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:	***2016.003a,bD***	(to be completed by ICTV officers)

Short title: 6 new species in the subfamily *Densovirinae*
(e.g. 6 new species in the genus *Zetavirus*)

Modules attached (modules 1 and 11 are required)	**2** ☒ **3** ☐ **4** ☐ **5** ☐ **6** ☐ **7** ☐ **8** ☐ **9** ☐ **10** ☐

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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)	Parvoviridae Study Group

ICTV Study Group comments (if any) and response of the proposer:

Date first submitted to ICTV:	23th July 2016
Date of this revision (if different to above):	28th July 2016

ICTV-EC comments and response of the proposer:

Family *Parvoviridae* is divided into two subfamilies, the *Parvovirinae* and *Densovirinae*, distinguished by the ability of constituent viruses to infect vertebrate versus invertebrate hosts respectively and by phylogeny. The classification of this family has not been updated since it was substantially rationalized and extended in 2014. Here we propose extending subfamily *Densovirinae*, introducing five new species into genus *Ambidensovirus*, three containing viruses that infect insects, one to accommodate a virus that kills decapod crayfish, and one for a major pathogen of asteroid sea stars in the Pacific Northeast. In addition, we propose the creation of a 6th new species in the subfamily for a well-documented virus that has an ambisense transcription strategy, and would thus be expected to cluster with members of genus *Ambidensovirus*, but which has an exceptionally compact genome, with alignments that make it difficult to determine its exact linkage at this time. The proposal does not confine this species to any existing genus within the subfamily *Densovirinae*.

As previously, phylogenetic analyses described here are based on the amino acid sequence of the conserved viral replication initiator protein, NS1, which occupies almost half of the viral genome and contains a highly-structured HuH family site-specific nickase module linked to an SF3 AAA+ helicase domain. Taxonomic linkages were determined using a pipeline platform called ViCTree, developed by Sejal Modha, Joseph Hughes and Andrew Davison, which automatically selects candidate virus sequences from GenBank, generates alignments, calculates a maximum likelihood tree and integrates the sequences into an existing phylogenetic tree. This ensures that databases are scanned effectively for new candidate viruses and renders analysis of large numbers of new and duplicated genomes manageable, and so will become increasingly important for the future. However, since the current parvovirus taxonomy (2014) was based on alignments that included insights from structural biology and was subjected to protracted Bayesian inference, the resulting trees do show some disparate branching patterns and probability values.

To assess the suitability of new genomes for classification as parvoviruses, the study group used the following virus definition, which was developed for restructuring the taxonomy in 2014.
In order for an agent to be classified in the family *Parvoviridae*, it must be judged to be an authentic parvovirus on the basis of having been isolated and sequenced or, failing this, on the basis of having been sequenced in tissues, secretions, or excretions of unambiguous host origin, supported by evidence of its distribution in multiple individual hosts in a pattern that is compatible with dissemination by infection. The sequence must be in one piece, contain all the nonstructural (NS) and virus particle (VP) coding regions, and meet the size constraints and motif patterns typical of the family.
All species proposed for inclusion in the *Densovirinae* contain viruses that conform to this definition.

MODULE 2: **NEW SPECIES**

Code	***2016.003aD***	(assigned by ICTV officers)

To create 5 new species within:

Genus:	***Ambidensovirus***
Subfamily:	***Densovirinae***
Family:	***Parvoviridae***
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.

