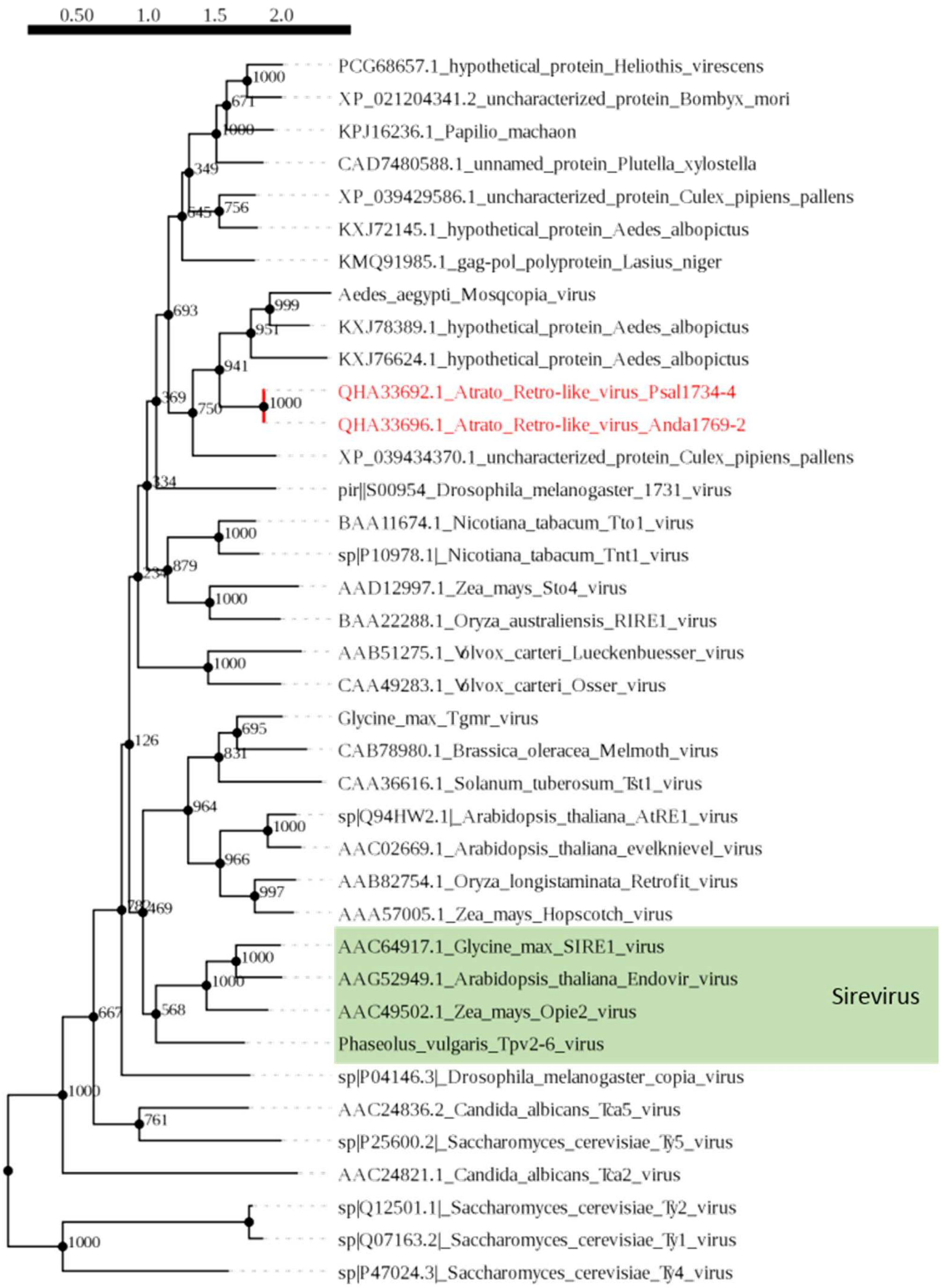


Figure 78: Phylogeny of the family Pseudoviridae estimated based on the AA sequence of the gag-pol polyprotein, (selected model of AA substitution: LG +G+I+F). A midrooted maximum likelihood tree including genera representatives from the ICTV species master list and yet unassigned Pseudo-like viruses is displayed in a rectangular view. Nodes show bootstrap values (bootstrap=1000) and branch length is shown in the upper left corner. Novel species found in this study are in red.

11.6.2 Viral genome structures

11.6.2.1 Legend for genomes structures

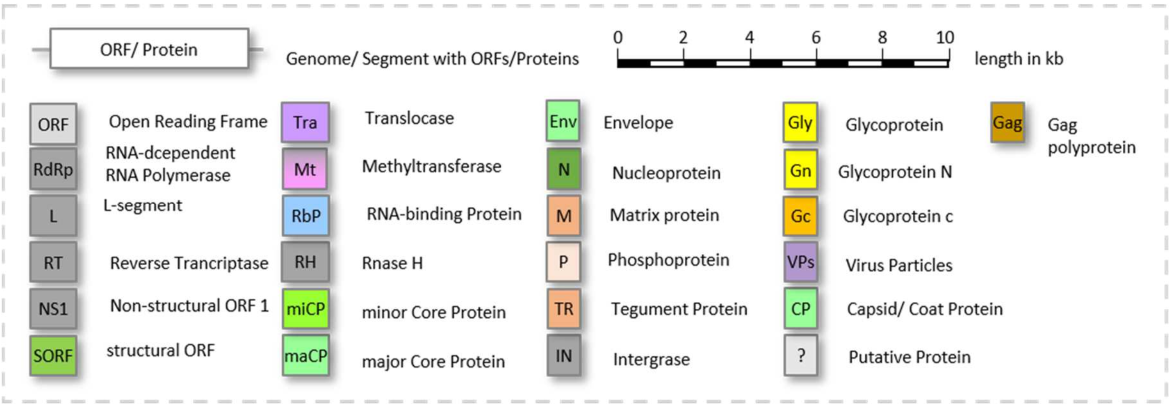
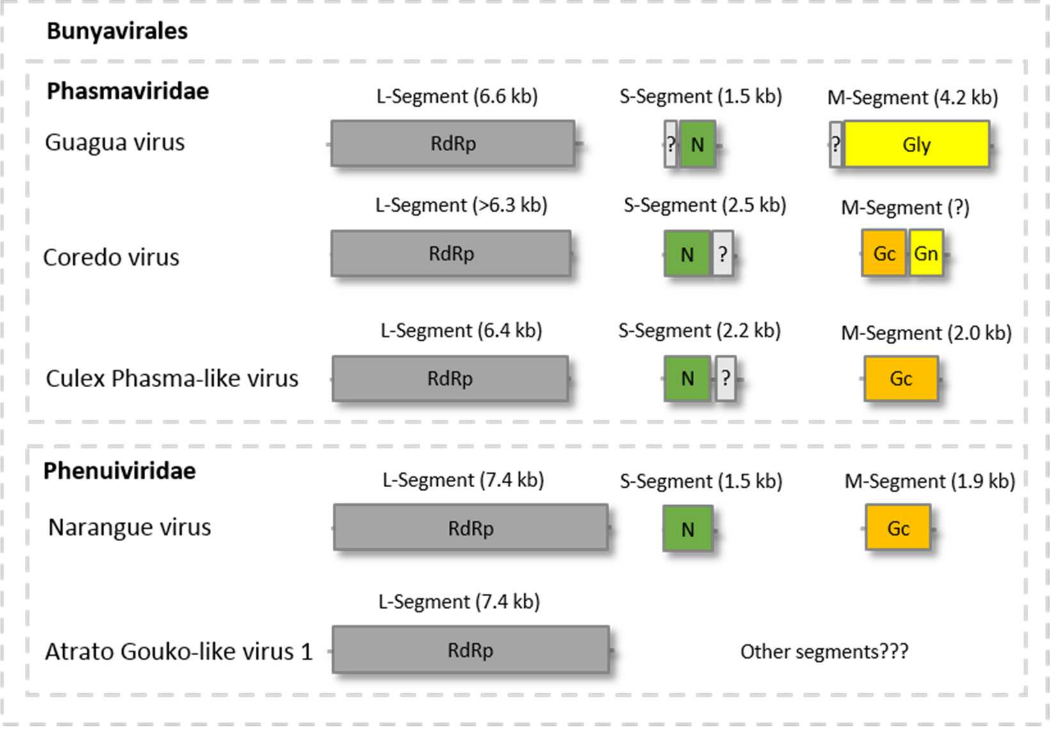


