

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

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| **Code assigned:** | **2020.013M** |  |
| **Short title:** Create two new species in the genus *Alphanucleorhabdovirus*, three new species in the genus *Betanucleorhabdovirus*, two new species in the genus *Cytorhabdovirus* and two new species in the genus *Varicosavirus* (*Mononegavirales*: *Rhabdoviridae*) | | |
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**List the ICTV Study Group(s) that have seen this proposal**

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| *Rhabdoviridae* Study Group |

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

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| Approved by all responding SG members (10 of 14) with minor revisions. |

**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 | 30 July 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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| 2020.013M.R.Plant\_rhabdoviruses\_9nsp.xlsx |

**Abstract**

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| Viruses classified in the family *Rhabdoviridae* infect vertebrates, invertebrates and plants. Nine new plant-infecting rhabdoviruses were discovered recently and their complete or coding-complete genomes determined. This proposal aims to taxonomically classify these 9 viruses in 9 new species in the genera *Alphanucleorhabdovirus*, *Betanucleorhabdovirus*, *Cytorhabdovirus* and *Varicosavirus*. |

**Text of proposal**

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| |  | | --- | | We propose the taxonomic classification of 9 novel plant-infecting rhabdoviruses in 9 new species in four established genera in the family *Rhabdoviridae*:  **Two new species in the genus *Alphanucleorhabdovirus***  1) **Peach virus 1 (PeV1)** was identified in peach (*Prunus persica* L.) trees from Shenzen, Guangzhou Province, China. The complete genome sequence of 13,949 nucleotides (isolate NSTT, MN520414) has been determined [1]. We propose to assign PeV1 to the new species *Peach alphanucleorhabdovirus* in the genus *Alphanucleorhabdovirus*.  The PeV1 genome is similar in organization to those of other alphanucleorhabdoviruses, containing the five canonical rhabdovirus structural protein genes (N, P, M, G and L) and an additional accessory gene between the P and M genes that likely encodes the viral cell-to-cell movement protein gene as in other members of the genus (**Figure 1**). Based on ML trees generated from complete L protein sequences, PeV1 falls in a well-supported cluster with other alphanucleorhabdoviruses (**Figure 2**). Genome sequence identity between PeV1 and other sequenced alphanucleorhabdoviruses ranges from 35.4–41.9%. Amino acid sequence identity between PeV1 and its closest relatives is 43.6% for N (maize Iranian mosaic virus), 41% for G (maize mosaic virus) and 42.7% for L (Physostegia chlorotic mottle virus).  2) Potato yellow dwarf virus (PYDV) is a pathogen of potato (*Solanum tuberosum*). Several strains of PYDV have been described based on vector specificity and symptom severity. Two strains distinguished by their differential transmission by leafhopper vectors *Aceratagallia sanguinolenta* and *Agallia constricta* have been studied in most detail. Historically they have been referred to as sanguinolenta yellow dwarf virus (SYDV) or PYDV-New York and **constricta yellow dwarf virus (CYDV)** or PYDV-New Jersey. A virus of the SYDV (PYDV-New York) strain is known as the exemplar isolate of PYDV and is classified in the type species *Potato yellow dwarf alphanucleorhabdovirus* in the genus *Alphanucleorhabdovirus*. The complete genome sequence (12,792 nucleotides, isolate PV-233, MT185675) of the CYDV (PYDV-New Jersey) strain was reported in 2017 [2] and shown to be sufficiently diverged to warrant its classification in a separate, new species, *Constricta yellow dwarf alphanucleorhabdovirus*.  The CYDV genome organization is the same as that of PYDV-New York (SYDV), containing the five canonical rhabdovirus structural protein genes (N, P, M, G and L) plus additional accessory genes X (unknown function; between N and P genes) and Y (movement protein gene; between P and M genes) [2] (**Figure 1**). Based on ML trees generated from complete L protein sequences, CYDV forms a well-supported cluster with other alphanucleorhabdoviruses, most closely related to PYDV, eggplant mottled dwarf virus and physostegia chlorotic mottle virus (**Figure 2**). Overall, the genomes of CYDV and PYDV-New York (SYDV) are 69% identical at the nucleotide level and amino acid sequence identity for the N, X, P, Y, M, G and L genes is 71%, 22%, 52%, 74%, 72%, 69% and 72%, respectively [2].  ----------  Viruses assigned to different species within the genus *Alphanucleorhabdovirus* have several of the following characteristics:   1. nucleotide sequence identity lower than 75% in the complete genome sequence; 2. occupy different ecological niches as evidenced by differences in hosts and/or arthropod vectors; and 3. can be clearly distinguished in serological tests or by nucleic acid hybridization.  * Both PeV1 and CYDV meet criteria A and B.   ----------  **Three new species in the genus *Betanucleorhabdovirus***  3) **Cardamom vein clearing virus (CdVCV)** was recently characterized in India and shown to be transmitted between cardamon (*Elettaria cardamomum*) plants by the aphid *Pentalonia caladii* [3]. The coding-complete genome (CCG) sequence (partial 3’ leader and 5’ trailer) of CdVCV is 13,392 nucleotides (isolate ATH, MN273311) and contains six ORFs in the order 3’-N-P-P3-M-G-L-5’ representing the five canonical rhabdovirus structural protein genes and the putative cell-to-cell movement protein gene P3 in the conserved location between P and M genes (**Figure 1**). The CCG nucleotide sequence of CdVCV is 46–55% identical to that of other betanucleorhabdoviruses with highest sequence identity with black currant associated nucleorhabdovirus (BCaRV; 55%) and datura yellow vein virus (DYVV) and the type member sonchus yellow net virus (SYNV) (52% each). Based on ML trees generated from complete L protein sequences (**Figure 2**), CdVCV forms a well-supported cluster with other betanucleorhabdoviruses including BCaRV, sowthistle yellow vein virus (SYVV) and SYNV.  4) **Apple rootstock virus A (ApRVA)** was isolated from apple (*Malus* spp.) trees in South Korea. The complete ApRVA genome consists of 14,043 nucleotides (MH778545) and contains seven ORFs encoding N, P, P3, M, G and L and an additional accessory protein P5 between the M and G genes [4] (**Figure 1**). The ApRVA genome is 47.5% identical to that of BCaRV. Based on ML trees generated from complete L protein sequences (**Figure 2**), ApRVA forms a well-supported cluster with other betanucleorhabdoviruses including BCaRV and SYNV [4].  5) **Zhuye pepper nucleorhabdovirus (ZPNPV)** was discovered among four novel viruses during high-throughput sequencing of the transcriptome and small RNAs of Green Sichuan Pepper (*Zanthoxylum armatum*) showing flower yellowing disease in China [5]. The complete genome of ZPNPV consists of 13,553 nucleotides (isolate ZPNu1, MH323437) and contains six ORFs in the conserved order 3’-N-P-P3-M-G-L-5’ (**Figure 1**). The ZPNPV genome is 69.5% and 67.5% identical to those of DYVV and SYNV, respectively. ZPNPV is most closely related to DYVV with amino acid sequence identities of 68% for N, 33.4% for P, 46.7% for P3, 37.9% for M, 49% for G and 49% for L. Based on ML trees generated from complete L protein sequences (**Figure 2**), ZPNPV forms a well-supported cluster with the betanucleorhabdoviruses, most closely related to DYVV and SYNV [5].  We propose to classify CdVCV, ApRVA and ZPNPV in the new species *Cardamon betanucleorhabdovirus*, *Apple betanucleorhabdovirus* and *Pepper betanucleorhabdovirus*, respectively, in the genus *Betanucleorhabdovirus*, family *Rhabdoviridae.*  --------  Viruses assigned to different species within the genus *Betanucleorhabdovirus* have several of the following characteristics:   1. nucleotide sequence identity lower than 75% in the complete genome sequence; 2. occupy different ecological niches as evidenced by differences in hosts and/or arthropod vectors; and 3. can be clearly distinguished in serological tests or by nucleic acid hybridization.  * CdVCV, ApRVA and ZPNPV meet criteria A and B.   ---------  **Two new species in the genus *Cytorhabdovirus***  6) **Trichosanthes associated rhabdovirus 1 (TrARV1)** was identified in the transcriptome of *Trichosanthes krilowii*, a flowering plant in the family Cucurbitaceae from Shenzhen, China [7]. The coding-complete TrARV1 genome sequence (partial 3’ leader and 5’ trailer) consists of 12,783 nucleotides (isolate Shenzhen, BK011194) and contains six ORFs in the conserved order 3’-N-P-P3-M-G-L-5’ (**Figure 1**). TrARV1 is most closely related to Wuhan insect virus 5 (WhIV5) and persimmon virus A (PVA). The TrARV1 CCG nucleotide sequence is 68.6% identical to that of WhIV5, 65.3% identical to PVA, 66% identical to CCyV1 and 67.6% identical to YmVA. In a BlastP search, TrARV1 N, P, G and L protein sequences are 61%, 48%, 54% and 61% identical to the cognate proteins of WhIV5, the most closely related cytorhabdovirus. Based on ML trees generated from complete L protein sequences (**Figure 2**), TrARV1 is placed within a subclade of cytorhabdoviruses, most closely related to WhIV5.  