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Diversity of large DNA viruses of invertebrates

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ABSTRACT

In this review we provide an overview of the diversity of large DNA viruses known to be pathogenic for invertebrates. We present their taxonomical classification and describe the evolutionary relationships among various groups of invertebrate-infecting viruses. We also indicate the relationships of the invertebrate viruses to viruses infecting mammals or other vertebrates. The shared characteristics of the viruses within the various families are described, including the structure of the virus particle, genome properties, and gene expression strategies. Finally, we explain the transmission and mode of infection of the most important viruses in these families and indicate, which orders of invertebrates are susceptible to these pathogens.

1. Introduction

Invertebrate DNA viruses span several virus families, some of which also include members that infect vertebrates, whereas other families are restricted to invertebrates. In this review we provide an overview of the large DNA viruses known to be pathogenic for invertebrate species. We define viruses as "large" when they have a particle length or diameter over 100 nm. It is important to note that arthropods, in particular insects, mites and ticks, may also serve as vectors for viruses that cause diseases in vertebrates and plants. Therefore, plant- and vertebrate-infecting viruses may also replicate in blood-feeding and plant-sucking arthropods. Most of these vector-borne viruses are RNA viruses and will not be considered further. Exceptions include the DNA viruses in the genus *Begomovirus* (family *Geminiviridae*), which are transmitted by whiteflies and infect a variety of plant species, but have a mutualistic rather than pathogenic relationship with their insect vector.

The large DNA viruses that are pathogenic to invertebrates can be divided in three main groups that share several characteristics within each group (see also Fig. 1 and Supplementary Table S1):

(a) Eukaryotic nucleo-cytoplasmic large DNA viruses (NCLDVs) with a double stranded (ds) linear or circular genome that complete all or part of their replication and capsid assembly in the cytoplasm. This group comprises viruses in the families *Poxviridae* (subfamily *Entomopoxvirinae*) and *Iridoviridae*. The viruses in the family *Ascoviridae* are also discussed as part of this group as their replication starts in the nucleus, which later degrades. All these viruses are discussed as one group as they share evolutionary relationships and are placed together in the proposed order *Megavirales* (Colson et al., 2013), that also contains viruses that infect protists (*Mimiviridae*) or the symbiotic algae of such protists (*Phycodnaviridae*).
(b) Enveloped viruses with rod-shaped nucleocapsids and a large circular dsDNA genome that replicate and assemble in the nucleus of infected cells. This group comprises viruses in the families *Baculoviridae*, *Nudiviridae*, *Hytrosaviridae* and *Nimaviridae*. We will also consider two not yet classified dsDNA viruses of Hymenoptera, Leptopilina boulardi filamentous virus and Apis mellifera filamentous virus in this group.
(c) Viruses with icosahedral capsids surrounded by an envelope and containing linear dsDNA. This group is formed by viruses in the family *Malacoherpesviridae* in the order *Herpesvirales* that infect ostreid and haliotid mollusks.

In the sections below we describe the general properties of the three known groups with large, autonomously replicating DNA viruses of invertebrates from a taxonomical perspective. For this, we have referred to the most recent Taxonomy Release from the International Committee on Taxonomy of Viruses (ICTV, 2015). We

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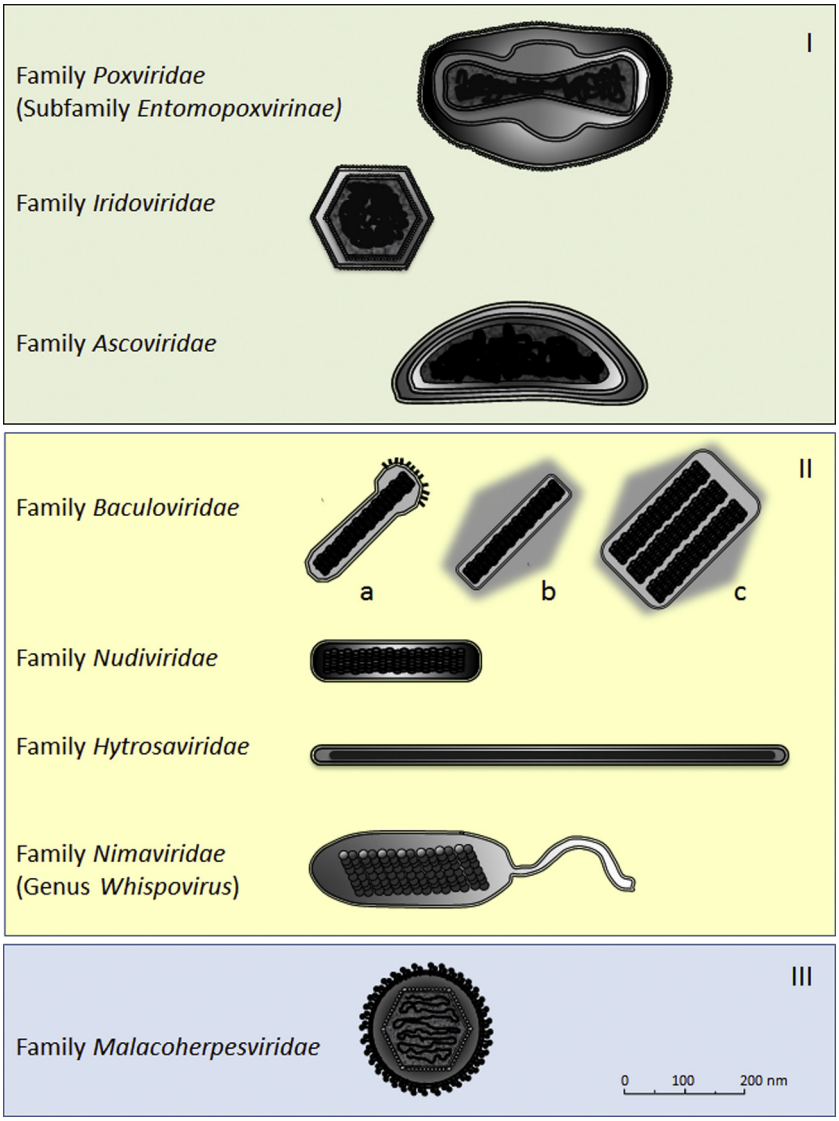

Fig. 1. Large invertebrate DNA virus diversity. Virus particle morphology is indicated per group. For the family *Baculoviridae* budded viruses (BV) (a) as well as occluded viruses (ODV) of GVs/SNPVs (b) and MNPVs (c) are depicted. The size of the occlusion body (OB, in gray) is not to scale (courtesy of Eugene Ryabov).

will describe the evolutionary relationships between the viruses in these groups and, where appropriate, their relationships to vertebrate-infecting viruses. We will also examine how some of the large DNA viruses of invertebrates relate to endogenous viral elements in the genomes of parasitic wasps that provide these wasps with the ability to produce polydnaviruses or virus-like particles. For detailed descriptions of the biological properties and how all these viruses may relate to the food chain, we refer to the respective papers elsewhere in this special issue of the Journal of Invertebrate Pathology. For an overview of the small DNA viruses in invertebrates, including viruses belonging to the subfamily *Densovirinae* in the family *Parvoviridae*, to the families *Bidnaviridae* and *Circoviridae* we refer to the respective manuscript by Tijssen et al. (this issue).

2. Nucleo-cytoplasmic large DNA viruses

2.1. Entomopoxviruses

2.1.1. General characteristics

Entomopoxviruses (EVs) represent a homogeneous group of large cytoplasmic dsDNA viruses that infect different orders of insects. They share significant morphological and molecular similarities with their vertebrate counterparts, the chordopoxviruses (ChPVs). The brick-shaped or ovoid mature EV virions (Figs. 1 and 2A) consist of three major components: an outer lipoprotein envelope, one or two lateral bodies and a centrally positioned electron dense core containing the genome. The size of EV particles varies from 250 to 400 nm in length and 150–250 nm in width. Their envelope is ornamented with globular prominent structures giving the particles a mulberry-like appearance. The lateral bodies occupy the space between the envelope and the central core, which consists of a thick coat containing a folded cable-like structure surrounding the viral DNA (Bergoin and Dales, 1971; Granados and Roberts, 1970). Three morphotypes of EVs are observed that differ in the shape of the core (Granados, 1981). EVs infecting coleopterans exhibit a kidney-shaped unilaterally concave core and a single lateral body located in its concavity (morphotype A). Those infecting lepidopterans and orthopterans possess a cylindrical core flanked by two discrete lateral bodies (morphotype B). EVs infecting dipterans possess a biconcave core flanked by two symmetrically positioned lateral bodies (morphotype C) resembling the typical dumbbell-shaped core of vertebrate poxviruses.

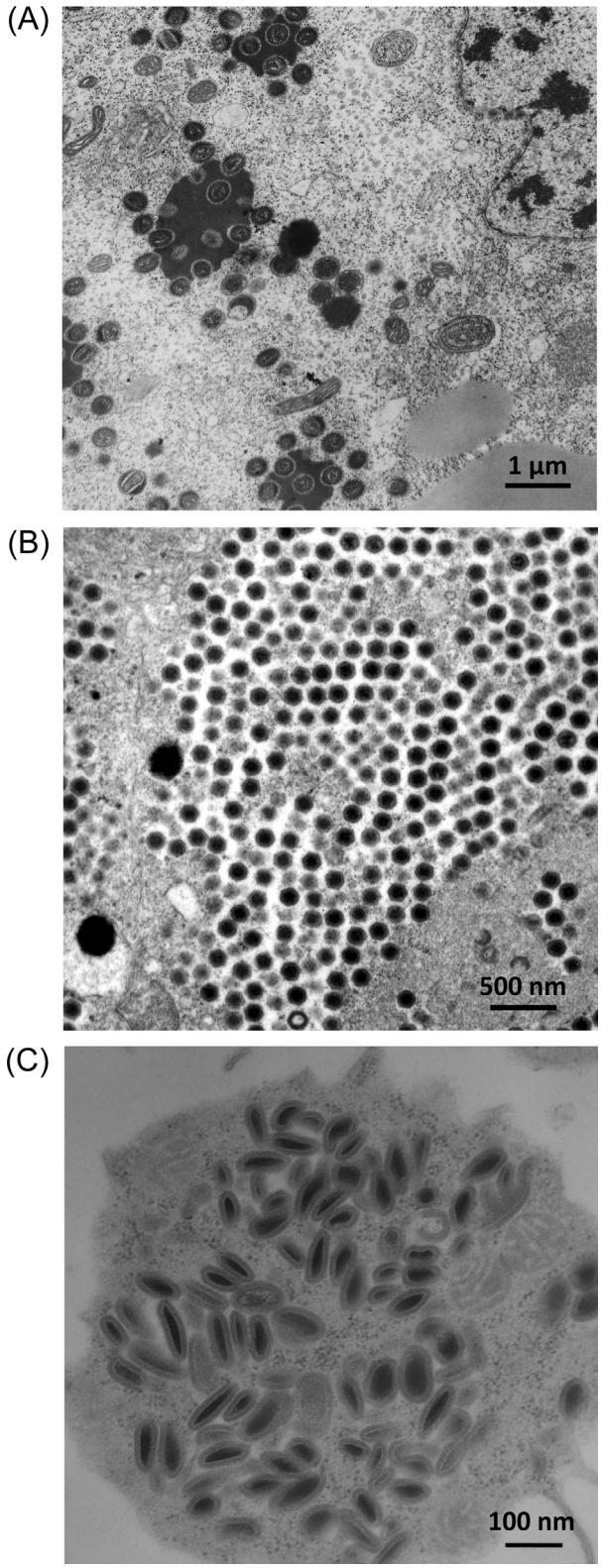

Fig. 2. Electron microscope images of cells infected with NCDLVs. (A) Entomopoxvirus: Amsacta moorei EV virions in the process of being occluded in spheroids (courtesy of Bob Granados). (B) Iridovirus: Invertebrate iridescent virus 3 (IIV-3) infecting *Aedes taeniorhynchus* fat body (courtesy of James Becnel). (C) Ascovirus: vesicle of HvAV-3f infecting *Heliothis virescens* larvae (courtesy of Xiao-Wen Cheng).

