This form should be used for all taxonomic proposals. Please complete all those modules that are applicable (and then delete the unwanted sections). For guidance, see the notes written in blue and the separate document "Help with completing a taxonomic proposal"

Please try to keep related proposals within a single document; you can copy the modules to create more than one genus within a new family, for example.

MODULE 1: **TITLE, AUTHORS, etc**

Code assigned:	***2013.003a-kI***	(to be completed by ICTV officers)

Short title: Creation of a new Family *Nudiviridae* including two new genera and three species
(e.g. 6 new species in the genus *Zetavirus*)

Modules attached (modules 1 and 9 are required)	**1** ☒	**2** ☒	**3** ☒	**4** ☐	**5** ☒
	6 ☐	**7** ☐	**8** ☐	**9** ☒	

Author(s) with e-mail address(es) of the proposer:

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Johannes.jehle@jki.bund.de.

List the ICTV study group(s) that have seen this proposal:

A list of study groups and contacts is provided at http://www.ictvonline.org/subcommittees.asp . If in doubt, contact the appropriate subcommittee chair (fungal, invertebrate, plant, prokaryote or vertebrate viruses)	Baculoviridae Study Group

ICTV-EC or Study Group comments and response of the proposer:

The authors belong to the Baculoviridae Study Group

Date first submitted to ICTV: 24.06.2013
Date of this revision (if different to above):

MODULE 2: **NEW SPECIES**

creating and naming one or more new species.

If more than one, they should be a group of related species belonging to the same genus. All new species must be placed in a higher taxon. This is usually a genus although it is also permissible for species to be "unassigned" within a subfamily or family. Wherever possible, provide sequence accession number(s) for one isolate of each new species proposed.

Code	***2013.003aI***	(assigned by ICTV officers)

To create 2 new species within:

Genus:	***Alphanudivirus*** **(new)**
Subfamily:	
Family:	***Nudiviridae*** **(new)**
Order:	

Fill in all that apply.

- If the higher taxon has yet to be created (in a later module, below) write "**(new)**" after its proposed name.
- If no genus is specified, enter "**unassigned**" in the genus box.

And name the new species:	**GenBank sequence accession number(s) of reference isolate:**
Oryctes rhinoceros nudivirus	EU747721
Gryllus bimaculatus nudivirus	EF203088

Reasons to justify the creation and assignment of the new species:

- Explain how the proposed species differ(s) from all existing species.
  - If species demarcation criteria (see module 3) have previously been defined for the genus, **explain how the new species meet these criteria**.
  - If criteria for demarcating species need to be defined (because there will now be more than one species in the genus), please state the proposed criteria.
- Further material in support of this proposal may be presented in the Appendix, Module 9

Oryctes rhinoceros nudivirus

Significant differences between the viruses of the proposed *Oryctes rhinoceros nudivirus* and other proposed species exist. Theses differences refer to genome size, gene content and gene arrangement, phylogenetic studies, tissue tropism, pathobiology, virus transmission.

The distinct properties associated with members of the *Oryctes rhinoceros nudivirus* genus are the following:

(i) infects larval and adult stages of the rhinoceros beetle *Oryctes rhinoceros*.
(ii) large, circular dsDNA genome of about 128 kb and 140 open reading frames
(iii) single rod-shaped nucleocapsid of 185 x 65 nm in enveloped virions of 220 x 120 nm
(iv) orally transmitted by infection of larvae and adult rhinoceros beetles *O. rhinoceros*
(v) initiation of infection in midgut epithelial cells followed by systemic spreading to other tissues including the fat body
(vi) diseased larvae become turgid and vitreous, often appearing beige, and waxen, dying within 1 to 4 weeks after infection
(vii) adult beetles most often develop chronic infection with virus replication limited to midgut.

Gryllus bimaculatus nudivirus

Significant differences between the viruses of the proposed *Gryllus bimaculatus nudivirus* species and other proposed species exist. These differences refer to genome size, gene content and gene arrangement, phylogenetic studies, tissue tropism, pathobiology, virus transmission.

The distinct properties associated with members of the *Gryllus bimaculatus nudivirus* are the following:

(i) infects larval and adult stages of *Gryllus bimaculatus* and several other species of crickets including *G. campestris*, *Teleogryllus oceanicus* and *T. commodus*
(ii) large, circular dsDNA genome of about 97 kb and 98 open reading frames
(iii) very low G+C content of the genome of only 28%
(iv) rod-shaped or irregularly ellipsoidal, enveloped virions of 90 x 180 nm
(v) virus replication occurs primarily in the nuclei of fat body cells which are greatly hypertrophied with numerous singly-enveloped virus particles being apparent, often arranged in large arrays
(vi) diseased nymphs of *G. bimaculatus* die of virus infection 3 to 12 weeks after infection
(vii) infected crickets are smaller in size than normal insects, appear lethargic and are sometimes crippled

MODULE 2: **NEW SPECIES**

creating and naming one or more new species.

If more than one, they should be a group of related species belonging to the same genus. All new species must be placed in a higher taxon. This is usually a genus although it is also permissible for species to be "unassigned" within a subfamily or family. Wherever possible, provide sequence accession number(s) for one isolate of each new species proposed.

Code	***2013.003bI***	(assigned by ICTV officers)

To create 1 new species within:

Genus:	***Betanudivirus (new)***
Subfamily:	
Family:	***Nudiviridae (new)***
Order:	

Fill in all that apply.
- If the higher taxon has yet to be created (in a later module, below) write "**(new)**" after its proposed name.
- If no genus is specified, enter "**unassigned**" in the genus box.

And name the new species:	**GenBank sequence accession number(s) of reference isolate:**
Heliothis zea nudivirus	JN418988.1

Reasons to justify the creation and assignment of the new species:

- Explain how the proposed species differ(s) from all existing species.
  - If species demarcation criteria (see module 3) have previously been defined for the genus, **explain how the new species meet these criteria**.
  - If criteria for demarcating species need to be defined (because there will now be more than one species in the genus), please state the proposed criteria.
- Further material in support of this proposal may be presented in the Appendix, Module 9

Two viruses Heliothis zea nudivirus 1 (HzNV-1) and Heliothis zea nudivirus 2 (HzNV-2) are considered to belong to the proposed species. Significant differences between the viruses of the proposed *Heliothis zea nudivirus* species and other proposed species exist. These differences refer to genome size, gene content and gene arrangement, phylogenetic studies, tissue tropism, pathobiology, virus transmission.

Heliothis zea nudivirus

The distinct properties associated with members of the *Heliothis zea nudivirus* are the following:

(i) infects larvae and adults (HzNV-2) of *Heliothis zea* or cell lines of different lepidopterans (HzNV-1 and HzNV-2)
(ii) large, circular dsDNA genome of 228-232 kb and about 113 open reading frames
(iii) single rod-shaped enveloped virions of 385-445 x 77-83 nm
(iv) transmission unknown (HzNV-1) or sexually (HzNV-2) upon mating attempts, peroral infection possible
(v) overtly infected male and female moths are sterile with malformed reproductive tissues
(vi) the malformed reproductive tissues of both males and females are the primary site of virus replication
(vii) virus particles released from infected cells fill the lumen of these oviduct tissues eventually coalescing with other material in the bursa to form vesicles which makes up a "virus plug".

MODULE 3: **NEW GENUS**

creating a new genus

Ideally, a genus should be placed within a higher taxon.

Code	***2013.003cI***	(assigned by ICTV officers)

To create a new genus within:

Subfamily:	
Family:	***Nudiviridae (new)***
Order:	

Fill in all that apply.

- If the higher taxon has yet to be created (in a later module, below) write "**(new)**" after its proposed name.
- If no family is specified, enter "**unassigned**" in the family box

naming a new genus

Code	***2013.003dI***	(assigned by ICTV officers)

To name the new genus: *Alphanudivirus*

Assigning the type species and other species to a new genus

Code	***2013.003eI***	(assigned by ICTV officers)

To designate the following as the type species of the new genus

Oryctes rhinoceros nudivirus (new)	Every genus must have a type species. This should be a well characterized species although not necessarily the first to be discovered

The new genus will also contain any other new species created and assigned to it (Module 2) and any that are being moved from elsewhere (Module 7b). **Please enter here the TOTAL number of species (including the type species) that the genus will contain:**

2

Reasons to justify the creation of a new genus:

Additional material in support of this proposal may be presented in the Appendix, Module 9

Significant differences between *Oryctes rhinoceros nudivirus* and *Gryllus bimaculytus nudivirus* (both to be considered as species belonging to the proposed genus *Alphanudivirus*) and the *Heliothis zea nudivirus* species (considered to belong to the proposed *Betanudivirus* genus) exist.

