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# Invertebrate RNA virus diversity from a taxonomic point of view



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## ABSTRACT

Invertebrates are hosts to diverse RNA viruses that have all possible types of encapsidated genomes (positive, negative and ambisense single stranded RNA genomes, or a double stranded RNA genome). These viruses also differ markedly in virion morphology and genome structure. Invertebrate RNA viruses are present in three out of four currently recognized orders of RNA viruses: *Mononegavirales*, *Nidovirales*, and *Picornavirales*, and 10 out of 37 RNA virus families that have yet to be assigned to an order. This mini-review describes general properties of the taxonomic groups, which include invertebrate RNA viruses on the basis of their current classification by the International Committee on Taxonomy of Viruses (ICTV).

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## 1. Introduction

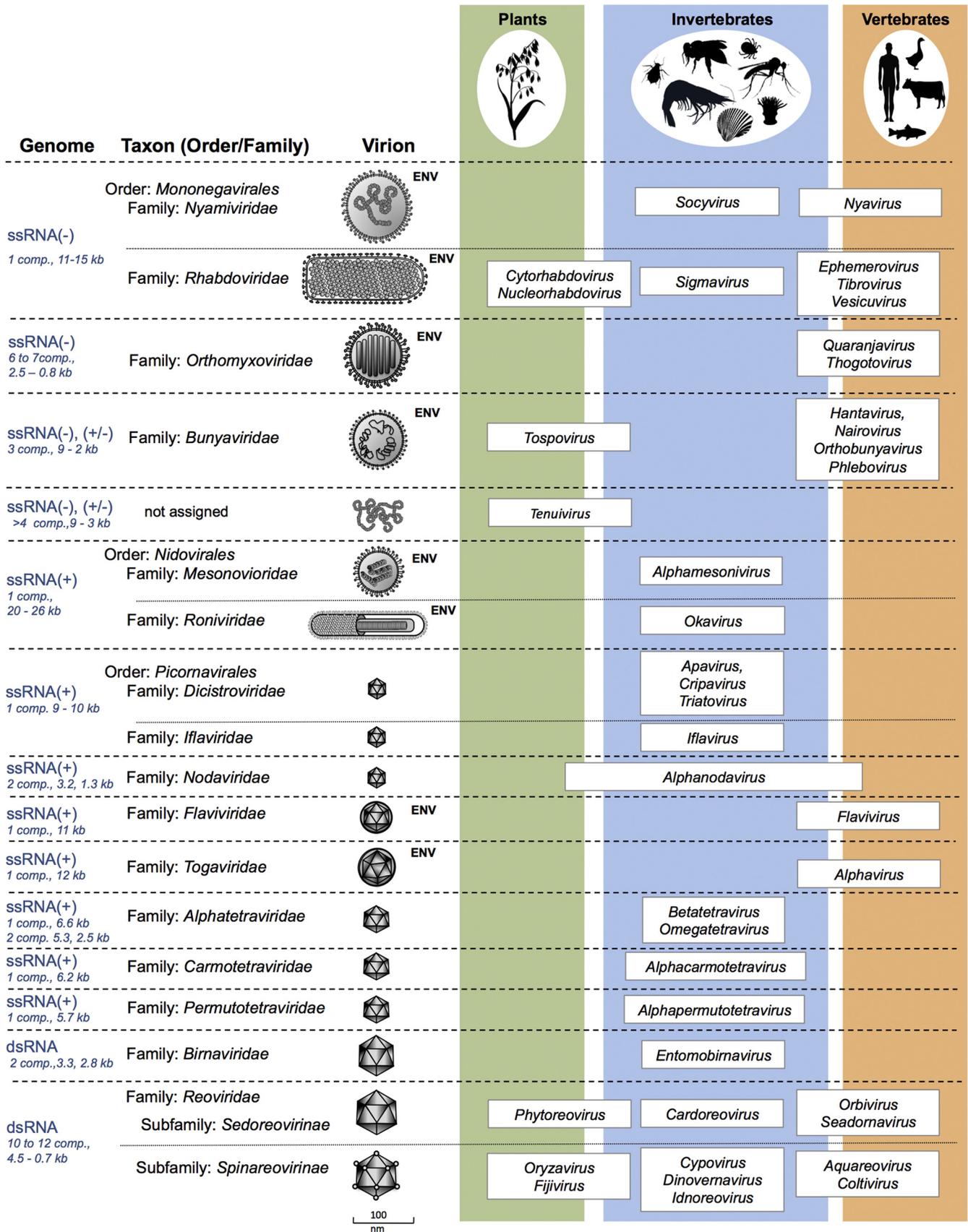
### 1.1. Principles of ICTV taxonomy

Invertebrates are hosts to diverse RNA viruses, some of which infect only invertebrates, while others can also infect vertebrates or plants, and some cases both vertebrates and plants (Fig. 1). Invertebrate RNA viruses have all possible types of encapsidated genetic material (a positive, negative and ambisense, single stranded RNA genome, or a double stranded RNA genome), marked differences in virion morphology and composition, high variability in genome structures and the mechanisms by which genes are expressed (Figs. 2–4). It is likely that currently only a small proportion of the existing diversity of invertebrate RNA viruses has been characterized, because the majority of known invertebrate RNA viruses have been found in a relatively small number of economically important species, such as hematophagous insects that vector human or livestock pathogens, or domesticated invertebrate species, such as prawns and honeybees. Therefore the number of RNA viruses of invertebrates is expected to grow to include a wider variety of species, in particular with the application of novel RNA sequencing technologies (Liu et al., 2011). Such an increase in virus genomic information necessitates a system of virus classification that facilitates the assignment of a novel virus to a taxonomic group or the creation of novel taxa. This article provides a brief overview of the principles of virus systematics and describes the distinctive features of the taxonomic groups, which include both

pathogenic and non-pathogenic RNA viruses that replicate in invertebrates.

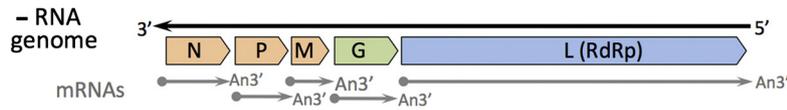
The universally accepted virus classification system is the responsibility of the International Committee on Taxonomy of Viruses (ICTV). The most recent report of this committee was published in 2012 (King et al., 2012), and was modified in an online update in 2014 (<http://www.ictvonline.org/>) (Adams et al., 2013). This classification is largely based on the viral genome organization (Figs. 2–4) and genetic relatedness among viruses, but it also takes into account virion characteristics and biological properties such as host range (King et al., 2012). Central to the ICTV virus classification is the concept of a virus species, which is defined as “a polythetic class of viruses that constitutes a replicating lineage and occupies a particular ecological niche”, i.e., the members of a virus species are collectively defined by a consensus group of properties (van Regenmortel and Mahy, 2004; Carstens, 2012). Importantly, this definition recognizes that within a particular virus species, multiple virus variants may be generated as a result of the high replication error rate of RNA genomes (Lauring and Andino, 2010), in addition to recombination and reassortment events between related variants (Holland and Domingo, 1998), and reflects that RNA viruses often exist as populations of non-identical but closely related variants, known as “quasispecies” (Domingo et al., 2012). In general, a group of virus species that share identical genome organization and high sequence homology form a genus, although the precise definition of a genus can vary between virus families. Similarly, there are no universally accepted species demarcation criteria applicable to all RNA virus species across all genera. Viruses are considered to be a different species if they have a distinct host range, have distinguishable serological

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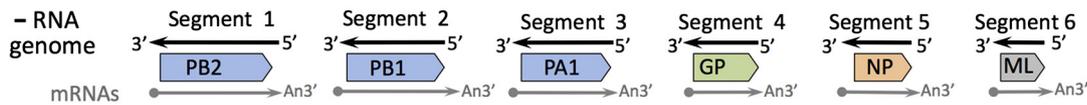


**Fig. 1.** Major genera of the RNA viruses that replicate in invertebrates. Schematic representation of virion morphology and the host range. Virion sizes are given according to the scale shown. ENV indicate enveloped virions. The lists of recognized virus species of the genera is given in [Table S1 \(Supplementary material\)](#).