EV-infected cells contain large (4–20 µm) paraspherical or ovoid cytoplasmic inclusions named spheroids (Amargier et al., 1964; Roberts and Granados, 1968; Vago and Bergoin, 1968). These inclusions consist of a proteinaceous crystalline lattice in which mature virions are occluded in a manner similar to the virions of baculoviruses and cypoviruses. Spheroids allow the virus to retain its infectivity in the environment, sometimes for months, between host generations. The major component of spheroids is a 110–115 kDa cysteine-rich protein termed spheroidin (Hall and Moyer, 1991, 1993; Sanz et al., 1994). Bipyramidal-shaped inclusions, 1–10 µm in length, named spindles, are frequently observed in EV infections. They are devoid of virions and consist of a crystal of a 36–44 kDa cysteine-rich protein termed fusolin that shares significant homology with the GP37 baculovirus glycoprotein (Dall et al., 1993; Gauthier et al., 1995; Gross et al., 1993). In lepidopteran EVs spindles may be occluded along with virions in the spheroid matrix (Arif and Kurstak, 1991; Bird, 1974).

2.1.2. Host range and taxonomy

EVs have been isolated from ~40 different species of insects, mostly from the orders Lepidoptera, Coleoptera, Orthoptera, and Diptera (Arif and Kurstak, 1991; Granados, 1981), but also from Blattoidea (Radek and Fabel, 2000) and Hymenoptera (Lawrence, 2002). The host spectrum of EVs seems to be restricted to species of the same or closely related genera as that of the host from which they were isolated (Levin et al., 1993; Takatsuka et al., 2010). EVs are named after the host species from which they were isolated. For example, the EVs isolated from *Melolontha melolontha* is named Melolontha melolontha entomopoxvirus (MMEV). All EVs are classified in the subfamily *Entomopoxvirinae* of the family *Poxviridae* (Skinner et al., 2011). They are assigned to three genera based on the shape of virion core and the insect order from which they were isolated: *Alphaentomopoxvirus* that infect Coleoptera, *Betaentomopoxvirus* that infect Lepidoptera and Orthoptera and *Gammaentomopoxvirus* that infect Diptera. Phylogenetic analysis of complete genome sequences (see below), resulted in the exclusion of the EV that infects the orthopteran *Melanoplus sanguinipes* from the genus *Betaentomopoxvirus* (Skinner et al., 2011).

2.1.3. Replicative cycle and pathology

The normal infection route of EVs involves the ingestion of spheroids by susceptible insect larvae. The spheroid matrix dissolves in the alkaline insect midgut releasing virions while partial digestion of the spindle crystal activates a chitinase domain of the fusolin sequence that degrades the peritrophic matrix to facilitate access of the large EV virions to midgut cells to initiate infection (Chiu et al., 2015; Mitsuhashi and Miyamoto, 2003; Takemoto et al., 2008. Virions enter midgut columnar cells by fusion of their envelope with microvilli membranes (Granados, 1973a). Virogenesis is initiated in cytoplasmic viral factories and parallels the steps observed in vaccinia-infected HeLa cells (Bergoin and Dales, 1971; Devauchelle et al., 1971; Granados and Roberts, 1970). In hemocytes, occlusion of mature virions in spheroids is seldom observed, and they are released in the hemolymph by budding (Devauchelle et al., 1971), whereas in fat body cells most of the virions are occluded (Bergoin et al., 1969).

EV infections are lethal for their hosts but the course of infection is very slow. Infected lepidopteran larvae can survive one to ten weeks (Bird et al., 1971; Granados, 1973b), whereas infected coleopterans may survive for several months (Hurpin, 1968). This slow speed of kill, combined with the fact that related poxviruses are serious human and animal pathogens, has discouraged their use as biocontrol agents. However, EVs are responsible for slow developing epizootics, which may affect the frequency of insect pest outbreaks (Harkrider and Hall, 1978). EV infection often delays or prevents pupation or adult emergence. This is due to EV-mediated changes in juvenile hormone and ecdysteroid titers that prevent metamorphosis (Palli et al., 2000).

Fat body cells and hemocytes are the primary sites of infection in most EVs (Amargier et al., 1964; Götz et al., 1969; Henry et al., 1969), although some EVs have a broader tissue tropism including silk gland, hypodermis, muscles, tracheal cells and reproductive organs (King et al., 1998; Radek and Fabel, 2000; Roberts and Granados, 1968). Typical signs of infection include the loss of mobility, flaccidity and whitening caused by destruction of adipose tissue and the accumulation of spheroids and spindles in the body cavity. The EV of the braconid parasitoid *Diachasmimorpha longicaudata* infects the poison gland apparatus of females but the virus also replicates in the hemocytes of its dipteran host, *Anastrepha suspense* (Lawrence, 2002, 2005).

2.1.4. Genome structure and gene content

The genome of EVs, like that of ChPVs, consists of a linear dsDNA molecule including a central region flanked by an inverted terminal repeat (ITR) at each extremity, consisting of short, tandemly repeated sequences. At the end of each ITR an incompletely base-paired hairpin loop covalently connects the two DNA strands, thus forming a continuous polynucleotidic chain. Adjacent to the hairpin of each extremity, a highly conserved concatemer resolution motif of ca. 20 nucleotides is essential for the resolution of concatemeric DNA molecules produced during replication (Moss, 2013). This motif, appropriately positioned in the ITR sequence of the Anomala cuprea entomopoxvirus (ACEV) genome, strongly suggests that EVs share the same mode of DNA replication as ChPVs (Mitsuhashi et al., 2014).

As the replicative cycle of all poxviruses occurs entirely in the cytoplasm (Moss, 2007), they must encode all the structural proteins and enzymes necessary for their own transcription, replication, virion assembly and release (Condit et al., 2006). These genes are located in the central region of all poxvirus genomes. The genes located towards either ends of the genome are much more variable among ChPV species, and the proteins they code for are mostly involved in virus-host interactions, such as host range, immunomodulation and pathogenicity (Johnston and McFadden, 2004; Stanford et al., 2007). Gene expression in EVs is believed to follow that observed in ChPVs, in which regulation occurs via a cascade of stage-specific transcription factors that recognize distinct early, intermediate and late promoters (Moss, 2007; Yang et al., 2010).

Only seven complete sequences of EV genomes have been published to date. These include the 245,717 bp sequence of ACEV, the only alphaentomopoxvirus (Mitsuhashi et al., 2014), the sequences of five betaentomopoxviruses, that range from 228,750 bp for Adoxophyes honmai entomopoxvirus (AHEV) to 307,691 bp for Choristoneura biennis entomopoxvirus (CBEV) (Bawden et al., 2000; Thézé et al., 2013), and the presently unclassified orthopteran Melanoplus sanguinipes entomopoxvirus (MSEV) that has a genome of 236,120 bp (Afonso et al., 1999). In line with the genome length, their predicted coding sequences range from 247 ORFs for AHEV to 334 ORFs for CBEV, corresponding to about 90% of their genome coding capacity. Their high A+T content (79–81.7%) distinguish them from most ChPV genomes whose A+T content ranges from 60 to 75%. The size of their ITRs varies from 5.6 kbp in Amsacta moorei entomopoxvirus (AMEV) to 23.8 kbp in CBEV.

A total of 148 orthologous genes are common to all five lepidopteran EVs (*Betaentomopoxvirus* core genes) and 104 genes are common to betaentomopoxviruses and the unassigned orthopteran MSEV (Thézé et al., 2013). The seven EV genomes contain, in their central region, the 49 highly conserved poxvirus core genes involved in functions including transcription (21 genes), replication (8 genes), and virion assembly and release (20 genes) (Mitsuhashi et al., 2014; Thézé et al., 2013; Upton et al., 2003). The 148 core genes of the betaentomopoxviruses have a high synteny conservation of the genes in the central region, whereas the genes located near the extremities show lower colinearity (Thézé et al., 2013). Contrary to most chordopoxvirus genomes, which share the same overall gene arrangement of the 49 poxvirus core genes (Gubser et al., 2004; Lefkowitz et al., 2006), gene parity plots failed to show any colinearity between vaccinia and EV genomes or between genomes from each of the EV genera (Mitsuhashi et al., 2014; Perera et al., 2010), highlighting significant divergence between the coleopteran, lepidopteran and orthopteran EV genomes. However, among the 241 haploid (single copy) genes of the ACEV genome, 152 (63.1%) are similar to those of the six other EV genomes, of which 137 are similar between ACEV and AMEV genomes and 128 between ACEV and MSEV genomes, implying that ACEV is phylogenetically closer to AMEV than MSEV (Mitsuhashi et al., 2014).

All EVs contain multiple copies of genes belonging to large gene families of unknown function including the myeloid translocation gene motif (MTG), 17K/kilA-N domain containing proteins, alanine-leucine-isoleucine motif subgroups 1 and 2 (ALI), and N1R/p28 gene family, that are predominantly localized in the terminal regions of the genome. The N1R/p28 proteins of ChPVs have been implicated in preventing cellular antiviral apoptotic responses (Brick et al., 2000; Nicholls and Gray, 2004).

2.1.5. Phylogeny

Phylogenetic analyses of a concatenated multiple alignment of the 49 poxvirus core genes of vaccinia (representative of *Chordopoxvirinae*) and *Entomopoxvirinae* clearly revealed two major monophyletic clades corresponding to the two subfamilies. The five lepidopteran EVs and the two coleopteran EVs were each grouped in a monophyletic lineage corroborating their status as five species in the *Betaentomopoxvirus* genus and two species in the *Alphaentomopoxvirus* genus, clearly separated from the orthopteran MSEV (Fig. 3A). Phylogenetic analysis of the highly conserved spheroidin gene, for which 14 sequences of lepidopteran, coleopteran and orthopteran EVs are available, has corroborated the distances between the EVs infecting these three insect orders (Thézé et al., 2013) and justified the creation of a new genus for orthopteran EVs, as previously suggested (Hernandez-Crespo et al., 2000). Furthermore, these phylogenetic trees clearly show that coleopteran EVs are closer to lepidopteran EVs than to orthopteran EVs. This pattern is in agreement with the phylogenetic distances of these three orders in the class *Insecta* and supports the concept of coevolution of EVs with their hosts.

