

**Part 1:** **TITLE, AUTHORS, APPROVALS, etc**

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| **Code assigned:** | **2020.005D** |  |
| **Short title:** Create two new genera and eight new species (*Lefavirales*: *Nudiviridae*) | | |
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**List the ICTV Study Group(s) that have seen this proposal**

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| *Baculoviridae* and *Nudiviridae* Study Group |

**ICTV study group comments and response of proposer**

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| The *Baculoviridae*/*Nudiviridae* Study Group are the proposers. |

**Authority to use the name of a living person**

|  |  |  |
| --- | --- | --- |
| **Taxon name** | **Person from whom the name is derived** | **Permission attached (Y/N)** |
|  |  |  |

**Submission dates**

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| Date first submitted to SC Chair | Jan 7, 2020 |
| Date of this revision (if different to above) | July 21, 2020 |

**ICTV-EC comments and response of the proposer**

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**Part 3:** **TAXONOMIC PROPOSAL**

**Name of accompanying Excel module**

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| 2020.005D.R.Nudiviridae\_2ngen\_8nsp.xlsx |

**Abstract**

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| Members of the family *Nudiviridae* are rod-shaped, large dsDNA viruses of arthropods that are related to, but distinct from, viruses of the family *Baculoviridae*. When family *Nudiviridae* was created in 2013 (<https://talk.ictvonline.org//taxonomy/p/taxonomy-history?taxnode_id=201853936>), it consisted of two genera and three species. Since then, several previously undescribed nudiviruses have been identified and sequenced. We propose the creation of new taxa for these nudiviruses, including five new species in genus *Alphanudivirus* and two new genera (*Gammanudivirus* and *Deltanudivirus*) containing two and one new species, respectively. |