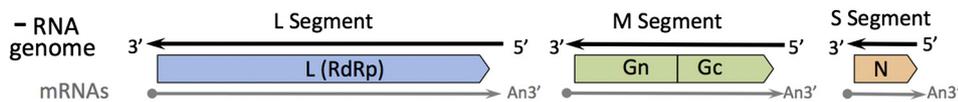
### A. Vesicular stomatitis Indiana virus, VSIV (*Mononegavirales*, *Rhabdoviridae*, *Vesiculovirus*, *Indiana vesiculovirus*)



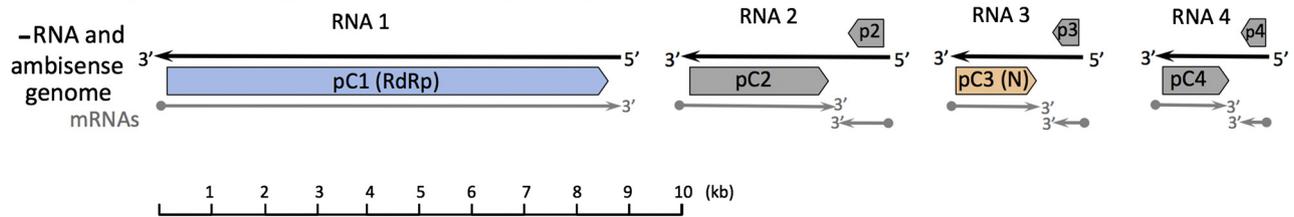
### B. Thogoto virus, THOV (*Orthomyxoviridae*, *Thogotovirus*, *Thogoto virus*)



### C. Hantaan virus, HTNV (*Bunyaviridae*, *Hantavirus*, *Hantaan hantavirus*)



### D. Rice stripe virus, RSV (*Tenuivirus*, *Rice stripe virus*)



**Fig. 2.** Genome organization and gene expression strategies of the major groups of invertebrate negative-strand RNA viruses. Genetic maps are given for the members of the following taxonomic groups: (A) order *Mononegavirales*, (B) family *Orthomyxoviridae*, (C) family *Bunyaviridae*, and (D) genus *Tenuivirus* (also has ambisense genomic RNA components). Full taxonomic classification is given for each virus genome. Genomic RNA strands are shown as thick black lines, block arrows show open reading frames (ORF) and the direction of translation; the ORFs are color-coded as follows: blue, RNA replication proteins; grey, non-structural proteins; light brown, structural non-membrane proteins; green, structural membrane proteins, glycoproteins. The mRNAs and the sub-genomic RNAs (sgRNA) involved in the gene expression are shown as grey arrows below the genome. The 5' cap structure shown as a dot at the 5' terminus of RNA, arrowhead indicate the 3' terminus, An3' - polyadenylated 3' terminus. The gene name abbreviations are explained in the article sections describing corresponding taxonomic groups.

characteristics and nucleotide (nt) or amino acid sequence similarity of a chosen gene below a threshold level, which is about 75–85% nt identity for the majority of RNA virus genera (King et al., 2012). Higher taxonomic categories are subfamilies, families and orders. Assignment of the lower taxa to a higher taxonomic levels reflects phylogenetic relationships between viruses. For example, grouping of virus families to an order is usually done on the basis of evolutionary relatedness of the genes involved in viral RNA replication, in particular the RNA-dependent RNA polymerase (RdRp) (Supplementary Table S1). Conversely, groups of viruses which lack significant genetic relatedness and/or have distinct genome organization and genome expression features are not grouped together. Such examples include the family *Reoviridae*, which is not assigned to an order, and the genus *Tenuivirus* which is not assigned to a family (Fig. 1; Supplementary Table S1). Although the ICTV classification is widely used, it has been only partially adopted by GenBank for the annotation of virus sequences, which usually provide order, family and genus information but does not require use of the ICTV approved virus species names (Benson et al., 2005).

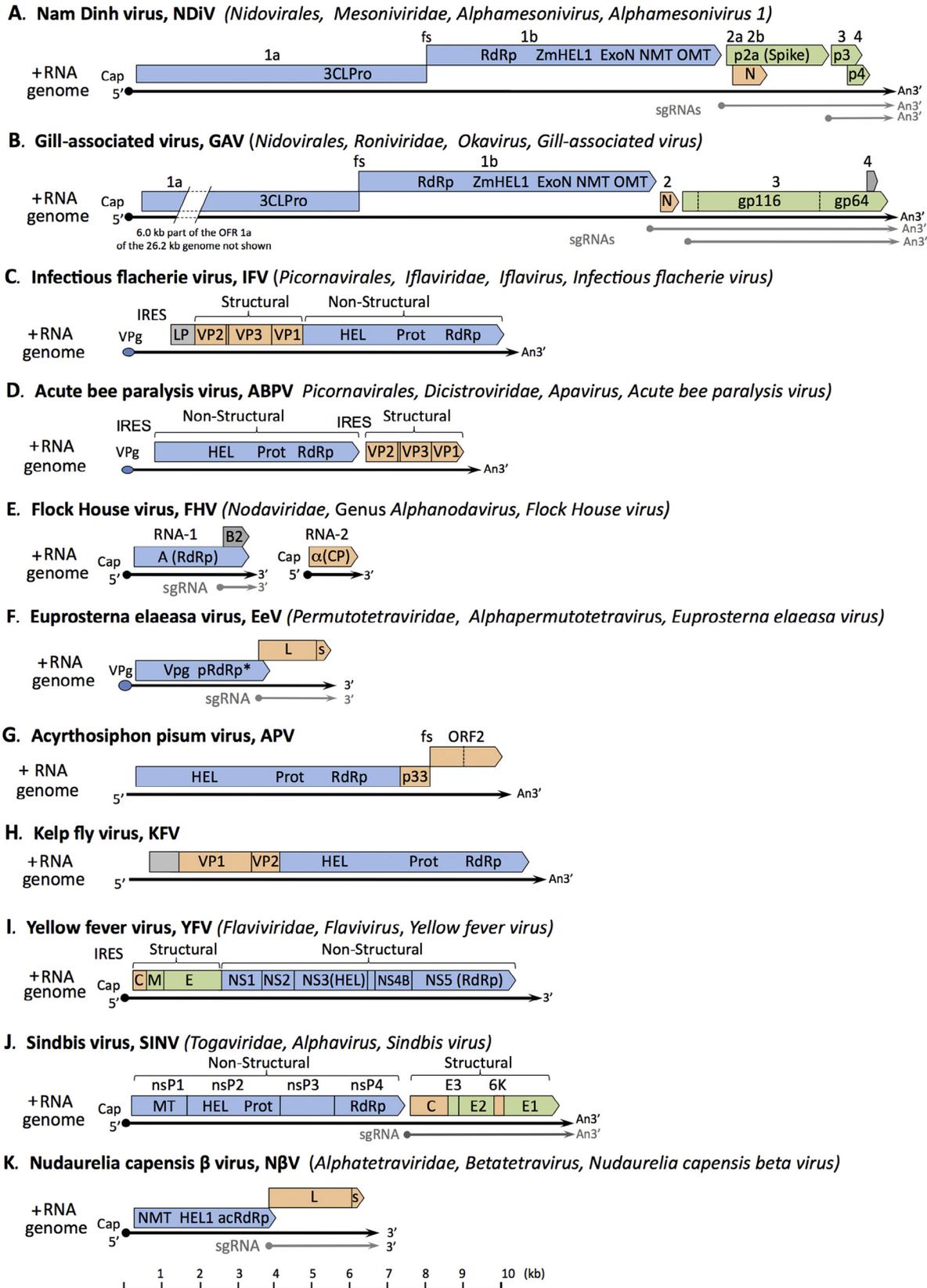
It should be noted, that the ICTV classification does not reflect evolutionary relationships between the orders of RNA viruses even when it is possible to trace the evolutionary history of RNA-dependent RNA polymerase (RdRp) genes, as in the case of positive strand RNA viruses (Koonin et al., 2008). For example, evolutionary relatedness of the RdRp of the family *Nodaviridae* and the order *Picornavirales* (Koonin et al., 2008), is not shown in the current ICTV taxonomy. Another reason why it might not be possible to

reconstruct the “evolutionary trees” of RNA viruses is the crucial role of horizontal gene transfer, between viral groups and even between viruses and their hosts, in the emergence of novel virus genomes. Individual genome segments, gene blocks and single genes may have independent evolutionary histories, making virus genomic evolution “network-like”, rather than “tree-like” (Koonin and Dolja, 2012). Therefore, although the ICTV taxonomy can be criticized for the lack of a firm basis in the principles of evolutionary biology (Peterson, 2014), it is probably the best compromise approach to the systematics of viruses as it stands today.