2.1.6. Relationships with other taxa

Studies on the evolutionary origins of ChPV-encoded proteins showed that capture of host genes by horizontal gene transfer (HGT), or progressive inactivation or loss of a number of genes originally present in an ancestral virus, have been a recurrent feature of poxvirus evolution and have allowed these viruses to overcome host antiviral defense mechanisms (Hendrickson et al., 2010; Hughes and Friedman, 2003; Iyer et al., 2006; Odom et al., 2009). Apart from their core genes, EVs also share a number of homologous, accessory genes probably acquired from their hosts (Bratke and McLysaght, 2008; Dall et al., 2001; Hughes and Friedman, 2003). A recent study comparing proteins of EVs and baculoviruses infecting the same lepidopteran hosts revealed 33 clusters of homologous genes shared by these two virus families (Thézé et al., 2015), including the DNA polymerase core protein and 32 genes of the accessory genome repertoire, independently acquired by HGT. These include homologs of essential cellular enzymes, genes involved in insect immune response, multicopy viral gene families and genes related to host ecology. Twenty of these genes are common to other large dsDNA virus families (Thézé et al., 2015). This convergence of gene acquisition between two virus families with divergent genomic structure, but overlapping

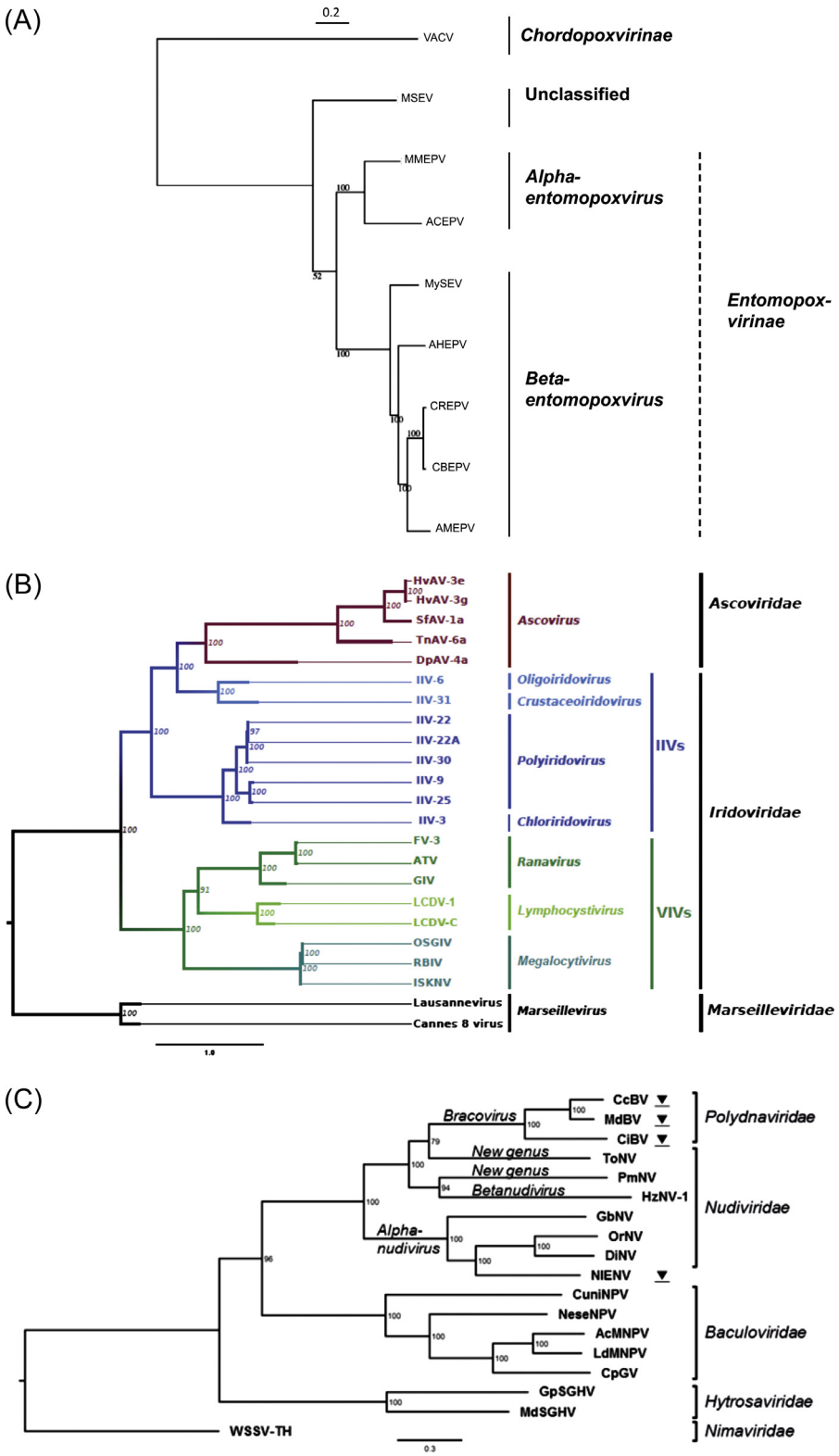

Fig. 3. Evolutionary relationships of large DNA viruses of invertebrates. For full names of the viruses included see Supplementary Table S2. (A) Phylogeny showing the relationship between entomopoxviruses. The tree was obtained from maximum likelihood inference analysis of aminoacid sequences derived from the 49 concatenated core genes of 9 entomopoxviruses. Vaccinia virus (VACV) was used as outgroup. Branch lengths are proportional to genetic distances and support for nodes is indicated as bootstraps (100 replicates). This phylogeny was kindly provided by F. Cousserans. (B) Phylogeny showing the relations between iridoviruses and ascoviruses based on nine core proteins. Numbers in italics at nodes indicate percentage bootstrap values (1000 replicates). Branch lengths are proportional to genetic distances. The taxonomic levels from genera to families are indicated (IIVs invertebrate iridescent viruses, VIV vertebrate iridoviruses). Two species of the NCLDV family *Marseilleviridae* were used as outgroups (reproduced by permission of Y. Bigot, adapted from Piégu et al. (2015)). (C) Phylogeny of large rod-shaped DNA viruses. The tree was obtained by ML inference analysis of a concatenated amino acid multiple alignment of 37 nudivirus-related genes. Numbers on the nodes indicate ML nonparametric bootstraps support (100 replicates). The white spot syndrome virus was defined as outgroup based on genome content. ▼: endogenous viral element derived from two independent nudivirus integration events, which led either to the bracovirus symbiosis or to the *Nilaparvata lugens* endogenous NV. The figure was reproduced from Bezier et al. (2015) with permission of A. Bezier and the American Society for Microbiology.

ecological niches and that infect the same hosts, illustrates the independent but similar evolution of viruses subjected to comparable selective forces from the host immune response.

2.2. Invertebrate iridescent viruses

2.2.1. General characteristics

The invertebrate iridescent viruses (IIVs, family *Iridoviridae*) are icosahedral particles that comprise a DNA and nucleoprotein core, an internal limiting double membrane and a viral capsid from which numerous flexible fibrils extend outwards (Jancovich et al., 2012). Particle size measurements vary depending on the sample preparation methods, but typically vary from 120 to 180 nm in ultrathin section (Figs. 1 and 2B). Measurements of vitrified particles of Invertebrate iridescent virus 6 (IIV-6, also known as Chilo iridescent virus), indicated a capsid diameter of 162–185 nm, depending on the axis of symmetry (Yan et al., 2000). The structure of IIV particles is complex with at least 54 virion associated proteins in IIV-6 (Ince et al., 2010) or 64 in Invertebrate iridescent virus 9 (IIV-9) (Wong et al., 2011). The capsid comprises 20 trisymmetrons and 12 pentasymmetrons located at the apices of the capsid (Yan et al., 2009). Both structures are mainly formed of hexavalent capsomers composed of two trimers of the highly conserved major capsid protein (51.4 kDa in IIV-6). A single fibril extends outwards from the center of the outer trimeric capsomer, which in the case of IIV-6 averages 35 nm in length. Additional proteins bind the capsomers together whereas dimeric zip proteins bind trisymmetrons and zip protein monomers bind trisymmetrons to pentasymmetrons, beneath which transmembrane anchor proteins connect pentasymmetrons and the internal lipid membrane (Yan et al., 2009).

The genome of IIVs comprises a linear molecule of dsDNA that is circularly permutated and terminally redundant. As such, the terminal sequences of the genome differ from one DNA molecule to another (circular permutation) and ~10% of the genome at the 5′ terminal is repeated at the 3′ terminal (terminal redundancy). Genome lengths are in the range 163.0–220.2 kbp in the nine IIV genomes sequenced to date (Piégu et al., 2014; Wong et al., 2011). There is a positive correlation between genome length and number of putatively functional open reading frames (ORFs); the larger genomes having around 200 ORFs and the smaller genomes, both isolated from mosquitoes, having 126–148 putative ORFs (Delhon et al., 2006; Huang et al., 2015). To date 26 core genes have been identified in members of the *Iridoviridae*, the majority of which are involved in replication (Eaton et al., 2007).

The route of infection in IIVs appears to depend on the host. Cannibalism, intraspecific predation and necrophagous consumption of IIV-infected tissues have been identified as efficient routes of transmission in some species (Carter, 1973; Fowler, 1989; Williams and Hernández, 2006). IIV particles are highly infectious when introduced into the insect hemocoel, which may occur during oviposition by hymenopteran parasitoids (Lopez et al., 2002), or by the action of entomopathogenic nematodes (Mullens et al., 1999; Muttis et al., 2015).

IIV particles enter the cell by endocytosis (Chitnis et al., 2008), a process better understood for vertebrate iridoviruses (Guo et al., 2012; Wang et al., 2014). Viral replication occurs in two phases: initially in the nucleus and subsequently in the cytoplasm. Gene expression occurs in a regulated temporal cascade. Numerous immediate early (IE, α) genes are expressed immediately following infection and are mainly, but not exclusively, involved in transcription, blocking cellular apoptosis, nucleoside metabolism, protein phosphorylation and transactivation factors for transcription for the delayed-early (DE, β) class of genes (D' Costa et al., 2001; Ince et al., 2008, 2013). DE genes include the viral DNA polymerase (*dpol*), protein kinase, and transactivation factors for regulation of late (L, γ) class genes that encode numerous structural proteins such as the major capsid protein, MCP (Ince et al., 2013). The abundance of IE and DE transcripts can remain high even late in infection and there is little correlation between transcription and the prevalence of particular proteins during the course of infection (D' Costa et al., 2001; Ince et al., 2015). Viral transcripts are not polyadenylated, but may have short upstream non-coding regions (Dizman et al., 2012). Virally encoded miRNAs may also be involved in post-transcriptional regulation of viral or host messages but this is poorly understood at present (Wong et al., 2011). The promoter region of IE and DE genes appear to differ in their organization in IIV-6, but for both temporal classes, critical sequences were located in the 20 nt region upstream from the transcription initiation site (Dizman et al., 2012; Nalçacioğlu et al., 2007).

Newly-synthesized viral DNA is transported from the nucleus to the cytoplasm, where large concatameric structures are formed (Goorha and Dixit, 1984), presumably within granular viral assembly sites. Late viral transcripts encode structural proteins that travel to assembly sites for virion formation. Packaging of viral DNA is believed to employ a "headful" mechanism involving concatameric DNA intermediates (Goorha and Murti, 1982). Progeny virions accumulate either in large, paracrystalline arrays in the cytoplasm, or bud from the plasma membrane and acquire an envelope. The virion assembly and budding processes have recently been described in detail for a vertebrate iridovirus (Liu et al., 2016).

The crystalline arrangement of IIV particles in infected cells is responsible for the iridescent hues of heavily infected invertebrates, the colors of which range from lavender or turquoise for most hosts, to green or orange for some mosquito species. This is the most obvious sign of patent IIV disease, which is usually lethal. In such individuals, crystalline arrays of particles are present in cells of almost all the tissues, particularly the fat body, epidermis, hemocytes, and muscles (Hall, 1985). Many individuals harbor inapparent infections (Williams, 1993), which can reduce the reproductive capacity and longevity of adult insects (Marina et al., 2003). IIV-6 particles are capable of inducing cellular apoptosis involving the JNK pathway (Paul et al., 2007), a process that is blocked by a viral inhibitor of apoptosis (*iap*) that is expressed immediately following infection (Ince et al., 2008). A protein extract of IIV-6 virions can also induce apoptosis due to the presence of a serine/threonine protein kinase (Chitnis et al., 2011).