**Text of proposal**

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| |  | | --- | | **New species in genus *Alphanudivirus***  New viruses have been discovered in drosophilid flies and determined to be nudiviruses on the basis of electron microscopy, sequence similarity, and phylogenetic relationships with other nudiviruses. These new viruses include Drosophila innubila nudivirus (DiNV) from *Drosophila innubila* [2, 8]; and Esparto virus (EV), Kallithea virus (KV), Mauternbach virus (MV), and Tomelloso virus (TV) from *D. melanogaster* [6, 7, 9] (Table 1). Phylogenetic inference from two sets of concatenated amino acid sequence alignments of core genes (Table 2) places these viruses into the same monophyletic clade as Oryctes rhinoceros nudivirus (OrNV) and Gryllus bimaculatus nudivirus (GbNV) of the genus *Alphanudivirus* (Figure 1), indicating that the drosophilid nudiviruses should be classified in this genus.  Species demarcation criteria based on Kimura-2-parameter (K2P) pairwise nucleotide distances have been developed for species of the family *Baculoviridae*, thanks to the availability of sequence data from a large number of baculoviruses [5, 10]. This body of sequence data has provided a picture of the typical degree of divergence among isolates of the same virus and of different viruses, which in turn has allowed researchers to set thresholds for segregating viruses into species. Although a similarly extensive body of sequence data does not exist for nudiviruses, baculoviruses and nudiviruses share many phenotypic and genetic similarities: they are both large dsDNA viruses of arthropods with circular genomes and replication that takes place in the nucleus, and viruses of both families have 21 core genes in common (Table 2). It is thus reasonable to expect that baculovirus pairwise distance thresholds for species demarcation will approximate thresholds for classifying nudivirus isolates into different species.  To evaluate this possibility, K2P pairwise distances were calculated for two sets of nudivirus nucleotide sequences: (1) *pif-2*, which encodes a virion envelope protein, and (2) *dnapol*, which encodes DNA polymerase B (Figure 2). These two data sets both include sequences from different isolates of some of the same viruses, thus allowing an examination of divergence among isolates of the same species as well as the divergence occurring between viruses of different nudivirus species.  Pairwise distances between *pif-2* sequences of isolates of the same virus ranged in 0.0–0.012 substitutions/nt site (Figure 2A, yellow-highlighted cells), while the distances for *dnapol* sequences of isolates of the same nudiviruses ranged in 0.001–0.018 substitutions/nt site (Figure 2B, yellow-highlighted cells). In comparison, baculovirus thresholds based on alignments of three gene sequences were selected to be <0.015 substitutions/nt site for isolates belonging to the same species [5], while a later analysis using concatenated alignments of all 38 baculovirus core genes calculated thresholds at <0.021 substitutions/nt site for isolates of the same baculovirus species [10]. Pairwise distances among *pif-2* sequences of OrNV-Ma07, GbNV, and HzNV-2, which are exemplars of different nudivirus species, range in 0.872–1.472 substitutions/nt site, while the distances among *dnapol* sequences of these three viruses range in 1.08–1.903 substitutions/nt site. In comparison, the thresholds for segregating baculoviruses into different species were set at >0.05 substitutions/nt site based on distances at three loci [5] and later at >0.072 substitutions/nt site based on distances among all the core genes [10]. Thus, K2P pairwise distances of nudivirus isolates from the same species and from different species appear to conform to the species demarcation thresholds determined for baculoviruses.  Pairwise distances between each drosophilid nudivirus and other nudiviruses ranged from 0.141 to 1.539 substitutions/nt site for *pif-2* comparisons (Figure 2A), and from 0.401 to 1.874 substitutions/nt site for *dnapol* comparisons (Figure 2B), and thus exceeded the thresholds set for baculovirus species demarcation. These results support the creation of a new alphanudivirus species for each of the drosophilid nudivirus isolates in Table 1.  **New genera in *Nudiviridae***  New nudiviruses have also been identified that occur outside the *Alphanudivirus* and *Betanudivirus* clades in core gene trees (Figure 1). These viruses include Tipula oleracea nudivirus (ToNV) from the marsh crane fly, *Tipula oleracea* (insect order Diptera) [1]; and two crustacean nudiviruses, Penaeus monodon nudivirus (PmNV) from the Asian tiger shrimp *Penaeus monodon* [11], and Homarus gammarus nudivirus (HgNV) from the European lobster, *Homarus gammarus* [3]. Sequence similarity, virion morphology, open reading frame (ORF) homolog content, and phylogenetic relationships of these viruses confirm that they should be classified in family *Nudiviridae*. Researchers working with these viruses have also suggested that new genera should be created for them [1, 3, 11]. Towards this end, we propose to create new species for these three viruses, and to classify them into two new genera (Table 1): *Gammanudivirus*, with type species *Penaeus monodon nudivirus* (for the first crustacean nudivirus to be described) and *Deltanudivirus*, with type species *Tipula oleracea nudivirus*. Differences between PmNV and HgNV in their respective hosts of origin and K2P pairwise nucleotide differences (Figure 3) indicate that HgNV should be classified into a separate species, *Homarus gammarus nudivirus* in the genus *Gammanudivirus*. Names of the new genera follow the convention established for the family, which involves the use of Greek alphabet-derived prefixes to denote different genera.  The nudivirus concatenated core gene phylogenies in Figure 1 place the viruses HgNV and PmNV into a monophyletic clade at a distal node and support the creation of genus *Gammanudivirus* for nudiviruses infecting arthropods of subphylum Crustacea. The branch lengths separating ToNV from other nudiviruses support the creation of a separate genus, *Deltanudivirus*, to accommodate this virus. While both ToNV and the drosophilid alphanudiviruses infect fly hosts of order Diptera, drosophilid flies are classified under suborder Brachycera (fruit flies, house flies, blow flies, robber flies), while crane flies such as *T. oleracea* are classified under the suborder Nematocera (mosquitoes, midges, crane flies, gnats).  To generate additional support for the creation of new genera, amino acid pairwise distances between nudiviruses in the same and different genera were calculated in MEGA X using the Jones-Taylor-Thornton (JTT) matrix-based model (Figure 3). The sequences used in this analysis are the translations of the ORFs for four nudivirus core genes, *fen-1*, *lef-8*, *lef-9*, and *pif-2*. The differences between intragenus and intergenus distances can be seen visually in the differences in the fill colors of cells from the red-yellow-green scale applied to Figure 3. In general, there was no overlap between the *intragenus* distances calculated for viruses classified in genus *Alphanudivirus* and new genus *Gammanudivirus*, and the *intergenus* differences between nudiviruses of different genera. Intergenus pairwise distances involving viruses of proposed genera *Gammanudivirus* and *Deltanudivirus* confirmed that these viruses should not be classified in the genera *Alphanudivirus* or *Betanudivirus*.  The LEF-9 pairwise distances of GbNV with other alphanudiviruses are higher in some instances than the distances between GbNV and viruses in the other genera. Although GbNV is classified as an alphanudivirus, its host range consists of field crickets from the hemimetabolous insect order Orthoptera, a more ancient lineage of insects than the holometabolous orders of the other hosts of *Alphanudivirus*. LEF-9 pairwise distances involving the GbNV sequence are consistent with GbNV being a more ancient anddivergent member of *Alphanudivirus*.  All nudiviruses possess a group of 32 core genes, and the degree of conservation of the order of these core genes along the genomes of different nudiviruses may also be a useful character for classification, to the extent that it reflects the degree of synteny among different genomes. Gene parity plots [4] comparing the order of nudivirus core genes were set up to evaluate core gene order in nudiviruses within the same genus (Figure 4) and between different genera (Figure 5). Blocks of core genes were conserved in terms of their relative positions in the genomes of viruses from the same genus (Figure 4), although genome sections where inversions had occurred were evident. The plot of core genes from OrNV and GbNV exhibited the least degree of conservation among alphanudiviruses, consistent with the relatively distant relationship of GbNV to the rest of the alphanudiviruses. The order of core genes in gammanudiviruses PmNV and HgNV was absolutely conserved. In contrast, there was little apparent conservation in the order of core gene ORFs when viruses from different genera were compared (Figure 5).  We conclude that the crustacean nudiviruses (PmNV and HgNV) and ToNV each requires classification into new genera (*Gammanudivirus* and *Deltanudivirus*, respectively) on the basis of phylogeny, host range, pairwise amino acid distances of selected genes, and core gene ORF synteny. | |

