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: *2009.001a-fF* (to be completed by ICTV officers)

Short title: Create the unassigned genus Dinodnavirus
(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:

Keizo Nagasaki (nagasaki@affrc.go.jp), Yuji Tomaru (tomaruy@affrc.go.jp)

Has this proposal has been seen and agreed by the relevant study group(s)? Please select answer in the box on the right	**Yes**

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

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

MODULE 2: **NEW SPECIES**

Part (a) to create and name one or more new species.

If more than one, they should be a group of related species belonging to the same genus (see Part b)

Code	***2009.001aF***	(assigned by ICTV officers)

To create 1 new species with the name(s):

Heterocapsa circularisquama DNA virus 01

Part (b) assigning new species to higher taxa

All new species must be assigned to a higher taxon. This is usually a genus although it is also permissible for species to be "unassigned" within a subfamily or family.

Code	***2009.001bF***	(assigned by ICTV officers)

To assign the species listed in section 2(a) as follows:

Genus:	***Dinodnavirus* (new)**
Subfamily:	***Unassigned***
Family:	***Unassigned***
Order:	***Unassigned***

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.

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.
- Provide Genbank accession numbers (not RefSeq accessions) for genomic sequences
- Further material in support of this proposal may be presented in the Appendix, Module 9

HcDNAV01 (*Heterocapsa circularisquama DNA virus 01*) is the only so-far-isolated nucleocytoplasmic large DNA virus infecting a bloom-forming dinoflagellate *Heterocapsa circularisquama*. In "Virus Taxonomy; 8th report of ICTV", this virus was shown as a tentative species "*Heterocapsa circularisquama* virus", which was unassigned in the family *Phycodnaviridae*. Since then, information on this dsDNA virus was intensively accumulated. Thus, at the present stage of study, it is considered rational to propose a new dinoflagellate-infecting dsDNA virus species "*Heterocapsa circularisquama DNA virus 01*"; further, a new genus "Dinodnavirus" where HcDNAV is included is also to be proposed. Just recently, HcDNAV's DNA polymerase gene was identified and sequenced. In spite of the host range (infecting marine alga "dinoflagellate") and morphological similarity to the other algal dsDNA viruses (the family *Phycodnaviridae*: ~0.2 µm icosahedral virion), phylogenetic analysis based on the deduced amino acid sequence of DNA polymerase, this virus was shown to be distantly related to phycodnaviruses ever reported. Instead, monophyly of *HcDNAV01* and "African swine fever virus (ASFV)" was supported with a bootstrap value of 98 %. This result suggests *HcDNAV01* is likely a member of the family *Asfaviridae*; however, considering its host range and morphology, it may be too rough-and-ready to determine the family to which *HcDNAV01* belongs (data not shown). The DNA polymerase gene sequence was registered with GenBank accession number AB522601.

MODULE 3: **NEW GENUS**

creating and naming a new genus

Code	***2009.001cF***	(assigned by ICTV officers)

To create a new genus to contain the species listed below

Heterocapsa circularisquama DNA virus 01

Code	***2009.001dF***	(assigned by ICTV officers)

To name the new genus: *Dinodnavirus*

assigning a new genus to higher taxa

Code	***2009.001eF***	(assigned by ICTV officers)

To assign the new genus as follows: Ideally, a genus should be placed within a higher taxon, but if not, write "**unassigned**" in the box below.

Subfamily:	***Unassigned***
Family:	***Unassigned***
Order:	***Unassigned***

If any of these taxa has yet to be created (in module 4, 5 or 6) please write "**(new)**" after its proposed name.

assigning type species and other species to a new genus

Code	***2009.001fF***	(assigned by ICTV officers)

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

Heterocapsa circularisquama DNA virus 01

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

Code		(assigned by ICTV officers)

To assign the following as additional species of the new genus:

Reasons to justify the creation of a new genus:

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

HcDNAV01 is the only so-far-isolated nucleocytoplasmic large DNA virus infecting a bloom-forming dinoflagellate *Heterocapsa circularisquama*. In "Virus Taxonomy; 8th report of ICTV", this virus was shown as tentative species "*Heterocapsa circularisquama* virus", which was unassigned in the family *Phycodnaviridae*. Since then, information on this dsDNA virus was intensively accumulated. Thus, at the present stage of study, it is considered rational to propose a new dinoflagellate-infecting dsDNA virus species "*Heterocapsa circularisquama DNA virus 01*"; further, a new genus "*Dinodnavirus*" where HcDNAV is included is also to be proposed. The type species of the new genus "*Dinodnavirus*" is "*Heterocapsa circularisquama DNA virus 01*". Just recently, its type B DNA polymerase gene was identified and sequenced. In spite of the host range (infecting dinoflagellates [commonly regarded as microalgae]) and morphological similarity to the other algal dsDNA viruses (the family *Phycodnaviridae*: ~0.2 µm icosahedral virion), phylogenetic analysis based on the deduced amino acid sequence of DNA polymerase revealed that *HcDNAV01* was distantly related to the phycodnaviruses ever reported; instead, monophyly of *HcDNAV01* and