2.2.2. Host range

Many insects and crustaceans have been reported with IIV infections, although most have not been subjected to characterization studies and their relationship to characterized IIVs remains unknown. Of the recorded host species, approximately half inhabit aquatic environments and half terrestrial habitats, many of which are soil-dwelling species (Williams, 2008). IIVs have been reported most frequently from mosquitoes, beetles and lepidopteran larvae with fewer records from the insect orders Orthoptera, Ephemeroptera, Hymenoptera, and Hemiptera (Williams, 2008). Host records from terrestrial isopods (Federici, 1980) have been recently augmented by reports from Italy and Japan that indicate that IIV-31 naturally infects multiple isopod species (Crustacea) in many parts of the world (Karasawa et al., 2012; Lupetti et al., 2013). A handful of reports of iridovirus-like particles in marine invertebrates may be similar to IIVs (Tang et al., 2007), or more closely related to vertebrate iridoviruses (Gregory et al., 2006), although the paucity of genomic information continues to hinder the classification of isolates from marine invertebrates.

The host range of individual IIVs depends on route of transmission, which is determined largely by the nature of the ecological interactions within each host-virus pathosystem (reviewed by Williams et al., 2005). IIV-3 naturally infects a single mosquito

species, although several insect cell lines (Becnel and Pridgeon, 2011) and a small number of additional mosquito species can be infected under laboratory conditions (Woodard and Chapman, 1968). IIV-6-like viruses have been reported naturally infecting several species of Lepidoptera and Orthoptera. However, intrahemocelomic injection of particles of IIV-6 results in lethal infection of many species across different orders or even different classes (Ohba and Aizawa, 1979). Under certain circumstances IIV-6 even appears capable of infecting reptiles and amphibians with pathological consequences (Marschang, 2011; Stöhr et al., 2016).

2.2.3. Evolutionary links among the IIVs

The IIVs are currently assigned to one of two genera: *Iridovirus* and *Chloriridovirus* (Jancovich et al., 2012). The *Iridovirus* genus comprises two virus species and 11 tentative species from insects and terrestrial isopods. Three distinct complexes of viruses have been identified within the *Iridovirus* genus (Webby and Kalmakoff, 1998; Williams and Cory, 1994): the polyiridovirus or group III complex comprises Invertebrate iridescent virus 1 (IIV-1) and related viruses (IIV-2, IIV-9, IIV-16, IIV-22, IIV-23, IIV-24, IIV-25, IIV-29, IIV-30, and *Anticarsia gemmatalis* IV), whereas the oligoiridovirus or group II complex comprises IIV-6, and the related virus IIV-21. Finally the custaceoiridovirus or group I complex comprises IIV-31 and *Popillia japonica* IV (Webby and Kalmakoff, 1998). In addition to the novel mosquito IIVs, several more IIVs have been described over the past decade that are likely to be incorporated into the official system of classification in future ICTV reports.

The *Chloriridovirus* genus currently comprises a single species *Invertebrate iridescent virus 3*. The IIV-3 virus was isolated from the mosquito *Aedes* (*Ochlerotatus*) *taeniorhynchus* (Delhon et al., 2006). The particles of IIV-3 are significantly larger than those of the members of the *Iridovirus* genus, although particle size does not appear to be a useful indicator of phylogenetic relationships among these viruses. Indeed, IIV-3 is more closely related to viruses in the polyiridovirus complex than to IIV-6 in the oligoiridovirus complex (Wong et al., 2011). Moreover recent genome sequence information from IIVs infecting *Culex* and *Anopheles* mosquitos has provided further evidence of the phylogenetic proximity of these viruses to IIV-3 and the polyiridoviruses (Huang et al., 2015; Muttis et al., 2012). A recent analysis of a set of nine core genes has now provided the highest resolution phylogenetic tree to date (Fig. 3B). These findings have led (Piégu et al., 2015) to propose that the *Iridovirus* genus should be discontinued and three new genera recognized in its place, each genus representing one of the IIV complexes, alongside the *Chloriridovirus* genus; a proposal that will doubtless be discussed in the ICTV study group on *Iridoviridae*.

2.2.4. Related viruses in other taxa

The relationship of the IIVs with other virus families has recently become far clearer thanks to the information provided by a number of genome sequencing studies. Phylogenetic analyses of the MCP, DNA polymerase, helicase, DNA primase and RNase III genes gave highly consistent results indicating that iridoviruses originated from a phycodnavirus-like ancestral virus and the vertebrate and invertebrate lineages of iridoviruses diverged soon after (Piégu et al., 2015). The giant amoeba-infecting viruses of the *Mimiviridae* (Yoosuf et al., 2012) and the newly proposed family *Marseilleviridae*, appear to have a similar origin (Piégu et al., 2015). Moreover it appears clear that ascoviruses subsequently diverged from the invertebrate iridoviruses before the differentiation of the oligoiridovirus and crustaceoiridovirus clades. As such, the ascoviruses, particularly DpAV-4a, are more closely related to IIV-6 and IIV-31 than to the IIVs in other clades. Given these relationships the family *Iridoviridae* has been included in the proposed new order of NCLDVs named Megavirales (Colson et al., 2013).

2.3. Ascoviruses

2.3.1. General characteristics

Ascoviruses are large dsDNA viruses that infect lepidopteran larvae and pupae, particularly species in the family Noctuidae (Federici, 1983). These viruses are transmitted during oviposition by hymenopteran parasitoids that act as vectors of ascoviruses. They are named according to host insect of origin and given a type number according to sequence of discovery and a letter indicating isolate (Federici et al., 2009). Ascovirus virions are ovoidal, bacilliform, or allantoid in shape (Figs. 1 and 2C) and measure approximately 130 nm in diameter and 200–400 nm in length, depending on virus species (Bigot et al., 2012). The virion comprises a complex inner particle containing a DNA and protein core surrounded by a lipid bilayer and covered by a characteristic layer of protein subunits. The inner particle is wrapped by an external lipid envelope to form the virion. Negatively stained virions exhibit a characteristic reticulate pattern, believed to occur due to the superimposition of inner particle protein subunits on the external envelope. Virions comprise at least 21 proteins of between 6 and 200 kDa, of which the most abundant are the MCP (50 kDa) and P64 (Tan et al., 2009a). The P64 protein is a basic protein, rich in arginine (19.8%), lysine (6.2%), serine (18.9%) and threonine (5.2%) residues that is unique to ascoviruses. In *Spodoptera frugiperda* ascovirus 1a, P64 appears to be involved in virion morphogenesis as it is present in virogenic stroma and is subsequently packaged with genomic DNA into the core of each virion (Tan et al., 2009b). This protein may have a multifunctional role with the amino-terminal domains involved in DNA-binding and genome condensation, whereas the carboxyl terminal domains involved in recruiting, or being recruited by, other structural proteins during virogenesis (Tan et al., 2009b).

2.3.2. Ascovirus genomes and genes

The ascovirus genome is a circular molecule of dsDNA of 150–190 kbp, with a G+C content of 42–60%, depending on species (Bigot et al., 2009). Variation in genome size is mainly due to differences in repeated structural elements and the number of copies of *bro* genes that can range from 3 to 23 copies in ascoviruses (Bigot et al., 2000). Each genome encodes 119–180 ORFs. Three main classes of gene functions stand out in ascoviruses: nucleotide metabolism (15 genes, including a δ-DNA polymerase that appears not to be present in the virion), lipid metabolism (5 genes) and apoptosis regulation (3 genes).

Transcripts of Trichoplusia ni ascovirus 2c (TnAV-2c) are polyadenylated, presumably through the action of the host polyA-polymerase (PAP), as these viruses do not encode a viral PAP (Cheng et al., 2007). The regulation of gene expression in ascoviruses has not been examined in detail with the notable exception of Heliothis virescens ascovirus 3e (HvAV-3e). In this virus a virally encoded microRNA regulates virus replication apparently by controlling transcriptional degradation of its predicted target, the viral DNA polymerase I (Hussain et al., 2008). Subsequent studies identified a viral RNase III that was capable of suppressing the host gene silencing antiviral defense mechanism, without which infection and DNA replication was not possible (Hussain et al., 2010). During analysis of the *mcp* gene promoter of Spodoptera exigua ascovirus 5a (SeAV-5a), a stem-loop structure was predicted in the 3′ downstream region of the gene that may be involved in transcription termination (Salem et al., 2008).

2.3.3. Infection cycle

Lepidopteran hosts become infected when a female parasitoid wasp inserts her ovipositor, contaminated by virions or virion-containing vesicles, into the body of the larva (Hamm et al., 1985). With a single notable exception (Diadromus pulchellus ascovirus

4a, DpAV-4a), the wasp itself is not infected by the virus, but acts as an efficient mechanical vector for the horizontal transmission of the virus. Braconid and ichneumonid wasps are the most frequent vectors. *Per os* infection of lepidopteran larvae has been achieved under laboratory conditions, but is inefficient and appears unlikely to be frequent in the field (Govindarajan and Federici, 1990; Hamm et al., 1986). Injected virions or vesicles attach to susceptible cells, enter possibly by endocytosis, and travel to the nucleus where they initiate a dramatic series of events that result from viral manipulation of apoptotic pathways (Bideshi et al., 2005; Smede et al., 2009). Infection is rapidly followed by nuclear and cellular hypertrophy, invagination and fragmentation of the nuclear envelope followed by invagination of the plasma membrane along planes lined with mitochondria. Small lipid vesicles appear from membranes apparently synthesized *de novo* which subsequently coalesce to form numerous virion-containing vesicles (>10^7 vesicles/ml), each 5–10 μm in diameter, that are shed into the hemolymph (Federici and Govindarajan, 1990). The vesicles result in a milky white hemolymph that is characteristic of ascovirus infection. Larvae also stop growing and may have difficulty shedding the cuticle during molting. Tissue tropisms vary among these viruses; the fat body is the principal organ infected by Spodoptera frugiperda ascovirus 1 (SfAV-1), whereas additional tissues such as the tracheal epithelium and epidermis are infected by viruses such as TnAV-2 (Bideshi et al., 2010). Infected insects can survive several weeks before death, during which time they are likely to provide a continuous source of inoculum for ovipositing parasitoids (Li et al., 2016).

Manipulation of cellular apoptosis is mediated through the expression of an executioner caspase, or caspase-like genes, as well as cathepsin B that may degrade mitochondrial outer membranes and/or mobilize other proapototic cellular proteins (Bideshi et al., 2005). The action of these proteins is likely to be modulated by the virus-encoded inhibitor of apoptosis (IAP) present in all ascoviruses except DpAV4a (Hussain and Asgari, 2008). The evidence for ascovirus manipulation of apoptosis and vesicle morphogenesis has been reviewed in detail elsewhere (Bideshi et al., 2010; Federici et al., 2009).