Origin of the new genus name:

Alphanudivirus (derived from Latin "nudus"), related to previous assumption that this virus group represent a non-occluded baculovirus, "Alpha-" Latin alphabet

Reasons to justify the choice of type species:

The first virus found and the most studied in term of prevalence, pathology, transmission and genome sequence.

Species demarcation criteria in the new genus:

If there will be more than one species in the new genus, list the criteria being used for species demarcation and explain how the proposed members meet these criteria.

Very distinct host range, viruses in *Oryctes rhinoceros nudivirus* infect beetles (Coleoptera) whereas virus of of *Gryllus bimaculatus nudivirus* infect crickets (Orthoptera). Both species differ in genome size (128 kb/140 ORF vs 97 kb/98 ORF), GC content (41% vs. 28%) and in virion size.

MODULE 3: **NEW GENUS**

creating a new genus

Ideally, a genus should be placed within a higher taxon.

Code	***2013.003fI***	(assigned by ICTV officers)

To create a new genus within:

Subfamily:	
Family:	***Nudiviridae (new)***
Order:	

Fill in all that apply.

- If the higher taxon has yet to be created (in a later module, below) write "**(new)**" after its proposed name.
- If no family is specified, enter "**unassigned**" in the family box

naming a new genus

Code	***2013.003gI***	(assigned by ICTV officers)

To name the new genus: *Betanudivirus*

Assigning the type species and other species to a new genus

Code	***2013.003hI***	(assigned by ICTV officers)

To designate the following as the type species of the new genus

Heliothis zea nudivirus (new)

Every genus must have a type species. This should be a well characterized species although not necessarily the first to be discovered

The new genus will also contain any other new species created and assigned to it (Module 2) and any that are being moved from elsewhere (Module 7b). **Please enter here the TOTAL number of species (including the type species) that the genus will contain:**

1

Reasons to justify the creation of a new genus:

Additional material in support of this proposal may be presented in the Appendix, Module 9

Significant differences between the *Heliothis zea nudivirus* species (considered to belong to the proposed genus *Betanudivirus*) and *Oryctes rhinoceros nudivirus* and *Gryllus bimaculytus nudivirus* (both to be considered as belonging to the proposed genus *Alphanudivirus*) exist.

Origin of the new genus name:

Betanudivirus (derived from Latin "nudus"), related to previous assumption that this virus group represent a non-occluded baculovirus, "Alpha-" Latin alphabet

Reasons to justify the choice of type species:

The first virus found and the most studied in term of prevalence, pathology, transmission and genome sequence.

Species demarcation criteria in the new genus:

If there will be more than one species in the new genus, list the criteria being used for species demarcation and explain how the proposed members meet these criteria.

MODULE 5: **NEW FAMILY**

creating and naming a new family

Code	***2013.003iI***	(assigned by ICTV officers)

To create a new family containing the subfamilies and/or genera listed below within the Order: Unassigned

If there is no Order, write "**unassigned**" here.
If the Order has yet to be created (in Module 6) please write **"(new)"** after the proposed name.

Code	***2013.003jI***	(assigned by ICTV officers)

To name the new family: *Nudiviridae*

assigning subfamilies, genera and unassigned species to a new family

Code		(assigned by ICTV officers)

To assign the following subfamilies (if any) to the new family:
You may list several subfamilies here. For each subfamily, please state whether it is new or existing.

- If the subfamily is new, it must be created in Module 4
- If the subfamily already exists, please complete Module 7 to 'REMOVE' it from its existing family

Code	***2013.003kI***	(assigned by ICTV officers)

To assign the following genera to the new family:
You may list several genera here. For each genus, please state whether it is new or existing.

- If the genus is new, it must be created in Module 3
- If the genus already exists, please state whether it is currently unassigned or is to be removed from another family. If the latter, complete Module 7 to 'REMOVE' it from that family

***Alphanudivirus* (new)**

***Betanudivirus* (new)**

The new family will also contain any other new species created and assigned to it (Module 3) and any that are being moved from elsewhere (Module 7b). **Please enter here the TOTAL number of unassigned species that the family will contain (those NOT within any of the genera or subfamilies listed above):**

Reasons to justify the creation of the new family:
Additional material in support of this proposal may be presented in the Appendix, Module 9

In the past, members of the proposed family *Nudiviridae,* were considered as so-called non occluded baculoviruses (NOB). Until the fifth ICTV report they were included as a subfamily (*Nudibaculovirinae*) in the family of *Baculoviridae*, because of their large dsDNA genome, replication in the nucleus and hypertrophy of infected cells. They were orphaned with the 6th ICTV report because no molecular data were available to prove their relationship with baculoviruses. During the last decade, genome sequences of HzNV-1, HzNV-2, OrNV and GbNV became available. The genome sequences indicated a distant relationship to baculoviruses. However, nudiviruses have only 15 of the 31 baculovirus core genes and are therefore considered to be sufficiently distinct from baculoviruses. Although some nudiviruses may form facultatively or obligatorily occlusion bodies, homology of the occlusion body

protein is not yet verified. Nudiviruses also differ from baculoviruses as they infect larval and adult stages.Wheras baculoviruses have been reported only from Lepidoptera, Hymenoptera and Diptera, nudiviruses have a considerably broader host range, including taxa from Lepidoptera, Coleoptera, Orthoptera and Crustacea. *Additional material see Appendix Modul 9-*

Origin of the new family name:

"Nudi-"; Latin "nudus", naked, in reference to the previously used name nudibaculovirus referring to rod shaped nucleocapsids lacking a protein matrix like polyhedrin or granulin.

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

References:

Annex:

Include as much information as necessary to support the proposal, including diagrams comparing the old and new taxonomic orders. The use of Figures and Tables is strongly recommended but direct pasting of content from publications will require permission from the copyright holder together with appropriate acknowledgement as this proposal will be placed on a public web site. For phylogenetic analysis, try to provide a tree where branch length is related to genetic distance.

Identifcation and History

Members of the proposed family *Nudiviridae* are a highly diverse group of rod-shaped, enveloped, and circular dsDNA viruses of arthropods, including insects and crustaceans (shrimp). Considering that they share a number of structural characteristics and replication aspects with insect baculoviruses, nudiviruses were classified in the past as "non-occluded baculoviruses" (NOBs). Due to the lack of convincing genetic data corroborating their relatedness to baculoviruses as well as different morphological characters, they were eventually removed from the family *Baculoviridae* (Mayo, 1995) and orphaned. Nudiviruses have also been referred to as intranuclear bacilliform viruses (IBVs) (Evans and Edgerton, 2002). Notably, unlike baculoviruses, nudiviruses generally lack occlusion bodies (OBs), though occluded nudiviruses may exist. Thus far, a variety of nudiviruses and nudivirus-like viruses have been reported from various host species belonging to Lepidoptera, Trichoptera, Diptera, Siphonaptera, Hymenoptera, Neuroptera, Coleoptera, Homoptera, Thysanura, Orthoptera, Acarina, Araneina, and Crustacea (Huger and Krieg, 1991). However some of these previously considered NOBs have been re-classified in the family *Hytrosaviridae* (Abd-Alla et al., 2009).

The well studied nudiviruses are the Oryctes rhinoceros nudivirus (OrNV), the Gryllus bimaculatus nudivirus (GbNV), the Heliothis zea nudivirus 1 (HzNV-1) and the Heliothis zea nudivirus 2 (HzNV-2) (Table 1).

Phylogenetic analyses using partial sequence analyses revealed that the occluded unassigned Penaeus monodon baculovirus (= PmSNPV, = Monodon baculovirus (MBV)) is also more closely related to nudiviruses than to baculoviruses (Wang and Jehle, 2009; Wang et al., 2001). Based on the phylogenetic analyses MBV should be considered as a nudivirus and the name Penaeus monodon nudivirus (PmNV) was proposed. However, due to the lack of complete genome data a species description is not yet possible. Therefore, this virus is considered as an unassigned nudivirus.

