

# Insect-Specific Viruses: An overview and their relationship to arboviruses of concern to humans and animals

Valéria L. Carvalho<sup>a,b</sup>, Maureen T. Long<sup>a,\*</sup>

<sup>a</sup> Department of Comparative, Diagnostic, and Population Medicine, University of Florida, College of Veterinary Medicine, 1945 SW 16th Ave, Gainesville, FL, 32608, USA

<sup>b</sup> Department of Arbovirology and Hemorrhagic Fevers, Evandro Chagas Institute, Ministry of Health, Rodovia BR-316, Km 7, S/n, Ananindeua, Para, 67030-000, Brazil

## ARTICLE INFO

### Keywords:

Insect-specific virus  
Arboviruses  
Transmission  
Evolution  
Biotechnological application

## ABSTRACT

The group of Insect-specific viruses (ISVs) includes viruses apparently restricted to insects based on their inability to replicate in the vertebrates. Increasing numbers of ISVs have been discovered and characterized representing a diverse number of viral families. However, most studies have focused on those ISVs belonging to the family *Flaviviridae*, which highlights the importance of ISV study from other viral families, which allow a better understanding for the mechanisms of transmission and evolution used for this diverse group of viruses. Some ISVs have shown the potential to modulate arboviruses replication and vector competence of mosquitoes. Based on this, ISVs may be used as an alternative tool for biological control, development of vaccines, and diagnostic platforms for arboviruses. In this review, we provide an update of the general characteristics of ISVs and their interaction with arboviruses that infect vertebrates.

## 1. Introduction

Arboviruses are viruses transmitted by hematophagous arthropods such as mosquitoes, phlebotomine sandflies, ticks, and other vectors. Most arboviruses are maintained in nature by sylvatic complex maintenance cycles, whereby hematophagous arthropods infect vertebrate hosts through biting. In addition, transovarial and venereal transmission can occur between arthropod vectors (WHO, 1985). Many of these viruses cause human diseases, which may arise endemically and epidemically. Some of them, such as Zika virus (ZIKV), dengue virus (DENV), yellow fever virus (YFV), chikungunya virus (CHIKV), West Nile virus (WNV), Oropouche virus (OROV) and Mayaro virus (MAYV) cause serious public health problems worldwide (Weaver and Reisen, 2010; Vasconcelos and Calisher, 2016).

Recent advancements in sequencing technology, phylogenetic analysis, and mosquito microbiome research has led to the discovery of a variety of RNA viruses associated with hematophagous insects, including the Insect-specific viruses (ISVs) (Bolling et al., 2015). ISVs are a diverse group, including viruses that naturally infect mosquitoes and phlebotomine sandflies and replicate in these insects both *in vivo* and *in vitro* but the hallmark characteristic is the inability to replicate in the vertebrates and their cells (Bolling et al., 2015; Vasilakis and Tesh,

2015). Phylogenetic analyses indicate that some ISVs are closely related to arboviruses, while others are more related to plant viruses.

## 2. Virus families including insect-specific viruses

Many of the recently discovered ISVs belong to the family *Flaviviridae*, genus *Flavivirus* (Guzman et al., 2018), which is likely biased toward surveillance for threats to human and animal health. Nonetheless, ISVs are ubiquitous and have been detected in many species of mosquitoes and phlebotomine sandflies. Most ISVs are included in the following viral families or taxon: *Flaviviridae*, *Togaviridae*, *Peribunyaviridae*, *Phenuiviridae*, *Rhabdoviridae*, *Mesoniviridae*, *Tymoviridae*, *Birnaviridae*, *Nodaviridae*, *Reoviridae*, *Parvoviridae*, *Iridoviridae*, *Permutotetraviridae*, *Iflaviridae*, *Orthomyxoviridae*, *Totiviridae* and the proposed taxon *Negevirus* (Bolling et al., 2015; Agboli et al., 2019) (Table 1). In this section, we will describe some virus families, including ISVs.

### 2.1. Family flaviviridae

The first ISV discovered was the cell fusing agent virus (CFAV), isolated 45 years ago from *Aedes albopictus* cells. The next two viruses as

\* Corresponding author.

E-mail addresses: [valeriacarvalho@ufl.edu](mailto:valeriacarvalho@ufl.edu) (V.L. Carvalho), [longm@ufl.edu](mailto:longm@ufl.edu) (M.T. Long).

<https://doi.org/10.1016/j.virol.2021.01.007>

Received 9 September 2020; Received in revised form 21 December 2020; Accepted 11 January 2021

Available online 9 February 2021

0042-6822/© 2021 Published by Elsevier Inc.

**Table 1**  
Representatives of insect-specific viruses described in the literature.

Family/Order/Taxon	Insect-specific virus	Reference
Family <i>Flaviviridae</i>	Cell-fusing agent virus (CFAV)	Stollar and Thomas (1975)
	Kamiti River virus (KRV)	Sang et al. (2003)
	Culex flavivirus (CxFV)	Hoshino et al. (2007)
	Palm Creek virus (PCV)	Hobson-Peters et al. (2013)
	Nhumirim virus (NHUV)	Pauvolid-Corrêa et al. (2015)
	Aedes flavivirus (AEFV)	Hoshino et al. (2009)
	Hanko virus (HANKV)	Huhtamo et al. (2014)
	Anopheles flavivirus (AnFV)	Ajamma et al. (2018)
	Culiseta flavivirus (CsFV)	Misencik et al. (2016)
	Ilomantsi virus (ILOV)	Huhtamo et al. (2014)
	Calbertado (CLBOV)	Bolling et al. (2011)
	Mercadeo virus (MECDV)	Carrera et al. (2015)
	Aedes galloisi flavivirus (AGFV)	Hoshino et al. (2012)
	Culex theileri flavivirus (CTFV)	Sadeghi et al. (2017)
	Lammi virus (LAMV)	Huhtamo et al. (2009)
	Chaoyang virus (CHAOV)	Lee et al. (2013)
	Nanay virus (NANV)	Evangelista et al. (2013)
	Marisma mosquito virus (MMV)	Vázquez et al. (2012)
	La Tina virus (LTNV)	Guzman et al. (2018)
	Quang Binh virus (QBV)	Crabtree et al. (2009)
Family <i>Flaviviridae</i>	Spanish Culex flavivirus (SCxFV)	Vázquez et al. (2012)
	Nounane virus (NOUV)	Junglen et al. (2009)
	Barkedji virus (BJV)	Kolodziejek et al. (2013)
	Yamadai flavivirus (YDFV)	Kuwata et al. (2015)
	Nienokoue virus (NIEV)	Junglen et al. (2017)
	Spanish Ochlerotatus flavivirus (SOcFV)	Vázquez et al. (2012)
	Kampung Karu virus (KPKV)	Guzman et al. (2018)
	Mediterranean Ochlerotatus flavivirus (MoFV)	Vázquez et al. (2012)
	Long Pine Key virus (LPKV)	Guzman et al. (2018)
	Nakiwogo virus (NAKV)	Cook et al. (2009)
	Aedes cinereus flavivirus (AeciFV)	Calzolari et al. (2012)
	Czech Aedes vexans flavivirus (Czech AeveFV)	Calzolari et al. (2012)
	Assam virus	Guzman et al. (2018)
	Mediterranean Culex flavivirus	Vázquez et al. (2012)
	Parramata River virus (PaRV)	McLean et al. (2015)
	Phlebotomine-associated flavivirus (PAFV)	Moureaux et al. (2010)
	Xishuangbanna flavivirus (XFV)	Fan et al. (2016)
	Sabethes flavivirus (SbFV)	Gravina et al. (2019)
	Donggang virus (DGV)	Genebank Accession No. NC_016997
Family <i>Togaviridae</i>	Ecuador Paraiso Escondido virus (EPEV)	Alkan et al. (2015)
	Eilat virus (EILV)	Nasar et al. (2012)
	Tai Forest alphavirus (TALV)	Hermanns et al. (2017)
	Mwinilunga alphavirus (MWAV)	Torii et al. (2018)
	Agua Salud alphavirus (ASALV)	Hermanns et al. (2020)
	Badu virus (BADUV)	Hobson-Peters et al. (2016)
	Phasi Charoen virus (PCLV)	Yamao et al. (2009)
Order <i>Bunyavirales</i>	Herbert virus (HEBV)	Marklewitz et al. (2013)
	Cumuto virus (CUMV)	Auguste et al. (2014)
	Ferak virus (FERV)	Marklewitz et al. (2015)
	Anadyr virus (ANADV)	Shchetinin et al. (2014)
	Kibale virus (KIBV)	Marklewitz et al. (2013)
	Gouleako virus (GOLV)	Marklewitz et al. (2011)

**Table 1 (continued)**

Family/Order/Taxon	Insect-specific virus	Reference
Family <i>Mesoniviridae</i>	Jonchet virus (JONV)	Marklewitz et al. (2015)
	Tai virus (TAIV)	Marklewitz et al. (2013)
	Bontag Baru virus (BBaV)	Vasilakis et al. (2014b)
	Houston virus (HOUV)	Charles et al. (2018)
	Hana virus (HanaV)	Zirkel et al. (2013)
	Dak Nong virus (DKNV)	Kuwata et al. (2013)
	Yichang virus (YCV)	Wang et al. (2017)
	Nse virus (NseV)	Zirkel et al. (2013)
	Meno virus (MenoV)	Zirkel et al. (2013)
	Nam Dinh virus (NDiV)	Nga et al. (2011)
Family <i>Reoviridae</i>	Moumo virus (MoumoV)	Zirkel et al. (2013)
	Cavally virus (CAVV)	Zirkel et al. (2011)
	Kamphaeng Phet virus	Vasilakis et al. (2014a,b)
	Aedes pseudoscutellaris reovirus (APRV)	Attoui et al. (2005)
	Cimodo virus (CMDV)	Hermanns et al. (2014)
	Fako virus (FAKV)	Auguste et al. (2015)
	Banna virus (BAV)	Song et al. (2017)
	Mangshi virus (MSV)	Wang et al. (2015)
	High Island virus (HISLV)	Sadeghi et al. (2017)
	Parry's Lagoon virus (PLV)	Harrison et al. (2016)
Family <i>Rhabdoviridae</i>	Ninarumi virus (NRUV)	Sadeghi et al. (2017)
	Arboretum virus (ABTV)	Vasilakis et al. (2014a)
	Culex tritaeniorhynchus rhabdovirus (CTRV)	Kuwata et al. (2011)
	Moussa virus (MOUV)	Quan et al. (2010)
	Puerto Almendras virus (PTAMV)	Vasilakis et al. (2014b)
	Aedes Anphevirus (AeAV)	Parry and Asgari (2018)
	Merida virus (MERDV)	Charles et al. (2016)
	Balsa almendravirus (BALV)	Contreras et al. (2017)
	Coot Bay virus almendravirus (CBV)	Contreras et al. (2017)
	Rio Chico virus almendravirus (RCHV)	Contreras et al. (2017)
Family <i>Tymoviridae</i>	Culex originated Tymovirus-like virus (CuTLV)	Wang et al. (2012)
Family <i>Birnaviridae</i>	Ek Balam virus (EkBV)	Charles et al. (2019)
	Culex Y virus (CYV)	Marklewitz et al. (2012)
Family <i>Iridoviridae</i>	Espirito Santo virus (ESV)	Vancini et al. (2012)
	Mosquito X virus	Huang et al. (2013)
	Anopheles minimus Iridovirus (AMIV)	Huang et al. (2015)
	Negev virus (NEGV)	Vasilakis et al. (2013)
	Ngewotan virus (NWTV)	Vasilakis et al. (2013)
	Piura virus (PIUV)	Vasilakis et al. (2013)
	Loreto virus (LORV)	Vasilakis et al. (2013)
	Brejeira virus (BREV)	Nunes et al. (2016)
	Santana virus (SANV)	Vasilakis et al. (2013)
	Dezidougou virus (DEZV)	Vasilakis et al. (2013)
Taxon <i>Negevirus</i>	Goutanap virus (GANV)	Kallies et al. (2014)
	Wallerfield virus (WALV)	Auguste et al. (2014)
	Tanay virus (TANAV)	Nabeshima et al. (2014)
	Uxmal virus (UVMV)	Charles et al. (2018)
	Cordoba virus (CDBV)	Nunes et al. (2017)
	San Bernardo virus (SBDV)	Nunes et al. (2017)
	Biratnagar virus (BIRV)	Nunes et al. (2017)
	Big Cypress virus (BCPV)	Nunes et al. (2017)
	Castlereas virus (CsV)	O'Brien et al. (2017)
	Okushiri virus (OKV)	Kawakami et al. (2016)
Family <i>Nodaviridae</i>	Bustos virus (BUSV)	Fujita et al. (2017)
	Mangle virus (MaV)	Wang et al. (2019)
	Ochlerotatus caspius negevirus (OCNV)	Carapeta et al. (2015)
	Culex univittatus negevirus (CUNV)	Carapeta et al. (2015)
	Mosinovirus (MoNV)	Schuster et al. (2014)
Family <i>Parvoviridae</i>	Aedes albopictus densovirus 2 (AalDV 2)	Jousset et al. (1993)
	Culex pipiens densovirus (CpDV)	Jousset et al. (2000)

(continued on next page)

Table 1 (continued)

Family/Order/Taxon	Insect-specific virus	Reference
	<i>Culex pipiens pallens</i> densovirus (CpPDV)	Zhai et al. (2008)
Family <i>Totiviridae</i>	Omono River virus (OmRV)	Isawa et al. (2011)
Family <i>Orthomyxoviridae</i>	Sinu virus (SINUV)	Contreras-Gutiérrez et al. (2017)
Family <i>Permutotereaviridae</i>	Sarawak virus (SWKV)	Sadeghi et al. (2017)
Family <i>Iflaviridae</i>	Shinobi tetravirus (SHTV)	Fujita et al. (2018)
	Armigeres iflavivirus (ArIFV)	Kobayashi et al. (2017)

well as this was designated as single-stranded positive-sense RNA viruses most closely related to the family *Flaviviridae* and genus *Flavivirus* (Simmonds et al., 2017). The CFAV was so called due to its ability to cause fusion of *A. albopictus* cells; yet, the virus did not replicate in the mammalian cell lines BHK-21, KB, and Vero (Stollar and Thomas, 1975). Fifteen years later, the CFAV genome was fully sequenced, showing that CFAV is distantly related to other flaviviruses (Cammisa-Parks et al., 1992).