2.3.4. Host range

Ascoviruses have been isolated from major pest species in the family Noctuidae including *Spodoptera frugiperda* (SfAV-1a–c), *Trichoplusia ni* (e.g. TnAV-2a, b; TnAV-6a), *Heliothis virescens* (HvAV-3a–g), *Helicoverpa armigera* (HaAV-7a), *Helicoverpa punctigera* (HpAV-8a) and *Spodoptera exigua* (SeAV-9a) (Bigot et al., 2012). Variants of these viruses have been isolated from natural populations of additional species of noctuids, such as *Autographa precationis*, *Helicoverpa zea* and *Hadula* (*Scotogramma*) *trifolii*. The geographical distribution of ascoviruses is likely to be worldwide and currently includes North America, Europe, Australia, China and Indonesia. SfAV-1a has been experimentally transmitted to various species in the genus *Spodoptera*, whereas the other ascoviruses appear to have a broader experimental host range, that comprises species in the family Noctuidae and some species in other families of Lepidoptera (Hamm et al., 1986, 1998). The prevalence of ascovirus disease in the field is positively correlated with the incidence of parasitism by insect parasitoids and may be common, especially in noctuid populations that are not frequently exposed to broad-spectrum insecticides (Cheng et al., 2005). DpAV-4a is unusual because it shares a symbiotic relationship with the ichneumonid parasitoid, *Diadromus pulchellus*, in which replication occurs in the ovaries (further described below).

2.3.5. Evolutionary links among ascoviruses

The family *Ascoviridae* comprises two genera, *Ascovirus*, of which the type species is *Spodoptera frugiperda ascovirus 1a* (Bigot et al., 2012) and the recently created *Toursvirus*, for which the type species is *Diadromus pulchellus ascovirus 4a* (ICTV, 2015). The *Ascovirus* genus contains two additional species at present: *Heliothis virescens ascovirus 3a* and *Trichoplusia ni ascovirus 2a*. Species status was initially defined based on genomic DNA hybridization studies, host range and tissue tropisms (Federici et al., 1990; Hamm et al., 1998), but later studies included phylogenetic analyses of the MCP and DNA polymerase (Cheng et al., 2005; Stasiak et al., 2000) that have now been extended to include additional genes (Piégu et al., 2015). Another four viruses are currently classified as tentative species: HaAV-7a, HpAV-8a, SeAV-9a and TnAV-6a (Bigot et al., 2012). SfAV-1a differs from other ascoviruses in DNA hybridization studies and replication is restricted to the fat body, but together with HvAV-3a and TnAV-2a, SfAV-1a forms a monophyletic clade (Fig. 3B). These three viruses also share a high degree of colinearity in gene organization.

Notably, DpAV-4a is the only ascovirus that replicates in the parasitoid ovary (Bigot et al., 1997a,b). During oviposition the wasp transmits the virus to pupae of the leek moth *Acrolepiopsis assectella* (Lepidoptera: Acrolepiidae), in which all tissues become infected and the host immune response is suppressed (Renault et al., 2002). The DpAV-4a genome is the farthest genetic distance from the other ascoviruses, it differs in gene composition, and does not share a common phylogenetic lineage with any other ascovirus characterized to date. This has led to the creation of the new *Toursvirus* genus within the *Ascoviridae* with DpAV-4a as the sole member (Bigot et al., 2009; Piégu et al., 2015).

2.3.6. Relationships with other taxa

As described in the section on iridescent viruses, ascoviruses appear to have diverged from IIVs prior to the differentiation of IIV-6 (oligoiridovirus clade) and IIV-31 (crustaceoiridovirus clade) from the larger polyiridovirus clade (Piégu et al., 2015; Stasiak et al., 2003). Specifically, DpAV-4a shows a close relationship with IIV-6, sharing a total of 64 genes, compared to 34 genes shared between DpAV-4a and the other ascoviruses (Bigot et al., 2009). However DpAV-4a and the other ascoviruses appear to have evolved in parallel with respect to virion structure, circular genome organization and cytopathology. A series of candidate genes have been identified as being differentially present or absent in the ascoviruses and IIVs that may account for the important differences in virion morphology, cytopathology and genome organization (Bigot et al., 2009). Evidence has been put forward for the evolution of ichnoviruses (family *Polydnaviridae*) from ascoviruses by symbiogenesis in the parasitoid wasp, apparently on more than one occasion (Bigot et al., 2008, 2009; Federici and Bigot, 2003). However, subsequent studies have concluded that ichnoviruses are likely derived from a common ancestral virus, possibly from a currently undescribed family of NCLDVs (Beliveau et al., 2015; Djoumad et al., 2013; Volkoff et al., 2010). See Section 3.3 for the evolutionary origin of polydnaviruses of braconid parasitoids (bracoviruses).

3. Large rod-shaped nuclear replicating DNA viruses

This group comprises the baculoviruses, nudiviruses, hytrosaviruses and white spot syndrome virus. All these viruses have rod-shaped, enveloped nucleocapsids, large circular dsDNA genomes, and they replicate in the nucleus of infected cells. They also share a number of genes, and, for at least the baculoviruses, nudiviruses and hytrosaviruses, a common evolutionary origin is assumed.

3.1. Baculoviruses

3.1.1. General characteristics

Baculoviruses (family *Baculoviridae*) received their name from their rod-shaped (*baculum* = rod) nucleocapsids. The explanation

Box 1 Baculovirus abbreviations.

AcMNPV - Autographa califonica MNPV
AgMNPV - Anticarsia gemmatalis MNPV
ChchNPV - Chrysodeixis chalcites NPV
HearNPV - Helicoverpa armigera NPV
NeseNPV - Neodiprion sertifer NPV
SeMNPV - Spodoptera exigua MNPV
ClanGV - Clostera anastomosis GV
CpGV - Cydia pomonella GV

for the abbreviations of virus names in this section is given in Box 1. Baculovirus nucleocapsids comprise dsDNA, packaged in capsid proteins. The nucleocapsids vary from 30–60 nm in width and 250–300 nm in length (Herniou et al., 2012). Most baculoviruses form two types of infectious virus particles, budded viruses (BVs) and occlusion derived viruses (ODVs) (see Fig. 1, IIa–c). In the course of infection, BV production precedes ODV synthesis. BVs contain a single copy of the viral genome encapsidated in viral proteins. The BV nucleocapsid (Fig. 1, IIa) is surrounded by an envelope obtained from the cell membrane during budding and modified by the inclusion of viral fusion proteins (either the F protein or GP64). ODVs are occluded in viral occlusion bodies (OBs) that are formed in the nucleus of infected cells (Fig. 4A), and are released upon liquefaction of the insect larvae. OBs are either polyhedral in shape containing many ODVs or granular with a single ODV inside, and are mainly composed of polyhedrin or granulin (Fig. 1, IIb–c). The names nucleopolyhedrovirus (NPV) and granulovirus (GVs) refer to this feature. ODVs may have one or several nucleocapsids within a single envelope depending on the species of baculovirus: single (S) or multiple (M) capsid NPVs and GVs (always a single nucleocapsid per ODV). ODVs obtain their envelope from the inner nuclear membrane. A tegument layer is located between the nucleocapsids and the envelope in ODVs.

The OBs are consumed by insect larvae during feeding and dissolve in the alkaline environment of the midgut, thereby releasing the ODVs. Subsequently, ODVs pass through the peritrophic matrix and fuse with the membrane of the microvilli of midgut epithelial cells, the initial site of infection. Upon entry the nucleocapsids are released and are transported along cytoplasmic actin filaments to the nucleus (Ohkawa et al., 2010), where the DNA is uncoated and transcription and genome replication subsequently occurs. Newly synthesized genomes are packaged and are transported to the basal site of the infected midgut cell to produce BVs, which are responsible for spreading the infection from cell to cell inside the insect body. Newly infected cells also produce BVs at first, but in the very late stage of infection, cells switch to ODV production and OB formation.

BV and ODV particles vary in protein and lipid composition. The lipid composition reflects the origin of the envelope of each particle (cell membrane or inner nuclear membrane) (Braunagel and Summers, 1994, 2007). The number of reported ODV proteins varies considerably between species, with for instance only 23 reported for HearNPV (Deng et al., 2007), 44 for AcMPNV and 73 for ClanGV (Zhang et al., 2015).

The protein composition of BVs is less well studied, but was quantified at 34 proteins in AcMNPV and 33 in AgMNPV and HearNPV (Hou et al., 2013) It is clear that several proteins, especially those that form the nucleocapsids, are shared between both types of virions, but there are also remarkable differences in protein composition (Braconi et al., 2014; Hou et al., 2013; Wang et al., 2010) and structure (Wang et al., 2016). Interestingly, for both ODVs and BVs, the host species in which the virus replicates affects the protein composition of these particles (Hou et al., 2016).

The genome of baculoviruses is a circular molecule of dsDNA that varies in size between 80 and 180 kDa. The G+C content varies per genome, but is typically around 40%. Each baculovirus genome is densely packed with ORFs on both strands of the DNA. Early and late genes are found scattered over the genome. Intergenic regions are, in general, short and promoters and/or 3′untranslated regions of flanking genes may overlap. Due to the dense ORF coverage, baculovirus genome size is roughly proportional to the number of ORFs. Genome size varies considerably in the more than 51 NPV and 19 GV genome sequences present in databases to date. So far, the smallest genomes have been identified in hymenopteran-infecting baculoviruses, e.g. NeseNPV with 81,755 kbp and 89 predicted ORFs (Lauzon et al., 2006). The smaller genome size in this group may, in part, be related to their inability to produce BVs.

A feature of almost all baculovirus genomes is the presence of homologous regions (*hrs*) that consist of repeats of short, often palindromic sequences. The nature and number of the repeat sequences varies between baculoviruses, but they are each typically 30–40 nt in length. An exception is ChchNPV, in which *hr* regions have not been found (van Oers et al., 2005). The *hrs* have been shown to function as origins of replication and enhancers in cell culture (Rodems and Friesen, 1993; Vanarsdall et al., 2005; Wu and Carstens, 1996).

Baculovirus genes are divided into four temporal classes: immediate early, delayed early, late and very late (Friesen and Miller, 1986; Rohel and Faulkner, 1984). The existence of a very late class is a special feature of baculoviruses. This class contains two highly expressed genes that play a role in OB formation and release: the *polh* or *gran* gene that encode the OB matrix protein in NPVs and GVs, respectively (Rohrmann, 1986), and the *p10* gene, which encodes a 10 kDa protein that forms fibrillar structures in the nucleus and cytoplasm of infected cells. These structures form a peri-nuclear cage and are associated with OB formation (Carpentier et al., 2008; Patmanidi et al., 2003) and disintegration of infected cell nuclei that leads to release of OBs (van Oers et al., 1993). See Carpentier and King (2009) for a review on the *p10* gene. The baculovirus-insect cell expression system is used intensively to produce subunit vaccines, virus-like particles and viral vectors, and is based on the utilization of the AcMNPV *polh* and *p10* promoters to drive the expression of foreign genes (van Oers et al., 2015).

Baculovirus early genes are characterized by a CAGT motif, often preceded by a TATA box (Pullen and Friesen, 1995). Both late and very late genes contain a TAAG signature that also harbors the mRNA start site (Kuzio et al., 1984; Morris and Miller, 1994). Early genes with typical cellular promoter motifs may also be present and may signal horizontal gene transfer events between host and virus. Early genes are transcribed by host polymerase II, although delayed early genes (e.g. the *39K* gene), require activation by immediate early gene products (Carson et al., 1988; Kovacs et al., 1991). In total, a set of 18 genes regulates late gene expression in AcMNPV, including a set of conserved genes (*lef-4*, *lef-8*, *lef-9*, *p43 helicase*) that probably encode the four subunits of the viral RNA polymerase (Guarino et al., 1998). Very late genes also require the very late factor 1 (VLF-1) (McLachlin and Miller, 1994; Todd et al., 1996).

