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**

|  |  |  |  |
| --- | --- | --- | --- |
| **Code assigned:** | ***2018.017P*** | | (to be completed by ICTV officers) |
| **Short title:** *Nectarine stem pitting associated virus*, a new species in genus *Luteovirus* | | | |
|  | | | |
| **Author(s):** | | | |
| Surapathrudu Kanakala, Elizabeth J. Carino, 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.” | | | |
|  | | | |
| Date first submitted to ICTV: | | | 2017 |
| Date of this revision (if different to above): | | | 6/6/2018 |

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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**

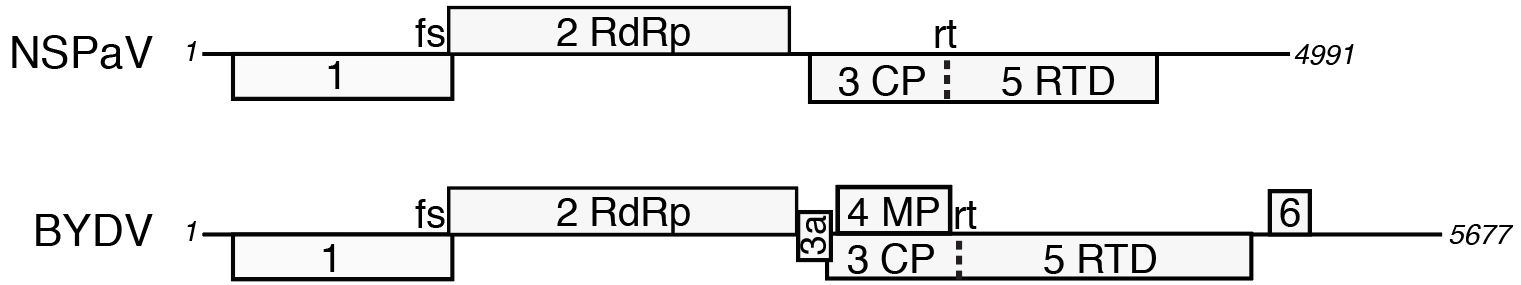
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| **Name of accompanying Excel module: 2018.017P.N.v1.Luteovirus\_nsp.xlsx** |

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.   Nectarine stem pitting associated virus (NSPaV) is a novel virus that has been recently identified in the United States, Japan, China, and Korea. Trunks of nectarine trees showed stem pitting on the woody cylinder after the removal of the bark when infected with NSPaV. Next-generation sequencing of overlapping RT-PCR products was used to determine the full genome sequence of NSPaV. The sequence of the 5'-end was determined by performing separate dATP and dGTP tailing reactions. Both reactions predicted the same 5'-end terminus with an additional 131 nt not found in the preliminary analysis of the NGS data. The untranslated region at the 3' terminus, as revealed from the cloned amplified products obtained by RACE and RT-PCR, was 671 nt in length. Two types of nectarine sources were used: i) budwood of nonsymptomatic nectarine trees, designated 12P42; and ii) budwood of a nectarine tree that exhibited stem pitting symptoms on the woody cylinder of the scion, designated SF04522E. These NSPaV isolates were compared with that identified previously by Bag et al. (2015).  The complete linear single-stranded positive sense RNA genome was found to be 4,991 nt in length composed of four open reading frames (ORFs). NSPaV genome organization was similar to members of the genus *Luteovirus* (family *Luteoviridae*) (Figure 1). ORFs 1 (nucleotides 131 to 1118) and 2 (nucleotides 1115 to 2674) could code for 334 and 519 amino acids long P1 and P2 proteins, respectively, which together would form the RdRp complex (Bag et al. 2015). ORF 2 was predicted to be synthesized by a -1 ribosomal frame shift during translation of ORF 1. ORFs 3 and 5 were identified after a small intergenic region of 65 nucleotides (2675-2739). ORF 3 is predicted to encode the CP and a fusion protein predicted to be responsible for insect transmission. ORF 5 is only about two-thirds the size of that of other luteoviruses. Unlike other luteoviruses, the NSPaV genome does not have the homolog of ORF 4, and no obvious homolog of ORF 3a was identified.  BLASTX analysis against the nucleotide database of NCBI showed high identity values to members of the *Luteoviridae* family. Full genome nucleotide sequences analyzed in pairwise comparisons against luteovirus revealed a 38-43% nucleotide identity for the whole genome and 26-39% amino acid identity for the coat protein (Table 1). Comparisons of NSPaV against poleroviruses showed 27-29 % nucleotide identity for the whole genome and 30-38% amino acid identity for the coat protein.    **Species demarcation criteria**  Currently, a virus is considered a new species within the *Luteoviridae* family if the amino acid sequence of any gene product differs by >10%. All ORFs differ from those of all other luteoviruses by much more than 10%. Therefore, the isolates SF04522E and 12P42 and NSPaV should be considered a single new virus species within genus *Luteovirus*, with the name *Nectarine stem pitting associated 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. |



**Figure 1**. Genome organization of nectarine stem pitting associated virus (NSPaV) and, for comparision, barley yellow dwarf virus (BYDV), the type member of genus *Luteovirus*. Standard 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).