Figure 79: Legend for virus genome structures.

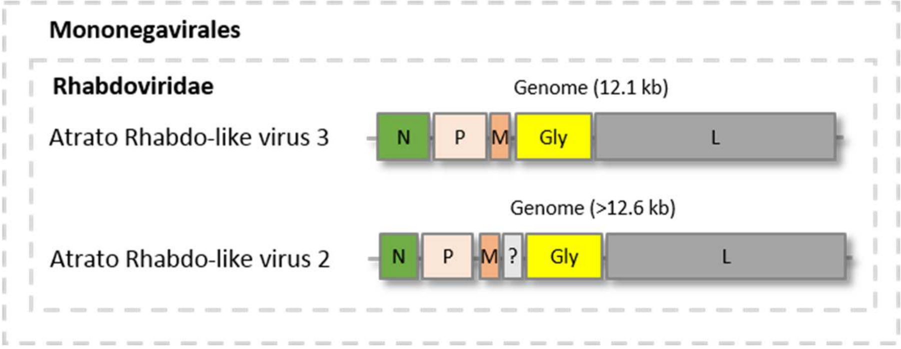
11.6.2.2 Bunyavirales genome structures



Phasmaviridae
https://viralzone.expasy.org/8356
Phenuiviridae
https://viralzone.expasy.org/7101

Figure 80: Genome structures of novel viruses of the order Bunyavirales. Viruses of the families Phasmaviridae and Phenuiviridae consist of the bunyavirus-typical segmented (-)ssRNA, linear genome with an L, M, and S segment. Guagua virus was assigned to the genus Feravirus family Phasmaviridae. In a proposal presented to the ICTV the creation of a new family designated Feraviridae was proposed. Ferak virus, former type species of the family was assigned to a new genus designated Orthoferavirus (Junglen et al. 7/18/2016). Coredo virus and Culex Phasma-like virus were assigned to the new genus Orthophasmavirus, family Phasmaviridae with Kigluaik phantom orthophasmavirus being the former type species of the genus (Junglen 7/18/2016). Visualizations of the Phenuviridae and Phasmaviridae virions and genomes are available at ViralZone (Hulo et al. 2011),(see links on the right of the figure).

11.6.2.3 Mononegavirales genome structures



Rhabdoviridae:
https://viralzone.expasy.org/2

https://talk.ictvonline.org/ictv-reports/ictv_online_report/negative-sense-rna-

Figure 81: Genome structures of novel viruses of the order Mononegavirales. Viruses of the family Rhabdoviridae consist of a linear or bisegmented, (-)ssRNA genome with at least 5 genes encoding structural proteins (N-P-M-Gly-L). ARLV2 and ARLV 2 could not be assigned to any existing genus. Therefore, new genera were proposed (see 6.2.2.4.1.2.2). Visualizations of the typical Rhabdoviridae virions and genomes are available at ViralZone (Hulo et al. 2011). A detailed description of the family Rhabdoviridae and the accepted genera is available on the ICTV website (see links on the right of the figure), (Walker et al. 2018).

11.6.2.4 Jingchuvirales genome structures

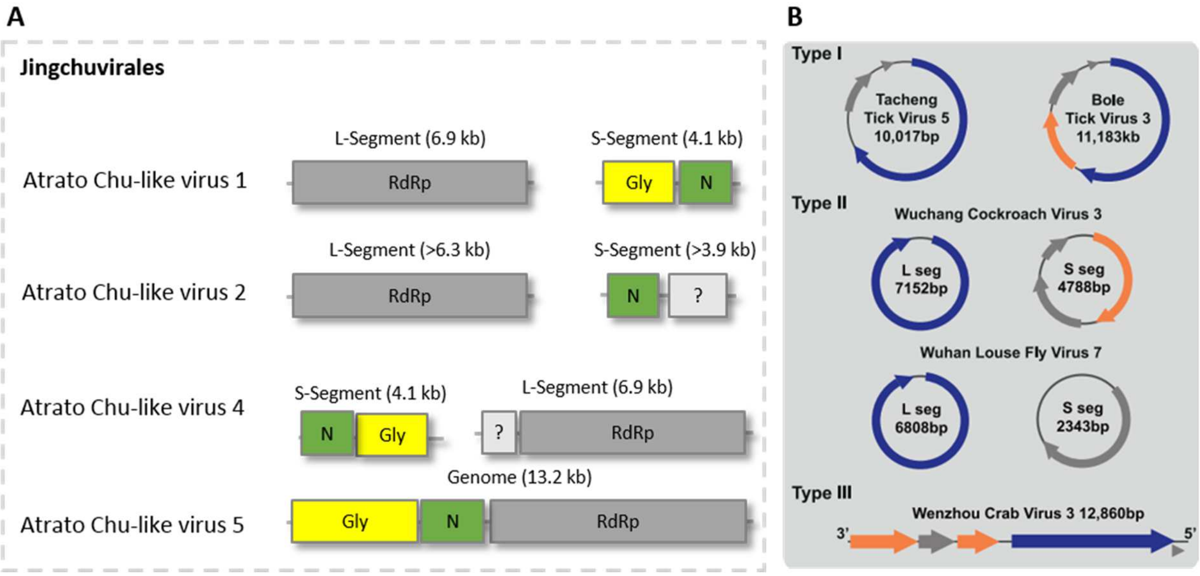


Figure 82: Genome structures of the members of the family Chuviridae, Order Jingchuvirales. (A) Novel viruses found in our study. It is supposed that Atrato Chu-like virus 1 is bipartite and linear like Chuvirus (former type species of the genus, not published, KX924630.1), Atrato Chu-like virus 4 (uncomplete) and 5 most probably are unsegmented and circular (type III) and Atrato Chu-like virus 2 is segmented and circular (type II). The circular genomic shape was not confirmed by 'around-the-genome' RT-PCR, but the segments show the typical sequence motives found in circular segments between the N and L ORF (see Li et al. 2015). (B) Three major types of putative Chuvirus genomes (circular, circular and segmented, and linear) are shown and are annotated with predicted ORFs: putative RdRp genes are shaded blue, putative Gly genes are shaded orange, and the remaining ORFs are shaded gray (figure from Li et al. 2015).

