

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

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| **Code assigned:** | **2020.023P** |  |
| |  | | --- | | **Short title:** Create four new species in the genus *Nanovirus* (*Mulpavirales*: *Nanoviridae)* | | | |
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**List the ICTV Study Group(s) that have seen this proposal**

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| *Nanoviridae* Study Group |

**ICTV study group comments and response of proposer**

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| Minor editorial comments, which have been incorporated into the proposal. |

**Submission dates**

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| Date first submitted to SC Chair (prepared by SC Chair, and first circulated on this date) | July 15, 2020 |
| Date of this revision (if different to above) | 31 July 2020 |

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

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**Part 3:** **TAXONOMIC PROPOSAL**

**Name of accompanying Excel module**

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| 2020.023P.R.Nanoviridae\_4nsp.xlxs |

**Abstract**

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| Four recently described viruses (cow vetch latent virus, milk vetch chlorotic dwarf virus, parsley severe stunt associated virus and Sophora yellow stunt virus) clearly exhibit the properties characteristic of members of the genus *Nanovirus*. Full sequences of all eight single-stranded circular DNA canonical genome components are available for all exemplar isolates except parsley severe stunt associated virus. In the latter case, only the DNA-U4 has not been identified. All viruses are unique, and show <80% nucleotide identity across their concatenated genomes with existing members of the genus *Nanovirus*. Therefore, we here propose creation of four new species in the genus *Nanovirus* to classify these new viruses. |

