

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

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| **Code assigned:** | ***2023.008M*** |  |
| **Short title:** Create twelve new species in the genus *Cytorhabdovirus*, subfamily *Betarhabdovirinae* (*Mononegavirales: Rhabdoviridae*) | | |
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**Author(s) and email address(es)**

|  |  |
| --- | --- |
| Bejerman N, Debat H, Dietzgen RG, Kondo H, Ramos-González P, Whitfield AE, Walker PJ, Freitas-Astúa J | bejerman.nicolas@inta.gob.ar;  debat.humberto@inta.gob.ar;  r.dietzgen@uq.edu.au; hkondo@rib.okayama-u.ac.jp;  plrg1970@gmail.com;  [awhitfi@ncsu.edu](mailto:awhitfi@ncsu.edu);  [peter.walker@uq.edu.au](mailto:peter.walker@uq.edu.au);  juliana.astua@embrapa.br |

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

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**Corresponding author**

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| Bejerman N |

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

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| ICTV *Rhabdoviridae* Study Group |

**ICTV Study Group comments and response of proposer**

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| Minor corrections regarding the creation of new species. |

**ICTV Study Group votes on proposal**

|  |  |  |  |
| --- | --- | --- | --- |
| **Study Group** | **Number of members** | | |
| **Votes support** | **Votes against** | **No vote** |
| ICTV *Rhabdoviridae* Study Group | 10 | 0 | 4 |

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

|  |  |
| --- | --- |
| **Is any taxon name used here derived from that of a living person (Y/N)** | N |

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

**Submission dates**

|  |  |
| --- | --- |
| Date first submitted to SC Chair | June 23, 2023 |
| 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**

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| N/A |

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

**Name of accompanying Excel module**

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| 2023.008M.N.v1.Cytorhabdovirus\_12nsp.xlxs |

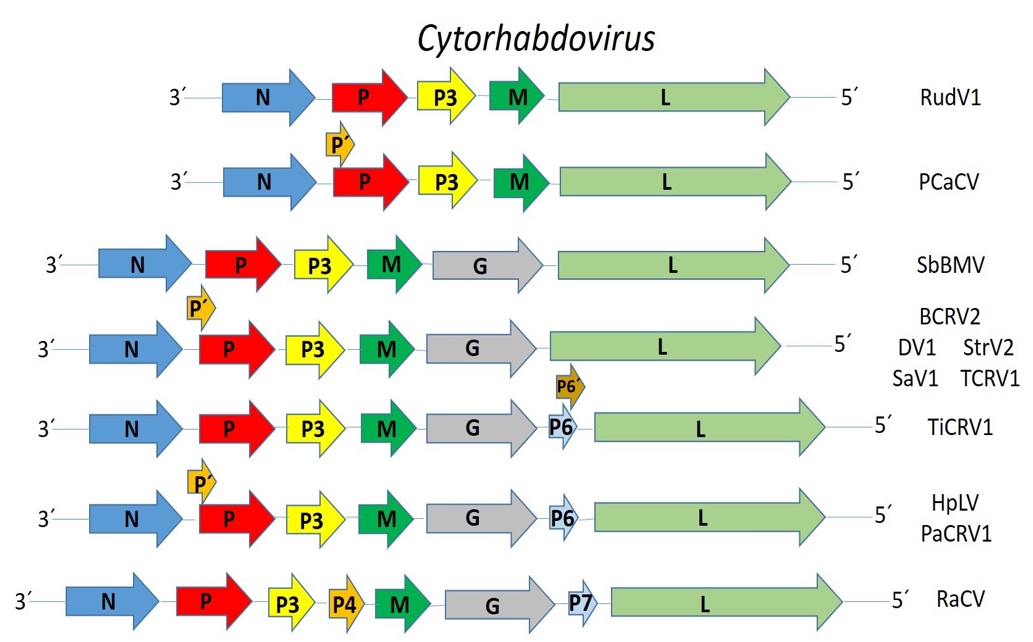
**Abstract**

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| Viruses classified in the family *Rhabdoviridae* infect vertebrates, invertebrates, and plants. Twelve new plant-infecting rhabdoviruses were discovered recently and their complete or coding-complete genomes were determined. This proposal aims to classify taxonomically these twelve viruses into twelve new species in the genus *Cytorhabdovirus.* |