**Supporting evidence**

Table 1. Current and proposed genera and species of family *Nudiviridae*, with associated exemplar isolates. Proposed new taxa are highlighted in red.

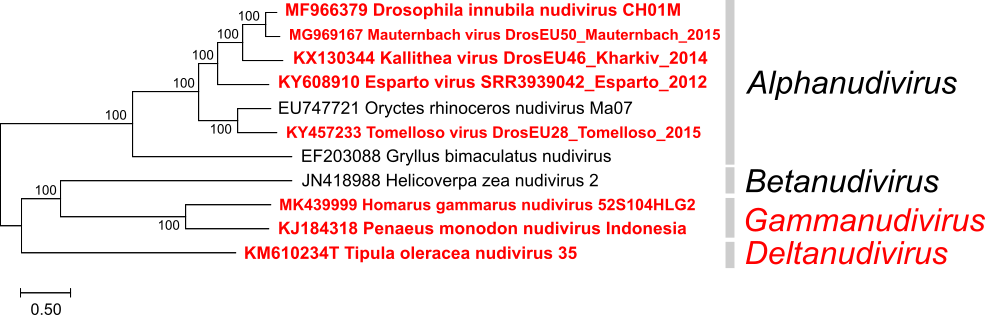
| **Genus** | **Species** | **Exemplar isolate** | **Abbreviation** |
| --- | --- | --- | --- |
| *Alphanudivirus* | ***Drosophila innubila nudivirus*** | Drosophila innubila nudivirus CH01M | DiNV-CH01M |
| ***Drosophila melanogaster nudivirus A*** | Kallithea virus DrosEU46\_Kharkiv\_2014 | KV- DrosEU46\_Kharkiv\_2014 |
| ***Drosophila melanogaster nudivirus B*** | Tomelloso virus DrosEU28\_Tomelloso\_2015 | TV- DrosEU28\_Tomelloso\_2015 |
| ***Drosophila melanogaster nudivirus C*** | Esparto virus SRR3939042\_Esparto\_2012 | EV- SRR3939042\_Esparto\_2012 |
| ***Drosophila melanogaster nudivirus D*** | Mauternbach virus DrosEU50\_Mauternbach\_2015 | MV- DrosEU50\_Mauternbach\_2015 |
| *Gryllus bimaculatus nudivirus* | Gryllus bimaculatus nudivirus | GbNV |
| *Oryctes rhinoceros nudivirus*a | Oryctes rhinoceros nudivirus Ma07 | OrNV-Ma07 |
| *Betanudivirus* | *Heliothis zea nudivirus*a | Helicoverpa zea nudivirus 2 | HzNV-2 |
| ***Gammanudivirus*** | ***Homarus gammarus nudivirus*** | Homarus gammarus nudivirus 52S104HLG2 | HgNV-52S104HLG2 |
| ***Penaeus monodon nudivirus*a** | Penaeus monodon nudivirus Indonesia | PmNV-Indonesia |
| ***Deltanudivirus*** | ***Tipula oleracea nudivirus*a** | Tipula oleracea nudivirus 35 | ToNV-35 |

aType species

Table 2. Core genes of nudiviruses

|  |  |  |
| --- | --- | --- |
| **Oryctes rhinoceros nudivirus (OrNV) ORF** | **Gene name** | **Inferred activity or function of encoded protein** |
| ORF1a | *dnapol* | DNA polymerase |
| ORF4a | *ac81* | Nucleocapsid envelopment |
| ORF15a | *vp39* | Major capsid protein |
| ORF16 | *fen-1* | FEN-1/FLAP endonuclease |
| ORF17a | *pif-2* | *Per os* infectivity factor |
| ORF18 | *GbNV\_gp67-like* | Unknown |
| ORF20a | *p47* | RNA polymerase subunit |
| ORF22a | *p6.9* | Nucleocapsid packaging/assembly |
| ORF30a | *vlf-1* | Very late gene expression factor |
| ORF33a | *pif-4* | *Per os* infectivity factor |
| ORF34a | *helicase* | DNA helicase |
| ORF41 | *11K-like* | Occlusion body component |
| ORF42a | *lef-4* | RNA polymerase subunit |
| ORF47 | *GbNV\_gp19-like* | Unknown |
| ORF52a | *lef-5* | Transcription initiation factor |
| ORF60a | *pif-1* | *Per os* infectivity factor |
| ORF61 | *GbNV\_gp51-like* | Unknown |
| ORF64a | *lef-8* | RNA polymerase subunit |
| ORF72a | *pif-6* | *Per os* infectivity factor |
| ORF75 | *integrase* | DNA processing |
| ORF76 | *GbNV\_gp58-like* | Unknown |
| ORF87a | *38k* | Nucleocapsid protein |
| ORF96a | *lef-9* | RNA polymerase subunit |
| ORF106a | *vp91/p95* | Nucleocapsid protein |
| ORF107a | *pif-3* | *Per os* infectivity factor |
| ORF108 | *helicase-2* | DNA helicase |
| ORF113a | *p33* | Sulfhydryl oxidase |
| ORF115a | *odv-e56/pif-5* | *Per os* infectivity factor |
| ORF117 | *tk2* | Thymidine kinase |
| ORF125 | *tk3* | Thymidine kinase |
| ORF126a | *p74/pif-0* | *Per os* infectivity factor |
| ORF137 | *tk1* | Thymidine kinase |

aAlso conserved among members of family *Baculoviridae*

1. **31 nudivirus core genes**
2. **20 nudivirus core genes also found in baculoviruses, with baculovirus outgroup**