#### 1.2. Taxonomic groups of invertebrate RNA viruses

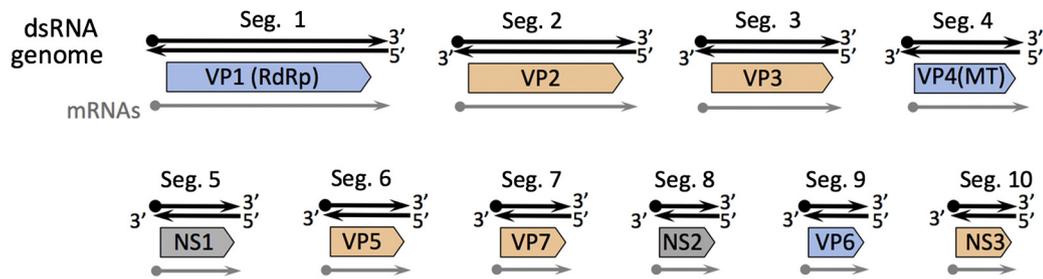
Invertebrate RNA viruses are represented in of three out of the four currently recognized RNA virus orders, *Mononegavirales* (2 families out of 5), *Nidovirales* (2 families out of 5), and *Picornavirales* (2 families out of 5). Among 37 RNA virus families, which are not currently assigned to an order, there are 10 families of invertebrate-infecting viruses. There is also one invertebrate virus genus among 10 RNA virus genera that have yet to be assigned to a family. Invertebrate RNA viruses of some genera can also infect vertebrates or plants, and often interactions of these viruses with their vertebrate or plant hosts are better studied compared with those in the invertebrate hosts.

Supplementary Table S1 presents all viral taxonomic groups recognized by the ICTV which include invertebrate RNA viruses, organized according to their order, family, subfamily and genus levels. For each genus, Supplementary Table S1 shows the type

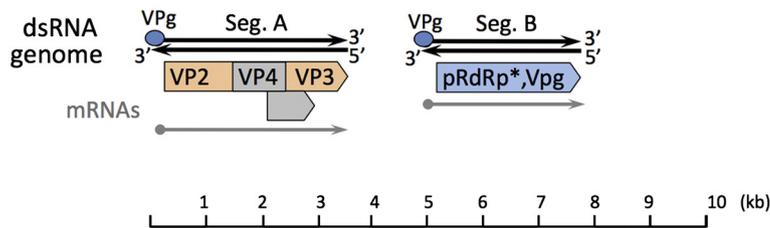


**Fig. 3.** Genome organization and gene expression strategies of the major groups of invertebrate positive-strand RNA viruses. Genetic maps are given for the members of the following taxonomic groups: order *Nidovirales*, (A) family *Mesoniviridae* and (B) family *Roniviridae*; order *Picornavirales*, (C) family *Iflaviridae* and (D) family *Dicistroviridae*; (E) family *Nodaviridae*; (F) family *Permutotetraviridae*; picorna-like viruses not classified by ICTV, (G) *Acyrthosiphon pisum* virus and (H) *Kelp fly* virus; (I) family *Flaviviridae*; (J) family *Togaviridae*; (K) family *Alphatetraviridae*. The genome structure elements are shown as in Fig. 2; also the vertical bars in the ORF block arrows represent proteolytic cleavage sites; Vpg - a protein covalently linked to the 5' end of the genomic RNA. Conserved proteins: RdRp - RNA dependent RNA polymerase, HEL - helicase, MET - methyltransferase. Other gene name abbreviations are explained in the article sections describing corresponding taxonomic groups. IRES - internal ribosomal entry signal, fs - frameshift signal.

### A. Bluetongue virus, BTV (*Reoviridae*, *Orbivirus*, *Bluetongue virus*)



### B. Drosophila X virus, DXV (*Birnaviridae*, *Entomobirnavirus*, *Drosophila X virus*)



**Fig. 4.** Genome organization and gene expression strategies of the major groups of invertebrate double-stranded RNA viruses. Genetic maps are given for the members of (A) the family *Reoviridae* and (B) the family *Birnaviridae*. RdRp - RNA dependent RNA polymerase; pRdRp\* - permuted RdRp, MT - methyltransferase. Seg. - segment.

species, type of genomic RNA, virion characteristics, and the GenBank accession numbers for the complete genome sequences of the type species, as well as the list of all recognized species in all listed genera. The sections below outline the general properties of these groups. In some cases, the ICTV recognized taxa are further grouped according to their genetic material into so-called “Baltimore classes” (Baltimore, 1971), or according to their RNA replication enzyme and genome expression strategies into so-called “supergroups” or “superfamilies” (Goldbach, 1987; Strauss and Strauss, 1988; Koonin et al., 2008; Holmes, 2009). More detailed descriptions of the specific biological properties of a number of pathogenic invertebrate RNA viruses and their impact on the food chain, are given in the papers elsewhere in this special issue of the Journal of Invertebrate Pathology.

## 2. Viruses with negative-stranded and ambisense single stranded RNA genomes

Invertebrate viruses of the order *Mononegavirales*, the families *Orthomyxoviridae*, *Bunyaviridae*, and the unassigned genus *Tenuivirus* share a number of common characteristics. These viruses have a single-stranded negative RNA genome which may have between one and eight components. Notably, while some of the genome components of *Bunyaviridae* and *Tenuivirus* are ambisense, the viral RNA-dependent RNA polymerase (RdRp) is always encoded by a negative strand component. The distinctive features of these viruses include that their genomic RNA is always associated with multiple monomers of the nucleoprotein (N protein), forming a highly structured filamentous ribonucleoprotein (RNP) or nucleocapsid. These nucleocapsids, rather than naked RNA molecules, are the active templates for transcription and replication (Kormelink et al., 2011; Ortin and Martin-Benito, 2015). The virus-encoded RNA-dependent RNA polymerase also forms part of the virions of all negative-stranded RNA viruses (Ruigrok et al., 2010). For all these viruses, with the exception of members of the *Tenuivirus* genus, the virions are enveloped, i.e. the nucleocapsids are surrounded by a lipid bilayer, which includes virus-encoded membrane proteins (Falk and Tsai, 1998; Ruigrok et al., 2011).

### 2.1. Order *Mononegavirales*

#### 2.1.1. General characteristics

Members of the order *Mononegavirales* have large enveloped virions containing a nucleocapsid with a negative-stranded non-fragmented linear RNA genome of 12–15 kb in length. The order includes two invertebrate-infecting families, *Nyamiviridae*, with spherical or pleomorphic virions 100–130 nm in diameter (Mihindukulasuriya et al., 2009), and *Rhabdoviridae*, with bacilliform or bullet-shaped virions, 45–100 nm width and 130–300 nm in length. In the virion, the lipid envelope containing the glycoprotein (G protein) interacts with the coiled ribonucleoprotein (RNP) via the matrix protein (M protein). The RNP core has a diameter of 13–20 nm, which, in the case of members of the *Rhabdoviridae*, is organized into a helical nucleocapsid of about 50 nm in diameter. The virion also contains a large RNA polymerase catalytic subunit (L protein) and phosphoprotein (P protein) which act together as a non-enzymatic cofactor during viral replication and transcription (Green and Luo, 2009).

The negative-stranded genomic RNA of the *Mononegavirales* contains at least five ORFs flanked by the 5'- and 3'-terminal untranslated regions in the conserved order; nucleoprotein (N) gene, phosphoprotein (P) gene, glycosylated matrix protein (M) gene, transmembrane glycoprotein (G) gene, RNA-dependent RNA polymerase (L) gene (Fig. 2A). This core set of five conserved genes is characteristic of the *Rhabdoviridae*, whereas the genomes of the *Nyamiviridae* have six ORFs with two separate ORFs in the place of the M gene (Lyles and Rupprecht, 2007; Mihindukulasuriya et al., 2009). Differences in genome organization between genera of the *Rhabdoviridae* (Fig. 1A) are primarily the results of extension and insertion of additional transcriptional units at all junctions of the core structural protein genes (N-P, P-M, M-G, and G-L, Fig. 2A) (Walker et al., 2015).

The virus particles enter the animal cell via the endocytic pathway in the case of infections in animal cells and subsequently fuse with a cellular membrane within the acidic environment of the endosome. Both receptor recognition and membrane fusion are mediated by viral transmembrane viral glycoprotein (G). Release of the nucleocapsid into the cytoplasm triggers primary

transcription performed by the virion-associated RNA polymerase (L protein), which occurs on the RNA-N protein complex (Albertini et al., 2012). Unlike the majority of rhabdoviruses, the members of the genus *Nucleorhabdovirus* replicate in the cell nucleus, similar to the family *Nyamiviridae* (Nyamanini virus), (Herrel et al., 2012). The viral RNA polymerase (L-protein) uses exclusively RNA-N protein complex as its template; no removal of the N protein takes place during transcription and replication of the viral RNA. As the viral RNA dependent RNA polymerase (the L protein) transcribes the negative genome template in the 3' to 5' direction, it stutters at the intergenic regions, leading to polyadenylation and mRNA termination, resulting in separate monocistronic capped mRNA for each of the viral genes in the N to L gene order (Fig. 2A). A proportion of RNA polymerase drops off the template at each intergenic region, leading to decreasing quantities of each of the transcripts in the N to L transcription sequence, with the lowest numbers of mRNA molecules produced for the L gene (Lyles and Rupprecht, 2007). After viral protein synthesis, infected cells show development of the inclusion bodies that contain the viral RNA synthesis machinery and become the predominant sites of the viral mRNA synthesis (Albertini et al., 2012). Genome replication includes production of the full-length positive sense copy of genomic RNA and then generation of its negative copies which could be encapsidated. The G protein is translocated across the endoplasmic reticulum membrane, the N-terminal signal peptide is removed and the G protein is glycosylated in the Golgi apparatus. Formation of the virus particles involves association of the viral nucleocapsids together with M proteins and membrane envelope containing the G protein, the mature particles are budded from the plasma membrane. In the case of nucleorhabdoviruses which replicate in cell nucleus, viral budding occurs from the inner nuclear envelope and mature particles are accumulated in the perinuclear space of infected cells (Jayakar et al., 2004).