Almost three decades passed between the CFAV isolation until the discovery of the second ISV, Kamiti River virus (KRV), isolated from *A. macintosh* mosquitoes in Kenya. KRV is more similar to CFAV, although both viruses were genetically distinct. Nonetheless, KRV was classified as an insect-specific flavivirus (ISF) able to replicate in C6/36 and AP-61 cells, but unable to replicate in the vertebrate cells (BHK-21 and LLC-MK2) or mice (Crabtree et al., 2003; Sang et al., 2003).

The third ISV described was the *Culex* flavivirus (CxFV), which was isolated from *Culex* spp. mosquitoes in 2007 in Japan with closes phylogenetic relationship to CFAV and KRV (Hoshino et al., 2007). CxFV replicated only in mosquito cell lines (C6/36 and AeA1-2) and did not show replication in either monkey or hamster cells (Vero and BHK-1).

Since then, many other insect-specific flaviviruses (ISFs) have been detected, some of them phylogenetically more distant from the first ISFs described. Based on their phylogenetic and antigenic relationship, ISFs are classified into two distinct groups. One group referred to as classical ISFs (cISF) includes the first ISVs discovered, such as CFAV, KRV, CxFV, which are phylogenetically more distant to other known flaviviruses. The second group, named dual-host affiliated ISFs (dISFs), comprises viruses that associate phylogenetically with mosquito/vertebrate flaviviruses, including arboviruses or dual host viruses, grouping within the mosquito-borne flavivirus clade (Blitvich and Firth, 2015) (Table 2). Phylogenetic analysis of the ISFs suggest that the group of cISF may represent possible ancestral of flaviviruses, because they are at the base of the phylogenetic tree of the genus *Flavivirus* and also, the cISFs are distinct from all other flaviviruses (Blitvich and Firth, 2015).

## 2.2. Family togaviridae

Four ISVs of single-stranded, positive-sense RNA belonging to the family *Togaviridae* are reported in the literature. The first insect-specific alphavirus (ISA) described was the Eilat virus (EILV), isolated from *Anopheles coustani* mosquitoes in the Negev desert of Israel demonstrating infection of mosquito cell lines such as *Aedes albopictus* (C6/36 and C7/10), *Culex tarsalis*, and *Phlebotomus papatasi* (PP-9), without the ability to infect the mammalian and avian cell lines: A6 (*Xenopus laevis*), Vero (African green monkey), BHK-21 (baby hamster kidney), HEK-293 (human embryonic kidney), NIH 3T3 (mouse fibroblast), duck embryo fibroblast (DEF) (Nasar et al., 2012). Based on sequencing, EILV grouped within the main clade of mosquito-borne alphaviruses phylogenetic tree with the most relatedness to the Western equine encephalitis antigenic complex.

The second ISA described was the Tai Forest alphavirus (TALV) detected in *C. decens* mosquitoes in the Ivory Coast. Phylogenetic analysis demonstrated that TALV is more related to EILV, forming a new clade of ISA within the alphaviruses phylogenetic tree (Hermanns et al.,

Table 2

Insect-specific flaviviruses according to their classification. Adapted from Guzman et al. (2018).

Classification	Insect-specific flavivirus
Classical ISFs (cISF)	Cell fusing agent virus Kamiti River virus Culex Flavivirus Hanko virus Aedes flavivirus Culex theileri flavivirus Quang Binh virus Nienokoue virus Nakiwogo virus Palm Creek virus Aedes cinereus flavivirus Aedes galloisi flavivirus Calbertado virus Czech Aedes vexans flavivirus Mercadeo virus Yamadai flavivirus Culiseta flavivirus Assam virus Mediterranean Culex flavivirus Parramatta River virus Phlebotomus-associated flavivirus Anopheles flavivirus Xishuangbanna flavivirus Sabethes flavivirus Nounane virus Barkedji virus Nhumirim virus Chaoyang virus Lammi virus Donggang virus Ilomantsi virus Marisma mosquito virus Nanay virus Ecuador Paraiso Escondido virus La Tina virus Kampung Karu virus Long Pine Key virus
Dual-host affiliated ISFs (dISFs)	

2017).

Recently, two other insect-specific alphaviruses (ISAs) consisting of the Mwinilunga alphavirus (MWAV) (Torii et al., 2018) and agua salud alphavirus (ASALV) (Hermanns et al., 2020). MWAV was isolated from *C. quinquefasciatus* mosquitoes in Zambia, whereas ASALV was detected in *C. declarator* in Panama. Phylogenetically, the MWAV grouped in the same clade with EILV and TALV, while the ASALV presented as a single deep rooting lineage, more closely related to EILV, TALV, and MWAV (Hermanns et al., 2020; Torii et al., 2018).

## 2.3. Families Peribunyaviridae and Phenuiviridae

The recently reclassified order *Bunyavirales* consists of two important families, *Peribunyaviridae* and the family *Phenuiviridae*, which contain viruses with negative-sense or ambi-sense single-stranded RNA and tripartite genomes (Hughes et al., 2020). The family *Peribunyaviridae* includes viruses of medical importance such as the OROV and La Crosse virus (LACV), which cause human disease outbreaks in the South and North Americas, respectively (Vasconcelos and Calisher, 2016). In the same way, the family *Phenuiviridae* comprises important pathogens such as the Rift Valley fever virus (RVFV) (Weaver and Reisen, 2010).

Recently described ISVs included the Badu virus (BADUV), a member of the family *Phenuiviridae*, and genus *Phasivirus*, isolated from *Culex* spp. mosquitoes captured in Northern Australia. The BADUV replicated in *Aedes* and *Culex* cell lines but failed to replicate in the cells like nonhuman primates (Vero), rodents (BHK-21), avians (DF-1 chicken embryo fibroblasts), and humans (SW-13 adenocarcinoma). Phylogenetic findings showed that BADUV grouped with previously described ISVs, the phasi charoen-like virus (PCLV), the Cumutu virus (CUMV),

and the Gouleako virus (GOLV). These viruses formed a cluster most related to the tick-born Uukuniemi complex, which cause diseases in humans (Hobson-Peters et al., 2016).

## 2.4. Taxon negevirus

The taxon *Negevirus* proposed by Vasilakis and colleagues (Vasilakis et al., 2013) includes ISVs with non-segmented, positive-sense, single-stranded RNA with genome sizes ranged from 7.039 nt to 10.054 nt in length (Nunes et al., 2017). These viruses have been detected in different parts of the world from several species of mosquitoes and phlebotomine sandflies; nonetheless, it is possible that these viruses infect other insects, even those that are non-hematophagous, which indicates the need to examine the occurrence of negevirus and possibly other ISVs from other viral families in other insects.

Like all other ISVs, negevirus have shown a lack of ability to replicate in the vertebrate cells such as African green monkey kidney (Vero), baby hamster kidney (BHK-21) and human embryonic kidney (HEK293), or laboratory animals but replicate in mosquito cell lines from *Drosophila melanogaster*, *Ae. albopictus* (C6/36 and C7/10), *Anopheles albimanus*, *An. gambiae*, *Culex tarsalis*, and *Phlebotomus papatasi* up to titers as high as  $10^{10}$  PFU/mL (Vasilakis et al., 2013). Negevirus described in the literature include the Negev virus (NEGV), Piura virus (PIUV), Loreto virus (LORV), Dezidougou virus (DEZV), Santana virus (SANV), ngewotan virus (NWTV), goutanap virus (GANV) (Kallies et al., 2014; Vasilakis et al., 2013). Many other viruses belonging to this taxon have been recently discovered (Table 1).

The negevirus studied present one or three open reading frames (ORFs), with most having three. ORF 1 (large) encodes the viral polymerase protein, whereas ORF 2 (medium) encodes the glycoprotein, and ORF 3 (small) encodes the membrane proteins (Fig. 1). Based on their phylogeny and genetic characteristics, the negevirus were classified by Kallies and colleagues in two main genera: *Nelorpivirus* and *Sandewavirus* (Kallies et al., 2014). Also, negevirus are more closely related to plant viruses in the genera *Cilevirus*, *Higrevirus*, and *Blunervirus*, suggesting that plants may play a role in the natural transmission cycle of negevirus (Nunes et al., 2017).

Some of the ISFs, mainly dISFs, and ISAs are often detected by generic RT-PCR used for arbovirus surveillance, as well, by immunofluorescence assay using polyclonal antibodies for alphaviruses and flaviviruses. On the other hand, negevirus thus far have been detected by observation of cytopathic effect in mosquito cell lines, with final

identification completed by sequencing. Reliance on generation of CPE, limits detection of these viruses especially in the absence of which. Recently, the development of monoclonal antibodies against Australian negevirus will assist in detection of these viruses (Colmant et al., 2020).

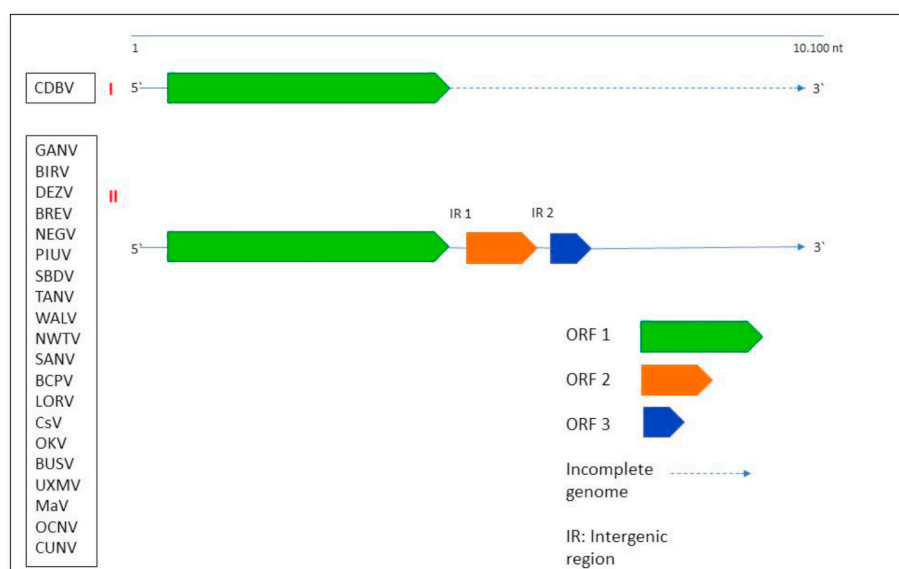
Even though several negevirus have been isolated and characterized, the knowledge about them is limited. Further studies are needed to understand better mechanisms of virus persistence, how they are acquired and transmitted by mosquitoes and sandflies, and what's their relationship with arboviruses and plant viruses.