7) **Yerba mate virus A (YmVA)** was detected in yerba mate (*Ilex paraguariensis*) in Argentina [8]. The complete genome of YmVA consists of 14,961 nucleotides (isolate Gob. Virasoro, MN781667). Notably, eight open reading frames were identified, including two novel accessory genes, in the order 3′-N-P-P3-P4-M-G-L-P8-5′. YmVA genomic organization is unique since is the first rhabdovirus identified so far that contains an accessory gene between the L gene and the 5´ trailer [8]. Furthermore, YmVA glycoprotein is remarkably short (155 aa) and appears to be the smallest reported rhabdovirus glycoprotein [8]. BlastP searches of the deduced proteins encoded by each predicted ORF identified ORFs 1 and 7 as coding for the N and L proteins, respectively. These two proteins are 30% and 46% identical to the aligned portion of partial N and L proteins encoded by the unassigned putative cytorhabdovirus Iranian citrus ringspot-associated virus (IrCRSaV, only partial genomic sequences are available). None of the other predicted proteins had significant matches with any GenBank entries [8]. In ML phylogenetic trees, YmVA clusters with members of the genus *Cytorhabdovirus*, close to a second subclade of cytorhabdoviruses, apparently forming a distinct evolutionary lineage [8]. Host range and arthropod vector are unknown.  We propose to classify TrARV1 and YmVA in the new species *Trichosanthes cytorhabdovirus* and *Yerba mate cytorhabdovirus* in the genus *Cytorhabdovirus*, family *Rhabdoviridae.*  --------  Viruses assigned to different species within the genus *Cytorhabdovirus*have several of the following characteristics:   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.  * TrARV1 and YmVA meet criteria A, B and C.   **Two new species in the genus *Varicosavirus***  8) **Red clover associated varicosavirus (RCaVV)** was identified by high-throughput sequencing during an aetiological study of diseased red clover (*Trifolium pratense*) [9]. The complete RCaVV genome is bi-segmented with RNA1 of 6320 nucleotides and RNA2 of 4062 nucleotides (isolate HZ2, MF918568 and MF918569). RNA1 contains one large ORF for the L polymerase protein while RNA2 contains three ORFs for proteins of 49K, 47K and 20K (**Figure 1**). In contrast, RNA2 of the type member lettuce big-vein associated virus (LBVaV) contains five ORFs (**Figure 1**). The 49K protein shares 17% amino acid sequence identity with the putative CP of LBVaV and the N protein of maize associated cytorhabdovirus [9]. The 47K and 20K proteins have no significant sequence similarities with any other proteins on GenBank. The L protein of RCaVV has low level sequence similarity with L protein of other rhabdoviruses. In an ML tree based on complete L protein sequences (**Figure 2**), RCaVV forms a well-supported cluster with the only other two known varicosaviruses, LBVaV and Alopecurus myosuroides varicosavirus 1 (AMVV1) – see below.  9) **Alopecurus myosuroides varicosavirus 1 (AMVV1)** was identified by transcriptome sequencing in non-symptomatic, herbicide resistant Black-grass (*Alopecurus myosuroides*) in the United Kingdom [10]. AMVV1 genome is bi-segmented; the coding-complete sequences of RNA1 of 6552 nucleotides and RNA2 of 4066 nucleotides (LN713933 and LN713934) were determined by high-throughput sequencing. RNA1 encodes two proteins, like the type member LBVaV, a small 81 aa protein and the L polymerase protein which shows ~40% (BlastP) aa sequence identity with the cognate proteins of LBVaV and RCaVV. RNA2 contains three ORFs (similar to RCaVV) coding for proteins of 801aa, 327aa and 170aa (**Figure 1**). BlastX analysis of RNA2 of AMVV1 found low-homology matches with the coat protein of LBVaV and RCaVV. In an ML tree based on complete L protein sequences, AMVV1 forms a well-supported cluster with the only other two known varicosaviruses, LBVaV and RCaVV (**Figure 2**).  We propose to classify RCaVV and AMVV1 in the new species *Trifolium varicosavirus* and *Alopecurus varicosavirus, respectively* in the genus *Varicosavirus*, family *Rhabdoviridae.*  --------  Since there is only one recognized species in the genus *Varicosavirus*, species demarcation criteria will need to be set. We propose that viruses assigned to different species within the genus *Varicosavirus*have a minimum nucleotide sequence divergence of 50% in the Lprotein.   * LBVaV, RCaVV and AMVV1 meet this criterion.   --------- | |

