

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

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| **Code assigned:** | **2020.004M** |  |
| **Short title:** Create three new species in the genus *Cytorhabdovirus (Mononegavirales*: *Rhabdoviridae)* | | |
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**List the ICTV Study Group(s) that have seen this proposal**

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| *Rhabdovirdae* SG |

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

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| The *Rhabdoviridae* SG has reviewed the proposal and supports this revised version. |

**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 |  |
| 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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| 2020.004M.R.Cytorhabdovirus\_3nsp.xlsx |

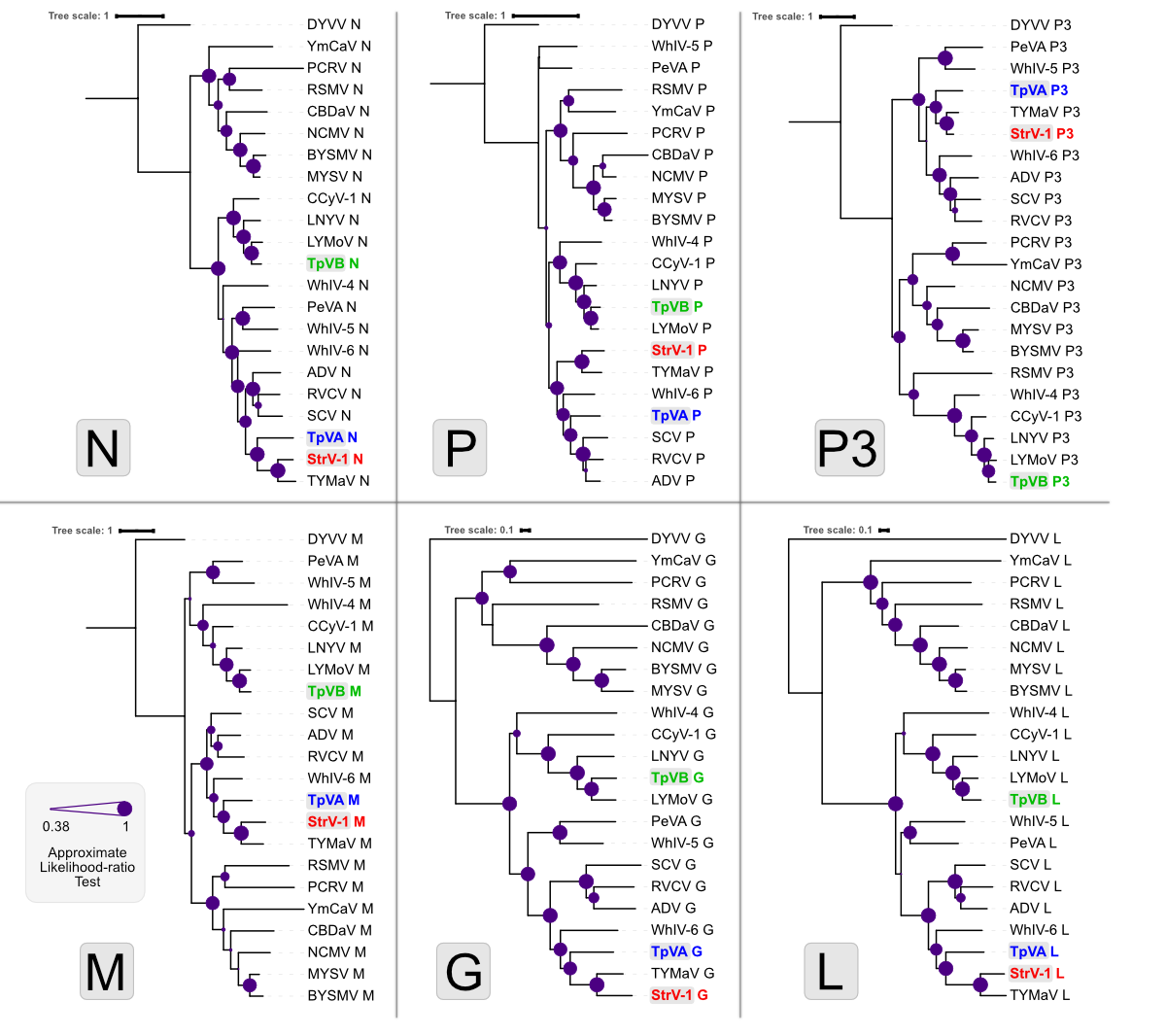
**Abstract**

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| Four novel cytorhabdoviruses infecting either strawberry (strawberry virus 1, StrV-1, and strawberry-associated virus 1, SaV-1) or red clover (Trifolium pratense virus A, TpVA, and Trifolium pratense virus B, TpVB) were described in 2019. Their molecular and biological properties were characterized. According to the species demarcation criteria, we propose that these four viruses should be classified as three new species in the genus *Cytorhabdovirus* and propose to:   * classify StrV-1 and SaV-1 in the new species *Strawberry cytorhabdovirus 1,* * TpVA in the new species *Trifolium pratense cytorhabdovirus A,* * TpVBin the new species *Trifolium pratense cytorhabdovirus B.* |