### 2.1.2. Host range and pathology

Members of the order *Mononegavirales* have a very wide host range, which includes vertebrates, plants and invertebrates (Fig. 1). Although most of the *Mononegavirales* which replicate in invertebrates also infect either plants or vertebrates, there are viruses which solely infect invertebrates, such as *Drosophila melanogaster sigmavirus*, genus *Sigmavirus* (Carpenter et al., 2007). This virus is vertically transmitted, both paternally and maternally, relying on its host survival and reproduction. Although it is essential for the virus to minimize its negative impact on the *Drosophila* host, the infected insects show reduced fitness. This could be connected to reduction of female fertility, egg viability, and possibly increased susceptibility to fungal infections (Longdon et al., 2012). Interestingly, the virus-infected flies become paralysed and die after exposure to a high concentration of CO<sub>2</sub>, possibly due to the effect of virus replication on the nervous tissues (Longdon et al., 2012).

Plant-infecting *Rhabdoviridae* include the families *Cytorhabdovirus* (type species: *Lettuce necrotic yellows virus*) and *Nucleorhabdovirus* (type species: *Potato yellow dwarf virus*), which are transmitted in a circulative propagative manner through plant phloem by aphids and leafhoppers (Dietzgen et al., 2006; Ghosh et al., 2008; Jackson et al., 2005). Potato yellow dwarf virus particles are acquired by *Aceratagallia* sp. leafhoppers from plants through feeding, the virus initially invades epithelial cells of midgut by receptor-mediated endocytosis. Then virus infection spreads into the nervous system, trachea and hemolymph, reaching the salivary glands and reproductive tissues. The salivary glands accumulate high levels of virus particles which are released by exocytosis and transmitted to new plant host during feeding. Infections of plants cells occurs through direct injection of the virus

particles to the cell cytoplasm and through mechanical damage caused by the insect vector's stylus (Hogenhout et al., 2003).

*Mononegavirales* include viruses that infect both ticks and birds, such as members of the *Nyamiviridae* family, *Nyamanini virus* and *Midway virus* (Mihindukulasuriya et al., 2009), and viruses that infect insects (*Culicoides* midges and sandflies) and cattle, such as members of the genera *Ephemerovirus*, *Tibrovirus* and *Vesiculovirus* in the family *Rhabdoviridae* (Dhillon et al., 2000; Gubala et al., 2011; Rodriguez et al., 2002). This include the *Bovine ephemeral fever virus*, the member of the *Ephemerovirus* family, which replicates in a variety of Diptera hosts including biting midges (*Culicoides* sp.) and mosquitoes (*Culex* sp.). Indeed epizootic dynamics of Bovine ephemeral fever virus (BEFV) is consistent with emergence of mosquito populations. Although it was shown that mosquitoes and midges could be infected with BEFV via artificial membrane feeding (Walker et al., 2012), infection in insect hosts remains poorly characterized (Nandi and Negi, 1999)

## 2.2. Family Orthomyxoviridae

### 2.2.1. General characteristics

*Orthomyxoviridae* have spherical or pleomorphic enveloped virions of 80–120 nm in diameter, with surface glycoprotein projections of 10–14 nm in length and 4–6 nm in diameter. Each virion contains six to eight ribonucleoproteins (RNP) of 50–150 nm in length. Structural proteins shared among all *Orthomyxoviridae* include a nucleoprotein (NP), which is associated with each single stranded RNA genome segment to form a RNP, a non-glycosylated matrix protein (M), an integral membrane glycoprotein (GP), a hemagglutinin, and three peptides forming the viral RdRp (PA, PB1, PB2) (Fig. 2B) (Palese and Shaw, 2007).

Virus particle entry involves binding of the viral glycoprotein (GP) to cell receptors (e.g. sialic acid in the case of vertebrates) and endocytosis. The fusion between viral and host membranes occurs in endosomes and is triggered by low pH. Following the release to the cytoplasm, viral RNPs are translocated into the cell nucleus where virion-associated polymerase (PA, PB1, PB2 peptides) synthesizes mRNAs on the negative-stranded genomic RNA templates (primary transcription). The 5' terminal cap of viral mRNA derived from the host mRNA, and the 3' terminus of the mRNAs are polyadenylated by repetitive copying of the polyuridine tract of the viral RNA template. Replication involves production of the full-length complementary RNA (cRNA) which are neither capped nor polyadenylated and act a templates for the negative genomic RNA synthesis. These replicative RNAs are associated with the nucleoprotein (N) and exist as RNPs. New enveloped virions are formed by budding, this involves translocation of the viral RNPs and matrix protein (M) to the regions of the plasma membrane containing the viral glycoprotein (GP) (Neumann et al., 2004).

### 2.2.2. Host range and pathology

Invertebrate viruses in this family are arboviruses in the genera *Quaranjavirus* and *Thogotovirus*. Viruses of these genera have seven genomic RNA segments from 0.8 to 2.4 kb in size (Allison et al., 2015). Both thogotoviruses (single species *Thogoto virus*) and quaranjaviruses (*Johnston Atoll virus* and *Quaranfil virus*) infect ticks, seabirds and mammals, including humans (Austin, 1978; Presti et al., 2009; Allison et al., 2015). No major pathological changes are observed in the ticks (*Rhipicephalus appendiculatus*) infected with Thogoto virus. The virus is concentrated in the tick brain early on in the blood-feeding process, with a proportion of virus located in the salivary glands. Lower levels of virus are found in the trachea, digestive tract and female but not in male sex organs (Booth et al., 1989).

### 2.3. Family Bunyaviridae

#### 2.3.1. General characteristics

This family comprises viruses with spherical or pleomorphic enveloped virions 80–120 nm in diameter, with 5–10 nm glycoprotein surface projections (Gn and Gc proteins), and three nucleocapsids. The virions contain viral helical ribonucleoproteins of 2–2.5 nm in diameter and 200–300 nm in length, which appear circular due to the base pairing between complementary terminal sequences of the genomic RNA. The virions also include a viral polymerase (L protein), which is essential to initiate virus transcription and the replication cycle in newly-infected cells. The single stranded RNA genome is tripartite and includes large (L), medium (M) and small (S) components with total length from 11 to 19 kb (Fig. 2C). The terminal nucleotides at the 3' and 5' ends of RNA each segment are complementary, resulting in the formation of a 'panhandle' structure acting as the promoter for both the transcription and replication. In all *Bunyaviridae* the L RNA segment, encodes the viral RdRp, the M segment encodes the glycoproteins Gn and Gc, and the S segments encodes the nucleoprotein (N) (Fig. 2D). The ORFs coding for these proteins are present on the genomic RNA segments in negative polarity. The S genomic RNA segment of the genus *Phlebovirus* and both S and M genomic RNA segments of *Tospovirus* (which replicates in arthropods and plants) are ambisense and also encode non-structural proteins in the positive polarity (de Haan et al., 1991; Goldbach and Kuo, 1996). Tospoviruses share high similarity with members of the *Tenuivirus* genus (type species *Rice stripe virus*, Fig. 2D), an unassigned genus of viruses, which also replicate in both insects (plant hoppers) and plants. Unlike the *Bunyaviridae*, the tenuiviruses do not possess an envelope and their virions consist of flexible filamentous nucleocapsids of 3–10 nm diameter and up to 700 nm in length (Falk and Tsai, 1998).

*Bunyaviridae* virus particle entry into the vertebrate cells involves binding of the glycoproteins Gn and Gc to unidentified cell receptors, endocytosis and fusion of viral and endosomal membranes. The nucleocapsid enters the cell cytoplasm where all stages of transcription and replication occur. The synthesis of mRNAs (Fig. 2C) (e.g. primary transcription), involves using host mRNA fragments containing the 5' cap as primers (Fig. 2D) (Garcin et al., 1995). Translation of the L and S segment mRNAs takes place by free ribosomes in the cytoplasm; translation of the M segment mRNAs by the membrane associated ribosomes. The glycoprotein precursor is co-translationally cleaved to produce Gn and Gc (Fig. 2D). Replication includes production of the full-length positive-strand copies of the genomic RNA, which act as templates for genomic RNA synthesis. Particle formation involves accumulation and glycosylation of Gn and Gc in the Golgi and budding into the Golgi membrane-derived vesicles. These vesicles containing enveloped virus particles are trafficked to the cell surface and, following the fusion of the vesicular membranes with the plasma membrane, the virus particles are released (Elliott, 2014).

