

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

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| **Code assigned:** | **2020.028P** |  |
| **Short title:** Create one new species in the genus *Marafivirus* (*Tymovirales*: *Tymoviridae*) | | |
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**Author(s) and email address(es)**

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**Author(s) institutional address(es) (optional)**

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**List the ICTV Study Group(s) that have seen this proposal**

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| *Tymoviridae* SG |

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

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| The ICTV *Tymoviridae* Study Group reviewed the proposal and made suggestions. The suggestions were addressed and revisions were made. R. Hammond, Study Group Chair. |

**Authority to use the name of a living person**

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| --- | --- | --- |
| **Taxon name** | **Person from whom the name is derived** | **Permission attached (Y/N)** |
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**Submission dates**

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| --- | --- |
| Date first submitted to SC Chair | July 29, 2020 |
| Date of this revision (if different to above) |  |

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

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**Part 2:** **NON-TAXONOMIC PROPOSAL**

**Text of proposal**

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

**Name of accompanying Excel module**

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| 2020.028P.R.Tymoviridae\_1nsp.xlsx |

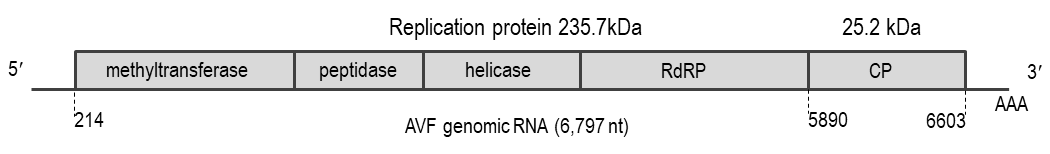
**Text of proposal**

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| |  | | --- | | Alfalfa virus F (AVF) was identified using a virion-associated nucleic acid (VANA) metagenomics-based approach in alfalfa (*Medicago sativa* L.) samples collected in Southern France (Nemchinov et al., 2018). Partial purification of the virus from alfalfa samples resulted in recovery of spherical virus particles ~ 30 nm in diameter, resembling T=3 isometric virions of marafiviruses (**Fig. 1**).  The nucleotide sequence of the viral genome was determined by *de-novo* assembly of VANA reads and by 5′/3′ RACE with viral RNA extracted from enriched viral particles or with total RNA, respectively. The full-length monopartite viral ssRNA genome consists of 6,797 nts, excluding the 3′-terminal poly(A) tail. The 5′ and 3′ untranslated regions are 213 and 194 nt long, respectively. AVF encodes a single precursor polyprotein 2129 amino acids in length containing conserved domains characteristic for viruses in the genus *Marafivirus*, family *Tymoviridae*: methyltransferase, protease/endopeptidase, RNA helicase superfamily 1, RNA dependent RNA polymerase (RdRp) and coat proteins (CP) (**Fig. 2**).  The G/C content of the virus genome is 48.3%. The AVF genome contains a conserved “marafibox”, a 16-nt consensus sequence of the subgenomic RNA promoter that is present in all known marafiviruses, at positions 5734-5749 nt (5′ GAGGGTGAATTGCTTC 3′). The CP-encoding sequences of AVF are in the same reading frame with the polyprotein and are located at its 3′- end. The AVF genome encodes two CPs: major (21kDa) and minor CPs (25kDa) that differ by an amino terminal extension in the minor CP and are found in the particles of marafiviruses in molar ratios of approximately 3:1 (major CP21 vs minor CP25), (Hammond and Ramirez, 2001; Edwards and Weiland, 2015). It is predicted that the coding region for the CP25 of AVF is located at 5890-6603 nt of the genome (**Fig. 2**). When this fragment was transiently expressed in *N. benthamiana* plants via a potato virus X-based vector (Nemchinov et al., 2018), TEM of crude leaf extracts revealed characteristic isometric virus-like particles ~ 30 nm in diameter resembling marafivirus virions.  Pairwise sequence alignments using different alignment programs indicated that AVF shares the greatest degree of overall sequence identity (72.8% - 75.4%) with Medicago sativa marafivirus 1 (MsMV1) that was computationally deduced from *Medicago sativa* L. transcriptomic data (Kim et al., 2018).The next closest virus, per PASC tool (Bao et al., 2014), is oat blue dwarf virus, isolate OBDV2r (50.4% identity, GenBank: GU396990), a member of the genus *Marafivirus*. MegaAlign program of the LaserGene suite (DNA-STAR, Madison, WI) predicted blackberry virus S (Genbank: NC038328), not yet ICTV-approved member of the genus *Marafivirus*, as the next closest genome with 50.3% identity, followed by the oat blue dwarf virus (49.3% identity; GenBank: NC\_001793). The criterion demarcating species in the genus *Marafivirus* for the overall genome sequence identity is less than 80% (Dreher et al., 2012).  On the amino acid level, AVF CP25 shares 82.7% identity with the corresponding portion of the MsMV1 polyprotein (query cover 100%, E-value=2e-126, GenBank: ATJ00054.1). Per BLASTp search, the next closest hit is CP of fig fleck-associated virus, an unassigned member of the family *Tymoviridae* (Elbeaino et al., 2011), (coverage 81%, E-value= 9e-57, identity 48.4%, GenBank: AUM57169.1). Less than 90% amino acid sequence identity among coat protein sequences is another criterion demarcating species in the genus *Marafivirus* (Dreher et al., 2012).  A phylogenetic tree, generated based on the alignment of the replication proteins, grouped AVF together with other ICTV-approved and tentative members of the genus *Marafivirus* (**Fig. 3**). AVF formed a subcluster with the most closely related MsMV1 and a sister-group with peach virus D, connected to other recognized members in the genus *Marafivirus*, including maize rayado fino virus.  Therefore, relying on the overall nucleotide sequence identity of less than 80%, identity of the capsid protein sequences less then 90%, phylogenetic relationship, and differential host range (*M. sativa*), AVF represents a distinct species in the genus *Marafivirus*, for which the name *Alfalfa virus F* is proposed. | |

**Supporting evidence**



**Figure 1.** Transmission electron microscopy (TEM) of partially purified AVF particles. Scale bar represents 200 nm. Arrows indicate AVF virions.



**Figure 2.** Genomic organization of alfalfa virus F (AVF). The putative protein products are indicated inside the boxes. RdRp, RNA-dependent RNA polymerase; CP, coat protein.



**Figure 3**. Molecular phylogenetic relationship of the AVF with members of the genus *Marafivirus* and selected members of the family *Tymoviridae*. The evolutionary history was inferred by using the Maximum Likelihood method based on the JTT matrix-based model. The percentage of trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. GenBank accession numbers are shown next to the virus names. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 22 amino acid sequences of the replication proteins (excluding CPs). There were a total of 1415 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar et al., 2016).

**References**

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