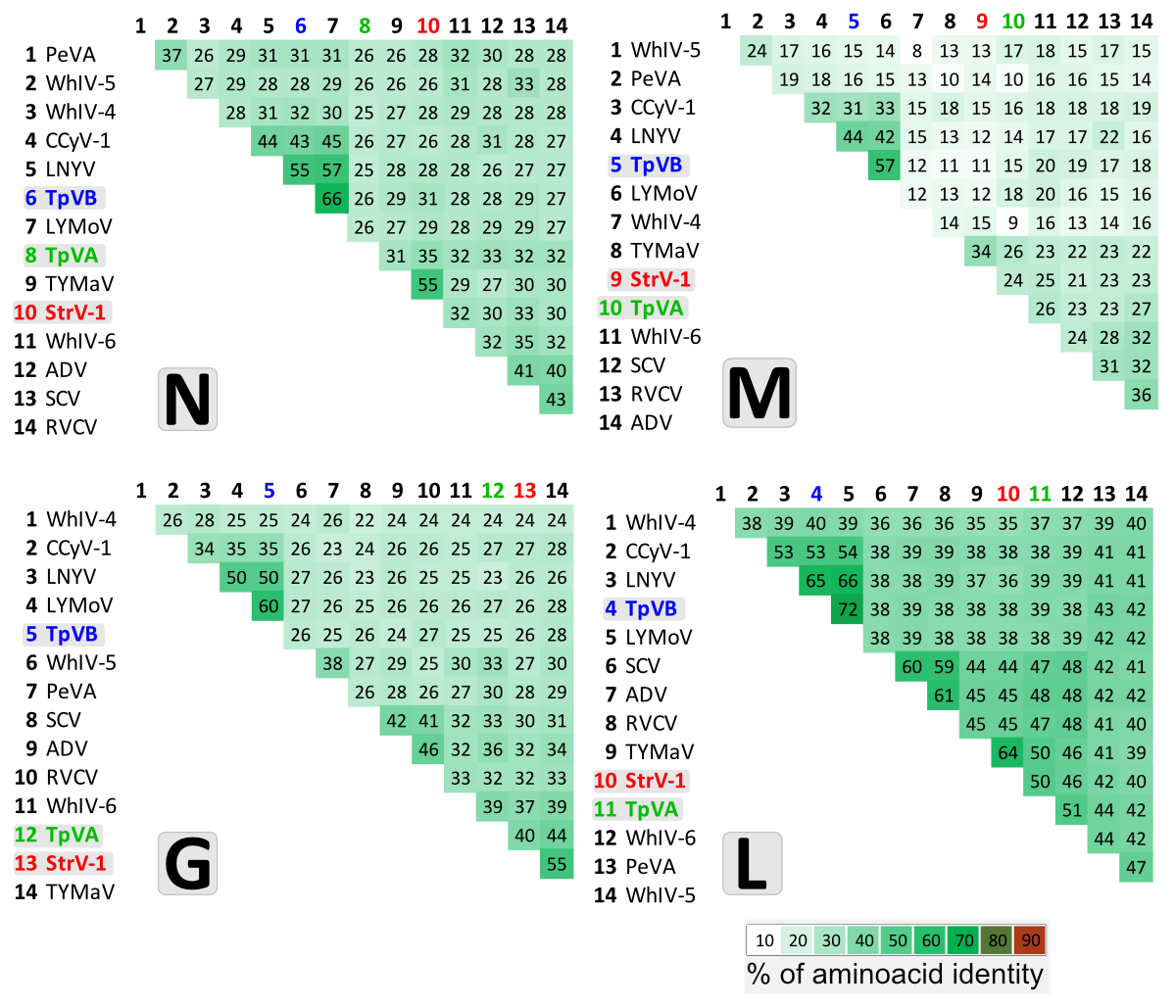
**Text of proposal**

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Three StrV-1 genotypes were identified in the Czech Republic (named CRM1A, CRM1B, and CRM1C). The Chinese isolate, SaV-1, shares 98.3% nucleotide (nt) sequence identity with the genome sequence of StrV-1 isolate CRM1A.  The complete genomes of StrV-1 and SaV1 are 14,162 (isolate CRM1, genotype A, MK211270), 14,028 (isolate CRM1, genotype B, MK211271), 14,255 (isolate CRM1, genotype C, MK211272), and 14,159 (SaV1, MK159261) nt long.  Five canonical CDSs are present, as in all known cytorhabdoviruses (N, P, M, G, and L), and in addition four accessory CDSs, P', P3, P6, P7 (Fig. 1). Notably, this is the first cytorhabdovirus with two small CDSs, P6 and P7, between G and L (compared to a single one in some other described cytorhabdoviruses, including SCV, another strawberry-infecting cytorhabdovirus).    **Figure 1** Genomic organization of StrV-1-CRM1, genotype A (MK211270). The nucleotide sequence is shown as a black line. Viral genome and complementary strand are labelled as *v* and *vc*, respectively. Open reading frames (ORFs, or CDSs) are drawn as grey arrows, and putative regulatory regions are shown as red vertical lines. Segments are drawn to scale.  Phylogenetic and sequence analyses that included other cytorhabdoviruses showed StrV-1 to be most closely related to tomato yellow mottle-associated virus (TYMaV) (Figs. 3 and 4 in supporting evidence). For instance, StrV-1 shared no more than 55% amino acid identities with other cytorhabdoviruses in the putative N, M, G, and L proteins (Fig. 4 in Supporting evidence). Overall shared nucleotide sequence identity between StrV-1 (MK211271) and TYMaV (NC\_034240) genomes is 55%.  Considering their similar host ranges, StrV-1 and SCV might share at least some of their arthropod vectors [2]. However, their sequence and genome organization differences clearly assign them to distinct species.  **Trifolium pratense virus A and Trifolium pratense virus B**  Trifolium pratense virus A (TpVA), and Trifolium pratense virus B (TpVB) are two new viruses identified in the Czech Republic infecting red clover (*Trifolium pratense*) [4]. Both viruses were initially detected using generic cytorhabdoviral primers and then sequenced using the Illumina platform. TpVA was further mechanically transmitted to *Nicotiana occidentalis* 37B and then to *Physalis floridana,* while the TpVB transmission attempts were not successful [4]. The virions of both TpVA and TpVB had cytoplasm-limited distribution [4].  The TpVA genome (12,430 nt, isolate 29/15/1, MH982250) was completely sequenced, while for TpVB (13,875 nt, isolate 1/2014, MH982249) there is the complete coding sequence.  The organization of TpVA and TpVB genomes is similar to that of other cytorhabdoviruses. There are eight and seven CDSs predicted for TpVA and TpVB, respectively. Five of them are canonical CDSs present in all known cytorhabdoviruses: N, P, M, G, and L. TpVA has P', P3, and P6 accessory CDSs and TpVB has P' and P3, but no P6 (Fig. 2).    **Figure 2** Genomic organization of TpVA (MH982250) and TpVB (MH982249). The nucleotide sequence is shown as a black line. Viral genome and complementary strand are labelled as *v* and *vc*, respectively. Open reading frames (ORFs, or CDSs) are drawn as grey arrows, and putative regulatory regions are shown as red vertical lines. Segments are drawn to scale.  Phylogenetic and sequence analyses that included other cytorhabdoviruses showed that TpVB is most closely related to lettuce yellow mottle virus (LYMoV) and lettuce yellow mottle yvirus (LYMoV), and TpVA is most closely related to a group of viruses that included StrV-1, TYMaV, and Wuhan insect virus 6 (Figs. 3, and 4). Furthermore, TpVA shared no more than 51% amino acid identity with other cytorhabdoviruses in any of the putative N, M, G, and L proteins (Fig. 4). TpVB shared no more than 72% amino acid identity with other cytorhabdoviruses in any of the putative N, M, G, and L proteins (Fig. 4).  Overall shared nucleotide identity between TpVA (MH982250) and TYMaV (NC\_034240), the most similar virus to TpVA, is 51%. Overall shared nucleotide identity for TpVB (MH982249) and LYMoV (NC\_011532) is 60%.  **Proposal**  Viruses assigned to different species within the genus *Cytorhabdovirus* have several of the following characteristics (according to [5] and the approved proposal 2016.017aM.A.v1.Cytorhabdovirus\_sp, https://talk.ictvonline.org/files/ictv\_official\_taxonomy\_updates\_since\_the\_8th\_report/m/plant-official/6691):   1. nucleotide sequence identity less than 75% for the complete genome sequence; 2. amino acid sequence identity in all cognate open reading frames less than 80%; 3. occupy different ecological niches as evidenced by differences in hosts and/or arthropod vectors.   Thus, based on the above-mentioned criteria and the provided data, we propose (Table 1)  to:   * classify **StrV-1** and SaV-1 in the new species *Strawberry cytorhabdovirus 1,* * classify **TpVA** in the new species *Trifolium pratense cytorhabdovirus A,* * classify **TpVB**in the new species *Trifolium pratense cytorhabdovirus B*  |  |  |  |  |  |  | | --- | --- | --- | --- | --- | --- | | **Table 1** Summary of three proposed species in the genus *Cytorhabdovirus* | | | | | | | **Species** | **Virus name(s)** | **Exemplar isolate** | **Exemplar accession number** | **Available sequence** | **Virus abbreviation** | | *Strawberry cytorhabdovirus 1* | strawberry virus 1 | CRM1B | MK211271 | Complete genome | StrV-1 | |  | strawberry-associated virus 1 |  |  | Complete genome | SaV-1 | | *Trifolium pratense cytorhabdovirus A* | Trifolium pratense virus A | 29/15/1 | MH982250 | Complete genome | TpVA | | *Trifolium pratense cytorhabdovirus B* | Trifolium pratense virus B | 1/2014 | MH982249 | Partial genome, complete coding sequence | TpVB | | |