The number of baculovirus core genes (conserved in all baculoviruses) is now 37 ORFs (Garavaglia et al., 2012). The lepidopteran-infecting viruses share 62 ORFs. Apart from the genes already mentioned above (except for *gp64*), other examples of conserved genes are the viral DNA polymerase and a set of structural genes involved in viral genomic DNA condensation and encapsidation (e.g. *p6.9*, *vp39*, *38K*, and *vp1054*). Additional proteins that play

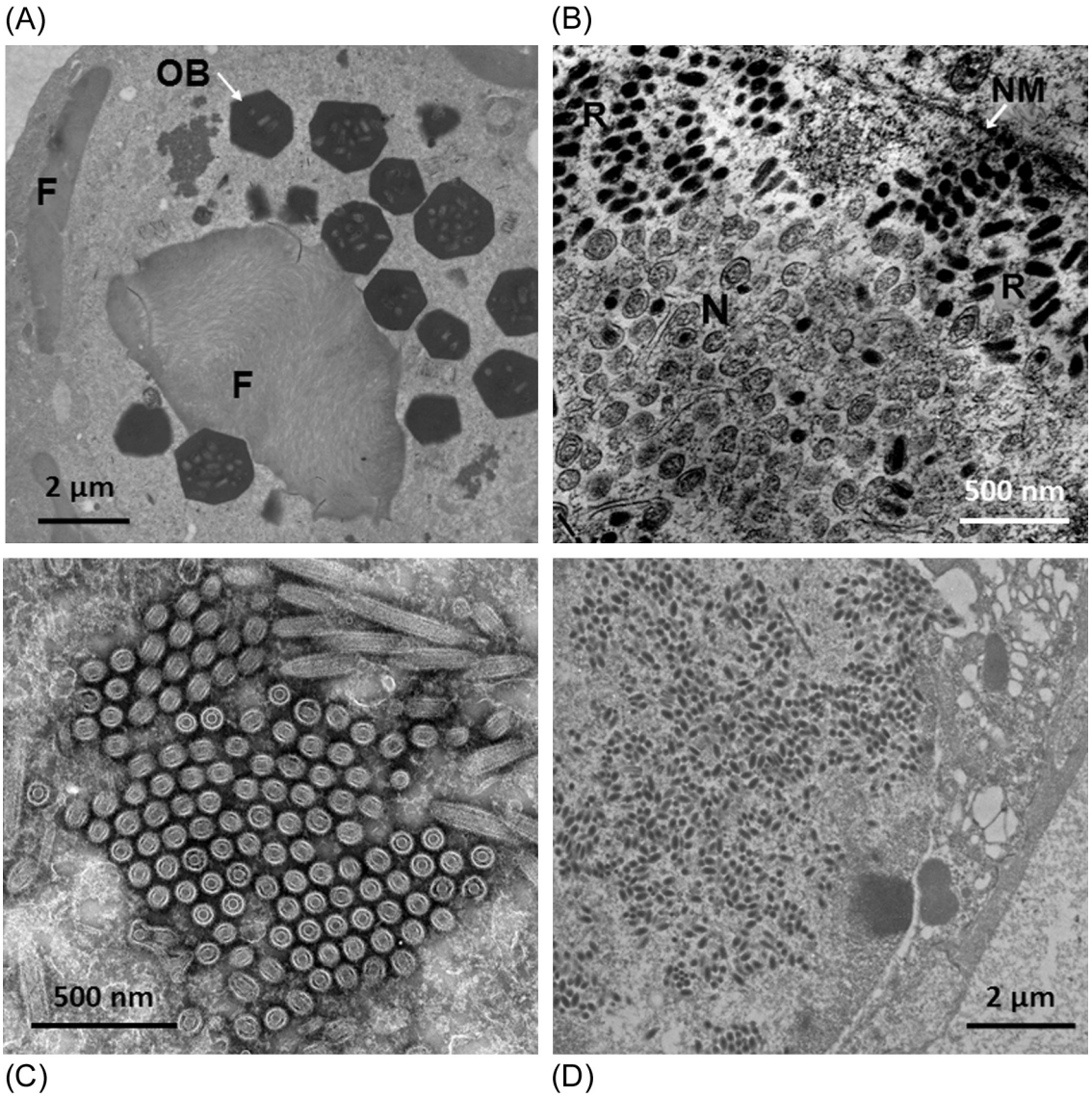

Fig. 4. Electron microscope images of cells infected with rod-shaped large nuclear DNA viruses. (A) Baculovirus: *Spodoptera frugiperda* cells infected with AcMNPV. Viral occlusion bodies (OB) carrying ODV particles are visible in the nucleus as well as a large fibrillar structure (F) comprising P10 protein. (B) Nudivirus: *Oryctes rhinoceros* fat body cells infected with OrNV. (N: nucleus; NM: nuclear membrane; R: rod-shaped viruses.) (C) Hytrosavirus: GpSGHV particles in the cytoplasm of salivary gland cells of *Glossina pallipides*. (D) Nimavirus: *Peneaus monodon* gill cell infected with WSSV. Figs. A, C and D were provided by Jan van Lent. Fig. B was adapted from Huger (2005) with permission of authors and publisher.

a role in particle assembly may be conserved only in certain genera leading to the hypothesis that viruses in other genera might have functional homologs. The VP80 protein, for instance, is only present in alphabaculoviruses, yet it is essential for both BV and ODV formation. VP80 is involved in the transport of progeny nucleocapsids towards the nuclear periphery along filamentous actin (Marek et al., 2011), a process that is likely to be crucial for other baculoviruses as well.

The absolute conservation of a set of seven genes coding for the *per os* infectivity factors (PIFs) is remarkable among the baculoviruses. These ODV envelope proteins are crucial for the initial infection of midgut epithelial cells. Several of these proteins form a complex presumably involved in binding to, and fusion with, midgut epithelial membranes (Dong et al., 2014; Fang et al., 2009; Gutierrez et al., 2004; Haas-Stapleton et al., 2004; Ohkawa et al., 2005; Peng et al., 2010; Pijlman et al., 2003; Sparks et al., 2011). The function of the individual proteins in this complex is still under investigation. The *pif* genes are also conserved in nudiviruses and hytrosaviruses, and several are also present in the whispovirus genome (see the respective sections below). Several *pif* genes were also detected recently in the unclassified Apis mellifera filamentous virus (Gauthier et al., 2015).

3.1.2. Host range

Baculovirus infections have been confirmed for insects in the orders Lepidoptera, Hemiptera and Diptera. The larval stages are the principal target of infection. Baculoviruses have a successful record as biological control agents of insect pests. Examples include CpGV against larvae of the apple codling moth, and HearNPV to control the cotton bollworm. Baculoviruses are named after the first insect species from which they were isolated, followed by the indications GV or NPV, based on OB composition and morphology. Some baculoviruses are completely specific to their host (e.g. SeMNPV only infects *Spodoptera exigua*), whereas others may infect closely related species such as HearNPV that infects *Heliothis* spp. and *Helicoverpa* spp. A third group can infect

and kill a broader range of Lepidoptera (e.g. AcMNPV), although susceptibility to these viruses can vary markedly among the different host species. Given these differences in host range, similar strains may be isolated from natural populations of different host species. A particular insect species may also be infected by more than one baculovirus. Both these phenomena can lead to confusion about virus identity. To determine whether an isolate belongs to an existing virus species, or whether it represents a new species, requires both biological and molecular information. A minimum set of specified genes (*polh* or *gran*; *lef-8*, *lef-9*) has to be analyzed and compared between the isolates using the so-called "Kimura 2" parameter. The critical value of this parameter for discriminating between two baculovirus species has been set at $\geqslant$0.050 (Jehle et al., 2006b).

Phylogenetic evidence for the co-evolution of baculoviruses and their insect hosts (Herniou et al., 2004), is reflected in the taxonomy of the family *Baculoviridae*, which is divided into four genera (Herniou et al., 2012; Jehle et al., 2006a). The NPVs that infect Lepidoptera are placed in the genus *Alphabaculovirus* that currently comprises 32 species (ICTV, 2015). *Autographa californica multiple nucleopolyhedrovirus* (Ayres et al., 1994) is the type species of the genus. The GVs that infect caterpillars are assigned to the genus *Betabaculovirus*, with 14 currently recognized species and *Cydia pomonella granulovirus* as the type species (Luque et al., 2001). The *Gammabaculovirus* genus comprises NPVs of sawflies (order Hymenoptera), with currently two recognized species and *Neodiprion lecontei nucleopolyhedrovirus* as the type species (Lauzon et al., 2004). The genus *Deltabaculovirus* currently has only one assigned species, *Culex nigripalpus nucleopolyhedrovirus*, named after the mosquito from which it was isolated (Afonso et al., 2001).

The viruses in the *Alphabaculovirus* genus fall into two phylogenetic clades representing the group I and group II NPVs (de Zanotto et al., 1993). An important difference between these two groups involves the occurrence of the GP64 envelope fusion protein which is only present in the BV of group I viruses (Oomens and Blissard, 1999). This protein was likely acquired by horizontal transfer from a co-infecting errantivirus (Pearson and Rohrmann, 2006). All other baculoviruses use the F protein to mediate BV cell escape and entry (IJkel et al., 2001; Pearson et al., 2000). The sawfly-infecting gammabaculoviruses do not encode an F homolog. They do not produce BVs and their replication is restricted to the host gut (Lauzon et al., 2004).

3.2. Nudiviruses

3.2.1. Properties

Non-occluded rod-shaped viruses have been found in arthropods of various orders and were previously considered to be non-occluded baculoviruses (Huger and Krieg, 1991). These viruses replicate in the nucleus of infected cells, but until recently, too little information was available to understand how these viruses should be classified. It is now clear, however, that the nudiviruses form a monophyletic group within the nuclear-replicating large DNA viruses that is distinct from the family *Baculoviridae*.

Oryctes rhinoceros nudivirus (OrNV) infects larvae and adult stages of the rhinoceros beetle (Coleoptera: Scarabaeidae) and is an orally transmitted virus (reviewed by Huger (2005)). The enveloped virions (220 $\times$ 120 nm) contain a single rod-shaped nucleocapsid of 185 $\times$ 65 nm (see Figs. 1 and 4B). Infection starts in the midgut epithelial cells and from there spreads systemically to other tissues, including the fat body. Diseased larvae die within 1–4 weeks. This virus has been applied successfully as a biological control agent in palm tree farming to control the rhinoceros beetle in southeast Asia and the Pacific region (Huger, 2005; Zelazny et al., 1992).

Gryllus bimaculatus nudivirus (GbNV) is a virus of nymphs and adult field crickets (Orthoptera: Gryllidae). Besides *G. bimaculatus*, hosts also include the orthopterans *G. campestris*, *Teleogryllus oceanicus* and *T. commodus* (Huger, 1985). The enveloped virions are rod-shaped or irregularly ellipsoidal, 90 $\times$ 180 nm. Nuclei of fat body cells are the main target for GbNV replication. The nuclei become greatly enlarged and may contain large numbers of enveloped virions. Infected *G. bimaculatus* nymphs die at 3–12 weeks after infection. Infected adults have a reduced size, show lethargic behavior, and are sometimes immobile (Huger, 1985). As such, orthopteran nudiviruses pose a potential threat to cricket rearing as prey for entomophagous spiders and reptiles kept as pets (Eilenberg et al., 2015).