African swine fever virus (ASFV) was supported with a bootstrap value of 98 %. This result suggests it is likely a member of the family *Asfaviridae*; however, considering the host range and morphology, it may be too rough-and-ready to determine the family to which the new genus “*Dinodnavirus*” belongs (data not shown). The DNA polymerase gene sequence of *HcDNAV01* was registered with GenBank accession number AB522601 (GenBank: AB522601.1).

Origin of the new genus name:

The host is *dino*flagellate: the genome is *DNA*. --> Dinodnavirus

Reasons to justify the choice of type species:

At present, *HcDNAV01* is the only member belonging to the genus *Dinodnavirus*.

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.
- Provide Genbank accession numbers (not RefSeq accessions) for genomic sequences of new species

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

References:

1. Ogata, H., Toyoda, K., Tomaru, Y., Nakayama, N., Shirai, Y., Claverie, J. -M., Nagasaki, K. (2009) Remarkable sequence similarity between the dinoflagellate-infecting marine girus and the terrestrial pathogen African swine fever virus. *Virol. J.* **6:** 178.
2. Nagasaki, K. (2008) Dinoflagellates, diatoms and their viruses. J. Microbiol. 46(3): 235-243.
3. Nagasaki, K., Tomaru, Y., Shirai, Y., Takao, Y., Mizumoto, H. (2006) Dinoflagellate-infecting viruses. J. Mar. Biol. Ass. U.K., 86: 469-474.
4. Wilson, W. H., Van Etten, J. L., Schroeder, D. S., Nagasaki, K., Brussaard, C., Delaroque, N., Bratbak, G., Suttle, C. (2005) Phycodnaviridae. In: Virus Taxonomy, VIIIth Report of the ICTV (eds. C. M. Fauquet, M. A. Mayo, J. Maniloff, U. Desselberger, L. A. Ball), Elsevier Academic Press, Chian, pp. 163-175.
5. Nagasaki, K., Shirai, Y., Tomaru, Y., Nishida, K., Pietrokovski, S. (2005) Algal viruses with distinct intraspecies host specificities include identical intein elements. Appl. Environ. Microbiol., 71(7): 3599-3607.
6. Tomaru, Y., Tanabe, H., Yamanaka, S., Nagasaki, K. (2005) Effects of temperature and light on stability of microalgal viruses, HaV, HcV and HcRNAV. Plankton Biol. Ecol., 52(1): 1-6.
7. Tomaru, Y., Nagasaki, K. (2004) Widespread occurrence of viruses lytic to the bivalve-killing dinoflagellate Heterocapsa circularisquama in the western coast of Japan. Plankton Biol. Ecol., 51(1): 1-6.
8. Nagasaki, K., Tomaru, Y., Tarutani, K., Katanozaka, N., Yamanaka, S., Tanabe, H., Yamaguchi, M. (2003) Growth characteristics and intra-species host specificity of a large virus infecting the dinoflagellate, Heterocapsa circularisquama. Appl. Environ. Microbiol., 69(5): 2580-2586.
9. Tarutani, K., Nagasaki, K., Itakura, S., Yamaguchi, M. (2001) Isolation of a virus infecting the novel shellfish-killing dinoflagellate Heterocapsa circularisquama. Aquat. Microb. Ecol., 23(2): 103-111.

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.