11.6.2.5 Tymovirales genome structures

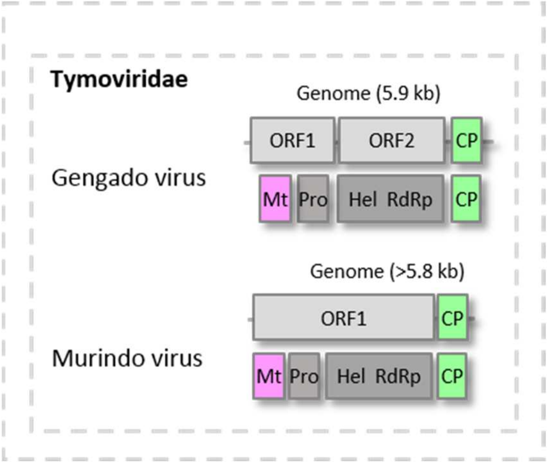


Figure 83: Genome structures of novel viruses of the order Tymovirales. Viruses of the family Tymoviridae consist of a monopartite, linear, (+)ssRNA genome encoding an Mt, Pro, Hel, RdRp, and CP. Gengado virus and Murindo virus could not be assigned to any existing genus. Therefore, new genera were proposed (see 6.2.2.4.2.1.1). Visualizations of the typical Tymoviridae virions and genome structures are available at ViralZone (Hulo et al. 2011). A detailed description of the family Tymoviridae and the accepted genera is available on the ICTV website (see links on the right of the figure).

Tymoviridae:
https://viralzone.expasy.org/54
https://talk.ictvonline.org/ictv-reports/ictv_9th_report/positive-sense-rna-viruses-2011/w/posrna_viruses/245/tymoviridae

11.6.2.6 Martellivirales genome structures

Figure 84: Genome structures of novel viruses of the order Martellivirales. Virgaviridae-like viruses consist of a monopartite, linear, (+)ssRNA genome that resembles the genome components and genome organization of the family Virgaviridae, in particular of the genus Tobamovirus. Most probably these viruses share the same ancestors as members of the family Virgaviridae, but belong to yet unassigned genera (Figure 51 and Figure 52). Vizualizations of the typical Tobamovirus virions and genome structures are available at ViralZone (Hulo et al. 2011). A detailed description of the family Virgaviridae and the accepted genera is available on the ICTV website (Adams et al. 2017), (see links on the right of the figure).

Tobamovirus:
https://viralzone.expasy.org/51

Virgaviridae:
https://talk.ictvonline.org/ictv-reports/ictv_online_report/positive-sense-rna-viruses/w/virgaviridae

11.6.2.7 Picornavirales genome structures

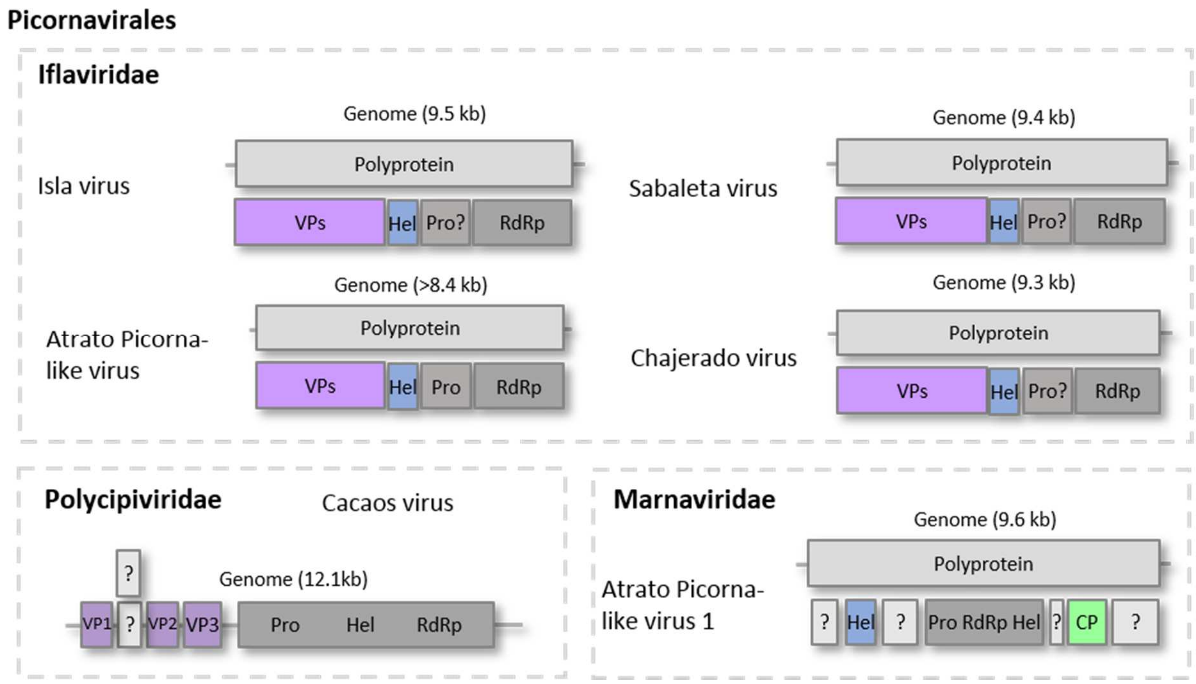


Figure 85: Genome structures of novel members of the order Picornavirales.
Viruses of the family Iflaviridae have a linear, non-segmented, (+)ssRNA genome with a single ORF coding a polyprotein (VPs-Hel-Pro-RpdP). Viruses of the family Polycipiviridae consist of a (+)ssRNA genome with a long 3' ORF coding a Pro, Hel, and RdRp and several small ORFs. APLV1 was not assigned to any family due to low AA similarity but shares a common ancestor (Figure 55) and similar genome structure with members of the family Marnaviridae. Visualization of the typical Iflaviridae, Polycipiviridae, and Marnaviridae virions and genome structures are available at ViralZone (Hulo et al. 2011). A detailed description of the families Iflaviridaa, Polycipiviridae, and Marnaviridae and the accepted genera is available on the ICTV website (see links on the right of the figure).