Name of new species:	**Representative isolate: (only 1 per species please)**	**GenBank sequence accession number(s)**
Asteroid ambidensovirus 1	sea star-associated densovirus	KM052275
Decapod ambidensovirus 1	Cherax quadricarinatus densovirus	KP410261
Hemipteran ambidensovirus 2	Dysaphis plantaginea densovirus 1	FJ040397
Hemipteran ambidensovirus 3	Myzus persicae densovirus 1	AY148187
Hymenopteran ambidensovirus 1	Solenopsis invicta densovirus	KC991097

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

Proposed new species and viruses	Accession #	Acronym	Reference
Asteroid ambidensovirus 1			
sea star-associated densovirus	KM052275	SSaDV1	1, 2
Decapod ambidensovirus 1			
Cherax quadricarinatus densovirus	KP410261	CqDV1	3
Hemipteran ambidensovirus 2			
Dysaphis plantaginea densovirus 1	FJ040397	DplDV 1	4
Dysaphis plantaginea densovirus 2	EU851411	DplDV2	4
Hemipteran ambidensovirus 3			
Myzus persicae densovirus 1	AY148187	MpDV1	5
Myzus persicae nicotianae densovirus	KT239104	MpnDV	6
Hymenopteran ambidensovirus 1			
Solenopsis invicta densovirus 1	KC991097	SiDV1	7

Viruses in proposed species are monophyletic with others in genus *Ambidensovirus* (see tree in module 11), and share a wide range of characteristics including a complex rearrangement of the typically monosense parvovirus genome that allows these viruses to co-ordinate bidirectional transcription. Viral genomes are also distinguished by the presence of three distinct blocks of open reading frame encoding their non-structural proteins, and are flanked by inverted terminal repeat sequences that fold into hairpin termini with a characteristic structure. Phospholipase A2 domains are encoded in the structural proteins of all members of the genus.

All viruses meet the demarcation criteria for new species (less than 85% identity to viruses in other current or proposed species) in the genus *Ambidensovirus* (as discussed in module 11). Phylogenetic trees (module 11) of this genus show deep branches, with new taxa proposed in two of these sectors. In one branch, NS1 proteins encoded by the new viruses Cherax quadricarinatus densovirus and sea star-associated densovirus show 33% identity to each other, and share 26% identity with those of Dysaphis plantaginea densoviruses and Myzus persicae densovirus (which are 29% identical to each other). These proteins are also 26% identical to those of Periplaneta fulginosa densovirus, a founder member of the genus, and at least 18% identical to those of all other members, placing them within the established range for genus identity in this subfamily. The final new virus, Solenopsis invicta densovirus, occupies a different branch of this taxon, encoding an NS1 protein that shares around 40% and 35% identity with those of founder members Acheta domestica densovirus and Plannococcus citri densovirus respectively, and at least >18% identity with NS1 proteins from all other genus members.

Currently, all viruses in this genus infect insect hosts, but both Cherax quadricarinatus densovirus and Sea star-associated densovirus have been purified and shown to infect, kill and multiply in healthy members of their respective decapod and asteroid hosts. These viruses are of economic and ecological importance respectively, because they have been associated with the mass death of their host population in aquaculture or in nature.

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	***2016.003bD***	(assigned by ICTV officers)

To create 1 new species within:

Genus:	**unassigned**
Subfamily:	***Densovirinae***
Family:	***Parvoviridae***
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.

Name of new species:	**Representative isolate: (only 1 per species please)**	**GenBank sequence accession number(s)**
Orthopteran densovirus 1	Acheta domestica mini ambidensovirus	KF275669

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 11

The nonstructural and structural proteins of Acheta domestica mini ambidensovirus (AdMADV) are encoded in the 5' halves of complementary DNA strands and are transcribed in opposite directions from inverted but otherwise identical promoters. To date, this gene organization is only found in viruses from genus *Ambidensovirus* (reference 8). However, the genome of AdMADV is 4,945 nucleotides in length, rather than the typical ~6,000 nucleotides, and its genomic termini are much smaller than expected (199 rather than ~545 nucleotides), although they do take the form of inverted terminal repeats with predicted structures that broadly resemble the telomeres of such viruses. Size discrepancies are of concern because viruses from genus *Iteradensovirus* typically do have genomes of ~5,000 nucleotides with inverted terminal repeats of a similar size (~ 250 nucleotides), although they lack the profound genetic rearrangements associated with ambisense transcription.