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| Virus sequence comparison | Whole genome | Replicase | Coat Protein |
| NSPaV (Bag et al. 2015) | 95-99% | 96-99% | 99% |
| NSPaV isolates SF04522E, 12P42 | 95% | 96% | 99% |
| NSPaV *vs* luteoviruses | 38-43% | 61-66% | 26-39% |
| NSPaV *vs* poleroviruses | 27-29% | 6-11% | 30-38% |
| NSPaV *vs* enamovirus | 28% | 8-9% | 24% |

**Table 1.** Percent nucleotide and amino acid identities between nectarine stem pitting associated virus (NSPaV) and members of the family *Luteoviridae*. The two NSPaV isolates were obtained from asymptomatic (12P42) and symptomatic nectarine trees (SF04522E). Values indicate percent nucleotide identities for whole-genome comparison whereas amino acid identities used replicase and coat protein comparisons.

PEMV-1\_NC\_003629

**(A)**

**NSPaV\_KT273409**

**NSPaV\_KP638562**

**NSPaV\_KT273410**

RSDaV\_NC\_010806

ScYLV\_NC\_000874

BYDV-Ker-II\_KC559092

BYDV\_Ker-II\_NC\_021481

BYDV-MAV\_NC\_003680

BYDV-PAS\_NC\_002160

BYDV-PAV\_NC\_004750

**Luteovirus**

BLRV\_NC\_003369

SbDV\_NC\_003056

CABYV\_NC\_003688

CLRDV\_NC\_014545

BWYV\_NC\_004756

TuYV\_NC\_003743

Polerovirus

Enamovirus

89

100

100

100

84

66

100

46

66

71

98

99

95

90

0.1

PEMV-1\_NC\_003629

**(B)**

**NSPaV\_KT273409**

**NSPaV\_KP638562**

**NSPaV\_KT273410**

RSDaV\_NC\_010806.1\_

BLRV\_NC\_003369

SbDV\_NC\_003056

BYDV\_Ker-II\_KC559092

BYDV\_Ker-II\_NC\_021481

BYDV-PAS\_NC\_002160

BYDV-MAV\_NC\_003680

BYDV-PAV\_NC\_004750

**Luteovirus**

TuYV\_NC\_003743

BWYV\_NC\_004756

CLRDV\_NC\_014545

CABYV\_NC\_003688

ScYLV\_NC\_000874

Polerovirus

Enamovirus

98

94

99

84

100

100

100

98

62

100

98

100

98

100

0.2

**Figure 2**. Phylogenetic analyses of the coat protein amino acid (A) and full genome (B) sequences of nectarine stem pitting associated virus (NSPaV) with members of the family *Luteoviridae.* Dendograms were constructed using the neighbour-joining method with 1000 bootstrap replications. Luteoviruses: bean leafroll virus (BLRV), barley yellow dwarf virus KerII (BYDV-KerII), barley yellow dwarf virus KerIII (BYDV-KerIII), barley yellow dwarf virus MAV (BYDV-MAV), barley yellow dwarf virus PAS (BYDV-PAS), barley yellow dwarf virus PAV (BYDV-PAV), rose spring dwarf-associated virus (RSDaV), and soybean dwarf virus (SbDV); poleroviruses: beet western yellows virus (BYWV), cucurbit aphid-borne yellows virus (CABYV), cotton leaf roll dwarf virus (CLRDV), potato leafroll virus (PLRV), pepper yellow leaf curl virus (PYLCV), sugarcane yellow leaf virus (ScYLV), and turnip yellows virus (TuYV); enamovirus: pea enation mosaic virus 1 (PEMV-1).

| **References:** |
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| |  | | --- | | Bag, S., M. Al Rwahnih, A. Li, A. Gonzalez, A. Rowhani, J. K. Uyemoto and M. R. Sudarshana (2015) Detection of a new luteovirus in imported nectarine trees: a case study to propose adoption of metagenomics in post-entry quarantine. Phytopathology 105:840-846.  Lu, M. G., C. Zhang, Z. X. Zhang, C. A. Wang and S. F. Li (2016) Nectarine stem-pitting-associated virus detected in peach trees in China. Plant Disease 101:513-513.  Villamor, D. E. V., T. A. Mekuria, S. S. Pillai and K. C. Eastwell (2016) High-throughput sequencing identifies novel viruses in nectarine: insights to the etiology of stem-pitting disease. Phytopathology 106:519-527.  Candresse, T., C. Faure, S. Theil and A. Marais (2016) First report of nectarine stem pitting-associated virus infecting *Prunus mume* in Japan. Plant Disease 101:393-393.  Jo, Y., J. K. Cho, H. Choi, S. Lian and W. K. Cho (2017) First report of nectarine stem pitting-associated virus and plum bark necrosis and stem pitting-associated virus infecting a peach cultivar in Korea. Plant Disease:PDIS-11-16-1634-PDN. | |