## 2.5. Family tymoviridae

The viruses included in the family *Tymoviridae* have positive-sense, single-stranded RNA with a genome of approximately 6 to 7.5 kb (ICTV, 2009). Member of this family are classically plant viruses, some of which cause severe agriculture losses. However, several ISVs have been classified as tymoviruses in recent publications. The first ISV in the family *Tymoviridae* described was the *Culex* originated Tymoviridae-like virus (CuTLV) isolated from *Culex* mosquitoes in China (Wang et al., 2012). The CuTLV replicated in the *Ae. albopictus* cell line C6/36, but did not replicate in the vertebrate cell lines, Vero and BHK-21 (Wang et al., 2012).

Recently, another ISV called the Ek Balam virus (EkBV) was detected from *Culex quinquefasciatus* in Mexico (Charles et al., 2019). These two viruses formed a basal clade of ISV in the Tymoviridae phylogenetic tree. Interestingly, EkBV and CuTLV grouped with a bat virus called bat tymo-like virus, which was detected from guano of *Myotis yumanensis*. It is unclear whether the bat tymo-like virus is an ISV or replicate in bats. A most likely hypothesis is that the virus was acquired through the consumption of virus-infected insects (Charles et al., 2019). The EkBV demonstrated replicate well in invertebrate cells such as *Anopheles gambiae* (Sua 4.0), C6/36 and *Culex tarsalis* (CT), but did not show replication in the vertebrate cell lines: Vero, Baby hamster kidney (BSR-T7/5) and duck embryonic fibroblast (DEF) (Charles et al., 2019).

These results reinforce the close relationship between some ISVs and plant viruses. Some aspects still need to be clarified, such as the link ISV-plant viruses, their evolution process, and whether these ISVs can also replicate in plants.



**Fig. 1.** Schematic representation of the genome organization of negevirus. I) The virus with one Open Reading Frame (ORF). II) Viruses with three ORFs. CDBV: Cordoba virus, GANV: goutanap virus, BIRV: Biratnagar virus, DEZV: Dezidougou virus, BREV: Brejeira virus, NEGV: Negev virus, PIUV: Piura virus, SBDV: San Bernardo virus, TANV: Tanay virus, WALV: wallerfield virus, NWTV: ngeowtan virus, SANV: Santana virus, BCPV: Big Cypress virus, LORV: Loreto virus, CsV: Castlere virus, OKV: Okushiri virus, BUSV: Bustos virus, UXMV: uxmal virus, MaV: Manglie virus, OCNV: Ochlerotatus caspius negevirus, CUNV: Culex univittatus negevirus. Adapted from (Nunes et al., 2017) (Single image, color).



### 3. Transmission

Arboviruses and ISVs infect hematophagous insects, but differently of the arboviruses that have transmission cycles involving invertebrate and vertebrate hosts, the ISVs do not appear involve vertebrate hosts in their cycles, since these viruses have not been detected from vertebrate hosts naturally. Also, they cannot replicate in the vertebrate cell lines and laboratory animals like newborn mice. However, the ISVs have been isolated from different species of mosquitoes and phlebotomine sandflies; furthermore, they can replicate in invertebrate cells like *A. albopictus* cells, clone C6/36, and C7/10, *C. quinquefasciatus* cells (Auguste et al., 2014; Kenney et al., 2014; Bolling et al., 2015; Guzman et al., 2018).

Several vertebrate cell lines have been used to observe replication of different ISVs such as Vero (monkey cells), BHK-21 (hamster cells), SW-13 (human cells), PS-EK (porcine cells), DF-1 (avian cells) but replication has not been detected (Bolling et al., 2015; Hermanns et al., 2020; Hoshino et al., 2007). Recent studies with the insect-specific alphavirus ASALV have supported the fact that the ISVs do not replicate in the vertebrate hosts showing that this virus has temperature-dependent replication in C6/36 cells since the number of copies of the genome virus decreased above 31 °C (Hermanns et al., 2020).

Recent studies have shown that the ISVs host restriction may occur at different levels of replication cycle due to the interaction between host, viral and environment factors (Elrefaey et al., 2020; Junglen et al., 2017). For example, the presence of incomplete structural genes may be causing the host restriction for some ISVs. Sequence alignment of the envelope protein of several ISVs and dual-host flaviviruses showed the lack of 8-residue sequence in the Domain III of ISVs, while it is conserved in the dual-host flaviviruses. This may implicate in the impediment of the virus particle internalization, once this is the function of the domain III of the envelope protein (McLean et al., 2015). Furthermore, aiming to study the host restriction through reverse genetics, the cISF Niénokoué virus (NIEV) was chimerized with the Yellow fever virus (YFV) (*Flavivirus*), whereby the YFV carried the envelope proteins of NIEV; the study demonstrated a block of entry, RNA replication and assembly in vertebrate cells (Junglen et al., 2017).

According to several studies, it seems that the major transmission mechanism used by the ISVs is the vertical transmission, which is the transmission from females to their offspring since the ISVs have been detected in all four stages of mosquito's life cycle. ISVs and arboviruses use vertical transmission for their maintenance in nature, including two mechanisms: the transovarial transmission, through which the viruses infect the germinal tissues of female mosquitoes. The second mechanism is the transovular, whereby the eggs are infected by the virus during their passage through the oviduct (Duggal et al., 2019; Rosen, 1988).

Field *C. pipiens* mosquitoes naturally infected with CxFV demonstrated that transovarial transmission is an efficient way to the maintenance of the virus; furthermore, CxFV was able to infect the ovaries of *C. pipiens* colonized in a laboratory (uninfected) since the replication of the virus in the ovaries and follicles is essential to the transovarial transmission (Saiyasombat et al., 2011). Evidence of vertical transmission was observed for the ISV *Aedes flavivirus* (AEFV); once adult male *A. albopictus* reared from field-larvae were found infected by the virus (Haddow et al., 2013). Other studies have supported vertical transmission as a means of ISVs maintenance, for example, CxFV was detected in eggs, larvae, and both male and female adult *C. pipiens* mosquitoes (Bolling et al., 2011, 2012). Otherwise, the ISF Palm Creek (PCV) could not be transmitted vertically to *C. annulirostris* progeny, since the offspring reared from infected adult mosquitoes were not infected (Hall-Mendelin et al., 2016).

Detection of the virus in both mosquito sexes suggests vertical transmission, but also can indicate venereal transmission, which is the transmission of the virus from a infected male mosquito to a female mosquito during copulation (Agarwal et al., 2017). Some studies have shown that venereal transmission can occur as one mechanism used for

ISVs, but it seems that it may have a minor role in comparison with the vertical transmission (Bolling et al., 2012; Saiyasombat et al., 2011).

The ISVs of the taxon *Negevirus* (Vasilakis et al., 2013) are more genetically related to plant viruses. This raises the hypothesis that the mosquitoes and phlebotomine sandflies acquire negevirus by feeding on plant juices like nectar or plant material in the aquatic environment during their first stages of life cycle (Vasilakis and Tesh, 2015). Based on this, other potential horizontal transmission routes are the possibility of infected larvae, and adults become a source of ISVs when they die. Also, ISVs may be transmitted by a mermithid vector to mosquito larvae in nature, since the infection rate of an ISV called mosquito Iridescent virus increased following the addition of parasitic mermithid of mosquito larvae *Strelkovimermis spiculatus* (Nematoda) (Agboli et al., 2019; Linley and Nielsen, 1968; Muttis et al., 2015).

Even with all the evidence showed here, we still need to understand more deeply the transmission routes used by ISVs, mainly because the majority of the studies have focused on ISFs, and as we know, there are many ISVs from other virus families and taxon that may present different mechanisms.

### 4. Evolution

Phylogenetic analyses have shown that some ISVs, mainly those included in the families *Flaviviridae* and *Togaviridae*, are closely related to human pathogenic arboviruses. Nonetheless, mammalian arboviruses can replicate in both vertebrate and invertebrate hosts, while the ISVs appear restrict to invertebrates. Thus, mammalian arboviruses may have evolved from ISVs to dual host viruses, suggesting that new arboviruses may emerge from ISVs (Öhlund et al., 2019). The opposite can also be assumed: arboviruses or dual-host viruses may have evolved becoming ISVs. Regarding ISVs and plant hosts, plant viruses may have evolved from ISVs that became capable of infecting plants, losing the need for an insect host; or still, plant viruses may have evolved gaining the ability of infect insects originating new ISVs (Bolling et al., 2015).

Studies have demonstrated that arthropods may have an essential role in the virus evolution because of the high abundance and diversity of arthropod-associated RNA viruses allowing for development of dual-host adaptation (Bolling et al., 2015; Cook et al., 2013; Li et al., 2015). Vertical transmission has been reported for ISVs, which indicates long-term coexistence in insect-hosts. Some ISVs have even integrated into their putative host's genomes (Ballinger et al., 2013; Crochu et al., 2004; Fort et al., 2012).

The findings of some studies support the hypothesis that arboviruses emerged from ISVs. For example, the BADUV is a possible ancestor of arboviruses from the *Phlebovirus* genus (Hobson-Peters et al., 2016). Furthermore, the ISAs EILV and TALV are basally related to human pathogenic alphaviruses belonging to the Western equine encephalitis complex, suggesting that these ISAs are ancestors to viruses in this complex (Hermanns et al., 2017; Nasar et al., 2012; Öhlund et al., 2019).

Also, cISFs, such as CFAV, KRV and CxFV, PCV, are in the base of the *Flavivirus* phylogenetic tree, indicating that these ISFs possibly gave rise to arboviruses within the genus (Blitvich and Firth, 2015; Öhlund et al., 2019). Otherwise, other ISF seem to have diverged from arboviruses or dual-host viruses. For example, the Nhumirim virus (NHUV), grouped phylogenetically within other dual-host mosquito flaviviruses, but did not replicate in mammalian cells (Kenney et al., 2014).

Insect-specific viruses of the family *Tymoviridae* and the taxon *Negevirus* are close phylogenetically to plant viruses, indicating those plant viruses may play a role in maintenance cycles of these ISVs. ISVs of the family *Tymoviridae* grouped as a basal clade in the *Tymoviridae* phylogenetic tree, suggesting that ISVs may be ancestors of these plant viruses (Charles et al., 2019). Regarding negevirus, they are also genetically related to plant viruses, but the evolutionary aspects, including the negevirus, are still not clear (Nunes et al., 2017).

The base composition and codon usage in viruses, especially RNA viruses, may be indicators of the evolutionary forces which determine

genomes. Would be very useful more studies including this aspects and ISVs due such information may clarify the evolutionary origins of these viruses and their profile favored by some host species, helping understand the host restriction presented by ISVs (Jenkins et al., 2001). For example, comparative analysis of the dinucleotide usage of NHUV and some CxSF, no Known vector and dual-host viruses (CxFV, Modoc virus: MODV and WNV, respectively) showed that NHUV and WNV presented similar CpG usage, what could suggest that NHUV is not an ISF, but may be a dual-host virus with a not recognized vertebrate host. Also, as already mentioned, is possible that NHUV have lost the ability to replicate in vertebrates, but still presents this codon usage (Blitvich and Firth, 2015).

## 5. Interaction insect-specific viruses and arboviruses

ISVs and arboviruses have been isolated from several species of mosquitoes and phlebotomine sandflies, sometimes sharing the same vector. For example, *Culex* mosquitoes captured in urban green areas and residential sites were found co-infected with the ISF CxFV and WNV (*Flavivirus*) (Newman et al., 2011). Since these viruses can share the same vector, one of the most important question is how these viruses interact in the insect host. In particular, what is the consequence of this interaction; does co-infection inhibit or enhance vertebrate arboviruses within the insect host?

Most published studies focus on the interaction in mosquito cells such as the lineage clone C6/36, while fewer have shown results *in vivo*. Differently of the mosquitoes (*in vivo*), C6/36 cells do not present a functional antiviral RNAi response (Bolling et al., 2015; Brackney et al., 2010), which can lead to different results. Despite this, some studies have demonstrated concordance of findings of both types of investigations (Romo et al., 2018).

As briefly described previously, *Culex* spp. mosquitoes captured in endemic areas of WNV transmission in the city of Chicago in the United States, were co-infected with the CxFV and the WNV, showing a positive ecological association between these two viruses. Since mosquitoes positive for WNV were four times more likely also to be infected with CxFV than were spatiotemporally matched WNV-negative pools, suggesting that the primary infection with CxFV may not exclude a secondary infection with WNV (Newman et al., 2011).

To further investigate these results, Newman and colleagues (Newman et al., 2017) compared the spatial and temporal distribution of CxFV during interepidemic and epidemic years for WNV in Chicago. Interestingly they found that CxFV and WNV were more prevalent in *Culex* mosquitoes during the epidemic year and that during both investigated periods, the infection rate of mosquitoes by CxFV was associated with environmental factors than coinfection with WNV. The authors suggested that these results reinforced the possibility that CxFV and WNV are ecologically associated. In contrast, a study investigated the interaction between CxFV and WNV in *Cx. quinquefasciatus* mosquitoes from the southeastern United States. It did not observe evidence of a positive association between these viruses (Crockett et al., 2012).