In the current analyses, AdMADV does not appear reliably monophyletic with viruses in either the *Ambidensovirus* or *Iteradensovirus* genera, and its nucleotide and protein identities fail to anchor it securely to a single genus. For example, its NS1 protein exhibits a range of pairwise identities of less than 20% with proteins encoded by all viruses in both genera. Further caution is urged by a recent GenBank submission, Diaphorina citri densovirus (KX165268), which does appear consistently monophyletic with AdMADV although the NS1 proteins show little more than 20% identity. Currently the Diaphorina virus is not being considered for classification because supporting biological or epidemiological data are lacking.

In light of these uncertainties, it is proposed that at present the well-characterized AdMADV virus should be included as part of a new species in subfamily *Densovirinae*, called

Orthopteran densovirus 1, without this taxon being confined to a specific genus.

Although relatively few viruses are currently classified in subfamily *Densovirinae*, they can be remarkably disparate from each other, showing forms of genomic organization that have never been encountered in subfamily *Parvovirinae*. Thus, although many more virus species have been identified in the vertebrate subfamily, they conform to a limited number of structural forms and the taxonomic organization of the subfamily is more readily apparent. It is hoped that future use of the direct GenBank pipeline collection system employed here may allow us to access more metagenomic data, and thus assemble a much broader array of candidate densovirus genomes than we might otherwise encounter. This would be biologically exciting, but might also allow us to expand and organize the taxonomy of this subfamily more securely.

MODULE 11: **APPENDIX**: supporting material

additional material in support of this proposal

References:

1. Hewson I, Button JB, Gudenkauf BM, Miner B, Newton AL, Gaydos JK, Wynne J, Groves CL, Hendler G, Murray M, Fradkin S, Breitbart M, Fahsbender E, Lafferty KD, Kilpatrick AM, Miner CM, Raimondi P, Lahner L, Friedman CS, Daniels S, Haulena M, Marliave J, Burge C, Eisenlord ME, Harvell CD. 2014. Densovirus associated with sea-star wasting disease and mass mortality. Proc. Natl. Acad. Sci. U.S.A. 111, 17278-17283.

2. Fuess LE, Eisenlord ME, Closek CJ, Tracy AM, Mauntz R, Gignoux-Wolfsohn S, Moritsch MM, Yoshioka R, Burge CA, Harvell CD, Friedman CS, Hewson I, Hershberger PK, Roberts SB. 2015. Up in arms: immune and nervous system response to sea star wasting disease. PLOS One 10, e0133053.

3. Bochow S, Condon K, Elliman J, Owens L. 2015. First complete genome of an *Ambidensovirus*; Cherax quadricarinatus densovirus, from freshwater crayfish *Cherax quadricarinatus*. Marine Genomics 24, 305-312.

4. Ryabov EV, Keane G, Naish N, Evered C, Winstanley D. 2009. Densovirus induces winged morphs in asexual clones of the rosy apple aphid, *Dysaphis plantaginea*. Proc. Natl. Acad. Sci. U.S.A. 106, 8465-8470.

5. van Munster M, Dullemans AM, Verbeek M, van den Heuvel JF, Reinbold C, Brault V, Clerivet A, van der Wilk F. 2003. A new virus infecting *Myzus persicae* has a genome organization similar to the species of the genus *Densovirus*. J. Gen. Virol. 84, 165-172.

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7. Valles SM, Shoemaker D, Wurm Y. Strong CA, Varone L, Becnel JJ, Shirk PD. 2013. Discovery and molecular characterization of an ambisense densovirus from South American populations of *Solenopsis invicta*. Biol. Control 67, 431-439.

8. Pham HT, Yu Q, Bergoin M, Tijssen. P. 2013. A novel ambisense densovirus, acheta domesticus mini ambidensovirus, from crickets. Genome Announc. 1, e00914-13.