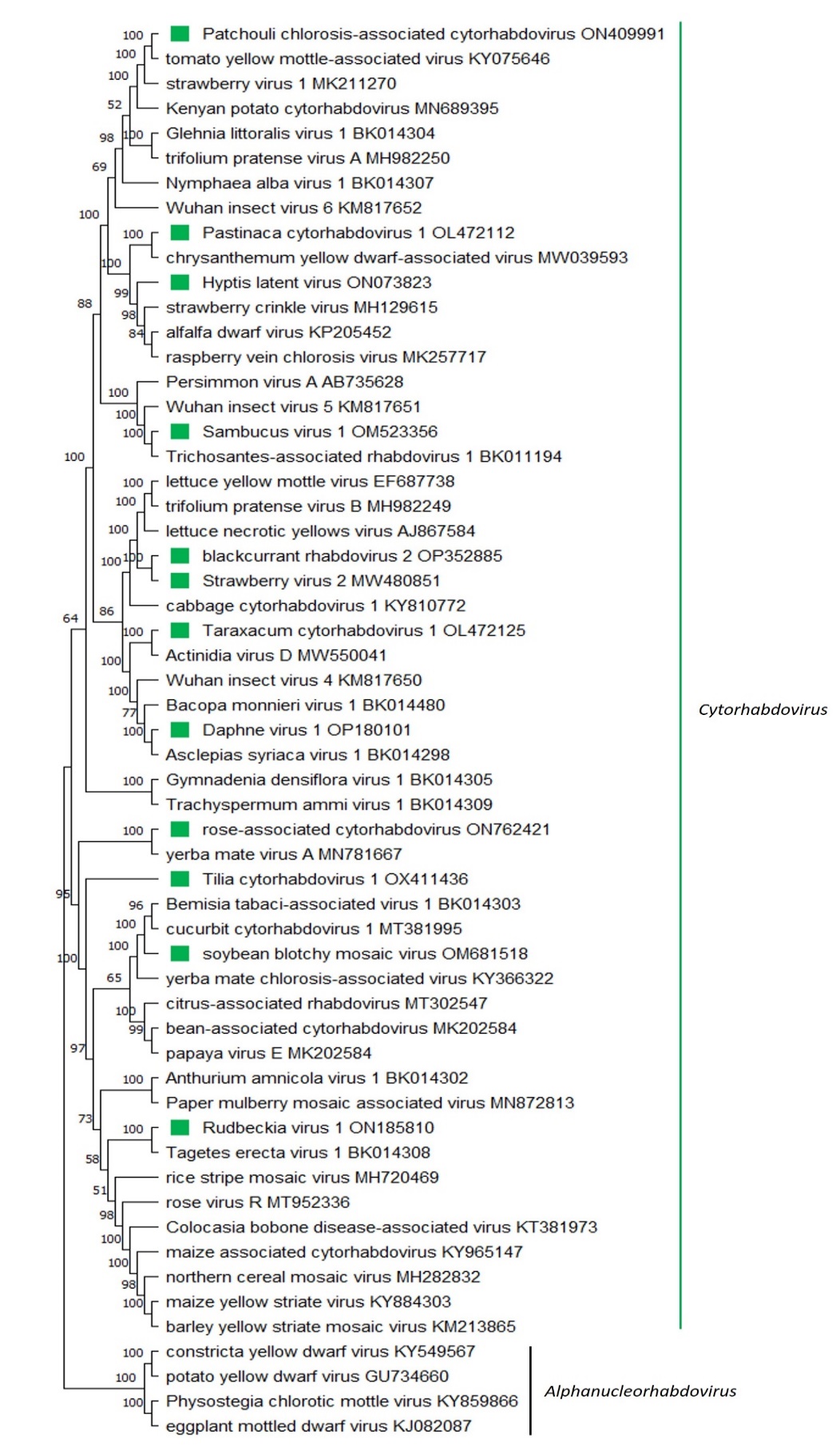
**Text of proposal**

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| |  | | --- | | We propose the taxonomic classification of twelve novel plant-infecting rhabdoviruses in twelve new species in the established genus *Cytorhabdovirus* in the subfamily *Betarhabdovirinae,* family *Rhabdoviridae*:  **1) Blackcurrant rhabdovirus 2 (BCRV2)** was identified in blackcurrant (*Ribes nigrum*) plants collected in the Czech Republic. The complete genome (CG) sequence of BCRV2 has 12,863 nucleotides (isolate KB1, OP352885) and contains seven ORFs in the order 3’-N-P´-P-P3-M-G-L-5’ [1] representing the five canonical rhabdovirus structural protein genes as well as the putative cell-to-cell movement protein gene P3 in the conserved location between P and M genes, and an overlapping ORF within the P-encoding ORF, which is named P´ (**Figure 1**). The CG nucleotide sequence of BCRV2 has the highest sequence identity with that of strawberry virus 2 (StrV2, 67%) [1], while the BCRV2 L protein amino acid sequence has the highest sequence identity with that of StrV2 (75%) [1]. Based on ML trees generated from complete L protein sequences BCRV2 forms a well-supported clade with the cytorhabdovirus StrV2 (**Figure 2**).  **2)** **Daphne virus 1 (DV1)** was identified in *Daphne odora* plants collected in South Korea. The complete genome (CG) sequence of DV1 has 13,206 nucleotides (isolate SK, OP180101) and contains seven ORFs in the order 3’-N- P´-P-P3-M-G-L-5’ [2] representing the five canonical rhabdovirus structural protein genes as well as the putative cell-to-cell movement protein gene P3 in the conserved location between P and M genes, and an overlapping ORF within the P-encoding ORF, which is named P´ (**Figure 1**). The CG nucleotide sequence of DV1 has the highest sequence identity with that of Asclepias syriaca virus 1 (AscSyV1, 57.4%) [2], while the DV1 L protein amino acid sequence has the highest sequence identity with that of AscSyV1 (57%) [2]. Based on ML trees generated from complete L protein sequences DV1 forms a well-supported clade with forms a well-supported clade with the cytorhabdovirus AscSyV1 (**Figure 2**).  **3)** **Hyptis latent virus (HpLV)** was identified in *Hyptis pectinata* plants collected in Ecuador. The complete genome (CG) sequence of HpLV has 13,765 nucleotides (isolate Prosperina, ON073823) and contains eight ORFs in the order 3’-N- P´-P-P3-M-G-P6-L-5’ [3] representing the five canonical rhabdovirus structural protein genes as well as the putative cell-to-cell movement protein gene P3 in the conserved location between P and M genes, an overlapping ORF within the P-encoding ORF, which is named P´, and the accessory protein P6 between the G and L genes (**Figure 1**). The CG nucleotide sequence of HpLV has the highest sequence identity with that of alfalfa dwarf virus (ADV, 53.4%), while the HpLV L protein amino acid sequence has the highest sequence identity with that of ADV (56.3%) [3]. Based on ML trees generated from complete L protein sequences HpLV is placed within a subclade of the cytorhabdoviruses, with ADV, raspberry vein chlorosis virus and strawberry crinkle virus (**Figure 2**).  **4)** **Pastinaca cytorhabdovirus 1 (PaCRV1)** was identified in *Pastinaca sativa* plants collected in Slovenia. The coding-complete genome (CCG) sequence of PaCRV1 has 13,072 nucleotides (isolate POR19SW, OL472112) and contains eight ORFs in the order 3’-N- P´-P-P3-M-G-P6-L-5’ [4] representing the five canonical rhabdovirus structural protein genes as well as the putative cell-to-cell movement protein gene P3 in the conserved location between P and M genes, an overlapping ORF within the P-encoding ORF, which is named P´, and the accessory protein P6 between the G and L genes (**Figure 1**). The CCG nucleotide sequence of PaCRV1 has the highest sequence identity with that of chrysanthemum yellow dwarf-associated virus (CYDaV, 61%) [4], while the PaCRV1 L protein amino acid sequence has the highest sequence identity with that of CYDaV (55.6%). Based on ML trees generated from complete L protein sequences PaCRV1 forms a well-supported clade with forms a well-supported clade with the cytorhabdovirus CYDaV (**Figure 2**).  **5)** **Patchouly chlorosis-associated cytorhabdovirus (PCaCV)** was identified in patchouly (*Pogostemon cablin*) plants collected in Pará, Brazil. The complete genome (CG) sequence of PCaCV has 12,913 nucleotides (isolate PA, ON409991) and contains six ORFs in the order 3’-N- P´-P-P3-M-L-5’ [5] representing four canonical rhabdovirus structural protein genes (N, P, M and L, the G gene was found to be defective), as well as the putative cell-to-cell movement protein gene P3 in the conserved location between P and M genes and the overlapping ORF within the P-encoding ORF, which is named P (**Figure 1**). The CG nucleotide sequence of PCaCV has the highest sequence identity with that of tomato yellow mottle-associated virus (TYMaV, 61.1%) [5], while the PCaCV L protein amino acid sequence has the highest sequence identity with that of TYMaV (73.9%) [5]. Based on ML trees generated from complete L protein sequences PCaCV forms a well-supported clade with forms a well-supported clade with the cytorhabdovirus TYMaV (**Figure 2**).  **6) Rose-associated cytorhabdovirus (RaCV)** was identified from the analysis of rose (*Rosa rubiginosa*) plants collected in Chongqing, China. The complete genome (CG) sequence of RaCV has 16,067 nucleotides (isolate SWU-X, ON762421), and contains eight ORFs in the order 3’-N-P-P3-P4-M-G-P7-L-5’ [6] (**Figure 1**). The CG nucleotide sequence of RaCV has the highest sequence identity with that of raspberry vein chlorosis virus (39.35%) [6], while the RaCV L protein amino acid sequence has the highest sequence identity with that of Yerba mate virus A (YmVA, 33.55%) [6]. Based on ML trees generated from complete L protein sequences, RaCV forms a well-supported clade with the cytorhabdovirus YmVA (**Figure 2**).  **7)** **Rudbeckia virus (RudV1)** was identified from the analysis of *Rudbeckia* sp. seeds from China. The complete genome (CG) sequence of RudV1 has 12,502 nucleotides (isolate PQ, ON185810), and contains five ORFs in the order 3’-N-P-P3-M-L-5’ [7] (**Figure 1**). The CG nucleotide sequence of RudV1 has the highest sequence identity with that of Tagetes erecta virus 1 (TaEV1; 51.7%) [7], while the RudV1 L protein amino acid sequence has the highest sequence identity with that of TaEV1 (56.8%) [7]. Based on ML trees generated from complete L protein sequences, RudV1 forms a well-supported clade with the cytorhabdovirus TaEV1 (**Figure 2**).  **8)** **Sambucus virus 1 (SaV1)** was identified in elderberry *(Sambucus nigra)* plants collected in the Czech Republic. The complete genome (CG) sequence of SaV1 has 12,622 nucleotides (isolate B15, OM523356) and contains seven ORFs in the order 3’-N- P´-P-P3-M-G-L-5’ [8] representing the five canonical rhabdovirus structural protein genes as well as the putative cell-to-cell movement protein gene P3 in the conserved location between P and M genes and an overlapping ORF within the P-encoding ORF, which is named P´(**Figure 1**). The CG nucleotide sequence of SaV1 has the highest sequence identity with that of Trichosanthes-associated rhabdovirus 1 (TrARV1, 63.5%) [8], while the SaV1 L protein amino acid sequence has the highest sequence identity with that of TrARV1 (69.5%) [8]. Based on ML trees generated from complete L protein sequences SaV1 is placed within a subclade of the cytorhabdoviruses, with TrARV1, Wuhan insect virus 5 and Persimmon virus A (**Figure 2**).  **9)** **Soybean blotchy mosaic virus (SbBMV)** was identified in soybean (*Glycine max*) plants collected in South Africa. The complete genome (CG) sequence of SbBMV has 13,425 nucleotides (isolate 21-0287, OM681518) and contains six ORFs in the order 3’-N- P-P3-M-G-L-5’ [9] representing the five canonical rhabdovirus structural protein genes as well as the putative cell-to-cell movement protein gene P3 in the conserved location between *P* and *M* genes (**Figure 1**). The CG nucleotide sequence of SbBMV has the highest sequence identity with that of Bemisia tabaci-associated virus 1(BeTaV1, 67%), while the SbBMV L protein amino acid sequence has the highest sequence identity with that of BeTaV1 (68.9%). Based on ML trees generated from complete L protein sequences SbBMV is placed within a subclade of the cytorhabdoviruses, with BeTaV1 and cucurbit cytorhabdovirus 1(**Figure 2**).  **10)** **Strawberry virus 2 (StrV2)** was identified in strawberry plants collected in USA. The complete genome (CG) sequence of StrV2 has 12,956 nucleotides (isolate CFRA9094, MW480851) and contains seven ORFs in the order 3’-N- P´-P-P3-M-G-L-5’ [10] representing the five canonical rhabdovirus structural protein genes as well as the putative cell-to-cell movement protein gene P3 in the conserved location between *P* and *M* genes and an overlapping ORF within the P-encoding ORF, which is named P´ (**Figure 1**). The CG nucleotide sequence of StrV2 has the highest sequence identity with that of blackcurrant cytorhabdovirus 2 (BCRV2, 67%), while the StrV2 L protein amino acid sequence has the highest sequence identity with that of BCRV2 (75%). Based on ML trees generated from complete L protein sequences StrV2 forms a well-supported clade with the cytorhabdovirus BCRV2 (**Figure 2**).  **11)** **Taraxacum cytorhabdovirus 1 (TCRV1)** was identified in *Taraxacum officinale* plants collected in Slovenia. The coding-complete genome (CCG) sequence of TCRV1 has 13,718 nucleotides (isolate PLE20SW, OL472125) and contains seven ORFs in the order 3’-N- P´-P-P3-M-G-L-5’ [4] representing the five canonical rhabdovirus structural protein genes as well as the putative cell-to-cell movement protein gene P3 in the conserved location between P and M genes and an overlapping ORF within the P-encoding ORF, which is named P´ (**Figure 1**). The CCG nucleotide sequence of TCRV1 has the highest sequence identity with that of Actinidia virus D (AcVD, 60%) [4], while the TCRV1 L protein amino acid sequence has the highest sequence identity with that of AcVD (54.4%). Based on ML trees generated from complete L protein sequences TCRV1 forms a well-supported clade with the cytorhabdovirus AcVD (**Figure 2**).  **12)** **Tilia cytorhabdovirus 1 (TiCRV1)** was identified in *Tilia cordata* plants collected in Germany. The coding-complete genome (CCG) sequence of TiCRV1 has 14,152 nucleotides (isolate E54634, OX411436) and contains eight ORFs in the order 3’-N-P-P3-M-G-P6-P6´-L-5’ [11] representing the five canonical rhabdovirus structural protein genes as well as the putative cell-to-cell movement protein gene P3 in the conserved location between P and M genes, and the accessory proteins P6 and P6´ between the G and L genes (**Figure 1**). The CCG nucleotide sequence of TiCRV1 has the highest sequence identity with that of Colocasia bobone disease-associated virus (CBDaV, 45.1%), while the TiCRV1 L protein amino acid sequence has the highest sequence identity with that of CBDaV (36.1%). Based on ML trees generated from complete L protein sequences TiCRV1 forms a well-supported clade with other cytorhabdoviruses (**Figure 2**).  We propose to classify BCRV2, DV1, HpLV, PaCRV1, PCaCV, RaCV, RudV1, SaV1, SbBMV, StrV2, TCRV1, and TiCRV1 in the new species *Cytorhabdovirus ribes*, *Cytorhabdovirus daphnis,* *Cytorhabdovirus hyptisis*, *Cytorhabdovirus pastinacae,* *Cytorhabdovirus pogostemi*, *Cytorhabdovirus betarosae*, *Cytorhabdovirus rudbeckiae*, *Cytorhabdovirus sambuci, Cytorhabdovirus glycinis,* *Cytorhabdovirus betafragariae, Cytorhabdovirus taraxaci* and *Cytorhabdovirus tiliae,* respectively, in the genus *Cytorhabdovirus,* subfamily *Betarhabdovirinae,* 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 less than 80% in proteins encoded by all the cognate open reading frames; 3. occupy different ecological niches as evidenced by differences in hosts and/or arthropod vectors.   BCRV2, DV1, HpLV, PaCRV1, PCaCV, RaCV, RudV1, SaV1, SbBMV, StrV2, TCRV1, and TiCRV1 meet criteria A, B and C. | |