Proposed Genus *Alphabaculovirus*

This genus would consist of two species, *Oryctes rhinocersos nudivirus* and *Gryllus bimaculatus nudivirus*.

Proposed species *Oryctes rhinoceros nudivirus*

A member, and type species, of the proposed species *Oryctes rhinoceros nudivirus* is the Oryctes rhinoceros nudivirus (OrNV), which is also known as *O. rhinoceros* virus (OrV). It was first discovered in 1963 in Malaysia as a natural pathogen of the beetle *O. rhinoceros* (Huger, 1966; 2005).

The enveloped virions of OrNV virions are rod-shaped and have a size of 220 x 120 nm (Payne, 1974; Payne et al., 1977). Purified nucleocapsids are 180 x 65 nm in size with slightly thickened caps on their longitudinal ends (Fig. 1). One end of the nucleocapsid is linked to a flexuous, tail-like appendage of about 270 x 10 nm.

Both, the larval stages and the adult beetles of *O. rhinoceros* can be infected by OrNV, which is transmitted perorally. After primary infection of the midgut epithelial cells, infection rapidly spreads to other tissues. Infected larvae become lethargic and cease feeding. Increasing vacuolation and disintegration of fat body cells and an increase of hemolypmph are typical symptoms. At the end stage of infection the larvae become turgid and vitreous, often beige and waxen. Infected larvae succumb from a lethal infection within 1-4 weeks. (Huger and Krieg, 1991). Also adult beetles may become lethally infected. However, most frequently they suffer from a symptomless chronic infection, often restricted to the midgut epithelial cells, which become heavily packed with newly synthesized viruses (Huger and Krieg, 1991). Then, the midguts are swollen and whitish. As infected midgut cells are continuously sloughed off, virus infected cells are excreted with their feces. This contributes to a very efficient spread of OrNV in beetle populations. Infected adults stop feeding and egg lying (Huger, 2005).

At the cellular level, it is thought that viruses enter the cell via pinocytosis (Crawford and Shehan, 1985). The first cytopathogenic effects of virus infection were nuclear hypertrophy, cell lysis may follow about 72-96 hours post infection (hpi). The first signs of virus replication occur 7 hpi. Between 12-36 hpi, virus replication has started and OrNV virions assemble in dense arrays close to the nuclear envelope (Fig. 2) (Crawford and Shehan, 1985).

The protein composition of the virion is not very well analyzed. Sofar, proteomic data are completely missing. The genome of OrNV is a circularly closed dsDNA molecule. Both the Philippine isolate PV505 and the Indian isolate KI were mapped by DNA restriction endonuclease analysis to 123-127 kb in length (Crawford et al., 1985; Mohan and Gopinathan, 1991).
The complete genome sequence of the 127,615 bp large genome was obtained for the isolate OrNV Ma07 (Wang et al., 2007; 2008; 2011) (Fig. 3). The G+C content was determined to be 42% (Wang et al. 2011). OrNV and other sequenced nudivirus genomes (GbNV, HzNV-1, see below) encode 20 out of 31 so-called baculovirus core genes. These core genes are associated with late and very late gene transcription (*p47*, *lef-8*, *lef-9*, *lef-4*, *vlf-1*, and *lef-5*), DNA replication (*dnapol* and *helicase*), virus structure (*p74*, *pif-1*, *pif-2*, *pif-3*, *ac68*, *vp91*, *vp39*, *38K*, *19kda*, and *odv-e56*), or have unknown functions (*ac81* and *p33*) (van Oers and Vlak, 2007) (Table 2). From the presence of these core gene homologues in nudiviruses and baculoviruses and from phylogenetic analyses it can be clearly concluded that nudiviruses and baculoviruses are two monophyletic sister groups which share a common ancestor (Wang et al., 2007a; Wang & Jehle, 2009).

Another feature of the OrNV and some other nudivirus genomes is the presence of a homologue to the *polyhedrin/granulin* (*polh/gran*) gene of Alpha-, Beta- and Gammabaculoviruses, which typically form occlusion bodies, while the nudiviruses are generally deficient of those. However, a facultative occurrence of occlusion bodies in midgut cells of OrNV infected rhinoceros beetles was reported (Huger and Krieg, 1991). Atypical occlusion bodies can be also occasionally observed with HzNV-2 (Raina et al., 2000; Rallis et al., 2002b). Also PmNV forms occlusion bodies, though its OB protein(s) seems not to be homologous to baculovirus polyhedrin (Chaivisuthangkura P et al., 2008).

Proposed species *Gryllus bimaculatus nudivirus*

Gryllus bimaculatus nudivirus (GbNV) infects nymphs and adults of several field crickets, such as *Gryllus bimaculatus*, *G. campestris*, *Teleogryllus oceanicus* and *T. commodus* (Huger, 1985). The virions are rod-shaped or irregularly ellipsoidal, enveloped and have a size of 90 x 180 nm (Fig. 4), The GbNV genome is 96,944 bp in length; 98 ORFs longer than 150 bp were predicted (Fig. 5). Its G+C content is 28%, thus significantly lower than that of other nudiviruses. Fourteen short direct repeat regions (*drs*) distributed throughout the genome and comprising about 0.6% of the genome sequence were identified. These *drs* are extremely AT rich (up to 98%) and contain 2 to 3 copies of short reiterated sequences of 11 to 42 bp in length

The GbNV is not very well studied, with only a few reports on its biology and structure of the virion (Huger and Krieg, 1991). Similar to other nudiviruses, GbNV replicates in the nuclei of the infected fat body cells; the infected cells are highly hypertrophoid. Infected early nymphs of *G. bimaculatus* succumb from infection between 3-12 weeks. Infected crickets are lethargic and smaller in size, sometimes even crippled (Huger & Krieg, 1991(Huger, 1985).

Proposed Genus *Betanudivirus*

The *Betanudivirus* genus would consist of one species *Heliothis zea nudivirus*

Proposed species *Heliothis zea nudiviruses*

The *Heliothis zea nudivirus* species would consist of two viruses *Heliothis zea* nudiviruses 1 (HzNV-1) and 2 (HzNV-2).

The *H. zea* nudivirus 1 is also known as IMC-HZ-I-NOV, baculovirus X or Hz-1 virus. It was first described in a persistent viral infection in the ovarian *H. zea* cell line IMC (Granados et al., 1978). HzNV-1 virions are 385-445 nm x 77-83 nm (Burand et al., 1983a) (Fig. 6; Fig.7). A physical map of the covalently closed dsDNA was constructed by Chao et al. (1990) and its size was estimated at 230 to 245 kb (Huang et al., 1982; Burand et al., 1983b), matching the genome size of 228,089 bp determined later by full genome sequencing (Cheng et al., 2002). The HzNV-1 genome encodes 154 putative open reading frames (ORFs) (Cheng et al., 2002). No homologous repeat regions (*hrs*) were found but a number of short tandem repeats of 21 to 75 bp are distributed throughout its genome sequence (Cheng et al., 2002). Again homologues to baculovirus genes, including 20 baculovirus core genes were identified (Cheng et al., 2002; Wang et al., 2007a) (Table 2).

The genome sequence of HzNV-1 is very similar to HzNV-2, also known as gonad-specific virus (GSV), *H. zea* reproductive virus and Hz-2V (for reviews see Burand, 1991; 2009; Wang et al. 2007c). HzNV-2 has a genome of 231,621 bp in size (Burand et al., 2011). The G+C content of both of the HzNV-2 and HzNV-1 genomes is 41.8%. HzNV-2 contains 113 predicted ORFs and shares a 94-95% sequence similarity with HzNV-1.

HzNV-1 is not perorally infectious but causes a persistent infection of the ovarian *H. zea* cell line IMC, as well as other lepidopteran cell lines, such as IPLB-1075 (*H. zea*), IPLB-SF-21 (*Spodoptera frugiperda*), IPLB-65Z (*Lymantria dispar*) and TN-368 (*Trichoplusia ni*) (Granados et al., 1978; Kelly et al., 1981; Ralston et al., 1981). Further experiments showed that HzNV-1 has a wide in vitro host cell spectrum but only Lepidopteran cell lines supported HzNV-1 replication. Interestingly, HzNV-1 caused acute infection and massive cytopathic effects (CPE) of TN-368 cell cultures of TN-368.

