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:** | ***2016.002aP*** | (to be completed by ICTV officers) |

**Short title:** Create one new species *Alfalfa enamovirus 1* in the genus *Enamovirus*, family *Luteoviridae*
(e.g. 6 new species in the genus *Zetavirus*)

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

**Author(s):**

N. Bejerman and S. Lenardon

**Corresponding author with e-mail address:**

Nicolas Bejerman; nicobejerman@gmail.com

**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) | Luteoviridae Study Group |

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

Date first submitted to ICTV: April 2016
Date of this revision (if different to above):

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

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 | ***2016.002aP*** | (assigned by ICTV officers) |
|---|---|---|

**To create 1 new species within:**

| | |
|---|---|
| Genus: | ***Enamovirus*** |
| Subfamily: | |
| Family: | ***Luteoviridae*** |
| 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)** |
|---|---|---|
| *Alfalfa enamovirus 1* | alfalfa enamovirus 1 (AEV-1) | KU297983 |
| | | |

**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 10

This proposal suggests that *Alfalfa enamovirus 1* should be considered a distinct species in the genus *Enamovirus*, family *Luteoviridae*.

Deep sequencing of small RNAs of alfalfa plants collected in the central region of Argentina showing dwarfism symptoms revealed the presence of a virus named alfalfa enamovirus 1 (AEV-1)[1] related to pea enation mosaic virus 1 (PEMV-1), the type member of the genus *Enamovirus*, family *Luteoviridae* [2].

Species demarcation criteria are not currently listed for enamoviruses; however we could apply the criteria used to demarcate species of the luteo and polerovirus genus [2] to demarcate species in the enamovirus genus which include:

- Differences in breadth and specificity of host range
- Differences in serological specificity with discriminatory polyclonal or monoclonal antibodies
- Differences in amino acid sequence identity of any gene product of greater than 10%.

Biological data and the complete genome sequence of AEV-1 support its assignment to a new species within the genus *Enamovirus*.

- Icosahedral particles
- A very weak reaction was detected when DAS and NC-ELISA test were performed in AEV-1 infected samples using PEMV 1 polyclonal antisera.

- Genome sequence resembling that one of PEMV-1 [3], but distinct from that one described for the luteoviruses and poleroviruses. The complete sequence of 5,726 nucleotides positive-sense RNA genome is available (KU297983) and shows five ORFs [1] as shown in Figure 1 (annex). Unlike poleroviruses, enamoviruses do not encode a putative P4 movement protein, and luteoviruses lack a P0 gene [2]. Therefore AEV-1 appears to be an enamovirus because it encodes a P0 gene, but does not encode a P4 gene (Annex, Figure 1).
- Nucleotide (nt) and amino acid (aa) sequence identities were determined doing comparisons between the AEV-1 ORFs and the predicted ORFs of the two complete PEMV-1 genome sequences available in GenBank (NC_003629 and HM439775) and those of the tentative enamovirus citrus vein enation virus (CVEV). The maximum nt sequence identity was 80.3, 80.1 and 49.6 %, respectively, whereas the maximum aa sequence identity was 82.7, 82.7 and 50.8 %, respectively [and Annex, Table 1). Therefore, the differences in aa sequence identity for each gene product were greater than 10 %, which, as was stated above, is one of the criteria used by the International Committee on Taxonomy of Viruses to demarcate species in the genera *Polerovirus* and *Luteovirus* [2].
- In a phylogenetic analysis based on the P1-P2 fusion protein aa sequence of viruses of the family *Luteoviridae*, AEV1 clustered with PEMV1 in the enamovirus complex (Annex, Figure 2).
- PEMV 1 infects several legume crops, including chickpea (*Cicer arietinum*), faba bean (*Vicia faba*), lentil (*Lens culinaris*) and pea (*Pisum sativum*) [4], but it does not infect alfalfa [5]. Whereas CVEV infects citrus [6] and AEV1 infects alfalfa [1]. Consequently, there is a difference between these three viruses regarding breadth and specificity of host range.

