* This Word module should be used for all taxonomic proposals.

Please complete **Part 1** and:

either **Part 3** for proposals to create new taxa or change existing taxa

or **Part 2** for proposals of a general nature.

Submit the completed Word module, together with the accompanying Excel module named in Part 3, to the appropriate ICTV Subcommittee Chair.

The Word module explains and justifies your proposal. The Excel module is a critical document that will be used to implement the proposed taxonomic changes once they are approved and ratified. If proposals presented in the Word module are not presented accurately in the Excel module, the taxonomic changes cannot proceed.

For guidance, see the notes written in blue, below, and the Help Notes in file Taxonomic\_Proposals\_Help\_2019.

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

|  |  |  |  |
| --- | --- | --- | --- |
| **Code assigned:** | ***2019.031M*** | |  |
| **Short title:** Split the genus *Nucleorhabdovirus,* creating three new genera (*Alphanucleorhabdovirus, Betanucleorhabdovirus* and *Gammanucleorhabdovirus*) comprising sixteen species, including six new species, in the family *Rhabdoviridae.* | | | |
|  | | | |
| **Author(s) and email address(es):** | | | |
| List authors in a single line *Archives of Virology* citation format (e.g. Smith AB, Huang C-L, Santos, F) | | Provide email address for each author in a single line separated by semi-colons | |
| ICTV *Rhabdoviridae* Study Group:  Freitas-Astúa J  Dietzgen RG  Walker PJ  Blasdell KR  Breyta R  Fooks AR  Kondo H  Kurath G  Kuzmin IV  Stone DM  Tesh RB  Tordo N  Vasilakis N  Whitfield AE  and  Ramos-González PL | | [Juliana.astua@embrapa.br](mailto:Juliana.astua@embrapa.br); [r.dietzgen@uq.edu.au](mailto:r.dietzgen@uq.edu.au);  peter.walker@uq.edu.au;  [kim.blasell@csiro.au](mailto:kim.blasell@csiro.au);  [Tony.Fooks@apha.gov.uk](mailto:Tony.Fooks@apha.gov.uk);  [hkondo@okayama-u.ac.jp](mailto:hkondo@okayama-u.ac.jp); [gkurath@usgs.gov](mailto:gkurath@usgs.gov);  [ivkuzmin@yandex.ru](mailto:ivkuzmin@yandex.ru); [David.stone@cefas.co.uk](mailto:David.stone@cefas.co.uk);  [rtesh@utmb.edu](mailto:rtesh@utmb.edu);  [ntordo@pasteur.fr;](mailto:ntordo@pasteur.fr;%20)  nivasila@utmb.edu;  [awhitfi@ncsu.edu](mailto:awhitfi@ncsu.edu);  plrg1970@gmail.com | |
| **Author(s) institutional address(es) (optional):**   |  | | --- | | Provide institutional addresses, each on a single line followed by author(s) initials (e.g. University of Woolloomooloo [SAB, HCL]) | | Embrapa Cassava and Fruits, Brazil [JFA]  University of Queensland, Australia [RGD, PJW]  University of Washington, WA, USA [RB]  CSIRO Australian Animal Health Laboratory, Geelong, Australia [KRB]  Animal and Plant Health Agency (APHA), Weybridge, Surrey, UK [ARF]  Okayama University, Kurashiki, Japan [HK]  Western Fisheries Research Center, Seattle, WA, USA [GK]  US Department of Agriculture, National Veterinary Services Laboratories [IVK]  Centre for Environment, Fisheries and Aquaculture Science, Weymouth, UK [DMS]  University of Texas Medical Branch, Galveston, TX, USA [RBT, NV]  Institut Pasteur de Guinée, Conakry, French Guiana [NT]  North Carolina State University, Raleigh, NC, USA [AEW]  Instituto Biológico, São Paulo, Brazil [PLRG] | | | | |
| **Corresponding author** | | | |
| Juliana Freitas-Astúa; [Juliana.astua@embrapa.br](mailto:Juliana.astua@embrapa.br) | | | |
| **List the ICTV study group(s) that have seen this proposal:** | | | |
| A list of study groups and contacts is provided at <http://www.ictvonline.org/subcommittees.asp> . If in doubt, contact the appropriate subcommittee chair (there are six virus subcommittees: animal DNA and retroviruses, animal ssRNA-, animal ssRNA+, fungal and protist, plant, bacterial and archaeal) | | ICTV *Rhabdoviridae* Study Group | |
| **ICTV Study Group comments (if any) and response of the proposer:** | | | |
|  | | | |
|  | | | |
| Date first submitted to ICTV: | | |  |
| Date of this revision (if different to above): | | |  |