**Supporting evidence**



**Figure 3** Phylogenetic analyses of the predicted protein sequences of the CRM isolate of **strawberry virus 1 (StrV-1, MK211271), Trifolium pratense virus A (TpVA, MH982250),** **Trifolium pratense virus B (TpVB, MH982249)** and other cytorhabdoviruses. Each tree is labelled with the corresponding CDS abbreviation. Multiple sequence alignments were constructed using the MUSCLE tool. The trees were constructed using maximum-likelihood method with minimum support and applying the Whelan and Goldman (WAG) substitution model. The acronyms are used as follows: alfalfa dwarf virus (ADV, NC\_028237); barley yellow striate mosaic cytorhabdovirus (BYSMV, NC\_028244); cabbage cytorhabdovirus 1 (CCyV-1, KY810772); colocasia bobone disease-associated virus (CBDaV, NC\_034551); datura yellow vein nucleorhabdovirus (DYVV, NC\_028231); lettuce necrotic yellows virus (LNYV, NC\_007642); lettuce yellow mottle virus (LYMoV, NC\_011532); maize yellow triate virus (MYSV, KY884303); northern cereal mosaic cytorhabdovirus (NCMV, NC\_002251); papaya cytorhabdovirus (PCRV, MH282832); Persimmon virus A (PeVA, NC\_018381); raspberry vein chlorosis virus (RVCV, MK257717); rice tripe mosaic virus (RSMV, NC\_040786); strawberry crinkle cytorhabdovirus (SCV, MH129615); tomato yellow mottle-associated virus (TYMaV, NC\_034240); Wuhan insect virus 4 (WhIV-4, NC\_031225); Wuhan insect virus 5 (WhIV-5, NC\_031227); Wuhan insect virus 6 (WhIV-6, NC\_031232); yerba mate chlorosis-associated virus (YmCaV, KY366322). A plant-infecting nucleorhabdovirus, DYVV, was used as outgroup. Scale bars refer to a phylogenetic distance expressed in amino acid substitutions per site. Support values are represented graphically. The figure is a modified version of Supplementary material from https://doi.org/10.3390/v11110982.



**Figure 4** Sequence amino acid identities between products of the N, M, G, and L genes (CDSs) of strawberry virus 1 (StrV-1, isolate CRM), Trifolium pratense virus A (TpVA), Trifolium pratense virus B (TpVB) and other cytorhabdoviruses. All other abbreviations are spelled out in Fig. 3.

**References**

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