HzNV-2 in contrast, can be infectious when fed to corn earworm larvae (*Heliothis zea*), however the main method for the horizontal transmission of the virus is via mating attempts by infected adults (Burand et al., 1996: Rana and Lupiani, 2006). Overtly infected male and female moths are sterile and show malformed reproductive tissues that appear as large "Y-shaped" structures (Burand et al., 1996, Rallis and Burand, 2002ab), a condition referred to as being agonadal. Although many of the reproductive tissues of both agonadal males and females are malformed some are completely absent including the ovaries and eggs of infected females and the accessory glands of males, which produce pheromonostatic peptide, a peptide known to inhibit the level of mating pheromone in female moths.

The malformed reproductive tissues of both males and females are the primary site of virus replication. Replication takes place in the nuclei of infected cells in these tissues where large arrays of enveloped nucleocapsids are visible prior to their entering the cytoplasm. Virus release appears to be the result of the lysis of the cell and nuclear membranes. However, budding of virus particles has been reported to occur from the membrane of infected Ld-652Y cells in culture (Lu and Burand, 2001; Rallis and Burand, 2002ab

Virus replication in the reproductive tissues in males results in the accumulation of virus particles in the lumen of the primary simplex and transfer of virus to females during mating attempts (Burand et al., 2004). The malformed reproductive tissues of females show considerable hyperplasia with the proliferation of cells in tissues comprising the remnants of the common and lateral oviducts. Virus particles released from infected cells fill the lumen of these oviduct tissues eventually coalescing with other material in the bursa to form vesicles which makes up a "virus

plug". This plug blocks the reproductive opening of agonadal female moths and the virus in this plug serves as a source inoculum for males attempting to mate with infected females (Burand et al., 2004). Virus-infected females produce more mating pheromone and are more attractive to males than uninfected females. Males attempting to mate with these females may become infected and can also transmit the virus to healthy females during subsequent matings (Burand et al., 2005; Burand and Tan, 2006).

The majority of HzNV-2 infected, feral *H. zea* moths are fertile, asymptomatic carriers of the virus (Lupiani et al, 1999). Infected, asymptomatic female moths appear to be the principle source of vertically transmitted virus and is passed by transovarian transmission to progeny insects inside eggs. Insects arising from these eggs maybe latently infected with some showing overt symptoms of the disease only as adult moths (Burand and Rallis, 2004; Hamm et al., 1996).

Molecular Phylogeny

Based on the presence of 20 baculovirus core genes, supertree analysis and super matrix analysis revealed that nudiviruses form a monophyletic clade which is most closely related to members of *Baculoviridae* and to *Hytrosaviridae* (Fig. 8, Fig.9). Nevertheless, based on their different morphology, gene content and phylogeny the establishement of a family separate from *Baculoviridae* and *Hytrosaviridae* is justified. The Nudiviruses branches into two clades, which represent the suggested genera *Alphanudivirus* and *Betanudivirus*

Gene content (see above) and phylogenetic analyses suggest that the nudiviruses form a mono-phyletic group of non-occluded dsDNA viruses. They diverged from a common ancestor of the baculoviruses lineages before this radiated into dipteran, hymennopteran and lepidopteran specific clades. They represent a highly diverse and phylogenetically ancient sister group of the baculoviruses, and have evolved into a variety of highly divergent host orders.

The following are demarcation criteria for classification of a candidate virus into the family Nudiviridae (Table 3) (Wang and Jehle, 2009):

1. Genome: large circular dsDNA
2. Genome organization and replication: a set of conserved core genes shared among members; propagation in the nuclei of infected host cells
3. Morphology: rod-shaped and enveloped virion

4. Biologic properties: transmission via per oral and/or per parenteral route; infection of larvae and/or adults; diverse tissue and cell tropisms

Clearly, these demarcation criteria might need to be updated when more biological properties such as virion properties, infection and replication strategies, as well as host range and virus ecology, become available.

References

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Tables

Table 1. The well studied nudiviruses (modified from Wang et al., 2007c)

Virus	Host	Host stage and/or tissue tropism	Size (nm)		Genome		
			Virus particles	Nucleocapsids	Size (kb)	GC%	ORFs
HzNV–1	*Heliothis zea* (?) (Lepidoptera: Noctuidae)	–	384–444×77–83	–	228,089	42	154
HzNV-2	*Heliothis zea* (Lepidoptera: Noctuidae)	Larvae and adults; reproductive tissues	415×80	500×25	231.621	42	113
OrNV	*Oryctes rhinoceros* (Coleoptera: Dynastidae)	Larvae and adults; midgut and fat body	200–235×100–120	160–180×50–65	127,615	42	139
GbNV	*Gryllus bimaculatus* (Orthoptera: Gryllidae)	Nymphs and adults; fat body	145–240×80–100	162×66	96,944	28	98

Table 2. Homologous genes conserved in nudiviruses (modified from Wang and Jehle, 2009). [To be completed for HzNV-2]

Gene name	OrNV	GbNV	HzNV-1	HzNV-2	PmNV	Function
dnapol	**1**	**12**	**131**	**18**	**n.d.**	DNA replication, repair, and recombination
helicase	**34**	**88**	**104**	**38**	**n.d.**	
helicase 2	108	46	60		n.d.	
integrase	75	57	144		+	
ligase	121	38	36	94	n.d.	
lef-3	59	86	–		n.d.	
rr1	51	82	95		n.d.	Nucleotide metabolism
rr2	102	63	73		n.d.	
tk	58	74	115		+	
tk	117	34	111		+	
tk	125	44	71		+	
tk	137	17	51		+	
p47	**20**	**69**	**75**		**n.d.**	Transcription
lef-4	**42**	**96**	**98**	**43**	**n.d.**	
lef-8	**64**	**49**	**90**	**51**	**n.d.**	
lef-9	**96**	**24**	**75**	**63**	**+**	
lef-5	**52**	**85**	**101**	**40**	**+**	
vlf-1	**30**	**80**	**121**	**28**	**+**	
p74	**126**	**45**	**11**	**106**	**n.d.**	Oral infectivity
pif-1	**60**	**52**	**55**	**82**	**n.d.**	
pif-2	**17**	**66**	**123**	**26**	**n.d.**	
pif-3	**107**	**3**	**88**	**53**	**n.d.**	
polh/gran	16	65	69		n.d.	Packaging, assembly, and morphogenesis
19kda	**33**	**87**	**103**	**39**	**n.d.**	
ac68	**72**	**55**	**74**		**n.d.**	
38K	**87**	**1**	**10**	**108**	**+**	
iap-3	134	98	138		n.d.	Inhibition of apoptosis
vp39	**15**	**64**	**89**		**n.d.**	Unknown function
vp91	**106**	**2**	**46**	**89**	**n.d.**	
odv-e56	**115**	**5**	**76**	**62**	**n.d.**	
ac81	**4**	**14**	**33**		**n.d.**	
ac92	**113**	**7**	**13**		**n.d.**	
	47	19	30		n.d.	
	76	58	143		n.d.	
	3	13	–		n.d.	

18	67	–	n.d.
22	72	–	n.d.
23	74	–	n.d.
24	75	–	n.d.
25	76	–	n.d.
27	78	–	n.d.
29	81	–	n.d.
39	93	–	n.d.
40	94	–	n.d.
41	95	–	n.d.
44	97	–	n.d.
45	23	–	n.d.
46	22	–	n.d.
53	84	–	n.d.
54	83	–	n.d.
61	51	–	n.d.
79	59	–	n.d.
80	60	–	n.d.
86	61	–	n.d.
90	28	–	n.d.
95	9	–	n.d.
104	62	–	n.d.
105	43	–	n.d.
114	6	–	n.d.
116	33	–	n.d.
118	35	–	n.d.
119	36	–	n.d.
120	37	–	n.d.
122	39	–	n.d.
123	41	–	n.d.
132	48	–	n.d.
6	–	109	n.d.
–	–	52	+
–	–	64	+
–	–	93	+
–	–	118	+
–	–	141	+

–: Absent; +: Present; n.d.: Not determined. The predicted ORFs in nudiviruses are presented in number. Homologues to baculovirus core genes are marked in bold face. PmNV is *Panaeus monodon* nudivirus

Table 3. The demarcation criteria of the baculoviruses and the nudiviruses. Modified from Wang and Jehle (2009).