#### 2.3.2. Host range and pathology

The *Bunyaviridae* viruses of the genera *Orthobunyavirus*, *Nairovirus*, *Phlebovirus*, and possibly *Hantavirus*, replicate in vertebrates and in hematophagous arthropods, including mosquitoes, midges, and ticks, which also serve as virus reservoirs (Horne and Vanlandingham, 2014; Yu and Tesh, 2014). Although *Bunyaviridae* cause acute infections in vertebrates, including some humans, no negative effects of *Bunyaviridae* on their insect and tick hosts have been reported (Horne and Vanlandingham, 2014).

Due to their medical importance, infections of a number of bunyaviruses, including California encephalitis orthobunyavirus - La Crosse virus (LACV), and Rift Valley fever phlebovirus, were studied in their insect vectors. In mosquitoes *Aedes triseriatus*,

following acquisition, LACV infects and replicates in midgut epithelial cells. Virus may then infect other epithelial cells, escape through modified basal lamina or an infected tracheal cell, or bud directly into the hemolymph from an infected muscle cell, from where it may disseminate further via the hemolymph or the tracheal system to secondary target organs, including ovaries and salivary glands (Horne and Vanlandingham, 2014).

### 3. Positive-stranded RNA viruses

Invertebrate viruses with positive stranded RNA genomes are extremely diverse. They may have enveloped and non-enveloped virions, employ different genome expression strategies and have different sets of core genes. The classification of positive stranded RNA viruses based on their genome expression strategies and the types of the key domains in the RNA replicases was first proposed about 30 years ago (Goldbach, 1987; Strauss and Strauss, 1988), and the main elements of this approach are still used in ICTV taxonomy. Currently, RNA viruses infecting invertebrates are classified in the orders *Nidovirales* and *Picornavirales*, and the families *Alphatetraviridae*, *Carmotetraviridae*, *Permutotetraviridae*, *Flaviviridae*, *Nodaviridae*, and *Togaviridae* (Table S1). A number of positive stranded RNA viruses of invertebrates possess sets of genes that are significantly different from those of other recognized groups and therefore remain unclassified (some of these viruses are listed in Supplementary Table S1). In some cases, ICTV taxa and unclassified viruses can be grouped according to characteristics of their RNA replicases and genome expression strategies, into so-called superfamilies or supergroups (Goldbach, 1987; Holmes, 2009).

#### 3.1. Order Nidovirales

##### 3.1.1. General characteristics

Viruses of the order *Nidovirales* have large enveloped virions and large non-partite RNA genomes that exceed 20 kb in length. The best studied viruses of this order are the *Coronaviridae* which can cause severe infections in humans (Peiris et al., 2003), although this order also includes viruses that exclusively infect invertebrates, such as the insect-infecting family *Mesoniviridae* (Lauber et al., 2012) and crustacean-infecting family *Roniviridae*. These virus families have morphologically different particles. *Gill-associated virus* (family *Roniviridae*, genus *Okavirus*) has enveloped rod-shaped virions 158–184 nm by 42–47 nm which bear 11 nm spikes, the envelope encloses tightly coiled tubular nucleocapsid with a diameter of about 25 nm and a 5–7 nm helical periodicity (Callinan et al., 2003; Cowley et al., 2004). Nam Dinh virus (NDiV) (family *Mesoniviridae*, genus *Alphamesonivirus*, *Alphamesonivirus 1*), has spherical enveloped virions 60–80 nm in diameter (Nga et al., 2011; Zirkel et al., 2013). These viruses have similar genome organization and a set of the conserved protein motifs involved in RNA replication characteristic of the *Nidovirales*, with large genomes that are encoded by two long overlapping ORF1a and ORF1b which are translated as a single polypeptide as a result of ribosomal –1 frameshifting event, i.e., shifting ribosome one nucleotide backward during translation without releasing nascent polypeptide to continue translation in different frame (Fig. 3A and B; fs). The ORF1a encodes a 3C-like cysteine protease domain (Fig. 3A and B; 3CLPRO) flanked by hydrophobic regions encoded by ORF1a. The ORF1b encodes (in a 5' to 3' order) an RNA-dependent RNA polymerase (RdRp), helicase (HEL), 3'–5' exoribonuclease (ExoN) and 2'-O-methyltransferase domains. It was suggested that the ExoN is involved in controlling RNA replication fidelity, making possible the existence of the extremely large RNA genomes in the viruses of this order (Fig. 3A and B).

(Nga et al., 2011). Expression of the 3' proximal ORFs coding for the nucleocapsid protein and the surface glycoproteins involve synthesis of the subgenomic RNA (sgRNAs) on the negative RNA template (Fig. 3A and B) (Cowley et al., 2002a).

Replication of *Nidovirales* involve convoluted membrane rearrangements and formation of large double-membrane vesicles, which are observed in vertebrate cells infected with the members of coronavirus and arterivirus families, and contain viral RNA replicase. The dsRNA, replication intermediate, predominantly localizes to the interiors of the large, 200–300 nm diameter, double-membrane vesicles in coronavirus-infected cells. It is uncertain how ribonucleotides and product RNAs would be exchanged with the cytosol if RNA synthesis occurs inside these double-membrane vesicles. It is possible that the coronavirus replication complex might use a protein channel. Alternatively, RNA synthesis might occur in the convoluted single membrane structures which appear to be the major accumulation sites of the viral replicase subunits and include compartments with open connections to the cytoplasm (den Boon et al., 2010).

### 3.1.2. Host range and pathology

Invertebrate *Nidovirales* infection may have different effects on their hosts. Yellow head virus (YHV) and Gill-associated virus (GAV), the members of the species *Gill-associated virus*, replicate in cultivated prawns *Penaeus monodon* in south-east Asia and Australia (Cowley et al., 2000; Sittidilokratna et al., 2008). Infections may be chronic or acute and transmission can occur horizontally and vertically, as suggested by the presence of GAV in both spermatophores and mature ovarian tissue, together with the high prevalence of chronic GAV infection (Cowley et al., 2002b). During acute infections, mortality is usually high and virus occurs in most tissues of ectodermal and mesodermal origin. Necrotic cells display intensely basophilic cytoplasmic inclusions (de Groot et al., 2012).

No pathologies and symptoms were reported in *Culex* spp. mosquitoes infected with Nam Dinh virus, a member of the species *Alphamesonivirus 1*, in the family *Mesoniviridae*, genus *Alphamesonivirus*, although virus preparations induced cytopathic effects in the C6/36 mosquito cell line (Nga et al., 2011).

## 3.2. Picornavirales and “picorna-like” supergroup

### 3.2.1. General characteristics

A number of invertebrate viruses with small isometric non-enveloped virions, about 30 nm in diameter, containing positive-stranded genomic RNA share key characteristics with picornaviruses of vertebrates, such as *Poliovirus* and *Foot and mouth disease virus*. These viruses are classified as members of the order *Picornavirales* (Le Gall et al., 2008). Invertebrate viruses of this order are assigned to the families *Dicistroviridae* and *Iflaviridae*, which are found only in arthropods. The family *Dicistroviridae* includes the genera *Aparavirus* (type species *Acute bee paralysis virus*) and *Cripavirus* (type species *Cricket paralysis virus*). The family *Iflaviridae* has a single genus *Iflavirus*, type species *Infectious flacherie virus*. The virus belonging to the type species infect the silkworm, *Bombyx mori*. RNA genome of the members of these families carry the protein covalently linked to the 5' terminus (Vpg) and poly(A) at the 3' terminus. They contain an extended ORF encoding, in 5' to 3' order, a conserved block of the non-structural domains typical for all *Picornavirales*. This includes a 2C RNA helicase, a chymotrypsin-like 3C protease and 3D RNA-dependent RNA polymerase (RdRp) (Fig. 3C and D) (Koonin et al., 1993). The genomes of both *Dicistroviridae* and *Iflaviridae* also encode a conserved block of three structural proteins with a jelly-roll domain typical of the capsid proteins of icosahedral picornaviruses (Le Gall et al., 2008), and the small structural protein

VP4. The major difference between *Iflaviridae* and *Dicistroviridae* genomes is in the arrangement of the non-structural and the structural genes. The *Iflaviridae* genomes contain a single ORF, the 5' proximal part of which encodes the structural proteins and the 3' proximal part of which encodes the conserved non-structural proteins (Fig. 3A). The genomes of *Dicistroviridae* contain two ORFs, the 5' proximal ORF encodes the non-structural block, and the 3' proximal encodes the structural proteins (Fig. 3D). The polypeptides encoded by these ORFs are proteolytically processed by the viral chymotrypsin-like 3C protease encoded by the non-structural block. Similar to other *Picornavirales* members, translation of the ORFs of *Dicistroviridae* and *Iflaviridae* involve internal ribosome entry signals, IRES, which are present at the 5' untranslated region (5'UTR), and the intragenic region of the *Dicistroviridae*.