MODULE 10: **APPENDIX**: supporting material

additional material in support of this proposal

**References:**


1. Bejerman, N., Giolitti, F., de Breuil, S., Trucco, V., Dietzgen, R.G., Lenardon, S., 2016. Complete genome sequence of a new enamovirus from Argentina infecting alfalfa plants showing dwarfism symptoms. Arch Virol DOI: 10.1007/s00705-016-2854-3.
2. Domier, L.L., 2011. Family *Luteoviridae*, in: King, A.M.Q, Adams, M.J., Carstens, E.B., Lefkowitz, E.J. (Eds), Virus Taxonomy, Ninth Report of the International Committee on Taxonomy of Viruses. Elsevier, Oxford, pp. 1045-1053.
3. Demler, S.A., de Zoeten, G.A., 1991. The nucleotide sequence and luteovirus-like nature of RNA 1 of an aphid non-transmissible strain of Pea enation mosaic virus. J Gen Virol 72:1819–1834.
4. Hagedorn, D.J., 1984. Compendium of pea diseases. The American Phytopathological Society, St Paul, p 60.
5. Larsen, R.C., Kaiser, W.J., Klein, R.E., 1996. Alfalfa, a non-host of pea enation mosaic virus in Washington State. Can J Plant Sci 76:521–524.
6. Vives, M.C., Velázquez, K., Pina, J.A., Moreno, P., Guerri, J., Navarro, L., 2013. Identification of a new enamovirus associated with citrus vein enation disease by deep sequencing of small RNAs. Phytopathol 103:1077–1083.

**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.

**Table 1**. Nucleotide (nt)/amino acid (aa) identities (%) of AEV-1 ORF´s compared with those of PEMV-1 and CVEV (taken from Fig. 1 panel D in Bejerman *et al*., 2016)

| | ORF O | ORF 1 | ORF 1-2 | ORF 3 | ORF 5 |
|---|---|---|---|---|---|
| PeMV-1-WSG | 75.2/66.3 | 77.5/71.4 | 80.3/79.3 | 75.3/82.7 | 69.3/67.9 |
| PeMV-1-ID | 76.9/69.0 | 77.3/71.6 | 80.1/78.5 | 75.1/82.7 | 70.6/68.5 |
| CVEV | 41.5/37.4 | 43.6/39.6 | 49.6/50.8 | 47.2/44.2 | 48.5/45.3 |

**Figure 1** (taken from Figure 1 panel C in Bejerman *et al*., 2016)
Putative genome organization of alfalfa dwarfism associated virus (AEV-1). Positions are marked at extremities