Demarcation criteria	Baculoviruses	Nudiviruses
Rod-shaped virion	+	+
Circular dsDNA genome	+	+
Replication in nucleus	+	+
Nucleus hypertrophy	+	+
Host range		
Holometabolous	+	+
Hemimetabolous	–	+
Host stage infected		
Larvae	+	+
Adults	–	+
Horizontal transmission		
Parenteral	–	+
Peroral	+	+
Vertical transmission	+/–	+/–
Occlusion body	+	+/–

Figures

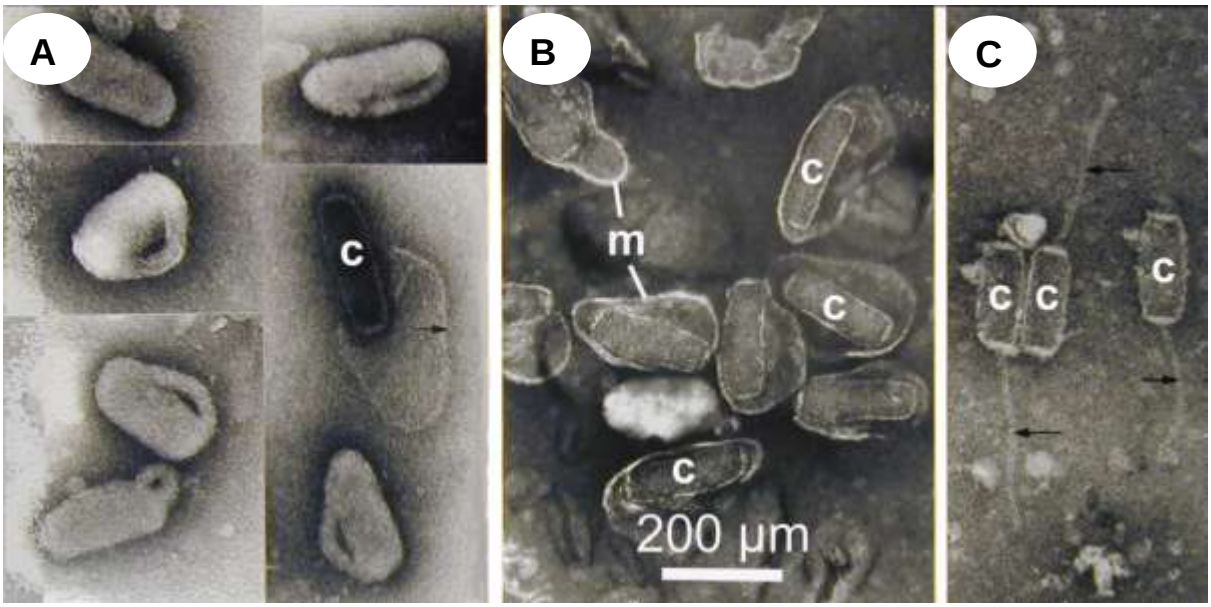

Fig. 1. Electron micrographs with structural details of *Oryctes* virus rods, negatively stained with phosphotungstic acid. (A) Virions unpenetrated by stain, often being artificially mug-shaped; middle right: the virus membrane (arrow) is shed off from the capsid (c). (B) Virions with longer penetration by stain, thus displaying the capsids (c) and the surrounding viral membrane (m). (C) Three capsids (c) showing the typical thread-like tail (arrows). Reprinted from Journal of Invertebrate Pathology 89, A. Huger, The *Oryctes* virus: Its detection, identification, and implementation in biological control of the coconut palm rhinoceros beetle, *Oryctes rhinoceros* (Coleoptera: Scarabaeidae), 78-84, Copyright 2005, with permission from Elsevier.

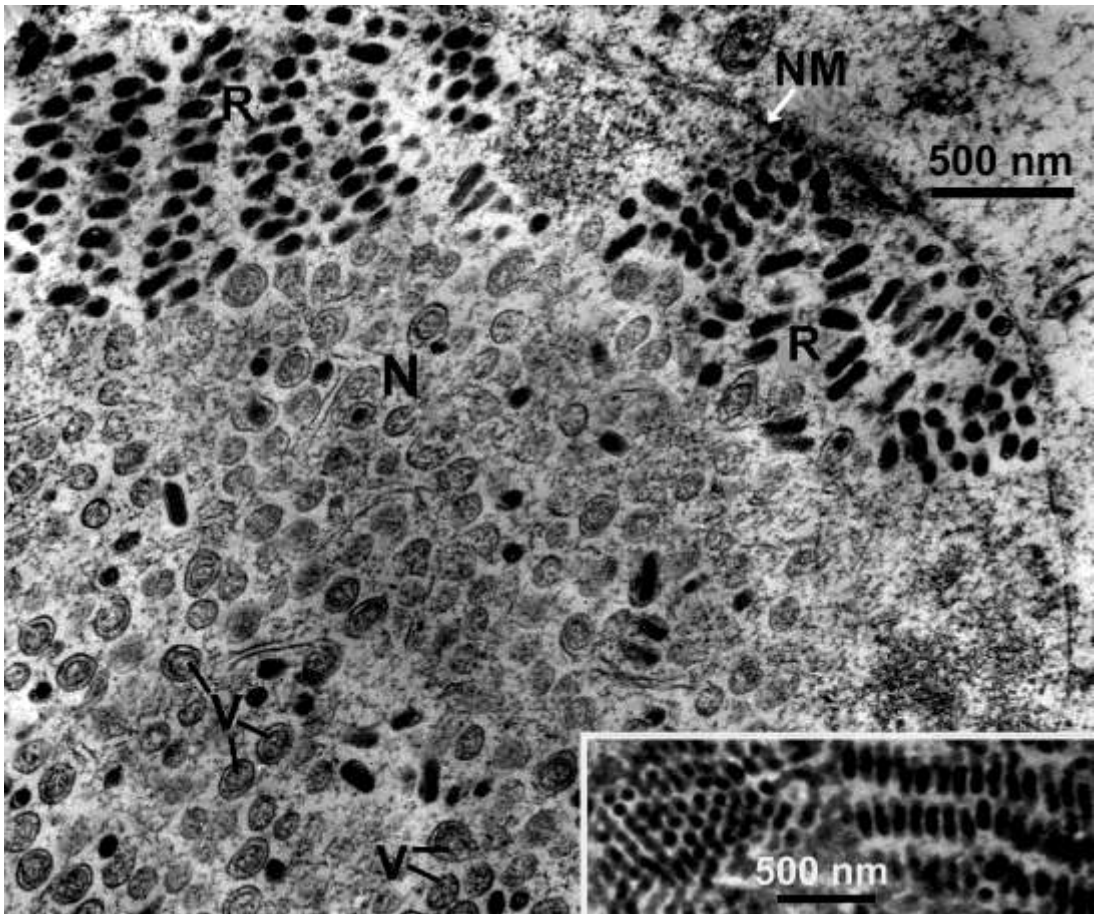

Fig. 2. Electron micrograph of a thin section showing part of the nucleus (N) of a fat body cell of a third instar rhinoceros beetle larva with heavy *Oryctes* virus infection. Note the accumulation of virus rods (R) at the nuclear periphery and the single- and double-membraned vesicles (V) in the nuclear center. NM, nuclear membrane. Inset: Virus rods in cross (left) and longitudinal (right) section arranged in a pseudocrystalline pattern. Reprinted from Journal of Invertebrate Pathology 89, A. Huger, The *Oryctes* virus: Its detection, identification, and implementation in biological control of the coconut palm rhinoceros beetle, *Oryctes rhinoceros* (Coleoptera: Scarabaeidae), 78-84, Copyright 2005, with permission from Elsevier.