The RNA-dependent RNA polymerases (RdRp) that are closely related to the RdRps of the *Picornavirales* (Fig. 3C and D), are also present in the genomes of viruses belonging to other RNA virus groups recognized by the ICTV, which is widely referred to as the “picorna-like supergroup” viruses (Koonin et al., 2008). The *Picornavirales*-type RdRp is not necessarily accompanied by the other conserved non-structural *Picornavirales* domains. The picorna-like supergroup viruses may have structural protein(s) and employ genome expression strategies different from those of members of the *Picornavirales*. This includes the families *Nodaviridae* and *Permutotetraviridae*, as well as a number of unclassified viruses (Fig. 3E–H).

The family *Nodaviridae* (genus *Alphanodavirus*), which includes Flock House virus (Fig. 3E), encodes only the RdRp domain of the picorna-like set typical for the *Picornavirales* and lacks the helicase, Vpg and 3C-Protease (Koonin et al., 2008) (Fig. 3E). Several unclassified insect RNA viruses have a RdRp domain similar to that of members of the *Nodaviridae*, these include Chronic bee paralysis virus (Olivier et al., 2008), another honeybee virus, Lake Sinai virus strain 1 (Runckel et al., 2011), and the mosquito Mosinivirus (Schuster et al., 2014). The viruses of the family *Permutotetraviridae*, for example Euprosterona elaeasa virus (Fig. 3F), have a non-canonical RdRp domain derived from the picorna-like supergroup, as a result of sequence permutation in the palm sub-domain of RdRp (Gorbalenya et al., 2002; Zeddarn et al., 2010; Ferrero et al., 2015). Both *Nodaviridae* and *Permutotetraviridae* families use subgenomic RNAs for expression of the 3' proximal ORFs (Fig. 3E and F).

The picorna-like supergroup includes several insect viruses with small isometric virions which are not yet classified by the ICTV, such as *Solenopsis invicta* virus 1 and the related viruses from fire ants (Valles et al., 2004, 2014; Valles and Hashimoto, 2009), *Acyrtosiphon pisum* virus (Fig. 3G) (van der Wilk et al., 1997), Kelp fly virus (Fig. 3H) (Hartley et al., 2005), and Nora virus isolated from *Drosophila melanogaster* (Habayeb et al., 2006), all of which possess the full set of picorna-like non-structural genes, but have structural genes which differ from those of *Picornavirales*.

Infection by the invertebrate picorna-like viruses is less characterized compared to those infecting vertebrates. The mechanisms of virus entry into the cell are unknown for *Iflaviridae* and *Dicistroviridae*, as well as for other picorna-like viruses with small non-enveloped particles. The viral RNA is infectious and acts as mRNA. Replication involves generation the full-length negative RNA which acts as a template for genomic RNA synthesis. Replication is likely to involve generation of long double-stranded RNA intermediates. It is likely that replication of *Iflaviridae* and *Dicistroviridae* in invertebrate cells is similar to that of the vertebrate-infecting *Picornavirales* members, including the family *Picornaviridae*. It was shown that replication of *Picornaviridae* RNA occurs in complexes associated with cytoplasmic membranes. These complexes contain proteins derived from the whole of the non-structural

region of the polyprotein, including helicase, protease and RNA polymerase (RdRp) domains. The short virus-encoded protein, VPg, acts as a transcription primer for both positive and negative strand RNA synthesis (Knowles et al., 2012).

Replication of *Nodaviruses* is associated with mitochondria. The protein A of Flock House virus (FHV) (Fig. 3E) contains, in addition to the RdRp domain, an N-terminal transmembrane domain targeting outer mitochondrial membranes (Miller et al., 2001). FHV induces ~50 nm vesicular invaginations between the inner and outer mitochondrial membranes. The interiors of these vesicles are the sites where protein A and newly synthesized FHV RNAs accumulate (den Boon et al., 2010).

### 3.2.2. Host range and pathology

Members of the order *Picornavirales* and other picorna-like viruses infect a range of species in terrestrial and aquatic environments. *Dicistroviridae* and *Iflaviridae* include 15 and 14 recognized species, respectively (Supplementary Table S1), and similar numbers of putative species. These species include viruses infecting insects belonging to a wide range variety of insect groups, including Lepidoptera, Hymenoptera, Hemiptera, as well as Acaria and crustaceans. These viruses could be transmitted vertically and horizontally, and cause different degrees of disease, ranging from asymptomatic to highly pronounced leading to host mortality, usually corresponding to low or high levels of virus in infected individual (Fannon and Ryabov, 2016). For example, Deformed wing virus and Israeli acute paralysis virus (the families *Iflaviridae* and *Dicistroviridae*, respectively), significantly affect honeybee health, and have been implicated in the global decline of honeybee colonies, thereby threatening pollination services and food security (Lanzi et al., 2006; Cox-Foster et al., 2007; Vanbergen, 2013). Picorna-like RdRp sequences have been identified in sea water (Culley et al., 2003), and it is likely that some of them originate from viruses infecting marine invertebrates. Indeed, Taura syndrome virus (family *Dicistroviridae*, genus *Aparavirus*) has a serious impact on shrimp farming (Mari et al., 2002). Picorna-like RdRp sequences have also been identified in bivalve mollusks (Kingsley et al., 2002). Infections by members of the *Picornavirales* and other picorna-like viruses may differ in their virulence in infected hosts, ranging from asymptomatic infection in honeybees infected with the Kakugo virus strain of *Deformed wing virus* (Fujiyuki et al., 2004), to a lethal infection, such as Infectious flacherie virus infection of silkworms (Isawa et al., 1998). Usually severe disease coincides with high levels of virus in the infected individuals. It is possible that the adaptation of the virus to the predominant route of transmission determines the severity of the disease, with vertically transmitted viruses usually causing asymptomatic infection or sublethal disease compared to horizontally transmitted viruses. For example, even related viruses, such as the members of the genus *Iflavirus* genus, such as Deformed wing virus and Sacbrood virus cause asymptomatic infection or death, respectively, when infected at the larval stage (Ryabov et al., 2016). Moreover, introduction of an efficient horizontal transmission route may result in selection of pathogenic strains in a virus population of low virulence, as observed in the case of *Deformed wing virus* following introduction of the mite *Varroa destructor* that can transmit the virus between honeybee hosts (Martin et al., 2012; Ryabov et al., 2014).

## 3.3. Family *Flaviviridae*

### 3.3.1. General characteristics

Invertebrate members of this family belong to the genus *Flavivirus* (type species *Yellow fever virus*). The genus *Flavivirus* comprises arboviruses that replicate in mosquitoes (e.g. Yellow fever virus, Dengue virus, Zika virus) and ticks (e.g. Tick-borne

encephalitis virus) and which can be transmitted to birds and mammals, including humans, causing severe diseases (Gould and Solomon, 2008). Flavivirus members have spherical enveloped virions of 50 nm in diameter. Mature virions contain two virus-encoded membrane proteins, E (hemagglutinin) and M, which are glycosylated. The genomic RNA, about 11 kb in length, encodes m7Gppp cap in the 5' section, and contains a single long ORF. The 5' proximal part encodes structural proteins M and E, whereas the 3' proximal part encodes non-structural proteins including NS3 with protease and RNA helicase domains, and NS5 with RdRp and methyltransferase domains (Fig. 3I). The polyprotein is processed by the virus-encoded NS5 protease (Chambers et al., 1990).

Virus entry into mosquito cells investigated for Dengue virus showed that following receptor binding which involves envelope glycoprotein (E), flaviviruses enter cells via receptor-mediated endocytosis through which the virus particles are transported to endosomes. Acidification of the endosome lumen promotes fusion of the viral membrane with the endosomal membrane. Subsequently, a fusion pore is formed, and the nucleocapsid is delivered into the cell cytoplasm. Viral replication is initiated after uncoating of the nucleocapsid in the cytoplasm of the cell (van der Schaar et al., 2007). RNA synthesis occurs in the cytoplasm in association with modified cellular membranes via synthesis of full-length negative-strand intermediates (Simmonds et al., 2012). *Flaviviridae* induce replication-associated membrane structures, observed in the cells infected with Hepatitis C virus, Yellow fever virus and Dengue virus (DENV). These vesicle packets consist of an outer bounding membrane surrounding a series of inner, ~90 nm vesicles containing most of the replication proteins, dsRNA and nascent RNA, indicating that these vesicles are the likely sites of genome replication (den Boon et al., 2010). Virion assembly, including acquisition of a glycoprotein-containing lipid envelope, occurs by budding through intra-cellular membranes. Viral particles are transported in cytoplasmic vesicles through the secretory pathway before they are released by exocytosis (Simmonds et al., 2012).