Marnaviridae:
https://viralzone.expasy.org/302

https://talk.ictvonline.org/ictv-reports/ictv_9th_report/positive-sense-rna-viruses-2011/w/posrna_viruses/232/marnaviridae

Iflaviridae:
https://viralzone.expasy.org/792

https://talk.ictvonline.org/ictv-reports/ictv_online_report/positive-sense-rna-viruses/w/iflaviridae
Polycipiviridae:
https://viralzone.expasy.org/8438

https://talk.ictvonline.org/ictv-reports/ictv_online_report/positive-sense-rna-viruses/w/polycipiviridae

11.6.2.8 Tolivirales genome structures

Figure 86: Genome structure of Hubei mosquito virus 4, member of the order Tolivirales.

11.6.2.9 Amarillovirales genome structures

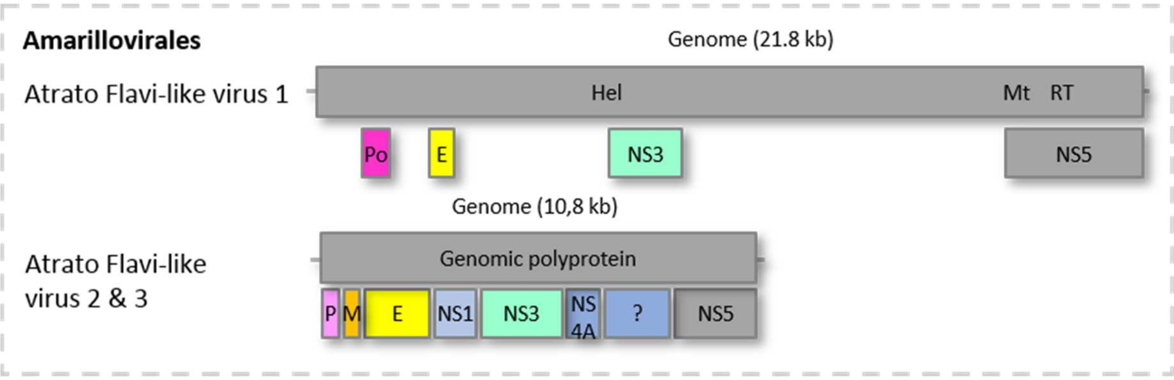


Figure 87: Genome structure of a novel Flaviviridae-like virus of the order Amarillovirales.
AFlv1 clusters with other recently discovered Flaviviridae-like viruses that differ from the typical Flaviviridae genome structure (Figure 61, Figure 62). The genomes are much longer and the identification of all the coded proteins was not possible. Most probably AFlv1 belongs to a yet undiscovered and undesignated genus or family. AFlv 2 and 3 cluster with recently discovered insect-specific Flaviviruses. Visualizations of the typical Flaviviridae virions and genome structures are available at ViralZone (Hulo et al. 2011). A detailed description of the family Flaviviridae and the accepted genera is available on the ICTV website (see links on the right of the figure).

Flaviviridae:
https://viralzone.expasy.org/43

https://talk.ictvonline.org/ictv-reports/ictv_online_report/positive-sense-rna-viruses/w/flaviviridae

11.6.2.10 Sobelivirales genome structures

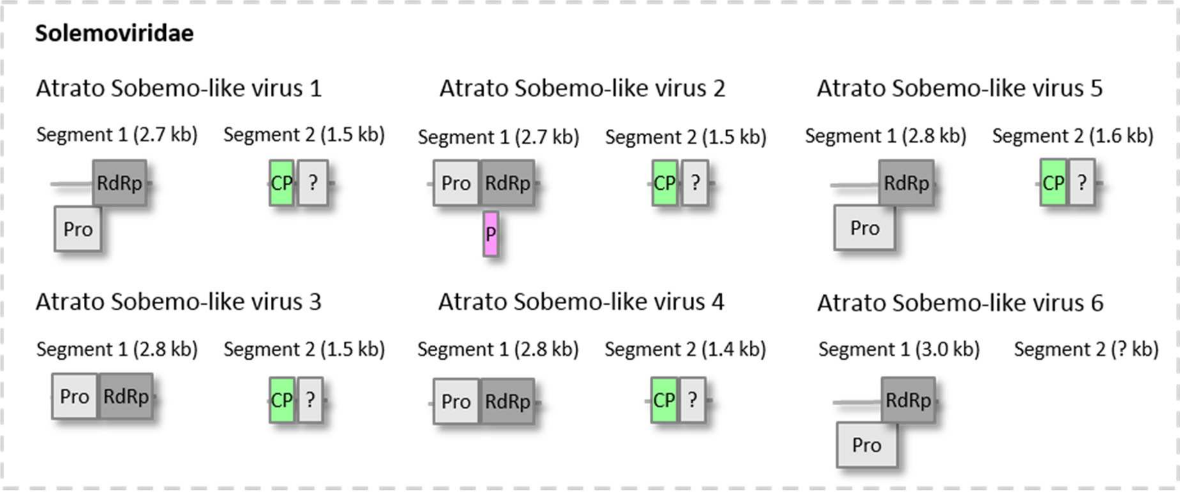


Figure 88: Genome structure of novel Solemo-luteoviridae-like viruses.
Most probably ASlvV 1-6 belong to a yet unassigned family of viruses that share a common ancestor with members of the families Solemoviridae and Luteovirida (Figure 63, Figure 64) but have a different genome composition. The members of the families Solemoviridae and Luteoviridae typically consist of a (+)ssRNA, linear, monopartite genome, whereas ASLV1-6 and their close relatives bear a bipartite genome. Visualizations of the typical Solemovirida and Luteoviridae virions and genome structures are available at ViralZone (Hulo et al. 2011). Detailed descriptions of the families Luteoviridae and Solemoviridae and the accepted genera are available on the ICTV website (see links on the right of the figure).

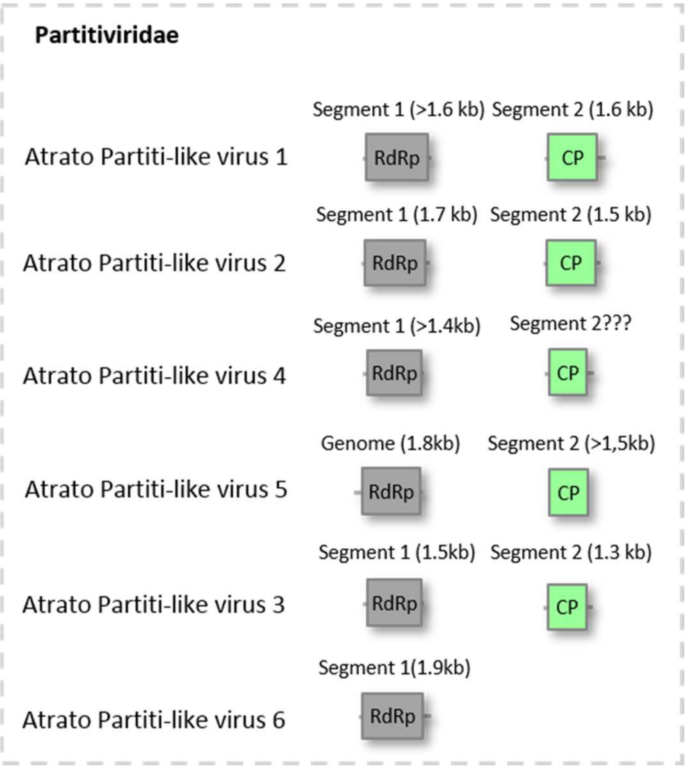
Solemoviridae:
https://viralzone.expasy.org/8460

https://talk.ictvonline.org/ictv-reports/ictv_online_report/positive-sense-rna-viruses/w/solemoviridae
Luteoviridae
https://viralzone.expasy.org/45

https://talk.ictvonline.org/ictv-reports/ictv_9th_report/positive-sense-rna-viruses-2011/w/posrna_viruses/265/luteoviridae