HcDNAV01 is an icosahedral virus 180 - 210 nm in diameter (Fig. 1: Tarutani et al., 2001) with a double-stranded DNA (dsDNA) genome ca. 356 kbp in length (Nagasaki et al., 2005); which was previously described as HcV (*Heterocapsa circularisquama* virus) in “the 8th Report of the International Committee on Taxonomy of Viruses” (Wilson et al., 2005). This virus is propagated within the cytoplasm of the host cell (Fig. 2) where the virion is associated with the viroplasmic regions. These features suggests the similarity of *HcDNAV01* to the other nucleocytoplasmic large DNA viruses infecting eukaryotic algae; thus, in “Virus Taxonomy: the 8th Report of the International Committee on Taxonomy of Viruses”, HcDNAV (=HcV) was introduced as a tentative member in the family *Phycodnaviridae* (Wilson et al., 2005). However, a recent phylogenetic analysis on its DNA polymerase amino acid sequence suggested its considerable similarity to African swine fever virus (ASFV) (Fig. 3); furthermore, the highly conserved motif I of the polymerase domain of DNA polymerase (generally YGDTDS) which includes a catalyticsite residue is “YSDTDS” in HcDNAV (Ogata et al., 2009). Thus, at the present stage of study, establishment of a new genus “*Dinodnavirus*” for *HcDNAV01* is most likely reasonable; still, determination of the family where the genus *Dinodnavirus* is included should require some more intensive analysis of *HcDNAV01* genome.

HcDNAV01 infects *H. circularisquama* in a wide range of temperatures (15-30 °C). The latent period and the burst size were respectively estimated at 40-56 h and 1,800-2,440 infectious units cell^{-1} using one-step growth experiments (Nagasaki et al., 2003).

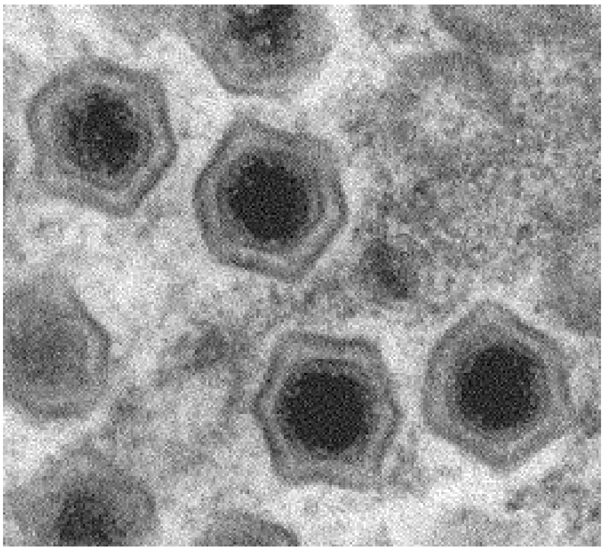

Fig. 1. Thin-section of *HcDNAV01* particles. The diameter is ca. 0.2 μm.

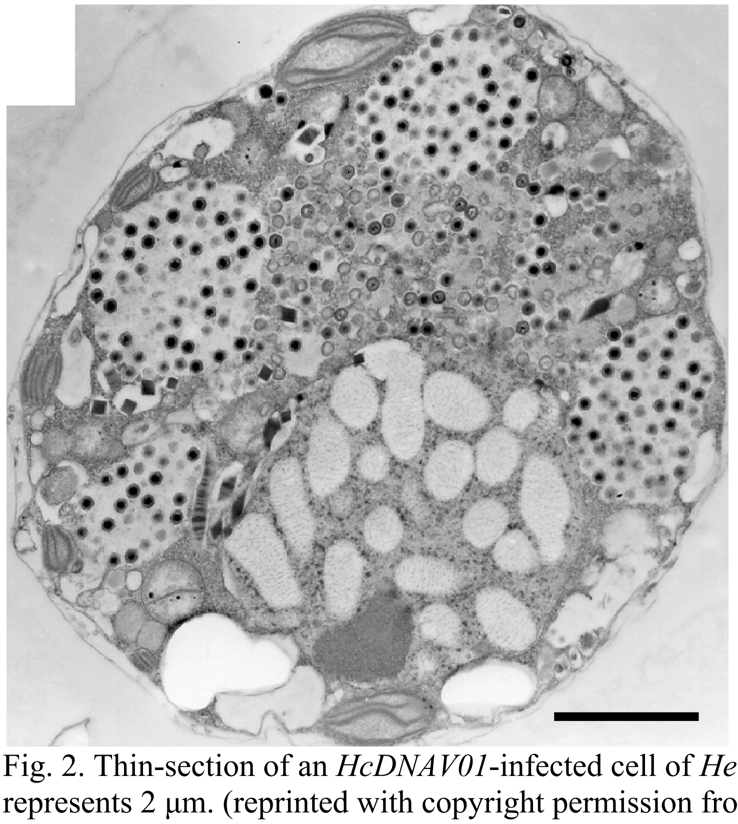

Fig. 2. Thin-section of an *HcDNAV01*-infected cell of *Heterocapsa circularisquama*. The bar represents 2 μm. (reprinted with copyright permission from the American Society for Microbiology: Nagasaki, K. et al. (2003) *Appl. Environ. Microbiol.*, **69(5):** 2580-2586. [published in March 2003])