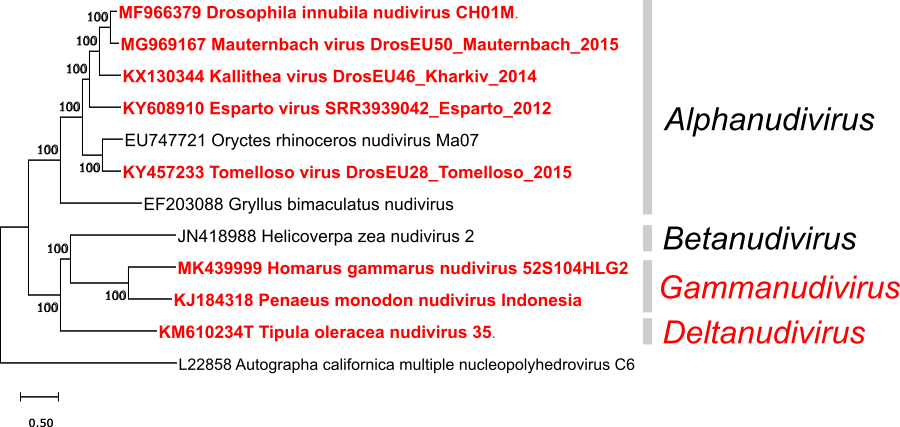
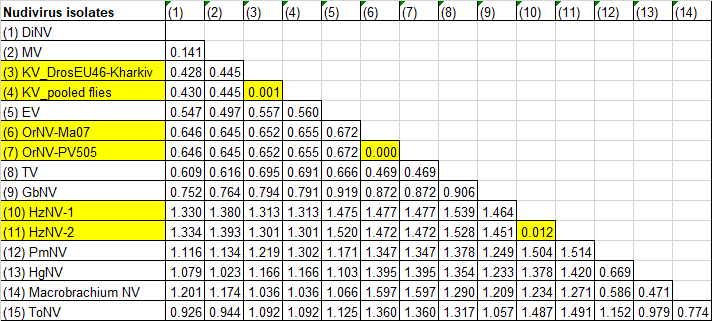


Figure 1. Phylogeny of family *Nudiviridae*. Amino acid sequences of nudivirus core genes (excluding *p6.9*; Table 2) were aligned individually by MUSCLE and concatenated prior to tree construction. (A) Midpoint-rooted tree showing the relationships among nudivirus isolates, which were inferred by maximum likelihood from the alignments of 31 core gene amino acid sequences with MEGA X using the Le\_Gascuel\_2008 (LG) substitution model and a discrete gamma distribution to account for evolutionary rate differences among sites. (B) Relationships among isolates were inferred by minimum evolution from the alignments of 20 core genes present in both nudiviruses and baculoviruses, using the baculovirus Autographa californica multiple nucleopolyhedrovirus as an outgroup. The tree was constructed with the Minimum Evolution method with distances computed using the JTT matrix-based method and modeled with a gamma distribution of rate variation among sites. In both trees, the percentage of 500 bootstrap replications in which the associated taxa clustered together is shown next to the branches. The names of proposed genera and isolates that are members of proposed species are in red type.

