This Word module should be used for all taxonomic proposals.

Please complete **Part 1** and:

either **Part 3** for proposals to create new taxa or change existing taxa

or **Part 2** for proposals of a general nature.

Submit the completed Word module, together with the accompanying Excel module named in Part 3, to the appropriate ICTV Subcommittee Chair.

For guidance, see the notes written in blue, below, and the help notes in file Taxonomic\_Proposals\_Help\_2018.

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

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| **Code assigned:** | ***2018.016P*** | (to be completed by ICTV officers) |
| **Short title:** New species *Citrus vein enation virus* in the genus *Enamovirus* |
|  |
| **Author(s):** |
| Surapathrudu Kanakala, W. Allen Miller |
| **Corresponding author with e-mail address:** |
| W. Allen Miller, wamiller@iastate.edu |
| **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 (there are six virus subcommittees: animal DNA and retroviruses, animal ssRNA-, animal ssRNA+, fungal and protist, plant, bacterial and archaeal) | *Luteoviridae* Study Group (Chair: W. Allen Miller, wamiller@iastate.edu) |
| **ICTV Study Group comments (if any) and response of the proposer:** |
| “I agree to accept it as a new species.” |
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| Date first submitted to ICTV: | 8 June 2018 |
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| **ICTV-EC comments and response of the proposer:** |
| First submission had figures directly from the published manuscript, by Villamor et al. (2016), which would have violated copyright rules. Here we drew our own version of the genome, calculated our own amino acid sequence similarities and made our own phylogenetic trees.  |

**Part 2:** **NON-STANDARD**

Template for any proposal regarding ICTV procedures, rules or policy, not involving the creation of new taxonomy.

| **Text of proposal:** |
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**Part 3:** **PROPOSED TAXONOMY**

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| **Name of accompanying Excel module: 2018.016P.N.v1.Enamovirus\_nsp2.xlsx** |

The taxonomic changes you are proposing should be presented on an accompanying Excel module, 2017\_TP\_Template\_Excel\_module. Please enter the file name of the completed module in this box.

**Supporting material:**

| additional material in support of this proposal |
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| Please explain the reasons for the taxonomic changes you are proposing and provide evidence to support them. The following information should be provided, where relevant:* **Species demarcation criteria**: Explain how new species differ from others in the genus and demonstrate that these differences meet the criteria previously established for demarcating between species. If no criteriahave previously been established, and if there will now be more than one species in the genus, please state the demarcation criteria you are proposing.
* **Higher taxa**:
	+ There is no formal requirement to state demarcation criteria when proposing new genera or other higher taxa. However, a similar concept should apply in pursuit of a rational and consistent virus taxonomy.
	+ Please indicate the **origin of names** assigned to new taxa at genus level and above.
	+ For each new genus a **type species** must be designated to represent it. Please explain your choice.