11.6.2.11 Durnavirales genome structures

11.6.2.11.1 Partitiviridae



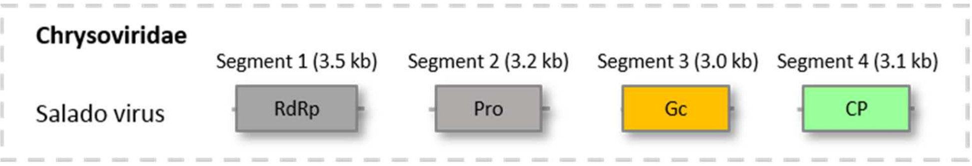
Partitiviridae
https://viralzone.expasy.org/168

https://talk.ictvonline.org/ictv-reports/ictv_online_report/dsrna-viruses/w/partitiviridae

Figure 89: Genome structure of novel Partiti-like viruses. Visualizations of the typical Partitiviridae virions and genome structures are available at ViralZone (Hulo et al. 2011). A detailed description of the family Partitiviridae and the accepted genera is available on the ICTV website (see links on the right of the figure).

11.6.2.12 Ghabrivirales genome structures

11.6.2.12.1 Chrysoviridae



Chrysoviridae
https://viralzone.expasy.org/166

https://talk.ictvonline.org/ictv-reports/ictv_online_r

Figure 90: Genome structures of novel members of the family Chrysoviridae. Viruses of the family Chrysoviridae consist of a multipartite, dsRNA genome, usually 4 monocistronic segments. Visualizations of the typical Chrysoviridae virions and genome structures are available at ViralZone (Hulo et al. 2011). A detailed description of the family Chrysoviridae and the accepted genera is available on the ICTV website (Kotta-Loizou et al. 2020), (see links on the right of the figure).

11.6.2.12.2 Totiviridae

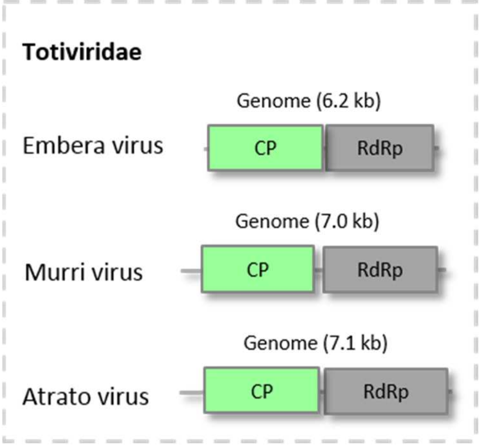
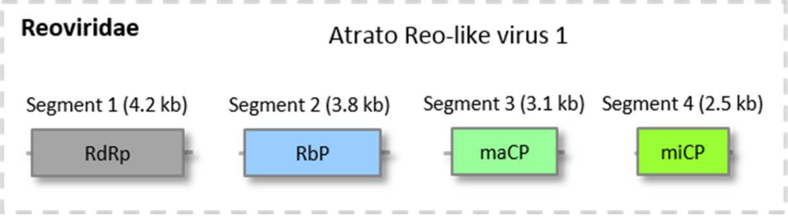


Figure 91: Genome structures of novel members of the order Ghabrivirales. Viruses of the family Totiviridae consist of a linear, dsRNA genome with two ORFs coding for an RdRp and a CP. Embera virus, Murri virus, and Atrato virus belong to yet unassigned new genera of the family Totiviridae (see 6.2.2.4.3.2.2) Visualizations of the typical Totiviridae virions and genome structures are available at ViralZone (Hulo et al. 2011). A detailed description of the family Totiviridae and the accepted genera is available on the ICTV website (see links on the right of the figure).

Totiviridae:
https://viralzone.expasy.org/161

https://talk.ictvonline.org/ictv-reports/ictv_9th_report/dsrna-viruses-2011/w/dsrna_viruses/191/totiviridae

11.6.2.13 Reovirales genome structures- Reoviridae



Phytovirus:
https://viralzone.expasy.org/628
Reoviridae:
https://talk.ictvonline.org/ictv-reports/ictv_9th_report/dsrna-viruses-2011/w/dsrna_viruses/188/reoviridae

Figure 92: Genome structure of a novel member of the order Reovirales. Viruses of the family Reoviridae consist of 9-12 segments of linear dsRNA, depending on the genus. ARLV1 was assigned to the genus Phytoreovirus and most probably has 12 segments (see Table 13), but only segments 1-4 are shown here, as no similar sequences are available at NCBI GenBank for proper identification of the proteins coded on the other segments. Visualization of the typical Phytoreovirus virion and genome structure is available at ViralZone (Hulo et al. 2011). A detailed description of the family Reoviridae and the accepted genera is available on the ICTV website (see links on the right of the figure).

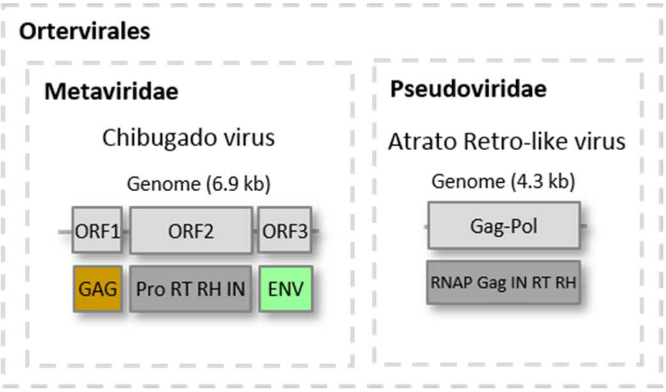
11.6.2.14 Piccovirales genome structures - Parvoviridae

Figure 93: Genome structures of novel members of the order Piccovirales. Viruses of the family Parvoviridae consist of a linear ssDNA genome with terminal hairpins. Atrato Denso-like virus and Atrato Denso-like virus 1 were assigned to the subfamily Densovirinae. Due to low AA similarity with other viruses a new genus was proposed (see Figure 71 and Figure 72). Visualizations of the typical Parvoviridae virions and genome structures are available at ViralZone (Hulo et al. 2011). A detailed description of the family Parvoviridae and the accepted genera is available on the ICTV website (Cotmore et al. 2019), (see links on the right of the figure).