Heliothis zea nudivirus 1 (HzNV-1) and Heliothis zea nudivirus 2 (HzNV-2) infect the corn earworm (Lepidoptera: Noctuidae). Both viruses belong to the same species (Jehle et al., 2013). The virus particles are single rod-shaped enveloped virions of 385–445 $\times$ 77–83 nm. HzNV-1 has been detected so far only in a cultured *H. zea* cell line (Kelly et al., 1981), and therefore its mode of transmission in the wild is unknown. HzNV-2 is sexually transmitted between adult moths, but these moths can also become orally infected. The main targets for HzNV-2 are the reproductive tissues of both female and male moths. In overt infections the gonads of both sexes show malformations and the insects are sterile (Rallis and Burand, 2002). In females, progeny virus accumulates in the lumen of the oviduct and together with other bursa-derived material forms a so-called "virus plug", the source of infection for males that try to mate with these females (Burand et al., 2004). Overtly infected females show prolonged calling behavior and produce more mating pheromone than healthy females. As a consequence, mating attempts by males increase (Burand et al., 2005) and males can subsequently transmit the disease to uninfected female mates. Most infected females develop asymptomatic infections and remain fertile (Hamm et al., 1996; Lupiani et al., 1999). These moths pass the infection to their offspring via the ovaries, but only a small percentage of the offspring develop overt infections (Burand and Rallis, 2004). The host population is likely to die out quickly when the percentage of overt infections increases, due to the appearance of high numbers of sterile individuals.

3.2.2. Genomic features and core genes

The size of nudivirus genomes varies considerably, with GbNV currently being the smallest at 97 kb (98 ORFs). The OrNV genome has a size of 128 kb and comprises 140 ORFs. HzNV genomes are the largest so far in this family, with 228 and 232 kb in length, and 152 and 113 predicted ORFs for HzNV-1 and HzNV-2, respectively. The G+C content of the genome various considerably for these viruses (28% in GbNV, versus 42% in HzNV-1, HzNV2 and OrNV) (reviewed by (Wang et al. (2007a)). A total of 32 core genes have now been assigned to the nudiviruses, of which 21 are shared with the viruses in the family *Baculoviridae* (Bezier et al., 2015). As biological studies on the function of nudivirus genes is almost completely lacking, gene functions are not discussed in detail here. One prominent class of genes shared with baculoviruses (but also the hytrosaviruses, and to a certain extent with white spot syndrome virus in shrimp, both discussed below) are the genes for the *per os* infectivity factors (PIFs). Little is known about the infection process of nudiviruses, but it is likely that the PIF proteins play a crucial role in the initial infection in the gut, as seen in baculovirus ODVs. The complete set of genes (*lef-4*, *lef-8*, *lef-9* and *p43*) needed to form the subunits of the baculovirus encoded RNA polymerase, required for late and very late gene expression, is present among the core nudivirus genes. Based on *in silico* studies, nudiviruses also appear to use the same TAAG promoter motif as baculoviruses to express late genes (Wang et al., 2011).

3.2.3. Taxonomy

The family *Nudiviridae* (derived from the Latin "*nudus*" = naked, which describes the non-occluded character of the viruses) currently comprises two genera: the genus *Alphanudivirus* with the two species *Oryctes rhinoceros nudivirus* and *Gryllus bimaculatus nudivirus*, and the genus *Betanudivirus*, with a single species *Heliothis zea nudivirus* (Jehle et al., 2013).

A virus that shares many characteristics with nudiviruses was recently detected in the crane fly *Tipula oleracea* (Diptera: Tipulidae), which appears to represent a novel virus species. The ToNV genome of 145.7 kbp and 131 predicted ORFs, is larger than that of the previously sequenced nudiviruses (Bezier et al., 2015). One of the ToNV genes is a close homolog of the *polh* gene of the baculovirus Tipula paludosa NPV, which might have been obtained by the nudivirus through horizontal gene transfer. The rational to place ToNV in the nudiviruses, despite the fact that it carries a *polh* gene and is an occluded virus, is that it shares 52 genes with other sequenced nudiviruses (Bezier et al., 2015). A metagenomic study has suggested the presence of a nudivirus in another dipteran, *Drosophila innubila* (DiNV) (Unckless, 2011). Information is lacking at this point in time to confirm that these sequences reflect the presence of an autonomously replicating virus. Despite the fact that DiNV and ToNV were both found in dipterans, they appear to be only distantly related to each other.

A virus previously named Monodon baculovirus or Penaeus monodon NPV, turned out to be more closely related to nudiviruses than to baculoviruses and has now been named Penaeus monodon nudivirus (PmNV) (Yang et al., 2014). The genetic distance between this virus and all the above mentioned nudiviruses from terrestrial hosts is greater than the distances among the above mentioned nudiviruses. Hence, PmNV may ultimately be placed in a separate genus. PmNV particles are occluded, but the occlusion body major protein is not homologous to polyhedrin or granulin of baculoviruses (Chaivisuthangkura et al., 2008). Recently, an additional virus was discovered in North Sea populations of the brown shrimp *Crangon crangon*, which may belong to the nudiviruses based on morphological characteristics and partial sequence information (K.S. Bateman and M.M. van Oers, unpublished data).

3.2.4. Endogenous nudivirus-derived elements

Integration of nudiviruses into insect genomes may have occurred several times during the evolution of insect species. Evidence exists for the integration of an ancient nudivirus into the genome of a wasp ancestral to the existing species of braconid wasps (Hymenoptera: Braconidae) about 100 million years ago (MYA) (Bezier et al., 2009; Herniou et al., 2013). These wasps have a collection of active gene clusters with strong homology to conserved nudivirus genes. The crane fly nudivirus ToNV is the closest known autonomous replicating relative of the endogenous nudivirus in the braconid wasp genomes. Expression of the endogenous nudivirus genes in the ovaries of female parasitic wasps results in the production of polydnavirus (bracovirus) particles that are co-injected with wasp eggs into moth larvae during oviposition (reviewed by Burke and Strand, 2012). These particles contain viral capsid proteins and a collection of dsDNA minicircles that form the bracovirus genome (Beck et al., 2007). Both the bracovirus proteins and the minicircles are encoded in the wasp genome (Chevignon et al., 2014). Gene expression products of the polydnavirus genome subsequently protect the developing wasp larvae against the host immune response. Unusually, a recent study reported the integration of polydnavirus minicircles into the genome of a lepidopteran host (Gasmi et al., 2015). In addition, gene sequences derived from nudivirus integration have recently been detected in the genome of the parasitic wasp *Venturia canescens* (Ichnomeunidae: Campopleginae) (Pichon et al., 2015). In this case only viral proteins packaged in liposomes are delivered with the wasp eggs to protect these against host immune responses during parasitism.

Parasitoid wasps belonging to the family Ichnomeunidae may also produce polydnaviruses. Indeed, there is now growing evidence for a common viral origin of genomic sequences in two ichneumonid subfamilies, Banchinae and Campopleginae (Beliveau et al., 2015). These ichneumonid endogenous viral elements, however, are not related to nudiviruses. As described in Section 2.3.6, evidence currently points towards an ancestral virus originating from within the NCLDVs.

Nudivirus-like sequences have also been identified in the genome of the brown planthopper *Nilaparvata lugens* (Hemiptera: Delphacidae), a plant sap-sucking insect (Cheng et al., 2014). Subsequent analysis detected these viral sequences in all brown planthopper populations collected over a wide geographical range in Asia. The integrated virus was named Nilaparvata lugens endogenous nudivirus (NlENV). Whether this planthopper, like the parasitoid wasps, also profits from the presence of the endogenous nudivirus is unknown. All 32 nudivirus core genes were present in the genome of the planthopper, but whether any nudivirus genes are expressed and, if so, in which tissues, is uncertain. In order to understand whether the gene conservation reflects a recent or an ancient but functional integration, additional *Nilaparvata* species and related species in the family Delphacidae need to be screened.

3.2.5. Host range and evolution

With new nudiviruses being identified frequently, it is too early to indicate precisely the range of arthropod taxa that are susceptible to nudivirus infections. Apart from the confirmed nudiviruses in the orders Coleoptera, Diptera, Lepidoptera, Orthoptera, Hymenoptera (endogenous viral elements) and in Crustacea, viruses with similar morphology have also been observed in the insect orders Siphonaptera, Thysanoptera, Trichoptera, Neuroptera, Homoptera, and in mites (Acarina) (Huger and Krieg, 1991). These reports may, or may not, represent infections by nudiviruses. Despite of the fact that species in several orders may be susceptible to both nudiviruses and baculoviruses, these virus families seem to have followed separate evolutionary lines after their original divergence estimated at around 310 MYA (Herniou et al., 2013; Thézé et al., 2011; Wang et al., 2007b), concurrent with the appearance of the main insect orders. Occasionally, horizontal gene transfers may have occurred between these virus groups after their segregation, as evidenced by the presence of the *polh* gene in the *Tipula*-infecting nudivirus.

Nudiviruses infect both larval and adult stages and sometimes replicate in reproductive tissues, e.g., the gonad specific virus HzNV-2. Given their intimate association with the gonads and gamete production, sexually transmitted nudiviruses may at some point in evolution have been integrated into genome of the insect germ line, giving rise to the integrated endogenous elements that are today found in braconid wasps, the ichneumonid *Ventura canescens* and the brown planthopper.

3.3. Hytrosaviruses

3.3.1. General properties

Hytrosaviruses are characterized by very large enveloped, rod-shaped virions (Figs. 1 and 4C) with a length of 500–1000 nm and a diameter of 50–100 nm (Abd-Alla et al., 2009). The virion of Glossina pallidipes salivary gland hypertrophy virus (GpSGHV) contains at least 61 proteins (Kariithi et al., 2013b). Of these, 33 homologs have been identified in Musca domestica salivary gland hypertrophy virus (MdSGHV) (Garcia-Maruniak et al., 2009). The circular dsDNA genome has a size of 120–190 kbp. Signs of disease

include overt salivary gland hypertrophy (SGH) in dipteran adults and gonadal anomalies that result in partial or complete sterility.

3.3.2. Host range

So far, hytrosaviruses have only been described in a few dipteran species. GpSGHV was first described in the 1970s (Jaenson, 1978) and can periodically cause colony collapse in tsetse fly production facilities (Abd-Alla et al., 2010). These flies are mass reared as part of a control program based on the sterile insect technique involving the release of massive numbers of sterile male flies to suppress natural populations of this vector of *Trypanosoma* spp. GpSGHV was also detected in wild populations of *G. pallidipes* and other species of tsetse flies in sub-saharan Africa (Kariithi et al., 2013a). The house fly hytrosavirus (MdSGHV) is distantly related to GpSGHV (Garcia-Maruniak et al., 2009). In the wild GpSGHV infections often seem to be inapparent (Kariithi et al., 2013c), whereas covert infections by MdSGHV have not been reported (Geden et al., 2011).

3.3.3. Taxonomy and evolutionary links

The family *Hytrosaviridae* got its name from the combined abbreviation of Hypertrophia and sialoadenitis (inflammation of the salivary glands). The family has two genera: *Glossinavirus* and *Muscavirus*, each with one species: *Glossina hytrosavirus* and *Musca hytrosavirus*, respectively (Abd-Alla et al., 2009). The Merodon equestris virus isolated from the large narcissus bulb fly (Diptera: Syrphidae) represents a tentative species in this family based on its morphology and biological characteristics. This virus is also associated with salivary gland hypertrophy and gonadal atrophy (Amargier et al., 1979) and was unofficially named MeSGHV (Abd-Alla et al., 2009). However, molecular data are required to determine the correct classification of this virus.