Another study that evaluated the interaction of CxFV and WNV *in vitro* and *in vivo* observed that C6/36 cells first infected with CxFV and by WNV 48 h later, resulted in significantly lower titers of WNV in the cells infected for both viruses, in comparison with the positive control (WNV only). CxFV growth titers in superinfected cells were similar to the positive control (CxFV only), which indicates that WNV did not interfere in the CxFV titers. Besides, *in vivo* experiments showed early suppression of WNV replication by persistent CxFV infection in *C. pipiens* mosquitoes (Bolling et al., 2012).

Kent and colleagues (Kent et al., 2010) observed that the growth of WNV in C6/36 cells and *C. quinquefasciatus* mosquitoes were not suppressed for the previous infection by CxFV. In contrast and surprisingly, WNV transmission was enhanced in *C. quinquefasciatus* mosquitoes from Honduras infected with both viruses (CxFV and WNV).

The ISF PCV, isolated from *Coquillettidia xanthogaster* mosquitoes collected in Australia, suppressed the replication in C6/36 cells of two flaviviruses of medical importance, the WNV and the Murray Valley encephalitis virus (MVEV) (Hobson-Peters et al., 2013). Also, *in vivo* experiments demonstrated that PCV decreased the WNV transmission in *C. annulirostris* mosquitoes from Australia (Hall-Mendelin et al., 2016). The ISF CFAV also demonstrated to interfere with DEN-1 and ZIKV (*Flavivirus*) replication in *A. aegypti* mosquitoes and C6/36 cells (Baidaliuk et al., 2019).

The ISF NHUV, isolated from *C. (Culex) chidesteri* mosquitoes in Brazil (Pauvolid-Corrêa et al., 2015), was also investigated for its potential to interact with human pathogenic arboviruses. The *in vitro* experiments in C6/36 cells showed that prior or concurrent infection by NHUV suppresses the replication of WNV, Saint Louis encephalitis virus (SLEV) (*Flavivirus*), and Japanese encephalitis virus (JEV) (*Flavivirus*), reaching over a million-fold and 10,000-fold reduction in titers (Kenney et al., 2014). Further reinforcing this, the *in vitro* and *in vivo* experiments demonstrated that NHUV interfered in WNV replication in C6/36 and C7-10 cells (both cell lines from *A. albopictus*). Also, the vector competence of *C. quinquefasciatus* mosquitoes of transmitting WNV was significantly lower when mosquitoes were previously inoculated by NHUV. Surprisingly, the authors did not observe differences in vector competence of *C. pipiens* mosquitoes (Goenaga et al., 2015).

Recently, another study assessed the interaction of NHUV with the ZIKV, dengue-2 virus (DENV-2) (*Flavivirus*) and CHIKV (*Alphavirus*) in C6/36 cells. The results of this study showed that NHUV caused 100,000-fold and 10,000-fold significant reductions in titer of ZIKV and DENV-2, respectively. This effect occurred both if NHUV was infected before or concurrent with infection of cells. However, only a 10-fold titer reduction was observed for CHIKV. NHUV interference may be restricted to viruses closely related to the *Flavivirus* genus, but further investigations are needed to clarify this hypothesis. Furthermore, in *A. aegypti* mosquitoes concomitantly exposed to NHUV and ZIKV had significantly lower ZIKV infection and transmission rates (Romo et al., 2018).

The interaction between arboviruses and ISVs from other viral families has also been studied. *A. albopictus* cells (C7-10) previously infected with the ISA EILV lead to homologous and heterologous interference. EILV delayed replication kinetics and reduced titers of the following superinfecting viruses by 10-10,000-fold: in Sindbis virus (SINV) (*Alphavirus*), Venezuelan equine encephalitis virus (VEEV) (*Alphavirus*), Eastern equine encephalitis virus (EEEV) (*Alphavirus*), Western equine encephalitis virus (WEEV) (*Alphavirus*) and CHIKV. Additionally, CHIKV dissemination was delayed in *A. aegypti* mosquitoes infected with EILV (Nasar et al., 2015a).

In some cases, the results of studies have been contradictory or inconclusive, suggesting that different species of mosquitoes, mosquitoes from different places, and different virus strains may influence the findings of experiments studying the interaction ISV-arbovirus.

## 6. Biotechnological application of insect-specific viruses

ISVs have value as possible agents of biological control of arbovirus transmission, vector control, vaccine, and diagnostic platforms. The use of ISVs as Biological Control agents is one of their possible applications in Public Health. Biological control tools include the use of natural predators or pathogenic agents aiming to minimize mosquito abundance or vector competence (Bolling et al., 2015). One aspect to consider is the ecological impact that could be generated with the decrease or elimination of some mosquito populations.

The bacteria *Wolbachia* is another example of agents used as a biological control for mosquitoes. *Wolbachia* is a maternally transmitted obligate intracellular bacteria that naturally infects several arthropod species (Hedges et al., 2008). The bacteria can manipulate insect reproduction using different mechanisms, including cytoplasmic incompatibility, besides uninfected females that mate with males carrying

*Wolbachia* can produce infertile eggs (Turelli and Hoffmann, 1995).

Based on this, *Wolbachia* reduces vector competence of mosquitoes in the transmission of arboviruses with importance in Public Health such as WNV, ZIKV, DENV and CHIKV mainly decreasing mosquitoes lifespan or blocking the virus replication (Glaser and Meola, 2010; Schultz et al., 2018; Xue et al., 2018). In light of recent findings, that *Wolbachia* can interact with ISV manipulating mosquito virome (Amuzu et al., 2018), it is crucial to investigate the interaction between *Wolbachia* and ISV (naturally present in some insects) or between the different components of the mosquito microbiome.

In the same way, since the ISVs suppress the replication of different arboviruses, their use as a potential biological control tool must be considered, although the relationship arbovirus-ISV should be more studied and comprised, allowing the possible future use of ISV as a biological control measure.

In addition to biological control of arbovirus transmission and vector control, another potential use of ISV is as a vaccine platform. For example, the ISA EILV, although genetically related to medically important alphaviruses, cannot replicate in the vertebrates due to blocks at attachment/entry and RNA replication (Nasar et al., 2012, 2015b). These safety characteristics are appealing to the development of vaccine candidates. Based on this, recombinant viruses (chimeras) composed of either EEEV, VEEV or CHIKV envelope proteins were generated with the safety properties of the EILV and the antigenic properties of pathogenic alphaviruses (Erasmus and Weaver, 2017). The chimeras EILV/VEEV and EILV/CHIKV were completely protective against lethal challenge of CHIKV and VEEV in mice models (Erasmus et al., 2017; Erasmus and Weaver, 2017).

ISVs can also be used as diagnostic platforms since the previously described chimeras showed the ability to bind antibodies from mice infected with EEEV, VEEV, and CHIKV due to their pathogenic counterparts (Erasmus et al., 2015). An IgM ELISA for CHIKV using EILV/CHIKV chimera as an antigen was developed, showing high sensitivity and specificity. This ELISA presented advantages when compared to traditional ELISA such as the safety of handle the chimera at biosafety level 1 laboratory, cheaper and efficient, high titers, fast production and they do not require concentration, purification, or inactivation (Erasmus et al., 2015; Erasmus and Weaver, 2017).

## 7. Conclusion

The growing number of ISVs discovered and characterized demonstrates that this group of viruses are diverse, worldwide spread, infect a variety of mosquitoes, and phlebotomine sandflies, probably have different mechanisms of evolution and to persist in nature. Despite this, the knowledge about ISVs is still limited, and some aspects need to be clarified, such as the mechanisms of transmission and evolution used for ISVs from different viral families since most evidence described in the literature is related to ISVs in the family *Flaviviridae*.

ISVs have shown modulate arboviruses replication in mosquitoes and their cells, decreasing virus titer and the competence of mosquitoes to transmit arbovirus, which highlights the potential use of ISVs as a tool for biological control of arboviruses. However, it seems that the modulation is dependent on the mosquito species, mosquito geographical origin, and virus strain. It is essential to study the possible modulation caused for other ISVs, never investigated, as well as to investigate the mechanisms of modulation. Besides, promising findings indicate the use of ISVs as a novel approach for the development of safer vaccines and diagnostic platforms for arboviruses.

In a world scenario of different arboviruses circulating epidemically and endemically causing serious problems in public health and no vaccines and drug treatment are available for most human pathogenic arboviruses, the ISVs appear as an alternative tool for arbovirus control efforts.

## Funding

This review is supported in part by the scholarship granted by the Brazilian Federal Foundation for Support and Evaluation of Graduate Education (Capes), in the scope of Procad-Amazonia-DRI, process number: 88881.369357/2019-01 as well as by the Fern-Audette Endowment and University of Florida.

## Author contribution

VLC: Conceptualization, Writing - Original Draft.  
MTL: Writing - Review & Editing, Supervision.

## Declaration of competing interest

The authors declare no conflict of interest.

## Acknowledgments

We thank to Instituto Evandro Chagas, especially the Program of Post-graduation in Virology, for the opportunity of developing collaborative work with University of Florida. We also thank to University of Florida, especially Sarah Beachboard for the writing review.

## References

- Agarwal, A., Parida, M., Dash, P.K., 2017. Impact of transmission cycles and vector competence on global expansion and emergence of arboviruses. *Rev. Med. Virol.* 27, 1–12. <https://doi.org/10.1002/rmv.1941>.
- Agboli, E., Leggewie, M., Altinli, M., Schnettler, E., 2019. Mosquito-specific viruses—transmission and interaction. *Viruses* 11, 1–26. <https://doi.org/10.3390/v11090873>.
- Ajamma, Y.U., Onchuru, T.O., Ouso, D.O., Omondi, D., Masiga, D.K., Villinger, J., 2018. Vertical transmission of naturally occurring Bunyamwera and insect-specific flavivirus infections in mosquitoes from islands and mainland shores of Lakes Victoria and Baringo in Kenya. *PLoS Neglected Trop. Dis.* 12, 1–16. <https://doi.org/10.1371/journal.pntd.0006949>.
- Alkan, C., Zapata, S., Bichaud, L., Moureau, G., Lemey, P., Firth, A.E., Grits, T.S., Gould, E.A., de Lamballerie, X., Depaquit, J., Charrel, R.N., 2015. Ecuador paraiso escondido virus, a new flavivirus isolated from new world sand flies in Ecuador, is the first representative of a novel clade in the genus flavivirus. *J. Virol.* 89, 11773–11785. <https://doi.org/10.1128/jvi.01543-15>.
- Amuzu, H.E., Tsyganov, K., Koh, C., Herbert, R.I., Powell, D.R., McGraw, E.A., 2018. *Wolbachia* enhances insect-specific flavivirus infection in *Aedes aegypti* mosquitoes. *Ecol. Evol.* 8, 5441–5454. <https://doi.org/10.1002/ece3.4066>.
- Attoui, H., Mohd Jaafar, F., Belhouichet, M., Biagini, P., Cantaloube, J.F., De Micco, P., De Lamballerie, X., 2005. Expansion of family Reoviridae to include nine-segmented dsRNA viruses: isolation and characterization of a new virus designated aedes pseudoscutellaris reovirus assigned to a proposed genus (Dinovernavirus). *Virology* 343, 212–223. <https://doi.org/10.1016/j.virol.2005.08.028>.
- Auguste, A.J., Carrington, C.V.F., Forrester, N.L., Popov, V.L., Guzman, H., Widen, S.G., Wood, T.G., Weaver, S.C., Tesh, R.B., 2014. Characterization of a novel Negev virus and a novel Bunyavirus isolated from *Culex* (Culex) declarator mosquitoes in Trinidad. *J. Gen. Virol.* 95, 481–485. <https://doi.org/10.1099/vir.0.058412-0>.
- Auguste, A.J., Kaelber, J.T., Fokam, E.B., Guzman, H., Carrington, C.V.F., Erasmus, J.H., Kamgang, B., Popov, V.L., Jakana, J., Liu, X., Wood, T.G., Widen, S.G., Vasilakis, N., Tesh, R.B., Chiu, W., Weaver, S.C., 2015. A newly isolated reovirus has the simplest genomic and structural organization of any reovirus. *J. Virol.* 89, 676–687. <https://doi.org/10.1128/jvi.02264-14>.
- Baidaliuk, A., Miot, E.F., Lequime, S., Moltini-Conclois, I., Delaigue, F., Dabo, S., Dickson, L.B., Aubry, F., Merklings, S.H., Cao-Lormeau, V.-M., Lambrechts, L., 2019. Cell-fusing agent virus reduces arbovirus dissemination in *Aedes aegypti* mosquitoes in vivo. *J. Virol.* 93, 1–17. <https://doi.org/10.1128/jvi.00705-19>.
- Ballinger, M.J., Bruenn, J.A., Kotov, A.A., Taylor, D.J., 2013. Selectively maintained paleoviruses in Holarctic water fleas reveal an ancient origin for phleboviruses. *Virology* 446, 276–282. <https://doi.org/10.1016/j.virol.2013.07.032>.
- Blitvich, B.J., Firth, A.E., 2015. Insect-specific Flaviviruses: A Systematic Review of Their Discovery, Host Range, Mode of Transmission, Superinfection Exclusion Potential and Genomic Organization. *Viruses*. <https://doi.org/10.3390/v7041927>.
- Bolling, B.G., Eisen, L., Moore, C.G., Blair, C.D., 2011. Insect-specific flaviviruses from *Culex* mosquitoes in Colorado, with evidence of vertical transmission. *Am. J. Trop. Med. Hyg.* 85, 169–177. <https://doi.org/10.4269/ajtmh.2011.10-0474>.
- Bolling, B.G., Olea-Popelka, F.J., Eisen, L., Moore, C.G., Blair, C.D., 2012. Transmission dynamics of an insect-specific flavivirus in a naturally infected *Culex pipiens* laboratory colony and effects of co-infection on vector competence for West Nile virus. *Virology* 427, 90–97. <https://doi.org/10.1016/j.virol.2012.02.016>.