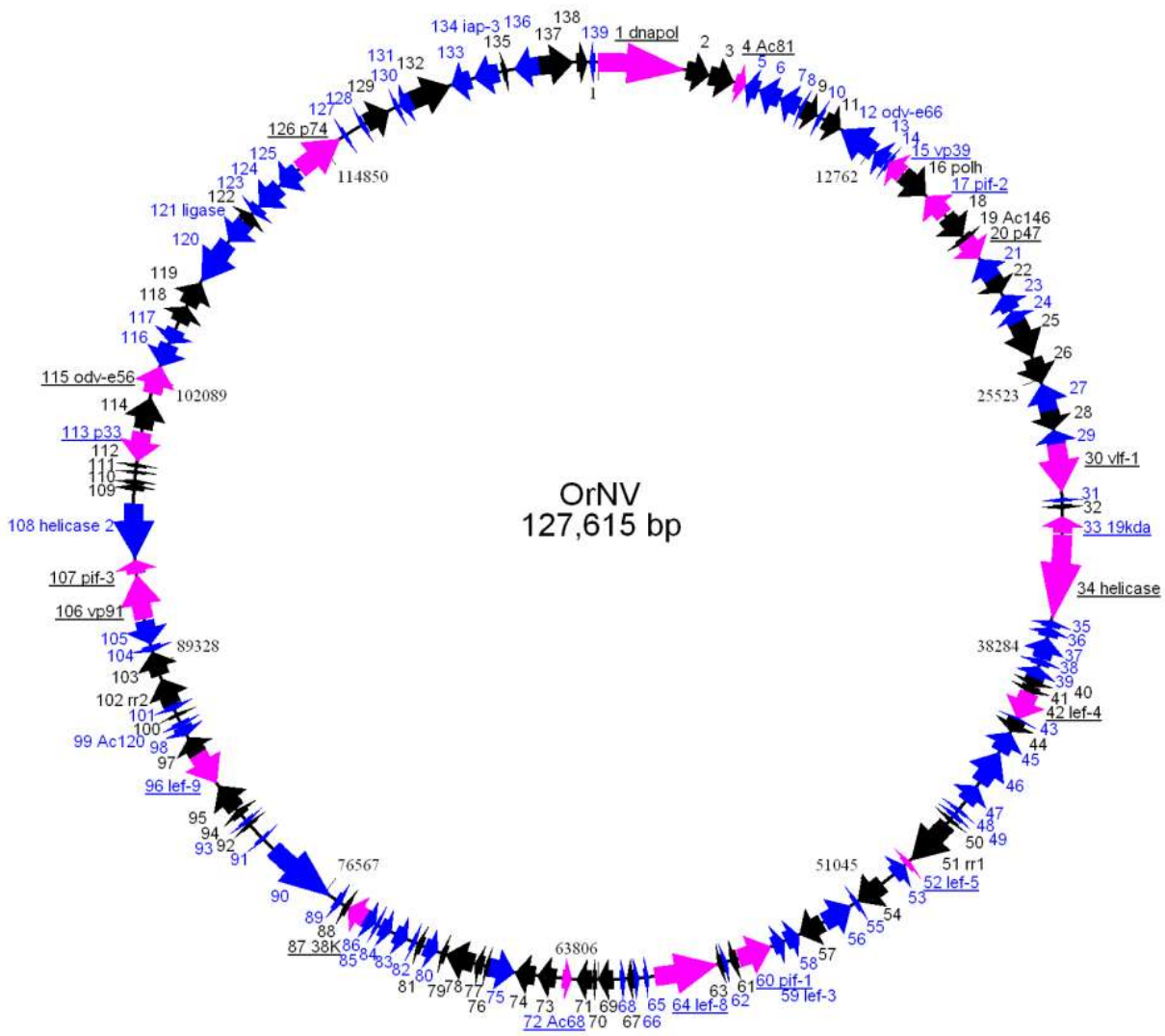

Fig. 3. The genome map of *Oryctes* NV. ORFs and their transcriptional directions are indicated in arrows. Black color, clockwise coding; blue color, counterclockwise coding; pink color, the 20 baculovirus core gene homologues.

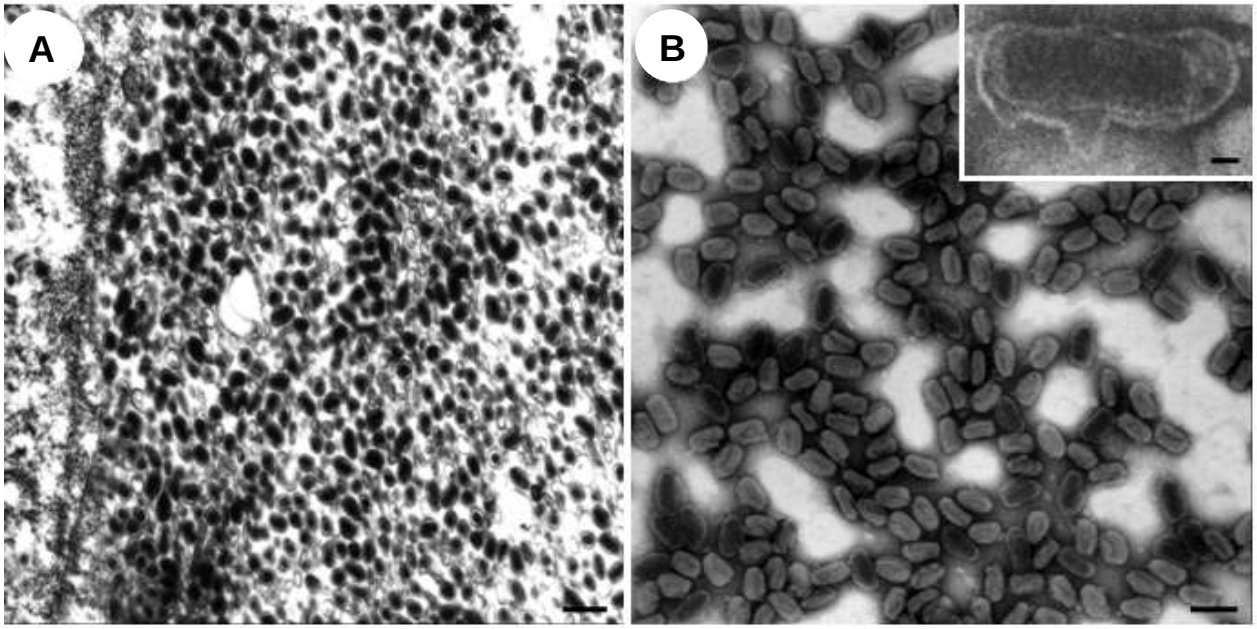

Fig 4. Electron micrographs of Gryllus bimaculatus nudivirus (GbNV) particles. A) Thin section of infected cell nucleus. B) Purified virions. Inset: Enlarged image of a typical virion showing the envelope and the rod-shaped nucleocapsid. Bar: 200 nm in A) and B) and 20 nm in inset. Courtesy of Dr. Alois M. Huger, JKI Darmstadt. Reprinted from Journal of Invertebrate Pathology 101, Y. Wang, J. A. Jehle, Nudiviruses and other large, double-stranded circular DNA viruses of invertebrates: New insights on an old topic, 187-193, Copyright 2009, with permission from Elsevier.