### 3.3.2. Host range and pathology

*Flaviviridae* are human and veterinary pathogens, causing morbidity and mortality associated with febrile illness, hemorrhagic fevers, and encephalitides. About half of known flaviviruses are mosquito-borne (e.g. DENV), a third are tick-borne (e.g. Omsk hemorrhagic fever virus), and the rest are zoonotic agents transmitted between rodents or bats without known arthropod vectors (Simmonds et al., 2012). The mosquito-borne flaviviruses have been further subdivided based on the main vector genus, with the *Culex*-borne viruses often associated with encephalitic disease in humans and the *Aedes*-borne viruses with hemorrhagic disease (Gaunt et al., 2001). A group which includes "insect-specific" flaviviruses (e.g. Cell fusing agent virus) that appear only to infect mosquitoes or mosquito cells is now recognized (Cook et al., 2012).

The arthropod-borne flaviviruses are maintained in nature by transmission between hematophagous arthropod vectors to vertebrate hosts, but in the arthropod vectors, the viruses may also be passed on trans-ovarially or vertically (Simmonds et al., 2012). When the mosquito ingests a DENV-infected blood meal, the virus first infects the midgut tissue, within which it replicates to produce more virus particles. It then spreads through the hemolymph to other tissues such as the trachea, fat body, and salivary glands, where it is further propagated through replication. Peak virus titers usually occur between 7 and 10 days post-infection in the midgut and between 7 and 17 days in the abdomen. Peak levels in the head and salivary gland occur later, at about 12–18 days after feeding (Salazar et al., 2007).

### 3.4. Alphavirus-like supergroup

#### 3.4.1. General characteristics

A supergroup of positive stranded RNA viruses, the Alphavirus-like viruses, which was recognized about 30 years ago (Goldbach, 1987; Strauss and Strauss, 1988), includes viruses that share key characteristics with *Sindbis virus* (the type species of the genus *Alphavirus*, family *Togaviridae*). Members of the genus *Alphavirus* have spherical enveloped particles of 40 nm in diameter containing capped positive stranded RNA of 11 kb in length. The virions are assembled into icosahedral particles of approximately 70 nm in diameter. The 40 nm particle core consists of 240 copies of the capsid protein (C) surrounding the genomic RNA. The nucleocapsid core is covered by a lipid bilayer containing heterodimers of the two viral glycoproteins, E2 and E1, which form a regular icosahedral surface lattice (Powers et al., 2012). The genomic RNA, which serves as mRNA, encodes conserved non-structural proteins which are involved in RNA replication and are encoded by the 5' proximal part of the genome in the 5' to 3' order. These proteins are nsP1 N7-methyltransferase (nsP1), superfamily 1 helicase and protease (nsP2), RNA dependent RNA polymerase (nsP4). Another distinctive feature of members of the *Togaviridae* is the expression of the 3' proximal ORFs, which encode the structural proteins, through subgenomic RNAs (Fig. 3J). The alphavirus subgenomic mRNA is translated into a single polyprotein which is cleaved by both viral and cellular proteases to produce which individual structural proteins. The glycoproteins (E1 and E2) are inserted into the endoplasmic reticulum during translation and are translocated to the plasma membrane. Upon generation of sufficient C protein, this protein assembles with the viral RNA to form the viral nucleocapsids which occurs in the cytosol. Budding through the plasma membrane leads to the acquisition of a lipid envelope containing the two membrane glycoproteins (E1, E2) (Powers et al., 2012).

Alphavirus-like viruses include members of the family *Alphatetraviridae*, genera *Betatetravirus* (type species *Nudaurelia capensis beta virus*, Fig. 3K) and *Omeгатetravirus* (type species *Nudaurelia capensis omega virus*), which have monopartite and bipartite genomes, respectively. Members of the *Alphatetraviridae* have an RNA replicase with three alphavirus-specific conserved enzymatic domains: N7-methyltransferase, superfamily 1 RNA helicase, and RNA-dependent RNA polymerase. This family has non-enveloped roughly spherical virions of about 40 nm in diameter. The structural proteins are expressed through subgenomic RNA in the case of *Betatetravirus*, or encoded by separate genomic RNA component in the case of bipartite members of the *Omeгатetravirus* genus.

#### 3.4.2. Host range and pathology

The members of the genus *Alphavirus* are arthropod-borne viruses which alternate between replication vertebrates and in hematophagous arthropods and transmitted via the bite of a persistently infected arthropod vector, usually a mosquito. In vertebrates, alphaviruses cause disease ranging from mild febrile illness to prolonged arthritis to encephalitis (Powers et al., 2012; Suhrbier et al., 2012). Alphaviruses are commonly referred to as 'Old World' and 'New World' viruses, with Old World viruses (such as Chikungunya virus, CHIKV, Ross River virus, Barmah Forest virus, O'nyong-nyong virus, ONNV, Sindbis virus, SINV, and related viruses) generally associated with rheumatic disease in humans and 'New World' viruses (which include Venezuelan, Eastern and Western equine encephalitis viruses) primarily associated with potentially fatal encephalitic disease (Suhrbier et al., 2012). SINV infects humans and avian hosts and are transmitted by *Culex* sp. mosquitoes. SINV infects both mosquitoes and mosquito cells. Persistently infected mosquito or *Drosophila* cells are morphologically not distinguishable from uninfected cells, though they show a reduction in the growth rate compared to uninfected. ONNV is

primarily transmitted by mosquitoes of the genus *Anopheles* (*An. gambiae* and *An. funestus*). No harmful effects of ONNV replication were observed in the insect host. Similarly, CHIKV replication has no negative impact on its *Aedes aegypti* vector (Atkins, 2013).

Unlike *Togaviridae* which infect mosquitoes and vertebrates, the members of the *Alphatetraviridae* family, infect exclusively insects of the order Lepidoptera, butterflies and moths (Powers et al., 2012). These include orally transmitted *Thoesa asigna virus* (TaV) which causes natural epizootics in the larvae of the moth *Setothosea asigna*, a major defoliating pest of oil and coconuts palms (Sugiharti et al., 2010).

## 4. Double-stranded RNA viruses

Invertebrate viruses with double-stranded RNA genomes are assigned to the families *Reoviridae* and *Birnaviridae*, which are not presently assigned to an order. These viral families have different types of non-enveloped virions, different sets of genes, differ in genome segment numbers, and, importantly, possess RdRp genes belonging to different evolutionary lineages.

### 4.1. Family Reoviridae

#### 4.1.1. General characteristics

Members of the *Reoviridae* have characteristic icosahedral non-enveloped virions with an overall diameter of 60–80 nm. The protein capsid has one, two or three layers of capsid proteins surrounding 10–12 linear dsRNA genomic fragments coding for individual viral proteins (Fig. 4A). The subfamily *Spinareovirinae* comprises viruses with large particles with up to 11 nm long protruding spikes at each of 12 icosahedral vertices. In contrast, members of the subfamily *Sedoreovirinae* have virions with a relatively smooth surface topography. The *Reoviridae* virion core is transcriptionally active and produces capped mRNAs. The virus core represents a multienzyme complex which is composed of two major proteins (VP3 and VP7 proteins in the case of *Bluetongue virus*, genus *Orbivirus*), forming a capsid shell composed of 120 protein subunits. The capsid shell contains 10–12 genomic RNA segments packed in liquid crystalline arrays in its interior. Each genomic RNA segment is associated with one of 12 RNA replicase subunits composed of two or three proteins. These polymerase complexes are likely to be associated with the capsid near the 5-fold axes of symmetry (Zhang et al., 2003; Tao and Ye, 2010). In the case of *Bluetongue virus*, the RNA replicase comprises three peptides: VP1, which contains a RdRp domain involved in mRNA production and replication of the genomic RNAs, VP4 methyltransferase, which is required for mRNA capping, and VP6 helicase (Boyce et al., 2004; Ramadevi et al., 1998).

The mode of entry of viruses into cells varies between genera but usually involves the loss of outer capsid components. The particle cores derived from the parental virions, represented by single or double layered particles (from subfamily *Spinareovirinae* or *Sedoreovirinae*, respectively), are released into the cell cytoplasm. These core particles are transcriptionally active, each containing the 10 segments of the genomic dsRNA within them, which are used as a template to synthesize an mRNA molecule of the same length as the positive strand in the dsRNA component. These mRNAs are capped by viral enzyme but not polyadenylated (Fig. 4A). All enzymatic activities required for the initiation of RNA synthesis, capping, and elongation of the product are present in the core. The newly synthesized mRNA molecules are extruded from the 12 vertices of the icosahedral core, while the parental dsRNA molecules remain in the core (Attoui et al., 2012).