- Bolling, B.G., Weaver, S.C., Tesh, R.B., Vasilakis, N., 2015. Insect-specific virus discovery: significance for the arbovirus community. *Viruses* 7, 4911–4928. <https://doi.org/10.3390/v7092851>.
- Brackney, D.E., Scott, J.C., Sagawa, F., Woodward, J.E., Miller, N.A., Schilkey, F.D., Mudge, J., Willusz, J., Olson, K.E., Blair, C.D., Ebel, G.D., 2010. C6/36 *Aedes albopictus* cells have a dysfunctional antiviral RNA interference response. *PLoS Neglected Trop. Dis.* 4, 24–27. <https://doi.org/10.1371/journal.pntd.0000856>.
- Calzolari, M., Zé-Zé, L., Rüzek, D., Vázquez, A., Jeffries, C., Defilippo, F., Osório, H.C., Kilian, P., Ruiz, S., Fooks, A.R., Maioli, G., Amaro, F., Tlustý, M., Figuerola, J., Medlock, J.M., Bonilauri, P., Alves, M.J., Sebesta, O., Tenorio, A., Vaux, A.G.C., Bellini, R., Gelbic, I., Sánchez-Seco, M.P., Johnson, N., Dottori, M., 2012. Detection of mosquito-only flaviviruses in Europe. *J. Gen. Virol.* 93, 1215–1225. <https://doi.org/10.1099/vir.0.040485-0>.
- Cammissa-Parks, H., Cisar, L.A., Kane, A., Stollar, V., 1992. The complete nucleotide sequence of cell fusing agent (CFA): homology between the nonstructural proteins encoded by CFA and the nonstructural proteins encoded by arthropod-borne flaviviruses. *Virology* 189, 511–524. [https://doi.org/10.1016/0042-6822\(92\)90575-A](https://doi.org/10.1016/0042-6822(92)90575-A).
- Carapeta, S., do Bem, B., McGuinness, J., Esteves, A., Abecasis, A., Lopes, A., de Matos, A. P., Piedade, J., de Almeida, A.P.G., Parreira, R., 2015. Negevirus found in multiple species of mosquitoes from southern Portugal: isolation, genetic diversity, and replication in insect cell culture. *Virology* 483, 318–328. <https://doi.org/10.1016/j.virol.2015.04.021>.
- Carrera, J.P., Guzman, H., Beltrán, D., Díaz, Y., López-Vergès, S., Torres-Cosme, R., Popov, V., Widen, S.G., Wood, T.G., Weaver, S.C., Cáceres-Carrera, L., Vasilakis, N., Tesh, R.B., 2015. Mercadeo virus: a novel mosquito-specific flavivirus from Panama. *Am. J. Trop. Med. Hyg.* 93, 1014–1019. <https://doi.org/10.4269/ajtmh.15-0117>.
- Charles, J., Firth, A.E., Loroño-Pino, M.A., García-Rejon, J.E., Farfan-Ale, J.A., Ian Lipkin, W., Blitvich, B.J., Briese, T., 2016. Merida virus, a putative novel rhabdovirus discovered in culex and ochlerotatus spp. Mosquitoes in the Yucatan peninsula of Mexico. *J. Gen. Virol.* 97, 977–987. <https://doi.org/10.1099/jgv.0.000424>.
- Charles, J., Tangudu, C.S., Hurt, S.L., Tumescheit, C., Firth, A.E., García-Rejon, J.E., Machain-Williams, C., Blitvich, B.J., 2019. Discovery of a novel Tymoviridae-like virus in mosquitoes from Mexico. *Arch. Virol.* 164, 649–652. <https://doi.org/10.1007/s00705-018-4098-x>.
- Charles, J., Tangudu, C.S., Hurt, S.L., Tumescheit, C., Firth, A.E., García-Rejon, J.E., Machain-Williams, C., Blitvich, B.J., 2018. Detection of novel and recognized RNA viruses in mosquitoes from the Yucatan Peninsula of Mexico using metagenomics and characterization of their in vitro host ranges. *J. Gen. Virol.* 99, 1729–1738. <https://doi.org/10.1099/jgv.0.001165>.
- Colmant, A.M.G., O'Brien, C.A., Newton, N.D., Watterson, D., Hardy, J., Coulbaly, F., Bielefeldt-Ohmann, H., Warrilow, D., Huang, B., Paramitha, D., Harrison, J.J., Hall, R.A., Hobson-Peters, J., 2020. Novel monoclonal antibodies against Australian strains of negevirus and insights into virus structure, replication and host restriction. *J. Gen. Virol.* 101546, 1–13. <https://doi.org/10.1099/jgv.0.001388>.
- Contreras-Gutiérrez, M.A., Nunes, M.R.T., Guzmán, H., Uribe, S., Gómez, J.C.G., Vasconcelos, J.D.S., Cardoso, J.F., Popov, V.L., Widen, S.G., Wood, T.G., Vasilakis, N., Tesh, R.B., 2017. Sinu virus, a novel and divergent orthomyxovirus related to members of the genus thogotovirus, isolated from mosquitoes in Colombia. *Virology* 501, 166–175. <https://doi.org/10.1016/j.gde.2016.03.011>.
- Contreras, M.A., Eastwood, G., Guzman, H., Popov, V., Savit, C., Uribe, S., Kramer, L.D., Wood, T.G., Widen, S.G., Fish, D., Tesh, R.B., Vasilakis, N., Walker, P.J., 2017. Almendraviruses: a proposed new genus of rhabdoviruses isolated from mosquitoes in tropical regions of the Americas. *Am. J. Trop. Med. Hyg.* 96, 100–109. <https://doi.org/10.4269/ajtmh.16-0403>.
- Cook, S., Chung, B.Y.W., Bass, D., Moureau, G., Tang, S., McAlister, E., Culverwell, C.L., Glucksmann, E., Wang, H., Brown, T.D.K., Gould, E.A., Harbach, R.E., De Lamballerie, X., Firth, A.E., 2013. Novel virus discovery and genome reconstruction from field rna samples reveals highly divergent viruses in dipteran hosts. *PLoS One* 8, 1–22. <https://doi.org/10.1371/journal.pone.0080720>.
- Cook, S., Moureau, G., Harbach, R.E., Mukwaya, L., Goodger, K., Ssenfuka, F., Gould, E., Holmes, E.C., de Lamballerie, X., 2009. Isolation of a novel species of flavivirus and a new strain of Culex flavivirus (Flaviviridae) from a natural mosquito population in Uganda. *J. Gen. Virol.* 90, 2669–2678. <https://doi.org/10.1099/vir.0.014183-0>.
- Crabtree, M.B., Nga, P.T., Miller, B.R., 2009. Isolation and characterization of a new mosquito flavivirus, Quang Binh virus, from Vietnam. *Arch. Virol.* 154, 857–860. <https://doi.org/10.1007/s00705-009-0373-1>.
- Crabtree, M.B., Sang, R.C., Stollar, V., Dunster, L.M., Miller, B.R., 2003. Genetic and phenotypic characterization of the newly described insect flavivirus, Kamiti River virus. *Arch. Virol.* 148, 1095–1118. <https://doi.org/10.1007/s00705-003-0019-7>.
- Crochu, S., Cook, S., Attoui, H., Charrel, R.N., De Chesne, R., Belhouchet, M., Lemasson, J.J., de Micco, P., de Lamballerie, X., 2004. Sequences of flavivirus-related RNA viruses persist in DNA form integrated in the genome of Aedes spp. mosquitoes. *J. Gen. Virol.* 85, 1971–1980. <https://doi.org/10.1099/vir.0.079850-0>.
- Crockett, R.K., Burkhalter, K., Mead, D., Kelly, R., Brown, J., Varnado, W., Roy, A., Horiuchi, K., Biggerstaff, B.J., Miller, B., Nasci, R., 2012. Culex flavivirus and West Nile virus in Culex quinquefasciatus populations in the southeastern United States. *J. Med. Entomol.* 49, 165–174. <https://doi.org/10.1603/me11080>.
- Duggal, N.K., Langwig, K.E., Ebel, G.D., Brault, A.C., 2019. On the fly: interactions between birds, mosquitoes, and environment that have molded West Nile virus genomic structure over two decades. *J. Med. Entomol.* 56, 1467–1474. <https://doi.org/10.1093/jme/tjz112>.
- Elrefaey, A.M., Abdelnabi, R., Rosas, A.L.R., Wang, L., Basu, S., Delang, L., 2020. Understanding the mechanisms underlying host restriction of insect-specific viruses. *Viruses* 12, 1–19. <https://doi.org/10.3390/v12090964>.