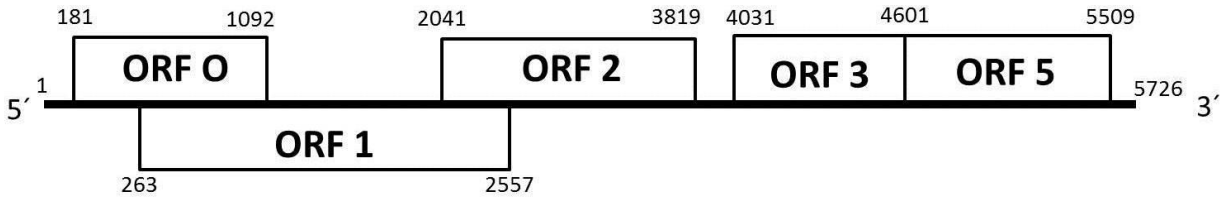
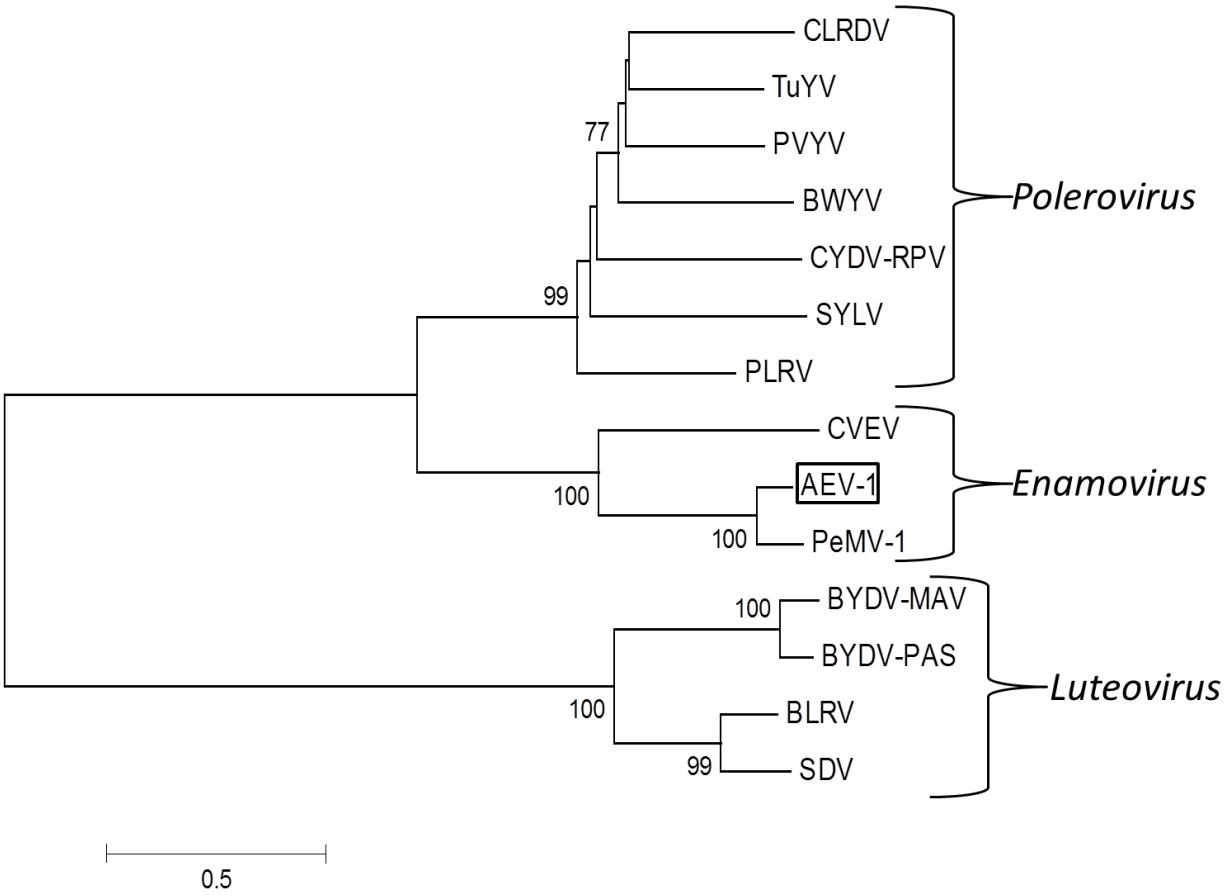



**Figure 2** (taken from Figure 2 in Bejerman *et al*., 2016)
Phylogenetic tree based on the alignment of the deduced amino acid sequences of the replicase protein (P1-P2) of AEV-1 and representative members of the family *Luteoviridae*. The tree was constructed in MEGA 6 using the Neighbor-joining method. The values on the branches show percentage of support out of 1000 bootstrap replications. The scale bar indicates the number of substitutions per base. The viruses used to construct the tree, and their accession numbers are: barley yellow dwarf virus-MAV (BYDV-MAV; NC_003680), barley yellow dwarf virus-PAS (BYDV-PAS; NC_002160), bean leafroll virus (BLRV; NC_003369), beet western yellows virus (BWYV; NC_004756), cereal yellow dwarf virus-RPV (CYDV-RPV; NC_004751), cotton leafroll dwarf virus (CLRDV; NC_014545), citrus vein enation virus (CVEV; NC_021564), pea enation mosaic virus 1 (PEMV 1; NC_003629), pepper vein yellows virus (PVYV; NC_015050), potato leafroll virus (PLRV; NC_001747), soybean dwarf virus (SDV; NC_003056), sugarcane yellow leaf virus (SYLV; NC_000874), turnip yellow virus (TuYV; NC_003743).


CLRDV
TuYV
77
PVYV
BWYV
Polerovirus
CYDV-RPV
99
SYLV
PLRV
CVEV
AEV-1
Enamovirus
100
100
PeMV-1
100
BYDV-MAV
BYDV-PAS
Luteovirus
100
BLRV
99
SDV
0.5