Parvoviridae:
https://viralzone.expasy.org/103

https://talk.ictvonline.org/ictv-reports/ictv_online_report/ssdna-viruses/w/parvoviridae

11.6.2.15 *Ortervirales*



Metaviridae:
https://talk.ictvonline.org/ictv-reports/ictv_online_report/reverse-transcribing-dna-and-rna-viruses/w/metaviridae

Figure 94: Genome structures of novel viruses of the order Ortervirales (formerly Retrovirales). Viruses of the family Metaviridae consist of a (+)ssRNA genome with one to three ORFs encoding a CP, N, P, RT, ribonuclease H, and integrase enzymes. ARlv could not be assigned to any existing genus. A detailed description of the family Metaviridae and the accepted genera is available on the ICTV website (see link on the right of the figure), (Llorens et al. 2020).

11.7 Sequences associated to non-viral agents found in mosquito and blood sample pools

Table 20: Contigs of non-viral agents derived from mosquito and blood sample pools.

NGS pool	Mosquito species	contig length (bps)	NCBI blastn Hit Description	Accession Hit	Per. Id.	Taxonomy
1731	Wyeomyia	2296	Wolbachia endosymbiont of Drosophila incompta strain wInc_Cu genome	CP011148.1	99%	Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; Anaplasmataceae; Wolbachieae; Wolbachia
		2048	Trypanosoma culicavium isolate CUL24 18S ribosomal RNA gene, partial sequence	HQ909083.1	97%	Eukaryota; Discoba; Euglenozoa; Kinetoplastea; Metakinetoplastina; Trypanosomatida; Trypanosomatidae; Trypanosoma
1738	Ma. titillans	1361	Wolbachia endosymbiont of Philaenus maghresignus gene for 16S ribosomal RNA, partial sequence	AB772263.1	99%	Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; Anaplasmataceae; Wolbachieae; Wolbachia
1740	An. darlingi	1663	Acinetobacter baumannii strain 17-84 chromosome, complete genome	CP059479.1	99%	Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Moraxellaceae; Acinetobacter; Acinetobacter calcoaceticus/baumannii complex
1741	Ps. funiculus	2605	Rickettsia sp. MEAM1 (Bemisia tabaci), complete genome	CP016305.1	96%	Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; Rickettsiaceae; Rickettsieae; Rickettsia

NGS pool	Mosquito species	contig length (bps)	NCBI blastn Hit Description	Accession Hit	Per. Id.	Taxonomy
1743	Ps. albipes	2528	Blechomonas keelingi isolate B07-22 18S small subunit ribosomal RNA gene, partial sequence	KF054130.1	90%	Eukaryota; Discoba; Euglenozoa; Kinetoplastea; Metakinetoplastina; Trypanosomatida; Trypanosomatidae; Blechomonas
		1501	Providencia rettgeri strain FDAARGOS_330 chromosome, complete genome	CP027418.1	99%	Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacterales; Morganellaceae; Providencia
		1155	Trypanosoma theileri OSK-16 genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, ITS3, ITS4, partial	LC618031.1	90%	Eukaryota; Discoba; Euglenozoa; Kinetoplastea; Metakinetoplastina; Trypanosomatida; Trypanosomatidae; Trypanosoma
		922	Providencia alcalifaciens strain FDAARGOS_408 chromosome, complete genome	CP023536.1	99%	Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacterales; Morganellaceae; Providencia
		712	Providencia rettgeri strain FDAARGOS_330 chromosome, complete genome	CP027418.1	100%	Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacterales; Morganellaceae; Providencia
		1802	Spiroplasma diabroticae 16S ribosomal RNA gene, partial sequence; 16S-23S ribosomal RNA intergenic spacer, 23S ribosomal RNA gene, and 23S-5S ribosomal RNA intergenic spacer, complete sequence; and 5S ribosomal RNA gene, partial sequence	GU908490.1	97%	Bacteria; Tenericutes; Mollicutes; Entomoplasmatales; Spiroplasmataceae; Spiroplasma
1745	Ps. albipes	2776	Spiroplasma monobiae MQ-1 chromosome, complete genome	CP025543.1	98%	Bacteria; Tenericutes; Mollicutes; Entomoplasmatales; Spiroplasmataceae; Spiroplasma
1746	An. darlingi	1912	Crithidia otongatchiensis isolate ECU-08 small subunit ribosomal RNA gene, partial sequence	KC205989.1	97%	Eukaryota; Discoba; Euglenozoa; Kinetoplastea; Metakinetoplastina; Trypanosomatida; Trypanosomatidae; Leishmaniinae; Crithidia
		1758	Crithidia fasciculata ribosomal DNA repeat (complete)	Y00055.1	96%	Eukaryota; Discoba; Euglenozoa; Kinetoplastea; Metakinetoplastina; Trypanosomatida; Trypanosomatidae; Leishmaniinae; Crithidia
		1699	Rickettsiella tipulae strain BBA296/BUR-D/1961 23S ribosomal RNA (rrl) gene, partial sequence	JF288933.1	98%	Bacteria; Proteobacteria; Gammaproteobacteria; Legionellales; Coxiellaceae; Rickettsiella
		1440	Spironema culicis 16S ribosomal RNA gene, partial sequence	AF166259.1	98%	Bacteria; Spirochaetes; Spirochaetales; Spirochaetaceae; Entomospira
		1319	Rickettsiella grylli 16S ribosomal RNA gene, partial sequence	U97547.1	98%	Bacteria; Proteobacteria; Gammaproteobacteria; Legionellales; Coxiellaceae; Rickettsiella

NGS pool	Mosquito species	contig length (bps)	NCBI blastn Hit Description	Accession Hit	Per. Id.	Taxonomy
		938	Rickettsiella tipulae strain BBA296/BUR-D/1961 23S ribosomal RNA (rrl) gene, partial sequence	JF288933.1	99%	Bacteria; Proteobacteria; Gammaproteobacteria; Legionellales; Coxiellaceae; Rickettsiella
		1185	Arcobacter suis CECT 7833 chromosome, complete genome	CP032100.1	99%	Bacteria; Proteobacteria; Epsilonproteobacteria; Campylobacterales; Campylobacteraceae; Arcobacter group; Arcobacter
		1061	Wolbachia endosymbiont of Mycopsylla fici isolate Syd 23S ribosomal RNA gene, partial sequence	KT273261.1	98%	Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; Anaplasmataceae; Wolbachieae; Wolbachia
		1162	Vorticella sp. 4 JG-2011 clone 34 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	JX178934	90%	Eukaryota; Sar; Alveolata; Ciliophora; Intramacronucleata; Oligohymenophorea; Peritrichia; Sessilida; Vorticellidae; Vorticella
1748	Ps. albipes	2701	Wolbachia endosymbiont of Culex quinquefasciatus Pel strain wPip 23S ribosomal RNA, complete sequence	NR_076253.1	99%	Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; Anaplasmataceae; Wolbachieae; Wolbachia
1750	An. darlingi	1425	Spironema culicis 16S ribosomal RNA gene, partial sequence	AF166259.1	98%	Bacteria; Spirochaetes; Spirochaetales; Spirochaetaceae; Entomospira
		1149	Brachiola algerae 16S rRNA gene, ITS1 and 23S rRNA gene, strain Undeen	AM422905.1	96%	Eukaryota; Fungi; Fungi incertae sedis; Microsporidia; Tubulinosematoidea; Tubulinosematidae; Anncaliia.
		397	Visvesvaria algerae small subunit ribosomal RNA gene, complete sequence	AF024656.1	99%	Eukaryota; Fungi; Fungi incertae sedis; Microsporidia; Visvesvaria
1751	Ps. albipes	1763	Gilliamella apicola strain wkB1 23S ribosomal RNA, complete sequence	NR_122000.1	95%	Bacteria; Proteobacteria; Gammaproteobacteria; Orbales; Orbaceae; Gilliamella
		1550	Vittaforma corneae ATCC 50505 28S ribosomal RNA rRNA	XR_552275.1	94%	Eukaryota; Fungi; Fungi incertae sedis; Microsporidia; Nosematidae; Vittaforma
		1158	Microsporidium sp. 1199 partial ssu gene for small subunit ribosomal RNA, isolate 1199	FN610845.1	94%	Eukaryota; Fungi; Fungi incertae sedis; Microsporidia; Microsporidium
		593	Gilliamella apicola strain wkB1, complete genome	CP007445.1	98%	Bacteria; Proteobacteria; Gammaproteobacteria; Orbales; Orbaceae; Frischella
1752	Cq. lynchi	2143	Wolbachia endosymbiont of Drosophila simulans wAu genome assembly, chromosome: 1	LK055284.1	99%	Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; Anaplasmataceae; Wolbachieae; Wolbachia
		1383	Rickettsia limoniae strain Gent 16S ribosomal RNA gene, partial sequence	AF322442.1	99%	Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; Rickettsiaceae; Rickettsieae; Rickettsia