- Erasmus, J.H., Auguste, A.J., Kaelber, J.T., Luo, H., Rossi, S.L., Fenton, K., Leal, G., Kim, D.Y., Chiu, W., Wang, T., Frolov, I., Nasar, F., Weaver, S.C., 2017. A chikungunya fever vaccine utilizing an insect-specific virus platform. *Nat. Med.* 23, 192–199. <https://doi.org/10.1038/nm.4253>.
- Erasmus, J.H., Needham, J., Raychaudhuri, S., Diamond, M.S., Beasley, D.W.C., Morkowski, S., Salje, H., Fernandez Salas, I., Kim, D.Y., Frolov, I., Nasar, F., Weaver, S.C., 2015. Utilization of an Eilat virus-based chimera for serological detection of chikungunya infection. *PLoS Neglected Trop. Dis.* 9, 1–16. <https://doi.org/10.1371/journal.pntd.0004119>.
- Erasmus, J.H., Weaver, S.C., 2017. Biotechnological applications of an insect-specific alphavirus. *DNA Cell Biol.* 36, 1045–1049. <https://doi.org/10.1089/dna.2017.4019>.
- Evangelista, J., Cruz, C., Guevara, C., Astete, H., Carey, C., Kochel, T.J., Morrison, A.C., Williams, M., Halsey, E.S., Forshey, B.M., 2013. Characterization of a novel flavivirus isolated from Culex (Melanoconion) ocosma mosquitoes from Iquitos, Peru. *J. Gen. Virol.* 94, 1266–1272. <https://doi.org/10.1099/vir.0.050575-0>.
- Fan, H., Zhao, Q., Guo, X., Sun, Q., Zuo, S., Wu, C., Zhou, H., An, X., Pei, G., Tong, Y., Zhang, J., Shi, T., 2016. Complete genome sequence of Xishuangbanna flavivirus, a novel mosquito-specific flavivirus from China. *Arch. Virol.* 161, 1723–1727. <https://doi.org/10.1007/s00705-016-2827-6>.
- Fort, P., Albertini, A., Van-Hua, A., Berthomieu, A., Roche, S., Delsuc, F., Pasteur, N., Capi, P., Gaudin, Y., Weill, M., 2012. Fossil rhabdoviral sequences integrated into arthropod genomes: ontogeny, evolution, and potential functionality. *Mol. Biol. Evol.* 29, 381–390. <https://doi.org/10.1093/molbev/msr226>.
- Fujita, R., Kato, F., Kobayashi, D., Murota, K., Takasaki, T., Tajima, S., Lim, C.K., Saijo, M., Isawa, H., Sawabe, K., 2018. Persistent viruses in mosquito cultured cell line suppress multiplication of flaviviruses. *Heliyon* 4, e00736. <https://doi.org/10.1016/j.heliyon.2018.e00736>.
- Fujita, R., Kuwata, R., Kobayashi, D., Bertuso, A.G., Isawa, H., Sawabe, K., 2017. Bustos virus, a new member of the negevirus group isolated from a Mansonia mosquito in the Philippines. *Arch. Virol.* 162, 79–88. <https://doi.org/10.1007/s00705-016-3068-4>.
- Glaser, R.L., Meola, M.A., 2010. The native Wolbachia endosymbionts of Drosophila melanogaster and Culex quinquefasciatus increase host resistance to west Nile virus infection. *PLoS One* 5, 1–11. <https://doi.org/10.1371/journal.pone.0011977>.
- Goenaga, S., Kenney, J.L., Duggal, N.K., Delorey, M., Ebel, G.D., Zhang, B., Levis, S.C., Enria, D.A., Brault, A.C., 2015. Potential for co-infection of a mosquito-specific flavivirus, nhumirim virus, to block west Nile virus transmission in mosquitoes. *Viruses* 7, 5801–5812. <https://doi.org/10.3390/v7112911>.
- Gravina, H.D., Suzukawa, A.A., Zanluca, C., Cardoso Segovia, F.M., Tschá, M.K., Martins da Silva, A., Faoro, H., da Silva Ribeiro, R., Mendoza Torres, L.P., Rojas, A., Ferrerira, L., Costa Ribeiro, M.C.V. da, Delfraro, A., Duarte dos Santos, C.N., 2019. Identification of insect-specific flaviviruses in areas of Brazil and Paraguay experiencing endemic arbovirus transmission and the description of a novel flavivirus infecting Sabethes belisarioi. *Virology* 527, 98–106. <https://doi.org/10.1016/j.virol.2018.11.008>.
- Guzman, H., Contreras-Gutiérrez, M.A., Travassos da Rosa, A.P.A., Nunes, M.R.T., Cardoso, J.F., Popov, V.L., Young, K.L., Savit, C., Wood, T.G., Widen, S.G., Watts, D. M., Hanley, K.A., Perera, D., Fish, D., Vasilakis, N., Tesh, R.B., 2018. Characterization of three new insect-specific flaviviruses: their relationship to the mosquito-borne flavivirus pathogens. *Am. J. Trop. Med. Hyg.* 98, 410–419. <https://doi.org/10.4269/ajtmh.17-0350>.
- Haddow, Andrew D., Guzman, H., Popov, V.L., Wood, T.G., Widen, S.G., Haddow, Alastair D., Tesh, R.B., Weaver, S.C., 2013. First isolation of Aedes flavivirus in the Western Hemisphere and evidence of vertical transmission in the mosquito Aedes (Stegomyia) albopictus (Diptera: Culicidae). *Virology* 440, 134–139. <https://doi.org/10.1016/j.virol.2012.12.008>.
- Hall-Mendelin, S., McLean, B.J., Bielefeldt-Ohmann, H., Hobson-Peters, J., Hall, R.A., Van Den Hurk, A.F., 2016. The insect-specific Palm Creek virus modulates West Nile virus infection in and transmission by Australian mosquitoes. *Parasites Vectors* 9, 1–10. <https://doi.org/10.1186/s13071-016-1683-2>.
- Harrison, J.J., Warrilow, D., McLean, B.J., Watterson, D., O'Brien, C.A., Colmant, A.M.G., Johansen, C.A., Barnard, R.T., Hall-Mendelin, S., Davis, S.S., Hall, R.A., Hobson-Peters, J., 2016. A new orbivirus isolated from mosquitoes in North-Western Australia shows antigenic and genetic similarity to corripata virus but does not replicate in vertebrate cells. *Viruses* 8, 1–15. <https://doi.org/10.3390/v8050141>.
- Hedges, L.M., Brownlie, J.C., O'Neill, S.L., Johnson, K.N., 2008. Wolbachia and virus protection in insects. *Science* 80 (322), 702. <https://doi.org/10.1126/science.1162418>.
- Hermanns, K., Marklewitz, M., Zirkel, F., Overheul, G.J., Page, R.A., Loaiza, J.R., Drosten, C., van Rij, R.P., Junglen, S., 2020. Agua Salud alphavirus defines a novel lineage of insect-specific alphaviruses discovered in the New World. *J. Gen. Virol.* 101, 96–104. <https://doi.org/10.1099/jgv.0.001344>.
- Hermanns, K., Zirkel, F., Kopp, A., Marklewitz, M., Rwegu, I.B., Estrada, A., Gillespie, T. R., Drosten, C., Junglen, S., 2017. Discovery of a novel alphavirus related to Eilat virus. *J. Gen. Virol.* 98, 43–49. <https://doi.org/10.1099/jgv.0.000694>.
- Hermanns, K., Zirkel, F., Kurth, A., Drosten, C., Junglen, S., 2014. Cimodo virus belongs to a novel lineage of reoviruses isolated from African mosquitoes. *J. Gen. Virol.* 95, 905–909. <https://doi.org/10.1099/vir.0.062349-0>.
- Hobson-Peters, J., Warrilow, D., McLean, B.J., Watterson, D., Colmant, A.M.G., van den Hurk, A.F., Hall-Mendelin, S., Hastie, M.L., Gorman, J.J., Harrison, J.J., Prow, N.A., Barnard, R.T., Alcock, R., Johansen, C.A., Hall, R.A., 2016. Discovery and characterisation of a new insect-specific bunyavirus from Culex mosquitoes captured in northern Australia. *Virology* 489, 269–281. <https://doi.org/10.1016/j.virol.2015.11.003>.
- Hobson-Peters, J., Yam, A.W.Y., Lu, J.W.F., Setoh, Y.X., May, F.J., Kurucz, N., Walsh, S., Prow, N.A., Davis, S.S., Weir, R., Melville, L., Hunt, N., Webb, R.I., Blitvich, B.J.,



