

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

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| **Code assigned:** | **2021.005P** |  |
| **Short title:** Create one new species (*Bromovirus SVS*)in the genus *Bromovirus* (*Martellivirales: Bromoviridae*) | | |
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**List the ICTV Study Group(s) that have seen this proposal**

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| *Bromoviridae* Study Group |

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

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**Submission dates**

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| Date first submitted to SC Chair | 21-May2021 |
| Date of this revision (if different to above) |  |

**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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| 2021.005P.A.v1.Bromovirus\_1ns.xlsx |

**Abstract**

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| High-throughput sequencing (HTS) of dsRNA combined with the RACE-PCR allowed the assembly of complete ss(+)RNA molecules of a novel virus infecting elderberry (*Sambucus nigra* L.). Detected tripartite genome consisted of RNA1 that contains ORF1a encoding the putative 1a protein, RNA2 with ORF2a encoding the putative 2a protein, and RNA3 that contains two ORFs, encoding the putative movement and coat proteins. The presence of typical bromovirus protein domains, together with the high-level identity with homologous bromovirus proteins, clearly assign the detected virus as a novel member of the genus *Bromovirus.* The name of species, *Bromovirus* SVS with the exemplar isolate sambucus virus S-B15, is proposed. |

**Text of proposal**

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| |  | | --- | | The novel bromovirus was detected during the study of elderberry virome using high-throughput (HTS) sequencing of dsRNA isolated from the elderberry (S*ambucus nigra* L*.)*, with the 5´- and 3´-ends sequenced by RACE-PCR analysis. The virus termed sambucus virus S (SVS) was shown to have three genomic RNA segments (RNA1 to RNA3; MF688613, MF688614, MF688615). RNA1, 3,241 nucleotides (nt) in length, contains ORF1a encoding protein 1a of 964 amino acids (aa), with the typical methyltransferase and helicase domains); RNA2, 2,810 nt in length, contains ORF2a encoding protein 2a of 813 aa (with the typical RdRp and DUF585 domains; and RNA3, 2,244 nt in length contains two ORFs, ORF3a encoding movement protein (MP) 300 aa in length and ORF3b encoding coat protein (CP) 190 aa in length. Both ORF3a and ORF3b are connected by a poly(A) stretch of 16-37 nt.  Comparisons of the SVS nucleotide and amino acid sequences showed similarity to the members of genus *Bromovirus.* Protein 1a of SVS-B15 showed 61.3-69.9% identity with the 1a protein of other bromoviruses, the highest identity was detected with the melandrium yellow fleck virus (MYFV) 1a (NC\_013266). SVS-B15 1a shared 23.8-43.2% identity with members of the other genera within the family *Bromoviridae*. The 2a protein shared 48.4-69.4% identity with homologous proteins of other bromoviruses, and 22.3-36.8 % with bromovirids outside the genus *Bromovirus*, respectively; the highest identity is shared again with MYFV 2a. The MP protein of SVS-B15 shared 47.1-59.7% identity to counterparts of bromoviruses; the highest identity is shared with the MP of cowpea chlorotic mottle virus (NC\_003542). The SVS-B15 CP showed 49.5-67.9% identity to the CPs of other bromoviruses, and 10.5-17.7% identity to the CPs of other bromovirids,respectively. The highest identity was detected between the CP of SVS-B15 and brome mosaic virus (NC\_002028). Phylogenetic analysis of nucleotide and amino acid sequences supported the assignment of B15 isolate to the genus *Bromovirus,* and the highly supported branching of B15 virus isolate between other bromoviruses in close relation with MYFV.  The detected identity fulfilling the species demarcation criteria and phylogenetic position of SVS-B15 clearly indicate that isolate B15 detected in elderberry tree by HTS (and confirmed by RT-PCR) represents a new species within the genus *Bromovirus*. The tripartite genome is showing the structure typical of bromoviruses, each genomic segment bearing the characteristic protein domains.  The creation of a new viral species within the genus *Bromovirus*, which contain SVS-B15 as the exemplar isolate is proposed. The species name *Bromovirus* *SVS* is proposed in agreement with the ICTV recommendation. | |

**Supporting evidence**

Table1. Nucleotide and amino acid (in parenthesis) identity [%] of sambucus virus S with the other species of genus *Bromovirus*.

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| **Exemplar isolates** | **ORF1a** (Met) | **ORF2a** (RdRp) | **ORF3a** (MP) | **ORF3b** (CP) |
| *Broad bean mottle virus* (NC\_004008) | 61.7 (63.6) | 54.6 (48.4) | 49.8 (47.1) | 55.1 (49.5) |
| *Brome mosaic virus* (NC\_002026) | 62.5 (65.7) | 59.1 (58.9) | 55.2 (49.7) | 64.2 (67.9) |
| *Cassia yellow blotch virus* (NC\_006999) | 60.5 (61.3) | 54.9 (50.3) | 58.2 (56.1) | 55.0 (54.7) |
| *Cowpea chlorotic mottle virus* (NC\_003543) | 63.2 (64.6) | 60.3 (59.7) | 64.2 59.7) | 62.7 (66.1) |
| *Melandrium yellow fleck virus* (NC\_013266) | 66.9 (69.9) | 66.6 (69.4) | 51.9 (49.5) | 60.2 (61.6) |
| *Spring beauty latent virus* (NC\_004120) | 61.2(62.9) | 57.5 (58.1) | 0,594 (53.7) | 61.3 (59.5) |

Figure 1: Schematic genome organization of sambucus virus S.

Conserved amino acid domains are indicated as follows: Mt: Methyltransferase domain, Hel: Helicase domain, RdRp: RNA dependent RNA polymerase domain, MP: movement protein domain , CP: coat protein domain; polyA – bromoviral polyA-stretch.