NGS pool	**Mosquito species**	**contig length (bps)**	**NCBI blastn Hit Description**	**Accession Hit**	**Per. Id.**	**Taxonomy**
1753	Cq. venezuelensis	2946	Erwinia amylovora CFBP1430 complete genome	FN434113.1	97%	Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacterales; Erwiniaceae; Erwinia
		2822	Helicobacter himalayensis strain YS1, complete genome	CP014991.1	94%	Bacteria; Proteobacteria; Epsilonproteobacteria; Campylobacterales; Helicobacteraceae; Helicobacter
		2600	Wolbachia sp. wRi, complete genome	CP001391.1	99%	Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; Anaplasmataceae; Wolbachieae; Wolbachia
1754	Ma. titillans	1587	Paratrypanosoma confusum isolate CUL13 small subunit ribosomal RNA gene, partial sequence	KF963538.1	95%	Eukaryota; Discoba; Euglenozoa; Kinetoplastea; Metakinetoplastina; Trypanosomatida; Trypanosomatidae; Paratrypanosoma
		1551	Wolbachia endosymbiont of Culex quinquefasciatus Pel strain wPip complete genome	AM999887.1	95%	Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; Anaplasmataceae; Wolbachieae; Wolbachia
		1465	Wolbachia endosymbiont of Aphrophora quadrinotata gene for 16S ribosomal RNA, partial sequence	AB772260.1	98%	Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; Anaplasmataceae; Wolbachieae; Wolbachia
		1291	Wolbachia endosymbiont of Culex quinquefasciatus Pel strain wPip 23S ribosomal RNA, complete sequence	NR_076253.1	99%	Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; Anaplasmataceae; Wolbachieae; Wolbachia
1755	Ma. titillans	1684	Gilliamella apicola strain wkB1 23S ribosomal RNA, complete sequence	NR_122000.1	94%	Bacteria; Proteobacteria; Gammaproteobacteria; Orbales; Orbaceae; Gilliamella
		1405	Wolbachia endosymbiont of Aphrophora quadrinotata gene for 16S ribosomal RNA, partial sequence	AB772260.1	99%	Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; Anaplasmataceae; Wolbachieae; Wolbachia
1756	Ma. titillans	1748	Helicobacter sp. 'Seymour B52 Bird-C' 23S ribosomal RNA gene, partial sequence	AY596231.1	93%	Bacteria; Proteobacteria; Epsilonproteobacteria; Campylobacterales; Helicobacteraceae; Helicobacter
		1107	Wolbachia endosymbiont of Culex quinquefasciatus Pel strain wPip 23S ribosomal RNA, complete sequence	NR_076253.1	99%	Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; Anaplasmataceae; Wolbachieae; Wolbachia
		868	Frischella perrara strain PEB0191, complete genome	CP009056.1	96%	Bacteria; Proteobacteria; Gammaproteobacteria; Orbales; Orbaceae; Frischella
		742	Gilliamella apicola strain wkB1, complete genome	CP007445.1	98%	Bacteria; Proteobacteria; Gammaproteobacteria; Orbales; Orbaceae; Gilliamella
1757	Ps. albipes	2442	Blechomonas keelingi isolate B07-22 18S small subunit ribosomal RNA gene, partial sequence	KF054130.1	89%	Eukaryota; Discoba; Euglenozoa; Kinetoplastea; Metakinetoplastina; Trypanosomatida; Trypanosomatidae; Blechomonas

NGS pool	Mosquito species	contig length (bps)	NCBI blastn Hit Description	Accession Hit	Per. Id.	Taxonomy
		1583	Spiroplasma floricola 23-6 chromosome, complete genome	CP025057.1	99%	Bacteria; Tenericutes; Mollicutes; Entomoplasmatales; Spiroplasmataceae; Spiroplasma
		1530	Encephalitozoon hellem ATCC 50504 chromosome VI, complete sequence	CP002718.1	99%	Eukaryota; Fungi; Fungi incertae sedis; Microsporidia; Unikaryonidae; Encephalitozoon
		1063	Trypanosoma theileri OSK-16 genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2,	LC618031.1	92%	Eukaryota; Discoba; Euglenozoa; Kinetoplastea; Metakinetoplastina; Trypanosomatida; Trypanosomatidae; Trypanosoma
1759	Cq. venezuelensis	1685	Wolbachia sp. wRi strain wRi 23S ribosomal RNA, complete sequence	NR_076629.1	99%	Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; Anaplasmataceae; Wolbachieae; Wolbachia
1761	Ps. albipes	2521	Providencia rettgeri strain FDAARGOS_330 chromosome, complete genome	CP027418.1	98%	Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacterales; Morganellaceae; Providencia
		1059	Asaia siamensis gene for 23S rRNA, partial sequence, strain: BCC 12268	AB255430.1	99%	Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales; Acetobacteraceae; Asaia
		1053	Leuconostoc ficulneum 23S rRNA gene, ITS2 and 5S rRNA gene, type strain CECT 5747T	AM773715.1	94%	Bacteria; Firmicutes; Bacilli; Lactobacillales; Lactobacillaceae; Fructobacillus
1767	An. darlingi	2069	Rickettsia sp. MEAM1 (Bemisia tabaci), complete genome	CP016305.1	99%	Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; Rickettsiaceae; Rickettsieae; Rickettsia
1768	Oc. serratus	2409	Serratia marcescens strain SmUNAM836, complete genome	CP012685.1	96%	Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacterales;Yersiniaceae; Serratia
		1113	Helicobacter trogontum strain ATCC 43966 23S ribosomal RNA gene, partial sequence	AY596250.1	94%	Bacteria; Proteobacteria; Epsilonproteobacteria; Campylobacterales; Helicobacteraceae; Helicobacter.
		1024	Vorticella sp. FG-2015 voucher SZ2010101001 large subunit ribosomal RNA gene, partial sequence	KM222177.1	93%	Eukaryota; Sar; Alveolata; Ciliophora; Intramacronucleata; Oligohymenophorea; Peritrichia; Sessilida; Vorticellidae; Vorticella
1769	An. darlingi	2092	Bifidobacterium adolescentis strain 1-11 chromosome, complete genome	CP028341.1	99%	Bacteria; Actinobacteria; Bifidobacteriales; Bifidobacteriaceae; Bifidobacterium
1770	An. darlingi	2279	Arcobacter suis strain CECT7833T 23S ribosomal RNA gene, partial sequence	MG196029.1	96%	Bacteria; Proteobacteria; Epsilonproteobacteria; Campylobacterales; Campylobacteraceae; Arcobacter group; Arcobacter
		1955	Rickettsiella tipulae strain BBA296/BUR-D/1961 23S ribosomal RNA (rrl) gene, partial sequence	JF288933.1	98%	Bacteria; Proteobacteria; Gammaproteobacteria; Legionellales; Coxiellaceae; Rickettsiella