The sites of viral mRNA synthesis, genome replication and particle assembly (virus inclusion bodies), occur in localized areas

of the infected cell cytoplasm. The mechanism of genome assembly and synthesis remains largely uncharacterized. For orthoreoviruses and rotaviruses it was demonstrated that the sets of capped mRNAs and certain NS proteins are incorporated into the precursors of progeny virus particles. These mRNAs are then used as templates for a single round of minus strand synthesis, thereby reforming the dsRNA genome segments of a progeny virus particle. The dsRNA genome segments are usually packaged in exactly equimolar ratios (i.e. one copy of each genome segment per particle). The selection of viral mRNAs for packaging is therefore thought to be highly specific, involving recognition signals on each mRNA species. The dsRNA within assembled particles has been shown, in at least some genera, to be packaged as a series of concentric and highly organized shells, which also have elements of icosahedral symmetry. A distinctive feature of insect-infecting members of the genus *Cypovirus* (e.g. *Bombyx mori cypovirus 1*) is that virus particles are embedded in a protein crystal, known as polyhedra which preserves virus infectivity in the environment (Coulibaly et al., 2007).

#### 4.1.2. Host range and pathology

The family *Reoviridae* comprises 102 species that infect a range of hosts, including vertebrates, several invertebrate groups, as well as plants, fungi and protozoa (Attoui et al., 2012; Brussaard et al., 2004; Hillman et al., 2004). The members of the *Reoviridae* that infect invertebrates are present in 11 out of 15 genera of this family. The genera with viruses that only infect invertebrates, include the subfamily *Sedoreovirinae*, genus *Cardoreovirus*, with the single species *Eriocheir sinensis reovirus*, and three genera in the subfamily *Spinareovirinae*: *Dinovernavirus* (type species, *Aedes pseudoscutellaris reovirus*), *Idnoreovirus* (type species, *Idnoreovirus 1*), and *Cypovirus* (type species *Cypovirus 1*). Arboviruses in the *Reoviridae*, which infect both hematophagous arthropods and vertebrates, include the members of two genera of the subfamily *Sedoreovirinae*: *Orbivirus* (type species, *Bluetongue virus*) and *Seadornavirus* (type species, *Banna virus*); and the *Spinareovirinae* tick-transmitted genus *Coltivirus* (type species, *Colorado tick fever virus*). Members of the *Reoviridae* are also present in aquatic ecosystems. Members of the genus *Aquareovirus* in the subfamily *Spinareovirinae* (type species, *Aquareovirus A*) replicate in fish, bivalve mollusks and crustaceans (Lupiani et al., 1995). Three genera replicate in insects and plants, including the genus *Phytoreovirus* in the subfamily *Sedoreovirinae* (type species *Wound tumor virus*), and the genera *Fijivirus* (type species *Fiji disease virus*) and *Oryzavirus* (type species *Rice ragged stunt virus*) the subfamily *Spinareovirinae* (Attoui et al., 2012).

The members of the different *Reoviridae* genera have distinct biological properties. The genus *Cypovirus* comprises the cytoplasmic polyhedrosis viruses of insects found in over 250 insect species. Cypoviruses have only been isolated from arthropods and do not infect vertebrate cells. The majority of *Cypovirus* infections produce chronic disease, usually without high larval mortality. A distinctive feature is the incorporation of the virus particles (about 70 nm in diameter) into large (several microns) structures, polyhedra, during the late stages of infection (Attoui et al., 2012; Belloncik and Mori, 1998). Cypoviruses are normally transmitted by ingestion of polyhedra on contaminated food materials. The polyhedra dissolve within the high pH environment of the gut and release occlusion derived virus, which then infect the cells lining the gut. Virus infection in larvae is generally restricted to the columnar epithelial cells of the midgut, although virus replication was also reported in the fat body (Attoui et al., 2012). The production of polyhedra gives the gut a characteristically creamy-white appearance. In infected cells the endoplasmic reticulum is progressively degraded, mitochondria enlarge and

the cytoplasm becomes highly vacuolated. In most cases, the nucleus shows few pathological changes. In the later stages of infection, cellular hypertrophy is common and microvillae are reduced or absent. Polyhedra are released by cell lysis into the gut lumen and excreted (Attoui et al., 2012; Belloncik and Mori, 1998).

The members of *Orbiviridae*, including Bluetongue virus (BTV), are infectious in both vertebrate and insect cells. Different orbiviruses infect a wide range of vertebrate hosts including ruminants (domesticated and wild), equids (domesticated and wild), rodents, bats, marsupials, birds, sloths and primates, including humans. Orbiviruses can also replicate in, and are primarily transmitted by, arthropod vectors (gnats, mosquitoes, sand flies, or ticks, depending on the virus). Infection of vertebrates *in utero* may also occur. Orbiviral RNA has been detected in *Culicoides* larvae, although trans-ovarial transmission has not been confirmed. Orbiviruses have also been detected in cell lines derived from tick eggs (Attoui et al., 2012).

#### 4.2. Family Birnaviridae

This family comprises non-enveloped, single-shell virions of about 65 nm in diameter, which are composed of a single type of capsid protein, VP2. *Birnaviridae* virions contain two double-stranded linear genomic RNA components, named A (3.1–3.6 kbp) and B (2.8–3.3 kbp). The RdRp is encoded by the component B (VP1 protein), which is present in virions and exists either in free form or as a Vpg protein covalently linked to the 5'-termini of the positive strands of both genomic RNA components (Magyar et al., 1998). The component A encodes VP2, a structural protein that form the virion shell, VP4 protease, which is involved in processing of the viral polypeptides, and VP3, which interacts with the genomic RNA forming thread-like ribonucleoprotein complexes. A remarkable feature of the *Birnaviridae* is the combination of double stranded RNA genome and the RdRp domain related to the RdRp of picorna-like viruses, particularly the permuted RdRp (Fig. 3E, pRdRp\*) of *Thosea asigna virus*, family *Permutotetraviridae*, genus *Alphapermutotetravirus* (Shwed et al., 2002; Gorbalenya et al., 2002; Koonin et al., 2015). There is one recognized invertebrate-infecting *Birnaviridae* species, *Drosophila virus X* (DVX), which is the sole member of the genus *Entomobirnavirus* (Chung et al., 1996). As a pathogen of a model host, DWX is widely used to study fundamental aspects of interactions between RNA viruses and insects, including antiviral resistance (Wang et al., 2006).

### 5. Conclusions

Invertebrates play major and diverse roles in natural and agricultural ecosystems, both terrestrial and aquatic. A growing body of evidence suggests that viruses, which are widespread in invertebrates, can significantly affect the health and population dynamics of their hosts. Examples of this include the effects of bee viruses on pollination services (Vanbergen, 2013), or shrimp viruses in aquaculture (Walker and Mohan, 2009). In addition, numerous arboviruses are vectored by hematophagous arthropods and transmitted to livestock and humans. Consequently, many of these viruses have major veterinary and medical importance (Weaver and Reisen, 2010). Similarly, crop production is greatly affected by plant viruses transmitted by their insect vectors in a propagative manner (Hogenhout et al., 2008). Knowledge of virus host range, pathogenicity, characteristics of the virions and genomic sequence information is essential for monitoring viruses in nature and developing approaches to control the prevalence of infection or minimize their negative impact.

The growing availability of RNA sequence information, in particular high-throughput Next Generation Sequencing (NGS), which is replacing Sanger technology, has made it possible to explore RNA virus diversity in an ever wider range of invertebrate species (Liu et al., 2011), as well as the adoption of metagenomic approaches for the analysis of environmental samples (Culley et al., 2003). These technical developments have resulted in dramatic increases in the abundance of RNA virus-derived genomic sequence information, but have also brought new challenges. Most important among these is the scarcity or complete lack of biologically relevant data, including the host range, pathological impact on hosts(s) and virion characteristics, for the viral sequences produced using high-throughput methods. Also, *in silico* assembly of relatively short NGS reads from viral populations can be hindered by the quasispecies structure of RNA virus populations (Prosperi et al., 2013), and could impact on the accuracy of the assembled virus sequences. The ICTV has defined standards for virus annotation (Brister et al., 2010), and for establishing new virus species and higher taxonomic groups (King et al., 2012), in an effort to resolve challenges related to the use of NGS. As such, maintaining and further developing the work of the ICTV expert study groups is essential for the virology community worldwide.

## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jip.2016.10.002>.

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