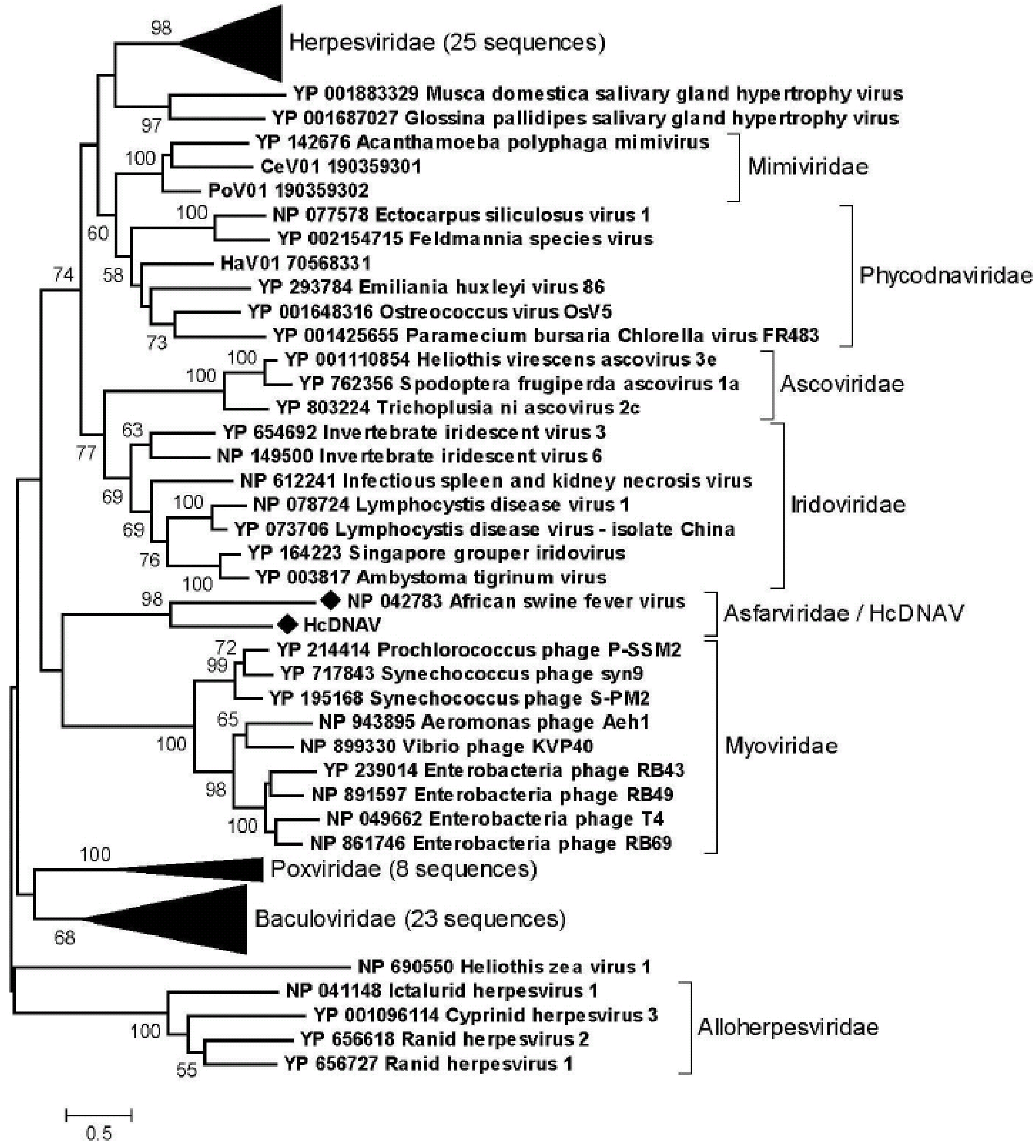

Fig. 3. Maximum likelihood tree of PolB amino acid sequences from diverse groups of viruses. The sequences of species *Heterocapsa circularisquama DNA virus 01* (HcDNAV) and African swine fever virus (ASFV) sequences are indicated by filled diamond marks. (reprinted with copyright permission from BioMed Central Ltd: Ogata, H. et al. (2009) *Virol. J.*, **6:** 178.) [published in October 2009])