**Text of proposal**

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| |  | | --- | | Four viruses that have recently been described clearly fall within the criteria described for classification in the genus *Nanovirus.* These classifications are based predominantly on molecular criteria. Genus demarcation criteria currently proposed within the family *Nanoviridae* include a nucleotide identity of the concatenated genome of <62% and the possession of six (*Babuvirus*) or eight (*Nanovirus*)genome components. Species demarcation within the *Nanovirus* genus is proposed as sequence identity of <80% (across all eight concatenated genomic DNAs).  This proposal presents the case for each of these viruses to be included as members of new species within the genus *Nanovirus.*   1. Cow vetch latent virus   A novel virus, cow vetch latent virus (CVLV [Sambuc 2010]) was identified from a single, symptomless plant of cow vetch (*Vicia cracca*) through a metagenomics study in the Rhône delta region of France (Gallet et al., 2018). The virus contained the eight single-stranded, circular DNA genome components with a structure and organization typical of nanoviruses (971-1010 nucleotides (nt) in length) and included the conserved CR-I, CR-II and nonanucleotide sequences. CVLV was phylogenetically related to, but distinct from, other members of the genus *Nanovirus* and the concatenated genome shared <72% nt identity with members of the genus *Nanovirus* (Figs 1 and 2)*.* It was also associated with two distinct alphasatellite molecules (MF535455, 2 MF535456).   1. Milk vetch chlorotic dwarf virus   From two plants of milk vetch (*Astragalus myriacanthus*) growing in Chaharmahal and Bakhtiari province, central Iran, a novel virus was isolated (Hassan-Sheikhi et al., 2020). The plants displayed marginal leaf chlorosis, little leaves and dwarfing symptoms and the virus thus named milk vetch chlorotic dwarf virus (MVCDV). The virus contained the eight single-stranded, circular DNA genome components with a structure and organization typical of nanoviruses and included the conserved CR-I, CR-II and nonanucleotide sequences. The two isolates of MVCDV, [G50] and [G53], shared 84.4-99.7% nt identity between each of their equivalent genome components.  A representative isolate, MVCDV [G53] has individual genome components 996-1006 nt in length and its concatenated genome shared <75% nt identity with members of the genus *Nanovirus.* It was phylogenetically related to, but distinct from, other members of the genus (Figs 1 and 2)*.* Three alphasatellite molecules were associated with MVCDV [G53]: MN273340.1 - MN273342.1.  In MVCDV [G50] two alphasatellites were found, MN273331.1 and MN273330.1 and an additional U1 component (MN273327.1), differing by ca 4% nt identity to the other U1 component. At times, additional copies of individual genome components with distinct sequences have been found in other nanovirus isolates and, at this stage, the biological significance of this situation is unclear.   1. Sophora yellow stunt virus   A virus was first identified in the wild perennial legume *Sophora alopecuroides* from Kerman, Iran, and later also recovered from chickpea (*Cicer arietinum*), lentil (*Lens culinaris*), liquorice (*Glycyrrhiza glabra*), esfand (*Peganum harmala*) and milk vetch (*Astragalus* sp.). All infected plants showed stunting and different degrees of leaf yellowing symptoms (Heydarnejad et al., 2017, Heydarnejad et al., 2018). The original isolate from *S. alopecuroides* (SYSV [Ta-1]; (Heydarnejad et al., 2017) contained four distinct DNA-R and two distinct DNA-C components in addition to a single copy each of the remaining six canonical nanovirus components. Nucleotide identity among the four DNA-R components was 64.8-76.5% and between the two DNA-C components was 71.0%. Thus this isolate may represent a mixed infection. It also contained 14 distinct alphasatellite molecules representing four *Alphasatellitidae* species. An additional isolate was also shown by a PCR and metagenomics sequencing approach to contain the eight canonical components and a second DNA-C and DNA-R. From a subsequent isolate from *S. alopecuroides* (SYSV [Har:H13:Soph:17]; Heydarnejad et al unpublished, 2018) available on GenBank, using a PCR approach only, just the eight canonical genome components were obtained and this is proposed as the exemplar isolate. The single-stranded, circular DNA genome components have a structure and organization typical of nanoviruses and includes the conserved CR-I, CR-II and nonanucleotide sequences. Individual components range in size from 978 to 997 nt. Genome components of other isolates of SYSV reported from Iran share the following nucleotide identities with the proposed exemplar isolate: DNA-R, 99.8%; DNA-C, 90.2-90.3%; DNA-N, 93.6-99.1%; DNA-S, 97.0-97.1% (Heydarnejad et al., 2018). The significance of the occurrence of additional distinct copies of some genome components is unknown..SYSV is phylogenetically related to, but distinct from, other members of the genus (Figs 1 and 2) and its concatenated genome shares less than 69.2% identity with members of the genus *Nanovirus.* SYSV was transmitted by the aphid vectors *Aphis craccivora and Acyrthosiphon pisum* (Heydarnejad et al., 2018).   1. Parsley severe stunt-associated virus   A novel virus, associated with a severe stunting disease, was isolated from parsley (*Petroselinum crispum*) from Germany (Vetten et al., 2019). In the two isolates of parsley severe stunt-associated virus (PSSaV) described therein, seven of the eight nanovirus canonical genome components were identified (both isolates lacked DNA U4), and in addition to the DNA-R presumed to encode the M-Rep protein, each had a separate, distinct DNA-R and a number of alphasatellite molecules (MK039138.1 -MK039142.1). PSSaV has a single-stranded, circular DNA genome (components 957-979 nucleotides in length) with a structure and organization typical of nanoviruses and includes the conserved CR-I, CR-II and nonanucleotide sequences. However, while the nanovirus nonanucleotide is typically perfectly conserved in nanoviruses (TA**G**TATTAC), PSSaV has a nonanucleotide identical to that of babuviruses (TA**T**TATTAC). PSSaV [Pa21] is proposed as the exemplar isolate of this virus, and its concatenated genome shares <63.8% identity with members of the genus *Nanovirus* (Figs 1 and 2)*.* Genome components have also been described for two isolates of PSSaV from Iran (Hasanvand et al., 2020);( Heydarnejad, unpublished). Interestingly, both isolates contained all genome components except DNA-U4, and two distinct DNA-R molecules were identified from each isolate. | |

**Supporting evidence**

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**Figure 1.** Maximum likelihood dendrogram showing nucleotide sequence relationships in the concatenated DNA components (DNA-C, -M, -N, -R and -S, and U-1, -2, -3 and -4 where present) from representative members of all assigned and tentative species of the genera *Nanovirus* and *Babuvirus* (family *Nanoviridae*). GenBank accession numbers and isolate details are shown in Tables 5 and 6. Vertical branch lengths are arbitrary and horizontal distances are proportional to the number of base substitutions per site (see scale bar). Evolutionary analyses were conducted in MEGA X, using the Muscle algorithm. The dendrograms were bootstrapped 500 times (scores are shown at nodes).

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**Figure 2.** Heat map illustrating pairwise distances between approved and proposed members of the family *Nanoviridae*, based on nucleotide sequences of the complete genomes (DNA-C, -M, -N, -R and -S, and U-1, -2, -3 and -4 where present) and using the Muscle algorithm in the program SDT 1.2

**References**

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