This proposal recommends that *Citrus vein enation virus* (CVEV) should be considered a distinct species in the genus *Enamovirus*, family *Luteoviridae*.Citrus vein enation virus (CVEV) is a novel virus that has been identified in Spain (Vives et al., 2013), China (Huang et al., 2015) and Japan (Nakazono-Nagaoka et al., 2017) by deep sequencing of small RNAs of citrus plants showing vein enation disease. The complete linear single stranded positive sense RNA genome was found to be 5,983 nucleotides in length composed of five potential open reading frames (ORF0, ORF1, ORF2, ORF3, and ORF5) in the positive-sense strand (Figure 1). The lengths of the 5’ UTR and 3’ UTR were 207 and 198 nt respectively. ORF0 (nucleotides 219 to 1283) potentially encodes a 39-kDa polypeptide of 354 amino acids. Amino acid sequence comparison with the homologous PEMV1 (L04573) and AEV1 (KU297983) ORF0 product showed 17% identity (Table 1). ORF1 (nt 208 to 2916) encodes for a conserved serine proteinase domain. ORF2 (nt 2,202 to 4,187) is predicted to be translated by a –1 ribosomal frameshift from ORF1 and encodes a 148-kDa fusion protein of 1,323 amino acids. ORF3 (nt 4,301 to 4,876) encodes a 21-kDa protein of 191 aa identified as the putative CP. Amino acid comparisons with the PEMV1 and AEV1 CPs showed 24% and 25% identity, respectively (Table 1). ORF5 (nt 4,877 to 5,785) is predicted to be expressed by readthrough of the CP amber stop codon as a 55-kDa fusion protein of 493 amino acids. Amino acid sequence comparisons with the PEMV1 and AEV1 ORF5 showed 38% identity (Table 1). In a phylogenetic analysis based on the complete genome sequences of luteovirids, CVEV clustered with PEMV1 and AEV1 in the enamovirus cluster (Figure 2). Moreover, like these other enamoviruses, CVEV lacks ORFs 4 and 3a, consistent with its classification in genus *Enamovirus*. **Species demarcation criteria**Species demarcation for the *Luteoviridae* include greater than 10% amino acid sequence divergence of any gene product from that of any other known luteovirid. Since all ORFs differ by more than 10%, CVEV is a distinct virus species. Enamoviruses from isolates VE1 (HF769486), PV52-IVIA (KF020314), RR-IVIA (KF055890) from Spain, STM1 (LC089853), STM2 (LC089853), IBK (LC089852), NGS (LC089851), YM1 (LC089850) from Japan, SM (KY303624) from China and JJ (LC360112) from South Korea are all 98-100% identical, so all would be considered members of the species *Citrus vein enation virus*.* **Supporting evidence**: The use of Figures and Tables is strongly recommended (note that copying from publications will require permission from the copyright holder). For phylogenetic analysis, try to provide a tree where branch length is related to genetic distance.
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**Figure 1**. Genome organizations of *Citrus vein enation virus* (CVEV, top) and *Pea enation mosaic virus 1* (PEMV1, the type member of genus *Enamovirus*), shown to scale. Standard luteovirid ORF numbering is used with some functions indicated: RdRp, RNA-dependent RNA polymerase; CP, coat protein; RTD, readthrough domain of CP; fs, -1 ribosomal frameshift site; rt, site of stop codon readthrough (vertical dashed line). Note the absence of ORFs 3a and 4, a common feature of enamoviruses.

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|   | ORF0 | ORF 1 | ORF 1-2 | ORF 3 | ORF5 | Genome |
| PEMV1 (L04573) | 17% | 23% | 37% | 24% | 38% | 47% |
| AEV1 (KU297983) | 17% | 24% | 38% | 25% | 38% | 47% |

**Table 1.** Percent nucleotide (genome) and amino acid (ORFs) identities between *Citrus vein enation virus* (CVEV) and those of PEMV1 and AEV1.

**Figure 2**. Phylogeny based on complete genome nucleotide sequences of CVEV and representative members of the family *Luteoviridae* with Genbank accession numbers shown. The tree was constructed with MEGA6 using the neighbour-joining method and 1000 bootstrap replications. The scale bar indicates the number of substitutions per site. Virus abbreviations: BYDV, barley yellow dwarf virus; SbDV, soybean dwarf virus; BLRV, bean leaf roll virus; PEMV1, pea enation mosaic virus 1; CVEV, citrus vein enation virus (proposed); ScYLV, sugarcane yellow leaf virus; PLRV, potato leaf roll virus; CYDV, cereal yellow dwarf virus; CABYV, cucurbit aphid-borne yellows virus; BChV, beet chlorosis virus and TuYV, turnip yellows virus.

 **CVEV\_LC089853**

 **CVEV\_HF679486**

 **CVEV\_LC089851**

 **CVEV\_LC360112**

 **CVEV\_LC089850**

 PEMV1\_L04573

 AEV1\_KU297983

**Enamoviruses**

 ScYLV\_AM072756

 CYDV\_L25299

 PLRV\_AY138970

 CABYV\_EU000535

 BChV\_AF352025

 TuYV\_X13063

Poleroviruses

 SbDV\_KJ786322

 BLRV\_AF441393

 RSDaV\_EU024678

 BYDV-KerII\_KC572000

 BYDV-PAS\_AF218798

 BYDV-PAS\_X07653

 BYDV-GAV\_AY220739

 BYDV-MAV\_D11028

Luteoviruses

98

60

99

98

99

98

100

100

99

99

93

97

100

100

56

33

100

0.5

| **References:** |
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| 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. Phytopathology 103:1077-1086.Huang, A.J., Zhen, S.O.N.G., Cao, M.J., Chen, H.M., Li, Z.A., Zhou, C. Y. (2015) The complete genome sequence of Citrus vein enation virus from China. Journal of Integrative Agriculture 14:598-601.Nakazono-Nagaoka, E., Fujikawa, T., Iwanami, T. (2017) Nucleotide sequences of Japanese isolates of citrus vein enation virus. Archives of Virology 162:879-883. |

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