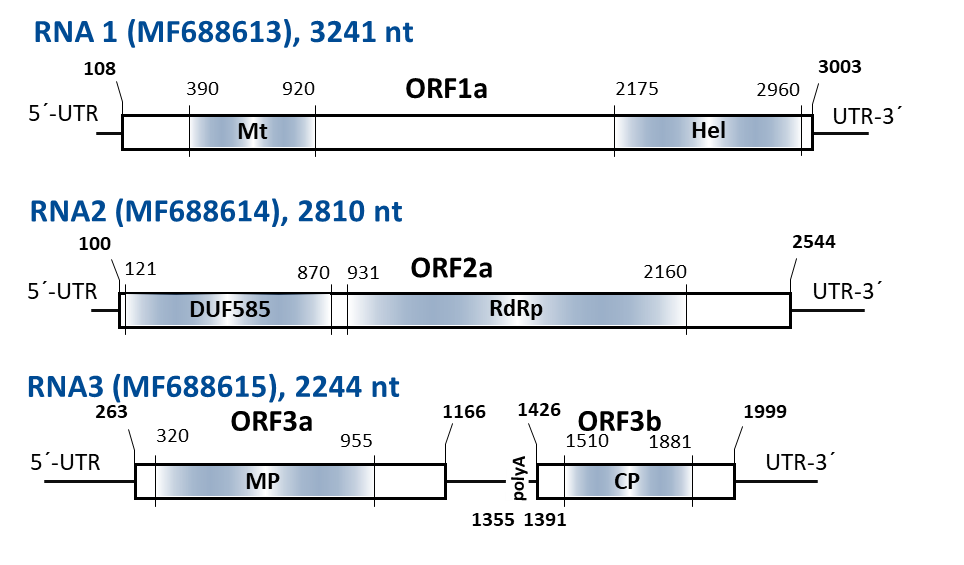


Figure 2: Neighbor-joining phylogenetic trees reconstructed using the 1a proteins of bromoviruses and other bromovirids.

*Bromovirus*

**sambucus virus S (SVS, MF688613)**

*Melandrium yellow fleck virus* (MYFV, NC\_013266)

*Broad bean mottle virus* (BBMV, NC\_004008)

*Brome mosaic virus* (BMV, NC\_002026)

*Cowpea chlorotic mottle virus* (CCMV, NC\_003543)

*Spring beauty latent virus* (SBLV, NC\_004120)

*Cassia yellow blotch virus* (CYBV, NC\_006999)

*Pelargonium zonate spot virus* (PZSV, NC\_003649)

*Cucumber mosaic virus* (CMV, NC\_002034)

*Alfalfa mosaic virus* (AMV, NC\_001495)

*Tobacco streak virus* (TSV, NC\_003844)

*Olive latent virus 2* (OLV2, NC\_003673)

*100*

*82*

*100*

*100*

*100*

0.20

Figure 3: Neighbor-joining phylogenetic trees reconstructed using the 2a (RdRp) proteins of bromoviruses and other bromovirids.

**sambucus virus S (SVS, MF688614)**

*Melandrium yellow fleck virus* (MYFV, NC\_013267)

*Broad bean mottle virus* (BBMV, NC\_004007)

*Cowpea chlorotic mottle virus* (CCMV, NC\_003541)

*Brome mosaic virus* (BMV, NC\_002027)

*Spring beauty latent virus* (SBLV, NC\_004121)

*Cassia yellow blotch virus* (CYBV, NC\_007000)

*Pelargonium zonate spot virus* (PZSV, NC\_003650)

*Cucumber mosaic virus* (CMV, NC\_002035)

*Alfalfa mosaic virus* (AMV, NC\_002024)

*Tobacco streak virus* (TSV, NC\_003842)

*Olive latent virus 2* (OLV2, NC\_003674)

*100*

*100*

*100*

*100*

*100*

*96*

*81*

0.20

*Bromovirus*

Figure 4: Neighbor-joining phylogenetic trees reconstructed using the coat proteins of bromoviruses and other bromovirids.

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**sambucus virus S (SVS, MF688615)**

*Melandrium yellow fleck virus* (MYFV, NC\_013268)

*Brome mosaic virus* (BMV, NC\_002028)

*Broad bean mottle virus* (BBMV, NC\_004006)

*Spring beauty latent virus* (SBLV, NC\_004122)

*Cowpea chlorotic mottle virus* (CCMV, NC\_003542)

*Cassia yellow blotch virus* (CYBV, NC\_007001)

*Pelargonium zonate spot virus* (PZSV, NC\_003651)

*Cucumber mosaic virus* (CMV, NC\_001440)

*Alfalfa mosaic virus* (AMV, NC\_002025)

*Tobacco streak virus* (TSV, NC\_003845)

*Olive latent virus 2* (OLV2, NC\_003671)

*75*

*60*

0.50

*Bromovirus*

Figure 2-4: Olive latent virus 2 proteins used as the outgroup to root the trees. The virus sequences are described by virus species name, virus acronym and GenBank accession number in parentheses. Bootstrap values > 60 % obtained from 500 replicates are shown. The novel bromovirus is marked in bold. Bars represent respectively 20% (1, 2) or 50% (3) aa-sequence divergence.

**References**

Šafářová D, Candresse T, Navrátil M (2018) Complete genome sequence of a novel bromovirus infecting elderberry (*Sambucus nigra* L.) in the Czech Republic. Arch Virol 163: 567–570 <https://doi.org/10.1007/s00705-017-3629-1>. PMID: **29094240**