9. Sievers F, Wilm A, Dineen DG, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Söding J, Thompson JD, Higgins DG. 2011. Fast, scalable generation of high-quality protein muliple sequence alignments using Clustal Omega. Mol. Syst. Biol. 7, 539.

10. Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30, 1312-1313.

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.

Taxon demarcation criteria

Demarcation criteria used in a previous taxonomic proposal for family *Parvoviridae* (2014) are retained here. The two subfamilies, *Parvovirinae* and *Densovirinae*, are separated by their respective vertebrate versus invertebrate host ranges, which is supported by sequence-based phylogeny. For both, the amino acid sequences of NS1 proteins from all viruses within a species are required to be at least 85% identical, while diverging by more than 15% from those of viruses in other species. In the subfamily *Parvovirinae*, viruses in the same genus are required to encode NS1 proteins that are usually greater than 30% identical to each other, but less than 30% identical to those of viruses from other genera. However, in subfamily *Densovirinae*, attributable sequences are generally only available for a small number of economically significant viruses, which likely fail to reflect the diverse nature of viruses that infect this immense group of potential host animals. Accordingly, the greater than 30% identity requirement within genera is applied less rigorously within this subfamily, in order to allow clustering of monophyletic viruses with conspicuously similar characteristics from host orders separated by large evolutionary distances.

Phylogenetic tree

Phylogenetic analyses presented here are based on the amino acid sequence of the NS1 protein, which carries conserved domains encoding both a site-specific HuH nickase and an SF3 AAA+ helicase. Potential viral sequences were assembled by implementing a new pipeline approach, called ViCTree, which searched GenBank for all available protein sequences reported to encode NS1 proteins from members of the *Densovirinae*. These were assembled and compared to 22 NS1 seed sequences from known members of the subfamily. Blast hits with a length of >100 and a coverage of 80 were collected, and sequences with 100% identity were clustered into 89 groups. These 89 sequences were aligned using ClustalO (ref 9), and a maximum likelihood tree reconstructed in RAxML (ref 10) using the JTT+Gamma protein model. Branch support was provided by bootstrapping 100 replicates. Possible new viral sequences that failed to meet the parvovirus definition in use (see module 1), together with many viruses that do not require the creation of new taxa, were removed from the tree to enhance clarity.

Figure Legend

The five recognized genera in subfamily *Densovirinae* are shown as colored blocks. A limited number of viruses from genera that are not affected by this proposal are shown to provide phylogenetic structure. Trees are midpoint rooted and branches labeled with the accession numbers and names of constituent viruses, with bold type indicating viruses that comprise proposed new species. Species names are indicated at the right of each branch as follows:
Genus *Ambidensovirus*: LA1: *Lepidopteran ambidensovirus 1*; DA1: *Diptera ambidensovirus 1*; HyA1: *Hymenopteran ambidensovirus 1*; OA1: *Orthopteran ambidensovirus 1*; HA1: *Hemipteran ambidensovirus 1*; HA2: *Hemipteran ambidensovirus 2*; HA3: *Hemipteran ambidensovirus 3*; DeA1: *Decapod ambidensovirus 1*; AA1: *Asteroid ambidensovirus 1*; BA1: *Blattodean ambidensovirus 1*; BA2: *Blattodean ambidensovirus 2*.
Unspecified genus:- OD1: *Orthopteran densovirus 1*.
Genus *Iterodensovirus*:- LI2: *Lepidopteran iteradensovirus 2*; LI3: *Lepidopteran iteradensovirus 3*; LI4: *Lepidopteran iteradensovirus 4*; LI5: *Lepidopteran iteradensovirus 5*.
Genus *Hepandensovirus*:- DeH1: *Decapod hepandensovirus 1*.
Genus *Brevidensovirus*:- DB1: *Dipteran brevidensovirus 1*; DB2: *Dipteran brevidensovirus 2*.
Genus *Penstyldensovirus*:- DeP1: *Decapod penstyldensovirus 1*.

Phylogenetic tree showing major branches and proposed new species in subfamily *Densovirinae*