NGS pool	Mosquito species	contig length (bps)	NCBI blastn Hit Description	Accession Hit	Per. Id.	Taxonomy
		1315	Rhodoferax ferrireducens strain T118 23S ribosomal RNA gene, complete sequence	NR_076409.1	98%	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Comamonadaceae; Rhodoferax
		1661	Tetracyclus sp. TN-2014 isolate B16Tetracyclus 18S small subunit ribosomal RNA gene, partial sequence	KJ577873.1	87%	Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Fragilariophyceae; Fragilariophycidae; Tabellariales; Tabellariaceae; Tetracyclus
		719	Rhodoferax saidenbachensis strain DSM 22694, complete genome	CP019239.1	99%	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Comamonadaceae; Rhodoferax
1771	An. nuneztovari	1423	Spironema culicis 16S ribosomal RNA gene, partial sequence	AF166259.1	98%	Bacteria; Spirochaetes; Spirochaetales; Spirochaetaceae; Entomospira
		1189	Acinetobacter bereziniae strain XH901, complete genome	CP018259.1	99%	Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Moraxellaceae; Acinetobacter
		1184	Polaromonas naphthalenivorans strain CJ2 23S ribosomal RNA gene, complete sequence	NR_076475.1	97%	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Comamonadaceae; Polaromonas
1772	Cq. venezuelensis	2627	Wolbachia sp. wRi, complete genome	CP001391.1	99%	Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; Anaplasmataceae; Wolbachieae; Wolbachia
1773	Culex	2740	Gilliamella apicola strain wkB1, complete genome	CP007445.1	96%	Bacteria; Proteobacteria; Gammaproteobacteria; Orbales; Orbaceae; Gilliamella
		1900	Spiroplasma floricola 23-6 chromosome, complete genome	CP025057.1	99%	Bacteria; Tenericutes; Mollicutes; Entomoplasmatales; Spiroplasmataceae; Spiroplasma
1774	blood samples	1184	IS4-like element ISAba1 family transposase [Acinetobacter baumannii]	WP_079785781.1	100%	Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Moraxellaceae; Acinetobacter; Acinetobacter calcoaceticus/baumannii complex
		947	hypothetical protein IST4103_06878 [Burkholderia cenocepacia]	CAB5352905.1	100%	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Burkholderiaceae; Burkholderia; Burkholderia cepacia complex.
1775	Psorophora	5769	Spiroplasma floricola 23-6 chromosome, complete genome	CP025057.1	93%	Bacteria; Tenericutes; Mollicutes; Entomoplasmatales; Spiroplasmataceae; Spiroplasma
		1174	Wolbachia endosymbiont of Drosophila simulans wAu genome assembly, chromosome: 1	LK055284.1	99%	Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; Anaplasmataceae; Wolbachieae; Wolbachia
		1128	Spiroplasma floricola 23-6 chromosome, complete genome	CP025057.1	99%	Bacteria; Tenericutes; Mollicutes; Entomoplasmatales; Spiroplasmataceae; Spiroplasma

NGS pool	Mosquito species	contig length (bps)	NCBI blastn Hit Description	Accession Hit	Per. Id.	Taxonomy
1777	blood samples	1294	Cylindrotheca closterium internal transcribed spacer 1, 5.8S ribosomal RNA gene, internal transcribed spacer 2, and 28S ribosomal RNA gene, complete sequence	AF289049.1	94%	Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Bacillariophyceae; Bacillariophycidae; Bacillariales; Bacillariaceae; Cylindrotheca
		1201	Arcobacter suis CECT 7833 chromosome, complete genome	CP032100.1	100%	Bacteria; Proteobacteria; Epsilonproteobacteria; Campylobacterales; Campylobacteraceae; Arcobacter group; Arcobacter
		1040	Acinetobacter johnsonii XBB1, complete genome	CP010350.1	99%	Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Moraxellaceae; Acinetobacter
1778	blood samples	2506	Acidovorax sp. JS42, complete genome	CP000539.1	97%	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Comamonadaceae; Acidovorax
		2372	Diaphorobacter sp. JS3050 chromosome, complete genome	CP053553.1	100%	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Comamonadaceae; Diaphorobacter
		1944	Arcobacter suis CECT 7833 chromosome, complete genome	CP032100.1	100%	Bacteria; Proteobacteria; Epsilonproteobacteria; Campylobacterales; Campylobacteraceae; Arcobacter group; Arcobacter
		1744	Plasmodium vivax isolate LV1 28S ribosomal RNA gene, partial sequence	JQ627143.1	99%	Eukaryota; Sar; Alveolata; Apicomplexa; Aconoidasida; Haemosporida; Plasmodiidae; Plasmodium
		1534	Xanthomonas campestris pv. campestris strain 3811 chromosome, complete genome	CP025750.1	100%	Bacteria; Proteobacteria; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Xanthomonas
		1332	Acidovorax sp. JS42, complete genome	CP000539.1	100%	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Comamonadaceae; Acidovorax
		1230	Cruoricaptor ignavus strain M1214 chromosome, complete genome	CP063145.1	98%	Bacteria; Bacteroidetes; Flavobacteriia; Flavobacteriales; Weeksellaceae; Cruoricaptor
		1205	Diaphorobacter sp. JS3050 chromosome, complete genome	CP053553.1	96%	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Comamonadaceae; Diaphorobacter
		1167	Acinetobacter baumannii strain VB473 chromosome, complete genome	CP050388.1	100%	Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Moraxellaceae; Acinetobacter
		1117	Acinetobacter sp. NEB 394 chromosome, complete genome	CP055277.1	99%	Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Moraxellaceae; Acinetobacter