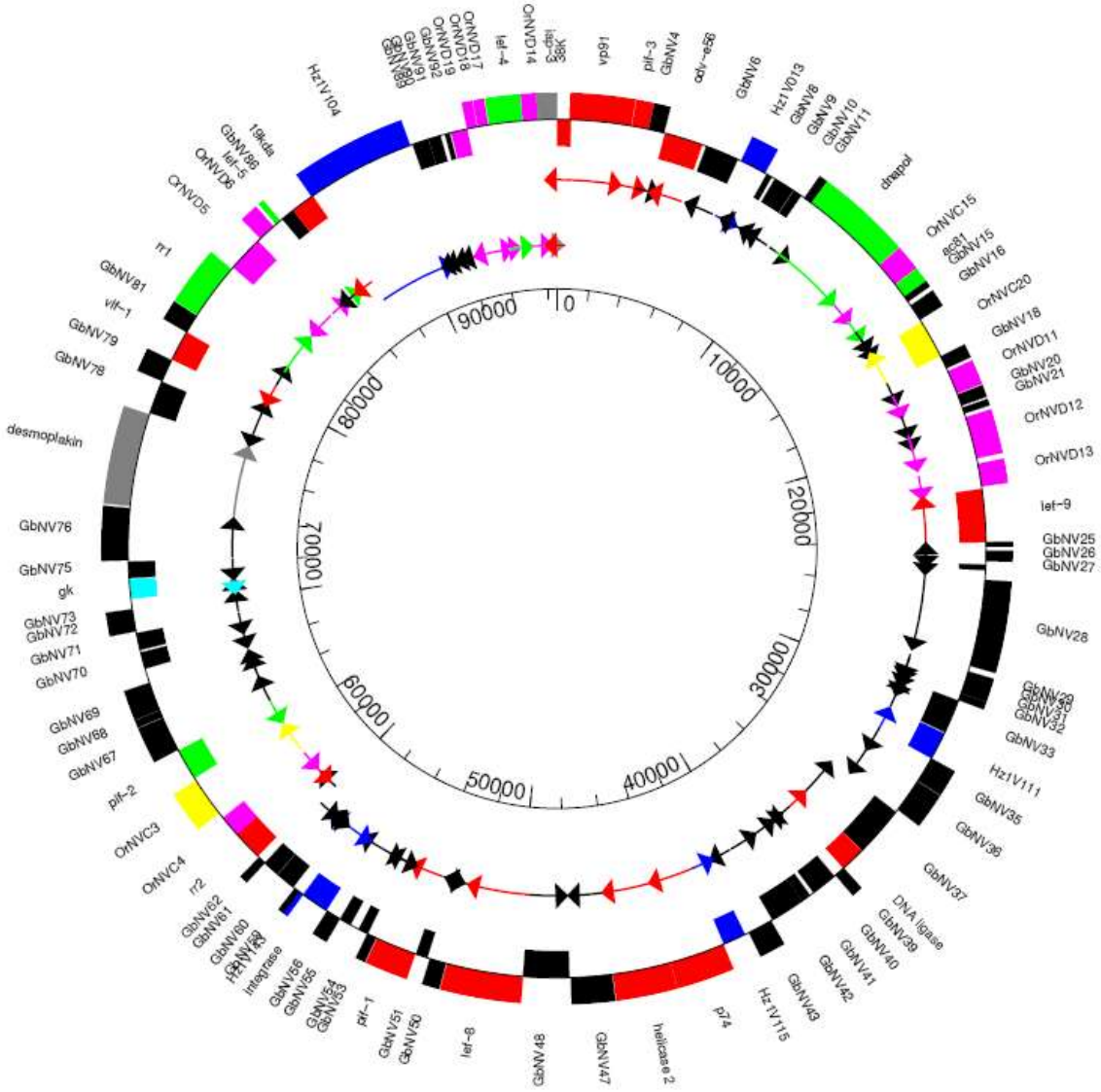

Fig. 5. Circular map of the genome of *Gryllus bimaculatus* nudivirus based on the sequence published by Wang et al. (2007), J. Virol. 81, 5395-5406.

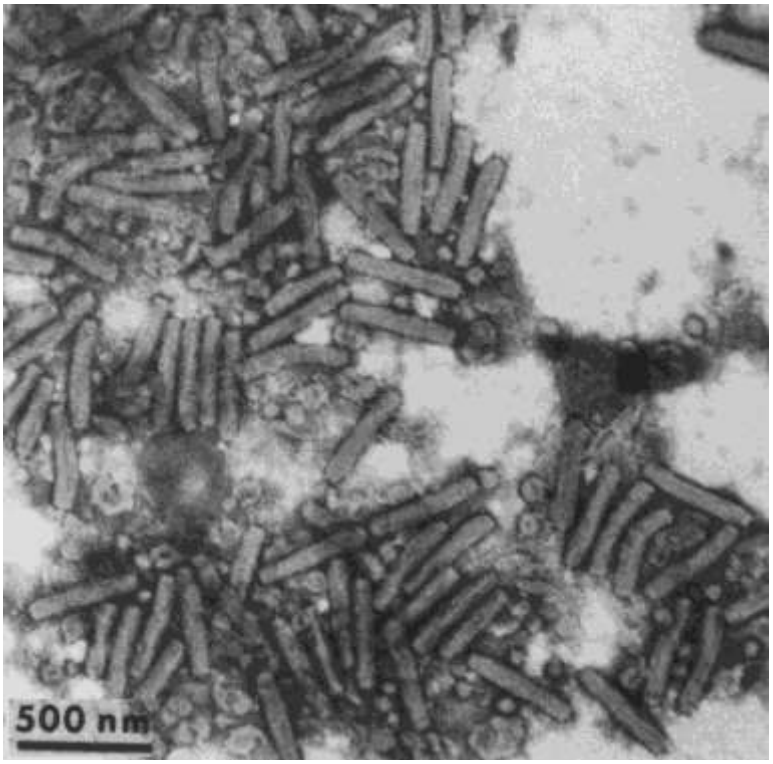

Fig. 6. Electron micrograph of *Heliothis zea* NV-1 virus particles Bar, 50 nm.

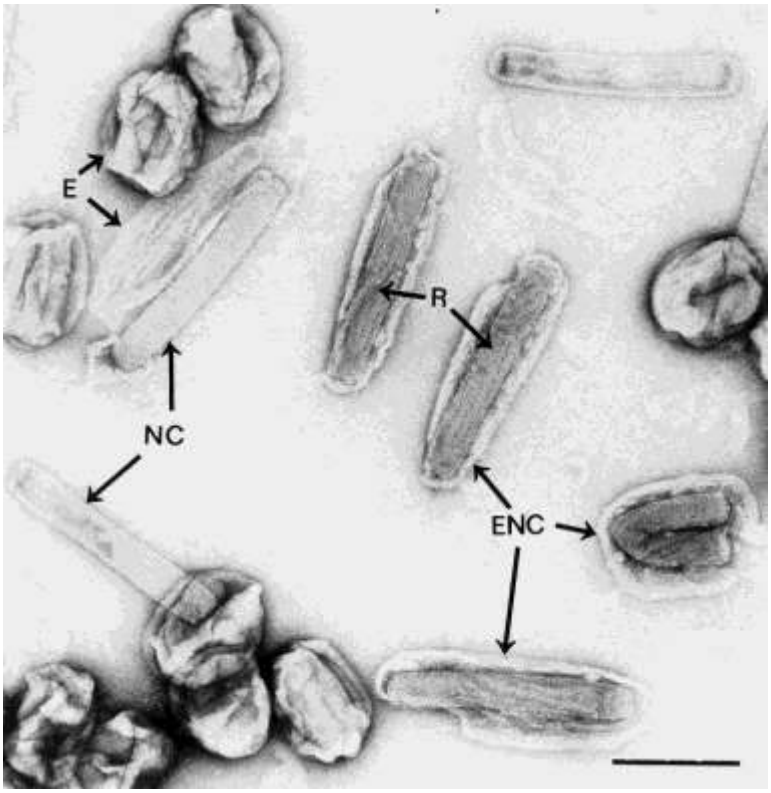

Fig. 7. Electron micrograph of *Heliothis zea* NV-2 virus particles purified by sucrose-gradient ultracentrifugation. The viral components found in this sample included rod-shaped, enveloped, nucleocapsids (ENC) and naked nucleocapsids (NC) as well as viral envelopes (E) and ‘‘rope-like’’ structures (R). Bar, 200 nm.