**Supporting evidence**

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**Figure 1**. Genome graphs depicting architecture and gene products of viruses to be included in proposed species within genus C*ytorhabdovirus*.Abbreviations: N: nucleoprotein; P: phosphoprotein; P´; protein P´ P3: putative cell-to-cell movement protein; P4: protein 4; M: matrix protein; G: glycoprotein; P6: Protein 6; P6¨: Protein 6´; P7: Protein 7 L: RNA-dependent RNA polymerase. Virus name abbreviations: blackcurrant rhabdovirus 2 (BCRV2), Daphne virus 1 (DV1), Hyptis latent virus (HpLV), Pastinaca cytorhabdovirus 1 (PaCRV1), Patchouly chlorosis-associated cytorhabdovirus (PCaCV), rose-associated cytorhabdovirus (RaCV), Rudbeckia virus 1 (RudV1), Sambucus virus 1 (SaV1), soybean blotchy mosaic virus (SbBMV), strawberry virus 2 (StrV2), Taraxacum cytorhabdovirus 1 (TCRV1), and Tilia cytorhabdovirus 1 (TiCRV1).

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**Figure 2.** AMaximum Likelihood (ML) phylogenetic tree of plant-infecting rhabdovirus L polymerase protein sequences. Amino acid sequences were aligned using MUSCLE. The resulting alignment was used to generate a phylogenetic tree using MegaX with the best-fit model LG + G + I +F. Ten viruses potentially belonging to the new species are indicated with green squares. Numbers at the nodes indicate bootstrap support (1000 replicates).

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