- Whelan, P., Hall, R.A., 2013. A new insect-specific flavivirus from northern Australia suppresses replication of West Nile virus and Murray Valley encephalitis virus in Co-infected mosquito cells. *PLoS One* 8, 1–12. <https://doi.org/10.1371/journal.pone.0056534>.
- Hoshino, K., Isawa, H., Tsuda, Y., Sawabe, K., Kobayashi, M., 2009. Isolation and characterization of a new insect flavivirus from *Aedes albopictus* and *Aedes flavopictus* mosquitoes in Japan. *Virology* 391, 119–129. <https://doi.org/10.1016/j.virol.2009.06.025>.
- Hoshino, K., Isawa, H., Tsuda, Y., Yano, K., Sasaki, T., Yuda, M., Takasaki, T., Kobayashi, M., Sawabe, K., 2007. Genetic characterization of a new insect flavivirus isolated from *Culex pipiens* mosquito in Japan. *Virology* 359, 405–414. <https://doi.org/10.1016/j.virol.2006.09.039>.
- Hoshino, K., Takahashi-Nakaguchi, A., Isawa, H., Sasaki, T., Higa, Y., Kasai, S., Tsuda, Y., Sawabe, K., Kobayashi, M., 2012. Entomological surveillance for flaviviruses at migratory bird stopover sites in Hokkaido, Japan, and a new insect flavivirus detected in *Aedes galloisi* (Diptera: Culicidae). *J. Med. Entomol.* 49, 175–182. <https://doi.org/10.1603/me11123>.
- Huang, Y., Li, S., Zhao, Q., Pei, G., An, X., Guo, X., Zhou, H., Zhang, Z., Zhang, J., Tong, Y., 2015. Isolation and characterization of a novel invertebrate iridovirus from adult *Anopheles minimus* (AMIV) in China. *J. Invertebr. Pathol.* 127, 1–5. <https://doi.org/10.1016/j.jip.2015.01.007>.
- Huang, Y., Mi, Z., Zhuang, L., Ma, M., An, X., Liu, W., Cao, W., Tong, Y., 2013. Presence of Entomobirnaviruses in Chinese mosquitoes in the absence of Dengue virus coinfection. *J. Gen. Virol.* 94, 663–667. <https://doi.org/10.1099/vir.0.048231-0>.
- Hughes, H.R., Adkins, S., Alkhovsky, S., Beer, M., Blair, C., Calisher, C.H., Dreibot, M., Lambert, A.J., De Souza, W.M., Marklewitz, M., Nunes, M.R.T., Shi, X., 2020. ICTV virus taxonomy profile: Peribunyaviridae. *J. Gen. Virol.* 101, 1–2. <https://doi.org/10.1099/JGV.0.001365>.
- Huhtamo, E., Cook, S., Moureau, G., Uzcátegui, N.Y., Sironen, T., Kuivaniemi, S., Putkuri, N., Kurkela, S., Harbach, R.E., Firth, A.E., Vapalahti, O., Gould, E.A., de Lamballerie, X., 2014. Novel flaviviruses from mosquitoes: mosquito-specific evolutionary lineages within the phylogenetic group of mosquito-borne flaviviruses. *Virology* 464 (465), 320–329. <https://doi.org/10.1016/j.virol.2014.07.015>.
- Huhtamo, E., Putkuri, N., Kurkela, S., Manni, T., Vaheri, A., Vapalahti, O., Uzcátegui, N. Y., 2009. Characterization of a novel flavivirus from mosquitoes in northern Europe that is related to mosquito-borne flaviviruses of the tropics. *J. Virol.* 83, 9532–9540. <https://doi.org/10.1128/JVI.00529-09>.
- ICTV 9th Report, 2009. Taxonomy release. [https://talk.ictvonline.org/ictv-reports/ictv\\_9th\\_report/positive-sense-rna-viruses-2011/w/posrna\\_viruses/238/tymovirales](https://talk.ictvonline.org/ictv-reports/ictv_9th_report/positive-sense-rna-viruses-2011/w/posrna_viruses/238/tymovirales). (Accessed 29 July 2020).
- Isawa, H., Kuwata, R., Hoshino, K., Tsuda, Y., Sakai, K., Watanabe, S., Nishimura, M., Satho, T., Kataoka, M., Nagata, N., Hasegawa, H., Bando, H., Yano, K., Sasaki, T., Kobayashi, M., Mizutani, T., Sawabe, K., 2011. Identification and molecular characterization of a new nonsegmented double-stranded RNA virus isolated from *Culex* mosquitoes in Japan. *Virus Res.* 155, 147–155. <https://doi.org/10.1016/j.virusres.2010.09.013>.
- Jenkins, G.M., Pagel, M., Gould, E.A., Zanotto, P.M.D.A., Holmes, E.C., 2001. Evolution of base composition and codon usage bias in the genus *Flavivirus*. *J. Mol. Evol.* 52, 383–390. <https://doi.org/10.1007/s002390010168>.
- Jousset, F.X., Baquerizo, E., Bergoin, M., 2000. A new densovirus isolated from the mosquito *Culex pipiens* (Diptera: Culicidae). *Virus Res.* 67, 11–16. [https://doi.org/10.1016/S0168-1702\(00\)00128-3](https://doi.org/10.1016/S0168-1702(00)00128-3).
- Jousset, F.X., Barreau, C., Boublik, Y., Cornet, M., 1993. A Parvo-like virus persistently infecting a C6/36 clone of *Aedes albopictus* mosquito cell line and pathogenic for *Aedes aegypti* larvae. *Virus Res.* 29, 99–114. [https://doi.org/10.1016/0168-1702\(93\)90052-0](https://doi.org/10.1016/0168-1702(93)90052-0).
- Junglen, S., Kopp, A., Kurth, A., Pauli, G., Ellerbrok, H., Leendertz, F.H., 2009. A new flavivirus and a new vector: characterization of a novel flavivirus isolated from *Uranotaenia* mosquitoes from a tropical rain forest. *J. Virol.* 83, 4462–4468. <https://doi.org/10.1128/JVI.00014-09>.
- Junglen, S., Korries, M., Grasse, W., Wieseler, J., Kopp, A., Hermanns, K., León-Juárez, M., Drosten, C., Kümmerer, B.M., 2017. Host range restriction of insect-specific flaviviruses occurs at several levels of the viral life cycle. *mSphere* 2, 1–15. <https://doi.org/10.1128/mSphere.00375-16>.
- Kallies, R., Kopp, A., Zirkel, F., Estrada, A., Gillespie, T.R., Drosten, C., Junglen, S., 2014. Genetic characterization of goutanap virus, a novel virus related to negevirus, cileviruses and higneviruses. *Viruses* 6, 4346–4357. <https://doi.org/10.3390/v6114346>.
- Kawakami, K., Kurnia, Y.W., Fujita, R., Ito, T., Isawa, H., Asano, S., Ichiro, Binh, N.D., Bando, H., 2016. Characterization of a novel negevirus isolated from *Aedes* larvae collected in a subarctic region of Japan. *Arch. Virol.* 161, 801–809. <https://doi.org/10.1007/s00705-015-2711-9>.
- Kenney, J.L., Solberg, O.D., Langevin, S.A., Brault, A.C., 2014. Characterization of a novel insect-specific flavivirus from Brazil: potential for inhibition of infection of arthropod cells with medically important flaviviruses. *J. Gen. Virol.* 95, 2796–2808. <https://doi.org/10.1099/vir.0.068031-0>.
- Kent, R.J., Crabtree, M.B., Miller, B.R., 2010. Transmission of West Nile virus by *Culex quinquefasciatus* say infected with *Culex* flavivirus Izabal. *PLoS Neglected Trop. Dis.* 4, 1–12. <https://doi.org/10.1371/journal.pntd.0000671>.
- Kobayashi, D., Isawa, H., Fujita, R., Murota, K., Itokawa, K., Higa, Y., Katayama, Y., Sasaki, T., Mizutani, T., Iwanaga, S., Ohta, N., Garcia-Bertoso, A., Sawabe, K., 2017. Isolation and characterization of a new iflavivirus from *armigeres* spp. Mosquitoes in the Philippines. *J. Gen. Virol.* 98, 2876–2881. <https://doi.org/10.1099/jgv.0.000929>.
- Kolodziejek, J., Pachler, K., Bin, H., Mendelson, E., Shulman, L., Orshan, L., Nowotny, N., 2013. Barkedji virus, a novel mosquito-borne flavivirus identified in *Culex* perexiguus mosquitoes, Israel, 2011. *J. Gen. Virol.* 94, 2449–2457. <https://doi.org/10.1099/vir.0.056200-0>.
- Kuwata, R., Isawa, H., Hoshino, K., Tsuda, Y., Yanase, T., Sasaki, T., Kobayashi, M., Sawabe, K., 2011. RNA splicing in a new rhabdovirus from *Culex* mosquitoes. *J. Virol.* 85, 6185–6196. <https://doi.org/10.1128/JVI.00040-11>.
- Kuwata, R., Satho, T., Isawa, H., Yen, N.T., Phong, T.V., Nga, P.T., Kurashige, T., Hiramatsu, Y., Fukumitsu, Y., Hoshino, K., Sasaki, T., Kobayashi, M., Mizutani, T., Sawabe, K., 2013. Characterization of Dak Nong virus, an insect nidovirus isolated from *Culex* mosquitoes in Vietnam. *Arch. Virol.* 158, 2273–2284. <https://doi.org/10.1007/s00705-013-1741-4>.
- Kuwata, R., Sugiyama, H., Yonemitsu, K., Van Dung, N., Terada, Y., Taniguchi, M., Shimoda, H., Takano, A., Maeda, K., 2015. Isolation of Japanese encephalitis virus and a novel insect-specific flavivirus from mosquitoes collected in a cowshed in Japan. *Arch. Virol.* 160, 2151–2159. <https://doi.org/10.1007/s00705-015-2488-x>.
- Lee, J.S., Grubaugh, N.D., Kondig, J.P., Turell, M.J., Kim, H.C., Klein, T.A., O'Guinn, M. L., 2013. Isolation and genomic characterization of Chaoyang virus strain ROK144 from *Aedes vexans nipponii* from the Republic of Korea. *Virology* 435, 220–224. <https://doi.org/10.1016/j.virol.2012.10.020>.
- Li, C.X., Shi, M., Tian, J.H., Lin, X.D., Kang, Y.J., Chen, L.J., Qin, X.C., Xu, J., Holmes, E. C., Zhang, Y.Z., 2015. Unprecedented genomic diversity of RNA viruses in arthropods reveals the ancestry of negative-sense RNA viruses. *Elife* 1–26. <https://doi.org/10.7554/eLife.05378>, 2015.
- Linley, J.R., Nielsen, H.T., 1968. Transmission of a mosquito iridescent virus in *Aedes taeniorhynchus*. *J. Invertebr. Pathol.* 12, 17–24. [https://doi.org/10.1016/0022-2011\(68\)90238-3](https://doi.org/10.1016/0022-2011(68)90238-3).
- Marklewitz, M., Gloza-Rausch, F., Kurth, A., Kümmerer, B.M., Drosten, C., Junglen, S., 2012. First isolation of an entomobirnavirus from free-living insects. *J. Gen. Virol.* 93, 2431–2435. <https://doi.org/10.1099/vir.0.045435-0>.
- Marklewitz, M., Handrick, S., Grasse, W., Kurth, A., Lukashev, A., Drosten, C., Ellerbrok, H., Leendertz, F.H., Pauli, G., Junglen, S., 2011. Gouleako virus isolated from west african mosquitoes constitutes a proposed novel genus in the family bunyaviridae. *J. Virol.* 85, 9227–9234. <https://doi.org/10.1128/JVI.00230-11>.
- Marklewitz, M., Zirkel, F., Kurth, A., Drosten, C., Junglen, S., 2015. Evolutionary and phenotypic analysis of live virus isolates suggests arthropod origin of a pathogenic RNA virus family. *Proc. Natl. Acad. Sci. U. S. A.* 112, 7536–7541. <https://doi.org/10.1073/pnas.1502036112>.
- Marklewitz, M., Zirkel, F., Rwegu, I.B., Heidemann, H., Trippner, P., Kurth, A., Kallies, R., Briesse, T., Lipkin, W.I., Drosten, C., Gillespie, T.R., Junglen, S., 2013. Discovery of a unique novel clade of mosquito-associated bunyaviruses. *J. Virol.* 87, 12850–12865. <https://doi.org/10.1128/JVI.01862-13>.
- McLean, B.J., Hobson-Peters, J., Webb, C.E., Watterson, D., Prow, N.A., Nguyen, H.D., Hall-Mendelin, S., Warrilow, D., Johansen, C.A., Jansen, C.C., van den Hurk, A.F., Beebe, N.W., Schmetz, E., Barnard, R.T., Hall, R.A., 2015. A novel insect-specific flavivirus replicates only in *Aedes*-derived cells and persists at high prevalence in wild *Aedes vigilax* populations in Sydney, Australia. *Virology* 486, 272–283. <https://doi.org/10.1016/j.virol.2015.07.021>.
- Misencik, M.J., Grubaugh, N.D., Andreadis, T.G., Ebel, G.D., Armstrong, P.M., 2016. Isolation of a novel insect-specific flavivirus from *Culex* melanura in the Northeastern United States. *Vector Borne Zoonotic Dis.* 16, 181–190. <https://doi.org/10.1089/vbz.2015.1889>.
- Moureau, G., Ninove, L., Izri, A., Cook, S., Lamballerie, X. De, 2010. Flavivirus RNA in phlebotomine sandflies. *Vector Borne Zoonotic Dis.* 10, 195–197. <https://doi.org/10.1089/vbz.2008.0216>.
- Mutts, E., Miceli, M.V., Urrutia, M.I., García, J.J., 2015. Transmission of a pathogenic virus (Iridoviridae) of *Culex pipiens* larvae mediated by the mermithid *Strelkovimermis spiculatus* (Nematoda). *J. Invertebr. Pathol.* 129, 40–44. <https://doi.org/10.1016/j.jip.2015.05.011>.
- Nabeshima, T., Inoue, S., Okamoto, K., Posadas-Herrera, G., Yu, F., Uchida, L., Ichinose, A., Sakaguchi, M., Sunahara, T., Buerano, C.C., Tadeno, F.P., Orbita, I.B., Natividad, F.F., Morita, K., 2014. Tanay virus, a new species of virus isolated from mosquitoes in the Philippines. *J. Gen. Virol.* 95, 1390–1395. <https://doi.org/10.1099/vir.0.061887-0>.
- Nasar, F., Erasmus, J.H., Haddow, A.D., Tesh, R.B., Weaver, S.C., 2015a. Eilat virus induces both homologous and heterologous interference. *Virology* 484, 51–58. <https://doi.org/10.1016/j.virol.2015.05.009>.
- Nasar, F., Gorchakov, R.V., Tesh, R.B., Weaver, S.C., 2015b. Eilat virus host range restriction is present at multiple levels of the virus life cycle. *J. Virol.* 89, 1404–1418. <https://doi.org/10.1128/JVI.01856-14>.
- Nasar, F., Palacios, G., Gorchakov, R.V., Guzman, H., Travassos Da Rosa, A.P., Savji, N., Popov, V.L., Sherman, M.B., Lipkin, W.I., Tesh, R.B., Weaver, S.C., 2012. Eilat virus, a unique alphavirus with host range restricted to insects by RNA replication. *Proc. Natl. Acad. Sci. U. S. A.* 109, 14622–14627. <https://doi.org/10.1073/pnas.1204787109>.
- Newman, C.M., Cerutti, F., Anderson, T.K., Hamer, G.L., Walker, E.D., Kitron, U.D., Ruiz, M.O., Brawn, J.D., Goldberg, T.L., 2011. *Culex* flavivirus and West Nile virus mosquito coinfection and positive ecological association in Chicago, United States. *Vector Borne Zoonotic Dis.* 11, 1099–1105. <https://doi.org/10.1089/vbz.2010.0144>.
- Newman, C.M., Krebs, B.L., Anderson, T.K., Hamer, G.L., Ruiz, M.O., Brawn, J.D., Brown, W.M., Kitron, U.D., Goldberg, T.L., 2017. *Culex* flavivirus during West Nile virus epidemic and interepidemic years in Chicago, United States. *Vector Borne Zoonotic Dis.* 17, 567–575. <https://doi.org/10.1089/vbz.2017.2124>.
- Nga, P.T., de Parquet, M.C., Lauber, C., Parida, M., Nabeshima, T., Yu, F., Thuy, N.T., Inoue, S., Ito, T., Okamoto, K., Ichinose, A., Snijder, E.J., Morita, K., Gorbalenya, A. E., 2011. Discovery of the first insect nidovirus, a missing evolutionary link in the