**Supporting evidence**

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**Figure 1**. Genome graphs depicting architecture and gene products of proposed viruses to be included in species within genera *Cytorhabdovirus*, *Alphanucleorhabdovirus*, *Betanucleorhabdovirus* and *Varicosavirus*. The predicted coding sequences are shown in orange arrow rectangles, and start and end coordinates are indicated. Reference genomes of type members of each genus are shown for comparisons corresponding to lettuce necrotic yellows virus (LNYV), potato yellow dwarf virus (PYDV), sonchus yellow net virus (SYNV) and lettuce big-vein associated virus (LBVaV).   Abbreviations: N: nucleoprotein; P: phosphoprotein; P3: putative cell-to-cell movement protein; M: matrix protein; G: glycoprotein; L: RNA dependent RNA polymerase; Y: putative movement protein. Virus name abbreviations: Yerba mate virus A (YmVA), Trichosanthes associated rhabdovirus 1 (TrARV1), peach virus 1 (PeV1), constricta yellow dwarf virus (CYDV), cardamom vein clearing virus (CdVCV), apple rootstock virus A (ApRVA), Zhuye pepper nucleorhabdovirus (ZPNRV), red clover associated varicosavirus (RCaVV), and Alopecurus myosuroides varicosavirus 1 (AMVV1).

**テキスト が含まれている画像

自動的に生成された説明**

**Figure 2.** AMaximum Likelihood (ML) phylogenetic tree of plant-infecting rhabdovirus L polymerase protein sequences. Amino acid sequences were aligned using MAFFT version 7 and then the alignment was filtered using Gblocks 0.91b. The resulting alignment (793 amino acid positions remaining) was subjected to generate a phylogenetic tree using PhyML 3.0 with the best-fit model LG + G + I +F. Nine viruses potentially belonging to the new species are shown in red font. The newly reported L protein sequence of sowthistle yellow vein virus (the HWY65 isolate of an assigned species *Sowthistle yellow vein betanucleorhabdovirus*) [11] was also included in this analysis. The tree is rooted to two animal rhabdoviruses as outgroup. Numbers at the nodes indicate bootstrap support (100 replicates).

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