Hytrosaviruses share several features with baculoviruses and nudiviruses, and based on the comparison of conserved genes, the hytrosaviruses cluster together in a separate clade (Fig. 2C). Other support for placing SGHVs in a separate family comes from the fact that the genomes of the two sequenced SGHVs (Abd-Alla et al., 2008; Garcia-Maruniak et al., 2009) are not co-linear with baculovirus, nudivirus or other NCLDV genomes. The recent annotation of an Ethiopian isolate of GpSGHV (GpSGHV-Eth) by proteomic and transcriptomic approaches allowed the putative function of 141 of the 174 ORFs to be identified (Abd-Alla et al., 2016). Among them, 68 have an appropriately upstream positioned TATAA-like box and/or (G/T/A)TAAG transcriptional signals similar to those of early, and late baculovirus genes, respectively. The GpSGHV-Eth genome contains homologs to 12 out of the 37 core genes of baculoviruses and nudiviruses including two genes involved in DNA repair and recombination, four genes involved in transcription (homologs to *lef-4*, *lef-5*, *lef-8* and *lef-9*), a homolog to desmoplakin, and five genes homologs to the baculovirus *per os* infectivity factors. These homologies suggest that hytrosaviruses share with baculoviruses and nudiviruses similar modes of entry and transcription of their late genes, which strongly support the hypothesis that they originate from a common ancestor (Jehle et al., 2013).

3.4. White spot syndrome virus

3.4.1. General properties

White spot syndrome virus (WSSV) particles are ovoid to baciliform in shape and contain a nucleocapsid of 300–350 nm in length and 65–70 nm in diameter (Lo et al., 2012), surrounded by a tegument layer and a trilaminar envelope. A characteristic thread-like (“*nima*”) fibril extends from one end of the virion (see Figs. 1 and 4D). WSSV replicates in the nucleus of infected cells, and progeny particles also assemble in the nucleus. WSSV has six major structural proteins: VP15 and VP664 in the nucleocapsid, VP24 and VP26 in the tegument, and VP19 and VP28 in the envelope, where the numbers refer to the mass of each protein. These virion proteins are not glycosylated (van Hulten et al., 2000, 2001b, 2002; Tsai et al., 2006).

The circular, dsDNA genome of WSSV has a size of approximately 300 kbp, depending on the strain (Hoa et al., 2012; van Hulten et al., 2001a). The genome contains nine homologous repeat regions and both strands are used equally to code for proteins. Several ORFs have been assigned to functional proteins, including proteins involved in deoxyribonucleotide synthesis (thymidylate synthetase, thymidine-thymidylate kinase, UTPase and ribonucleotide reductase subunits) (Tsai et al., 2000; Van Hulten and Vlak, 2001). Homologs of a small number of *pif* genes (*pif-1*, *pif-2*, and *p74*) are present in the WSSV genome (van Hulten et al., 2001a), but their role in the infection process is not known. In microarray studies, 79% of the ORFs was found to be expressed (Marks et al., 2005), although most of the ~180 non-overlapping ORFs encode proteins of unknown function.

In silico analysis indicated a common degenerate motif (ATNAC) in the upstream regions of late genes, whereas early genes were preceded by TATA motifs (Marks et al., 2006). The proteins that build the virion often are translated from polycistronic mRNAs, using internal ribosome entry sites.

3.4.2. Host range

WSSV was originally isolated from cultured penaeid shrimp, but can infect a large number of freshwater and marine crustaceans, including crayfish and crab species. WSSV infections were first described in Taiwan, but from there quickly spread along Asian coasts, but now it is present on a global scale. With its almost 100% mortality for cultured shrimp, WSSV is a major problem in marine aquaculture. The gills are the primary site of infection in contaminated water but the virus is also transmitted by feeding on diseased conspecifics (cannibalism) or other infected animals. There may also be a role for polychaetes as an additional reservoir of disease (Desrina et al., 2013).

3.4.3. Taxonomy

Phylogenetically, WSSV differs from all other large, invertebrate dsDNA-virus (Lo et al., 2012) (Fig. 3C), although some genes show homology with baculoviruses, nudiviruses and hytrosaviruses, including some *pif* genes. *White spot syndrome virus* is so far the only member of the family *Nimaviridae* and is placed in the genus *Whispovirus*.

3.5. Filamentous viruses of hymenopterans

Hymenopterans have been observed to be infected by two filamentous viruses the taxonomic status of which has yet to be determined. The Leptopilina boulardi filamentous virus (LbFV) is associated with superparasitism behavior of *L. boulardi* wasps (Patot et al., 2009; Varaldi et al., 2006). LbFV stimulates female wasps to lay eggs in *Drosophila* larvae that already have been parasitized. This superparasitism results in horizontal virus transmission between wasp larvae. LbFV also seems to play a crucial role at the population level in allowing co-existence of different wasp species (Patot et al., 2012). The virus appears to replicate in the nucleus of cells lining the oviduct, where LbFV nucleocapsids also assemble (Varaldi et al., 2006). The width of these particles is approximately 45 nm, their length may be over 1 μm. In a later stage of infection the nucleocapsids acquire an envelope as they travel to the cytoplasm, where they accumulate near the lumen of the oviduct. The mutual interaction of LbFV and the parasitic wasp may have originated from a parasitic virus that was sexually

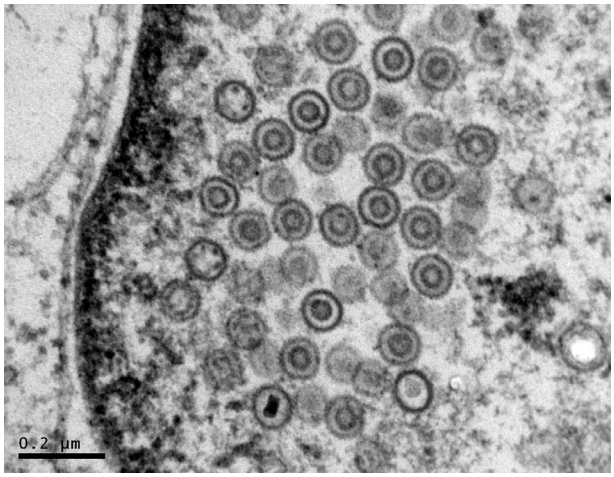

Fig. 5. Electron microscope image of the Pacific oyster *Crassostrea gigas* infected with an ostreid herpesvirus (courtesy of Kelly Bateman).

transmitted, similar to that of integrated nudiviruses (Bezier et al., 2009). Studies in progress indicate that the genome comprises a single, circular dsDNA molecule. Putative structural proteins include two PIFs for which the closest homologs are present in hytrosaviruses (Personal communication J. Varaldi).

The Apis mellifera filamentous virus (AmFV) is also a large enveloped filamentous DNA virus (3150 × 40 nm) that folds into loops within a 450 nm × 170 nm rod-shaped virion. The dsDNA core is encapsidated by two major nucleoproteins and wrapped in a tri-laminate virion envelope. The hemolymph of severely infected adult honeybees becomes milky-white due to the large number of virions present. The AmFV genome is a dsDNA molecule of ~ 498,500 bp with a GC content of 50.8%. The genome encodes 247 ORFs, distributed on both strands, which cover 65% of the genome (Gauthier et al., 2015). Of these, 28 display significant homologies with proteins present in NCLDVs. 13 ORFs had strong similarity with baculovirus domains including PIFs (pif-1, pif-2, pif-3 and p74) and BRO proteins. *Varroa* mites may be capable of vectoring this virus as next generation sequencing indicated the presence of AmFV sequences in mite populations from the USA (Gauthier et al., 2015). AmFV may belong to a new virus family.

4. Mollusk-infecting herpesviruses

4.1. General properties

The mollusk-infecting herpesviruses share clear morphological and genomic similarities with herpesviruses from vertebrates. The core consists of the viral genome packaged as a single linear dsDNA molecule (Figs. 1 and 5). Cryo-EM studies have revealed an icosahedral capsid with $T = 16$ configuration, comprising 161 capsomers: 150 hexamers and 11 vertex pentamers, consisting of the major capsid protein (150 kDa), and a ring-like portal protein complex at the twelfth vertex. The capsid has an overall diameter of 116 nm and is surrounded by an amorphous protein tegument that is enveloped by a lipid bilayer embedded with various glycoproteins to produce a virion of 200 nm diameter (Davison et al., 2005).

4.2. Classification and genome characteristics

The mollusk herpesviruses are assigned to the family *Malacoherpesviridae* in the order *Herpesvirales* that includes two additional families and three subfamilies of viruses from vertebrate hosts. The *Malacoherpesviridae* currently comprises two genera, *Ostreavirus* (type species: *Ostreid herpesvirus 1*, OsHV-1), and *Aurivirus* (type species: *Haliotid herpesvirus 1*, HaHV-1) (ICTV, 2015). The linear ds DNA genome of OsHV-1 is 207,439 bp in size and is composed of two invertible unique regions of 167.8 and 3.4 kbp, each flanked by inverted repeats (7.6–9.8 kbp) separated by an additional unique sequence of 1.5 kbp (Davison et al., 2005). A total of 124 ORFs were identified including helicases, primases, inhibitors of apoptosis, deoxyuridine triphosphate, RING-finger proteins, membrane associated proteins and an ATPase subunit of the terminase gene, which together with the viral δ-DNA polymerase, have been used for phylogenetic analyses (Savin et al., 2010).

4.3. Host range and pathology

OsHV-1 has a broad host range and has been reported infecting various species of oyster (Ostreoidea), clams and scallops, although the principal susceptible species is the Pacific oyster, *Crassostrea gigas* (Arzul et al., 2001). Infections are associated with sporadic mass mortality events in larval and juvenile (spat) bivalves in many parts of the world, although adults may also suffer lethal or inapparent infections. A rapid rise in water temperature has been identified as a common factor in triggering OsHV-1 outbreaks (Renault et al., 2014). Signs of OsHV-1 infection include a reduction in feeding, circular swimming behavior and loss of motility in larvae and gaping behavior or a protracted closing reaction in juveniles. Numerous other pathological changes have been described at the cellular level in larvae, juveniles and adults (Jenkins et al., 2013; Renault et al., 2002). Surprisingly, given the frequency of mass mortality events attributed to OsHV-1, the virus has not been studied in detail. The lack of a susceptible bivalve cell line has been the principal limitation. As a result, various PCR or LAMP-based detection techniques have been developed as diagnostic tools for the detection of OsHV-1 infections in natural and farmed oyster populations (Oden et al., 2011; Ren et al., 2010).

In contrast, HaHV-1 appears to have a more restricted host range, having only been reported from diseased abalone (*Haliotis* spp.) in Taiwan and Australia (Chang et al., 2005). HaHV-1 is neurotropic and causes lethal ganglioneuritis with hemocyte infiltration. Various strains have been identified and outbreaks are an issue of increasing concern in farmed and natural abalone in Australia (Corbeil et al., 2016). PCR and LAMP diagnostic techniques are available (Chen et al., 2014; Corbeil et al., 2010). Phylogenetic analysis revealed the distant relationship between HaHV-1 and OsHV-1 (Savin et al., 2010), which has led to the classification of HaHV-1 as the sole member of the recently recognized *Aurivirus* genus in the *Malacoherpesviridae* family.

5. Concluding remarks

With increasing genomic sequence information becoming available that provides new insights into the evolutionary links between various invertebrate DNA viruses, the classification of these viruses has seen a large number of changes over recent years. Interesting recent findings include the endogenous viral elements derived from ancient nudiviruses or an ancestral NCLDVs that have opened the way to the evolution of polydnaviruses in parasitoid wasps. We also note that several viruses are outsiders in the taxa in which they are currently classified. However, a persisting lack of information or inability to identify closely related viruses as for instance seen for WSSV, currently precludes a more appropriate classification. We hope that this review will assist in understanding the taxonomy of the DNA viruses described in this special issue and will draw attention to the importance of correctly classifying newly discovered invertebrate viruses.

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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.2016.08.001.

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