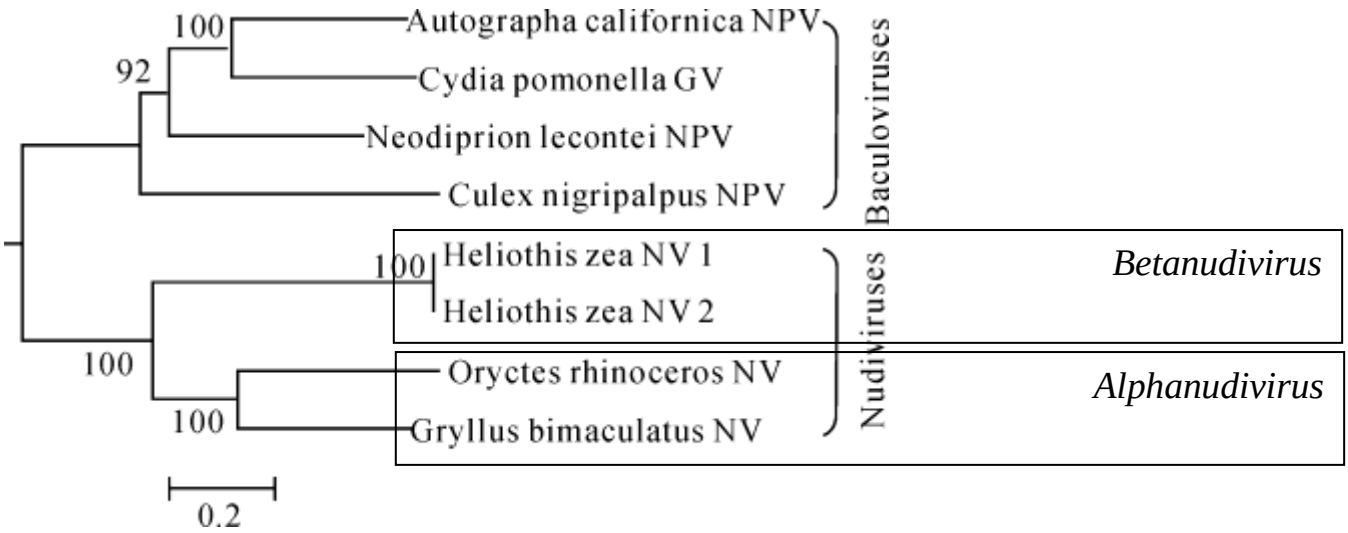

Fig. 8. The midpoint rooted neighbour-joining (NJ) phylogenetic tree based on 1789 sites of concatenated amino acid sequences of the *lef-4*, *lef-5*, *dnapol* and *ac81* genes from GbNV, OrNV, HzNV-1, HzNV-2 and 4 selected baculoviruses. Gaps and missing data are excluded for the analyses. The robustness of the tree was tested using bootstrap analyses (1000 replicates) and the percent values (NJ) are given next to the nodes. Minimal evolution (ME) and maximum parsimony (MP) analyses revealed a similar tree topology. The groups of baculoviruses and nudiviruses are indicated on the tree. The scale bar represents a distance of 20%. (modified from Wang and Jehle, 2009).

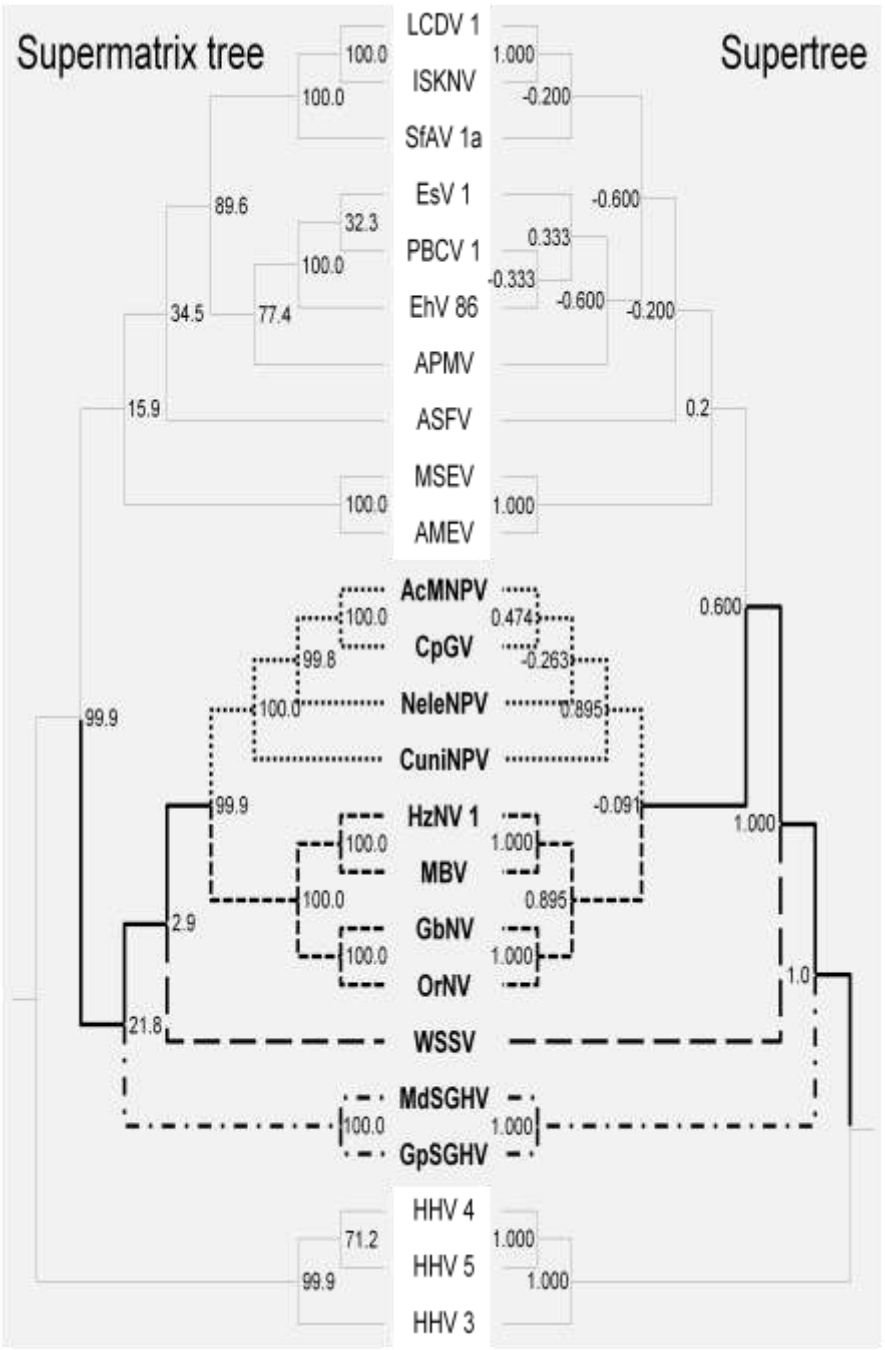

Fig. 9. Combined data trees based on the 20 conserved baculovirus core gene sequences: (A) ML supermatrix tree derived from a simultaneous analysis of the concatenated sequences and (B) weighted MRP supertree of the 20 gene trees in Wang et al, 2011. The latter represents the 50% majority-rule consensus of 71 equally most parsimonious solutions. Nodal support is given as non-parametric bootstrap frequencies (n = 1000) determined from the supermatrix data set / degree of support among the informative source trees for a given node as measured by the rQS index. Branch lengths in (A) are proportional to the average number of substitutions per site per unit time. GenBank accession numbers for these viral genomes and virus full names are listed as follows: NC_001623 (Autographa californica NPV, AcMNPV), NC_002816 (Cydia pomonella GV, CpGV), NC_005906 (Neodiprion lecontei NPV, NeleNPV), NC_003084 (Culex nigripalpus NPV, CuniNPV), NC_004156 (Heliothis zea NV 1, HzNV-1), NC_009240 (Gryllus bimaculatus NV, GbNV), EU747721 (Oryctes rhinoceros NV, OrNV), NC_010356 (Glossina

pallidipes SGHV, GpSGHV), NC_010671 (Musca domestica SGHV, MdSGHV), NC_003225 (Shrimp white spot syndrome virus, WSSV), NC_001659 (African swine fever virus, ASFV), NC_002520 (Amsacta moorei EV, AMEV), NC_001993 (Melanoplus sanguinipes EV, MSEV), NC_008361 (Spodoptera frugiperda AV 1a, SfAV-1a), NC_001824 (Lymphocystis disease virus 1, LCDV-1), NC_003494 (Infectious spleen and kidney necrosis virus, ISKNV), NC_006450 (Acanthamoeba polyphaga mimivirus, APMV), NC_000852 (Paramecium bursaria Chlorella virus 1, PBCV-1), NC_007346 (Emiliania huxleyi virus 86, EhV-86), NC_002687 (Ectocarpus siliculosus virus 1, EsV-1), NC_001348 (Human herpesvirus 3, HHV-3), NC_001347 (Human herpesvirus 5, HHV-5), and NC_007605 (Human herpesvirus 4, HHV-4).