**A) *pif-2* distances**



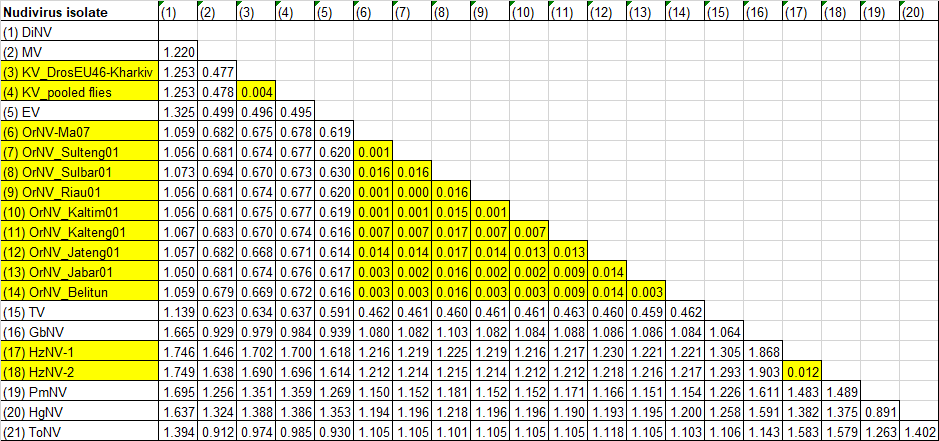
**B) *dnapol* distances**

Figure 2. Pairwise nucleotide distances (substitutions/site) between the sequences of nudivirus *pif-2* (A) and DNA polymerase (*dnapol*) (B) open reading frames. Distances were estimated with MEGA X using the Kimura-2-parameter model. Isolates of the same nudivirus species and the pairwise distances between their *pif-2* or *dnapol* sequences are highlighted in yellow, and are <0.021 substitutions/site, the threshold for classifying baculoviruses into the same species based on concatenated core gene alignments [10]. Pairwise distances between the sequences of all the other viruses analysed were >0.072, the threshold for assigning baculoviruses to different species based on concatenated core gene alignments [10]. Sequences used in this analysis derived either from complete genome sequences with GenBank accession numbers listed in Figure 1, or were derived from the following partial or complete virus sequences: KV\_pooled flies (KP714102, KP714105); Macrobrachium NV *pif-2* (JQ804993); OrNV-PV505 *pif-2* (AH015832); *dnapol* of OrNV isolates Belitun (MK241548), Jabar01 (MK241547), Jateng01 (MK241546), Kalteng01 (MK241545), Kaltim01 (MK241544), Riau01 (MK241543), Sulbar01 (MK241542), and Sulteng01 (MK241541); and HzNV-1 (AF451898).

| **A) LEF-9 (above diagonal line) and LEF-8 (below diagonal line) distances**    **B) PIF-2 (above diagonal line) and FEN-1 (below diagonal line) distances**    Figure 3. Pairwise distances of predicted amino acid sequences encoded by the nudivirus core genes *lef-8* and *lef-9* (A) and *pif-2* and *fen-1* (B). Amino acid sequences were aligned with MUSCLE, and pairwise distances were calculated using the James-Taylor-Thornton (JTT) matrix as implemented in MEGA X, with rate variation among sites modeled with a gamma distribution inferred for each data set. A red-yellow-green color scale was applied separately to each set of pairwise distances to visualize the differences among pairwise distances between viruses of the same genus and viruses of different genera. Names of virus isolates classified in *Alphanudivirus* are highlighted in light blue, while the two virus isolates classified in new genus *Gammanudivirus* are highlighted in light purple. |
| --- |



Figure 4. Gene parity plots comparing the core gene ORF order of viruses belonging to the same genus, including alphanudiviruses (OrNV x TV, OrNV x MV, and OrNV x GbNV) and gammanudiviruses (PmNV x HgNV). Each point in a plot represents one of the 32 nudivirus core gene ORFs, in the order in which they appear in the genomes being compared with the *dnapol* gene set as the first ORF. ORFs in the same order in the genomes being compared, or in a reverse order, form a straight diagonal line.



Figure 5. Gene parity plots comparing the core gene ORF order of viruses belonging to different genera. Plots shown compare the order of core genes of an alphanudivirus with a betanudivirus (OrNV x HzNV-2), a gammanudivirus (OrNV x PmNV), and deltanudivirus (OrNV x ToNV). Also shown are comparisons of a gammanudivirus with a betanudivirus (PmNV x HzNV-2) and a deltanudivirus (PmNV x ToNV), and a comparison between a deltanudivirus and a betanudivirus (ToNV x HzNV-2).

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