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:** | *2009.003a,bP* | (to be completed by ICTV officers) |

**Short title:** 1 new species in the genus Carlavirus
(e.g. 6 new species in the genus *Zetavirus*)

| | | | | | |
|---|---|---|---|---|---|
| **Modules attached**<br>(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:**

Mike Adams on behalf of the Flexiviridae Study Group mike.adams@bbsrc.ac.uk

| | |
|---|---|
| Has this proposal has been seen and agreed by the relevant study group(s)?<br>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: May 2009
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 | ***2009.003aP*** | (assigned by ICTV officers) |

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

*Helleborus net necrosis virus*

---

**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 | ***2009.003bP*** | (assigned by ICTV officers) |

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

| | |
|---|---|
| Genus: | ***Carlavirus*** |
| Subfamily: | |
| Family: | ***Betaflexiviridae* (awaiting ratification)** |
| Order: | ***Tymovirales* (awaiting ratification)** |

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

Throughout the family, species are recognized because of their specific natural host range, serological and molecular differences; distinct species have less than *ca*. 72% identical nt or 80% identical aa between their CP or polymerase genes (Adams et al., 2004; Martelli et al., 2007). Assignment to genera depends on genome organization, particle ultrastructure and biological characteristics including the mode of transmission.

The following details (from Eastwell et al., 2009) show that Helleborus net necrosis virus (HeNNV) clearly satisfies the criteria to be recognized as an independent species in the genus *Carlavirus*.

HeNNV was consistently associated with 'black death' disease of *Helleborus* spp., in the USA. The virus has filamentous particles approx 800 x 17 nm. Amplification with degenerate primers for carlaviruses yielded a product of approximately 3,000 bp from diseased plants and, building on this, the full genome sequences of two isolates were determined (FJ196835-6). The genome was predicted to contain 6ORFs typical of a member of the genus *Carlavirus*. The two isolates had 93% identical amino acids in their replication protein showing that they belonged to the same species. Analyses of the replication protein and the coat protein show that it is not closely related to any of the existing species. The closest sequences in the databases were those for Poplar mosaic virus (47% aa identity) in the replication protein and

Shallot latent virus (55-56% aa identity) in the coat protein. Phylogenetic analyses of these two genes (Appendix Figs 1 and 2) confirm the placement of HeNNV within the genus.

## MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

### References:

Adams MJ, Antoniw JF, Bar-Joseph M, Brunt AA, Candresse T, Foster GD, Martelli GP, Milne RG, Zavriev SK, Fauquet CM (2004) The new plant virus family Flexiviridae and assessment of molecular criteria for species demarcation. Arch. Virol. 149:1045-1060.

Eastwell KC, du Toit LJ, Druffel KL. (2009). Helleborus net necrosis virus: a new *Carlavirus* associated with 'black death' of *Helleborus* spp. Plant Dis. 93: 332-338.

Martelli G, Adams MJ, Kreuze JF, Dolja VV (2007) Family Flexiviridae: a case study in virion and genome plasticity. Ann. Rev. Phytopathol. 45:73-100

### 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.

Fig. 1 Phylogenetic tree of the replication protein of the fully sequenced members of the genus *Carlavirus* with Apple stem pitting virus (genus *Foveavirus*) as an outgroup. The tree was generated from a codon-aligned nucleotide sequence using MEGA4 (Neighbor-Joining, Maximum Composite Likelihood method with 10,000 bootstrap replicates. Bootstrap values shown when >60%. The position of the two isolates of Helleborus net necrosis virus (HeNNV) within the genus is highlighted.

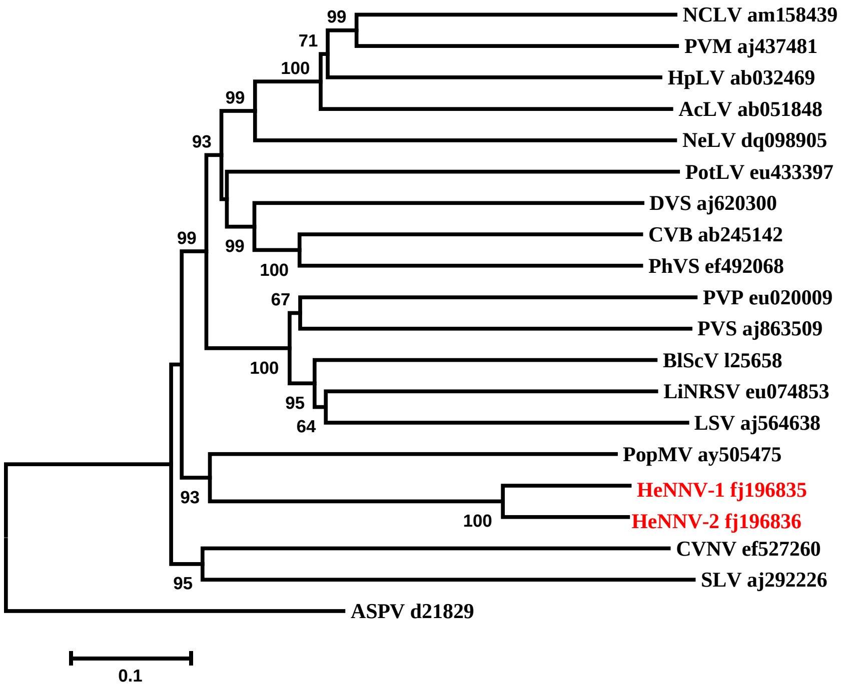

Fig. 2 Phylogenetic tree of the coat protein of the members of the genus *Carlavirus* with Apple stem pitting virus (genus *Foveavirus*) as an outgroup. The tree was generated from a codon-aligned nucleotide sequence using MEGA4 (Neighbor-Joining, Maximum Composite Likelihood method with 10,000 bootstrap replicates. Bootstrap values shown when >60%. The position of the two isolates of Helleborus net necrosis virus (HeNNV) within the genus is highlighted.