- emergence of the largest rna virus genomes. *PLoS Pathog.* 7 <https://doi.org/10.1371/journal.ppat.1002215>.
- Nunes, M.R.T., Contreras-Gutierrez, M.A., Guzman, H., Martins, L.C., Barbirato, M.F., Saviti, C., Baltaj, V., Uribe, S., Vivero, R., Souza, J.D., Oliveira, H., Neto, J.P.N., Carvalho, V.L., Silva, S.P., da Cardoso, J.F., Oliveira, R.S. de, Lemos, P. da S., Wood, T.G., Widen, S.G., Vasconcelos, P.F.C., Fish, D., Vasilakis, N., Tesh, R.B., 2017. Genetic characterization, molecular epidemiology, and phylogenetic relationships of insect-specific viruses in the taxon Negevirus. *Virology* 504, 152–167. <https://doi.org/10.1016/j.virol.2017.01.022>.
- Nunes, M.R.T., Silva, S.P., Carvalho, V.L., Vasconcelos, J.M., Da Silva, D.E.A., Oliveira, L. F., Nunes Neto, J.P., Rodrigues, S.G., Azevedo, R.S.S., Monteiro, H.A.O., Cardoso, J. F., Guzman, H., Tesh, R., Vasconcelos, P.F.C., Vianez-Júnior, J.L.S.G., Martins, L.C., 2016. Emergence of new insect-restrictive viruses in the Amazon region. *Genome Announc.* 3, 2–3. <https://doi.org/10.1128/genomeA.00131-15>.
- O'Brien, C.A., McLean, B.J., Colmant, A.M.G., Harrison, J.J., Hall-Mendelin, S., van den Hurk, A.F., Johansen, C.A., Watterson, D., Bielefeldt-Olmann, H., Newton, N.D., Schulz, B.L., Hall, R.A., Hobson-Peters, J., 2017. Discovery and characterisation of castlereia virus, a new species of Negevirus isolated in Australia. *Evol. Bioinf.* 13, 10–12. <https://doi.org/10.1177/1176934317691269>.
- Öhlund, P., Lundén, H., Blomström, A.L., 2019. Insect-specific virus evolution and potential effects on vector competence. *Virus Gene.* 55, 127–137. <https://doi.org/10.1007/s11262-018-01629-9>.
- Parry, R., Asgari, S., 2018. Aedes anphevirus: an insect-specific virus distributed worldwide in Aedes aegypti mosquitoes that has complex interplays with Wolbachia and dengue virus infection in cells. *J. Virol.* 92, 1–19. <https://doi.org/10.1128/jvi.00224-18>.
- Paulovici-Corrêa, A., Solberg, O., Couto-Lima, D., Kenney, J., Serra-Freire, N., Brault, A., Nogueira, R., Langevin, S., Komar, N., 2015. Nhumirim virus, a novel flavivirus isolated from mosquitoes from the Pantanal, Brazil. *Arch. Virol.* 160, 21–27. <https://doi.org/10.1007/s00705-014-2219-8>.
- Quan, P.-L., Junglen, S., Tashmukhamedova, A., Conlan, S., Hutchison, S.K., Kurth, A., Ellerbrok, H., Egholm, M., Briese, T., Leendertz, F.H., Lipkin, W.I., 2010. Moussa virus: a new member of the Rhabdoviridae family isolated from Culex decens mosquitoes in Côte d'Ivoire. *Virus Res.* 147, 17–24. <https://doi.org/10.1161/CIRCULATIONAHA.110.956839>.
- Romo, H., Kenney, J.L., Blitvich, B.J., Brault, A.C., 2018. Restriction of Zika virus infection and transmission in Aedes aegypti mediated by an insect-specific flavivirus. *Emerg. Microb. Infect.* 7, 1–13. <https://doi.org/10.1038/s41426-018-0180-4>.
- Rosen, L., 1988. Further observations on the mechanism of vertical transmission of flaviviruses by Aedes mosquitoes. *Am. J. Trop. Med. Hyg.* 39, 123–126. <https://doi.org/10.4269/ajtmh.1988.39.123>.
- Sadeghi, M., Popov, V., Guzman, H., Phan, T.G., Vasilakis, N., Tesh, R., Delwart, E., 2017. Genomes of viral isolates derived from different mosquito species. *Virus Res.* 242, 49–57. <https://doi.org/10.1016/j.jgde.2016.03.011>.
- Saiyasombat, R., Bolling, B.G., Brault, A.C., Bartholomay, L.C., Blitvich, B.J., 2011. Evidence of efficient transovarial transmission of Culex flavivirus by Culex pipiens (Diptera: Culicidae). *J. Med. Entomol.* 48, 1031–1038. <https://doi.org/10.1603/me11043>.
- Sang, R.C., Gichogo, A., Gachoya, J., Dunster, M.D., Ofula, V., Hunt, A.R., Crabtree, M.B., Miller, B.R., Dunster, L.M., 2003. Isolation of a new flavivirus related to Cell fusing agent virus (CFAV) from field-collected flood-water Aedes mosquitoes sampled from a dambo in central Kenya. *Arch. Virol.* 148, 1085–1093. <https://doi.org/10.1007/s00705-003-0018-8>.
- Schultz, M.J., Tan, A.L., Gray, C.N., Isern, S., Michael, S.F., Frydman, H.M., Connor, J.H., 2018. Wolbachia wStri blocks Zika virus growth at two independent stages of viral replication. *mBio* 9, 1–15. <https://doi.org/10.1128/mBio.00738-18>.
- Schuster, S., Zirkel, F., Kurth, A., van Cleef, K.W.R., Drosten, C., van Rij, R.P., Junglen, S., 2014. A unique nodavirus with novel features: mosinovirus expresses two subgenomic RNAs, a capsid gene of unknown origin, and a suppressor of the antiviral RNA interference pathway. *J. Virol.* 88, 13447–13459. <https://doi.org/10.1128/jvi.02144-14>.
- Shchutin, A., Lvov, D., Alkhovsky, S., Shchelkanov, M., Aristova, V., Morozova, T., Gitelman, A., Deryabin, P., Botikov, A., 2014. Complete genome analysis of the Batai virus (BATV) and the new Anadyr virus (ANADV) of the Bunyamwera group (Bunyaviridae, Orthobunyavirus) isolated in Russia. *Vopr. Virusol.* 59, 16–22.
- Simmonds, P., Becher, P., Bukh, J., Gould, E.A., Meyers, G., Monath, T., Muerhoff, S., Pletnev, A., Rico-Hesse, R., Smith, D.B., Stapleton, J.T., 2017. ICTV virus taxonomy profile: Flaviviridae. *J. Gen. Virol.* 98, 2–3. <https://doi.org/10.1099/jgv.0.000672>.
- Song, S., Li, Y., Fu, S., Lei, W., Guo, X., Feng, Y., Gao, X., Li, X., Yang, Z., Xu, Z., He, Y., Wang, H., Zhou, H., Wang, B., Lu, X., Liang, G., 2017. Genome sequencing and phylogenetic analysis of Banna virus (genus Seadornavirus, family Reoviridae) isolated from Culicoides. *Sci. China Life Sci.* 60, 1372–1382. <https://doi.org/10.1007/s11427-017-9190-6>.
- Stollar, V., Thomas, V.L., 1975. An agent in the Aedes aegypti cell line (Peleg) which causes fusion of Aedes albopictus cells. *Virology* 64, 367–377. [https://doi.org/10.1016/0042-6822\(75\)90113-0](https://doi.org/10.1016/0042-6822(75)90113-0).
- Torii, S., Orba, Y., Hang'ombe, B.M., Mweene, A.S., Wada, Y., Anindita, P.D., Phongphaew, W., Qiu, Y., Kajihara, M., Mori-Kajihara, A., Eto, Y., Harima, H., Sasaki, M., Carr, M., Hall, W.W., Eshita, Y., Abe, T., Sawa, H., 2018. Discovery of Mwinilunga alphavirus: a novel alphavirus in Culex mosquitoes in Zambia. *Virus Res.* 250, 31–36. <https://doi.org/10.1016/j.virusres.2018.04.005>.
- Turelli, M., Hoffmann, A.A., 1995. Cytoplasmic incompatibility in *Drosophila simulans*: dynamics and parameter estimates from natural populations. *Genetics* 1319–1338. <https://doi.org/10.1016/b978-0-12-205750-2.50010-4>.
- Vancini, R., Paredes, A., Ribeiro, M., Blackburn, K., Ferreira, D., Kononchik, J.P., Hernandez, R., Brown, D., 2012. Espirito santo virus: a new birnavirus that replicates in insect cells. *J. Virol.* 86, 2390–2399. <https://doi.org/10.1128/jvi.06614-11>.
- Vasconcelos, P.F.C., Calisher, C.H., 2016. Emergence of human arboviral diseases in the Americas, 2000–2016. *Vector Borne Zoonotic Dis.* 16, 295–301. <https://doi.org/10.1089/vbz.2016.1952>.
- Vasilakis, N., Forrester, N.L., Palacios, G., Nasar, F., Savji, N., Rossi, S.L., Guzman, H., Wood, T.G., Popov, V., Gorchakov, R., Gonzalez, A.V., Haddow, A.D., Watts, D.M., da Rosa, A.P.A.T., Weaver, S.C., Lipkin, W.I., Tesh, R.B., 2013. Negevirus: a proposed new taxon of insect-specific viruses with wide geographic distribution. *J. Virol.* 87, 2475–2488. <https://doi.org/10.1128/jvi.00776-12>.
- Vasilakis, N., Castro-Llanos, F., Widen, S.G., Aguilar, P.V., Guzman, H., Guevara, C., Fernandez, R., Auguste, A.J., Wood, T.G., Popov, V., Mundal, K., Ghedin, E., Kochel, T.J., Holmes, E.C., Walker, P.J., Tesh, R.B., 2014a. Arboretum and Puerto Almendras viruses: two novel rhabdoviruses isolated from mosquitoes in Peru. *J. Gen. Virol.* 95, 787–792. <https://doi.org/10.1099/vir.0.058685-0>.
- Vasilakis, N., Guzman, H., Firth, C., Forrester, N.L., Widen, S.G., Wood, T.G., Rossi, S.L., Ghedin, E., Popov, V., Blasdel, K.R., Walker, P.J., Tesh, R.B., 2014b. Mesoniviruses are mosquito-specific viruses with extensive geographic distribution and host range. *Virus Res.* 11, 1–12. <https://doi.org/10.1186/1743-422X-11-97>.
- Vasilakis, N., Tesh, R.B., 2015. Insect-specific viruses and their potential impact on arbovirus transmission. *Curr. Opin. Virol.* 15, 69–74. <https://doi.org/10.1016/j.coviro.2015.08.007>.
- Vázquez, A., Sánchez-Seco, M.P., Palacios, G., Molero, F., Reyes, N., Ruiz, S., Aranda, C., Marqués, E., Escosa, R., Moreno, J., Figuerola, J., Tenorio, A., 2012. Novel flaviviruses detected in different species of mosquitoes in Spain. *Vector Borne Zoonotic Dis.* 12, 223–229. <https://doi.org/10.1089/vbz.2011.0687>.
- Wang, J., Li, H., He, Y., Zhou, Y., Meng, J., Zhu, W., Chen, H., Liao, D., Man, Y., 2015. Isolation and genetic characterization of Mangshi virus: a newly discovered Seadornavirus of the Reoviridae family found in Yunnan Province, China. *PloS One* 10. <https://doi.org/10.1371/journal.pone.0143601>.
- Wang, L., Lv, X., Zhai, Y., Fu, S., Wang, D., Rayner, S., Tang, Q., Liang, G., 2012. Genomic characterization of a novel virus of the family Tymoviridae isolated from mosquitoes. *PloS One* 7, 1–8. <https://doi.org/10.1371/journal.pone.0039845>.
- Wang, Y., Guo, X., Peng, H., Lu, Y., Zeng, X., Dai, K., Zuo, S., Zhou, H., Zhang, J., Tong, Y., 2019. Complete genome sequence of a novel negevirus isolated from Culex tritaeniorhynchus in China. *Arch. Virol.* 164, 907–911. <https://doi.org/10.1007/s00705-018-04133-5>.
- Wang, Y., Xia, H., Zhang, B., Liu, X., Yuan, Z., 2017. Isolation and characterization of a novel mesonivirus from Culex mosquitoes in China. *Virus Res.* 240, 130–139. <https://doi.org/10.1016/j.virusres.2017.08.001>.
- Weaver, S.C., Reisen, W.K., 2010. Present and Future Arboviral Threats. *Antiviral Research.* <https://doi.org/10.1016/j.antiviral.2009.10.008>.
- World Health Organization (WHO) Scientific Group, 1985. *Arthropod-Born and Rodent-Born Viral Diseases*. WHO Technical Report Series, Geneva n° 719.
- Xue, L., Fang, X., Hyman, J.M., 2018. Comparing the effectiveness of different strains of Wolbachia for controlling chikungunya, dengue fever, and Zika. *PLoS Neglected Trop. Dis.* 12, 1–24. <https://doi.org/10.1371/journal.pntd.0006666>.
- Yamamoto, T., Eshita, Y., Kihara, Y., Satho, T., Kuroda, M., Sekizuka, T., Nishimura, M., Sakai, K., Watanabe, S., Akashi, H., Rongsriyam, Y., Komalamisra, N., Srisawat, R., Miyata, T., Sakata, A., Hosokawa, M., Nakashima, M., Kashige, N., Miake, F., Fukushi, S., Nakauchi, M., Saijo, M., Kurane, I., Morikawa, S., Mizutani, T., 2009. Novel virus discovery in field-collected mosquito larvae using an improved system for rapid determination of viral RNA sequences (RDV ver4.0). *Arch. Virol.* 154, 153–158. <https://doi.org/10.1007/s00705-008-0285-5>.
- Zhai, Y.G., Lv, X.J., Sun, X.H., Fu, S.H., Gong, Z. Da, Fen, Y., Tong, S.X., Wang, Z.X., Tang, Q., Attoui, H., Liang, G.D., 2008. Isolation and characterization of the full coding sequence of a novel densovirus from the mosquito Culex pipiens pallens. *J. Gen. Virol.* 89, 195–199. <https://doi.org/10.1099/vir.0.83221-0>.
- Zirkel, F., Kurth, A., Quan, P.L., Briese, T., Ellerbrok, H., Pauli, G., Leendertz, F.H., Lipkin, W.I., Ziebuhr, J., Drosten, C., Junglen, S., 2011. An insect nidovirus emerging from a primary tropical rainforest. *mBio* 2, 21–23. <https://doi.org/10.1128/mBio.00077-11>.
- Zirkel, F., Roth, H., Kurth, A., Drosten, C., Ziebuhr, J., Junglen, S., 2013. Identification and characterization of genetically divergent members of the newly established family Mesoniviridae. *J. Virol.* 87, 6346–6358. <https://doi.org/10.1128/jvi.00416-13>.