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

**Part 2:** **PROPOSED TAXONOMY**

|  |
| --- |
| **Name of accompanying Excel module:** 2019.031M.A.v1.Nucleorhabdovirus\_splitgen.xlsx |

**Supporting material:**

Viruses classified in the family *Rhabdovirida*e are highly diverse in the hosts they infect, including vertebrates, invertebrates and plants. Currently, plant rhabdoviruses are classified into four genera *Nucleorhabdovirus*, *Cytorhabdovirus*, *Dichorhavirus* and *Varicosavirus.* Nucleorhabdoviruses have been distinguished from members of the other genera by their unsegmented genome and by using the nucleus of infected cells as their intracellular site of replication and assembly. This proposal addresses the splitting of genus *Nucleorhabdovirus* in three new genera *Alphanucleorhabdovirus, Betanucleorhabdovirus* and *Gammanucleorhabdovirus*. This reclassification has become necessary since phylogenetic analyses of new rhabdovirus genomes have consistently shown that nucleorhabdoviruses do not form a monophyletic clade upon complete L protein sequence alignments (Fig. 1). From the ten assigned species within genus *Nucleorhabdovirus*, six will be accommodated in genus *Alphanucleorhabdovirus,* three in genus *Betanucleorhabdovirus* and one in genus *Gammanucleorhabdovirus.* Finally, six currently unassigned rhabdoviruses will be assigned to six new species within the first two new genera (three to each).

**Genus *Alphanucleorhabdovirus***

From the ten species currently assigned within the genus *Nucleorhabdovirus,* six will be moved to the new genus *Alphanucleorhabdovirus*.Therefore, we propose that the species *Eggplant mottled dwarf nucleorhabdovirus, Maize Iranian mosaic nucleorhabdovirus, Maize mosaic nucleorhabdovirus, Potato yellow dwarf nucleorhabdovirus, Rice yellow stunt nucleorhabdovirus* and *Taro vein chlorosis nucleorhabdovirus* shall be reassigned as *Eggplant mottled dwarf alphanucleorhabdovirus, Maize Iranian mosaic alphanucleorhabdovirus, Maize mosaic alphanucleorhabdovirus, Potato yellow dwarf alphanucleorhabdovirus, Rice yellow stunt alphanucleorhabdovirus* and *Taro vein chlorosis alphanucleorhabdovirus*. We also propose *Potato yellow dwarf alphanucleorhabdovirus* as the type species of the new genus, since it was the type species of the genus *Nucleorhabdovirus* and more biological and molecular data are available for this virus.

In addition to those reassigned species, we propose the creation of three new species within the genus *Alphanucleorhabdovirus* to accommodate the following viruses:

**Wheat yellow striate virus (WYSV)**

WYSV was identified as the causal agent of unusual yellow stripes on leaves and slight dwarfism on wheat plants (*Triticum aestivum* L.) in Hancheng, Shaanxi Province, Northwest China (Liu et al., 2018). Viruliferous leafhoppers of the species *Psammotettix alienus* were allowed to infect wheat seedling from where bullet-shaped viral particles were observed in the nucleus of the infected leave cells. Virions possess 180–210 nm length and 35–40 nm width (Liu et al., 2018). The full-length negative-sense RNA genome of WYSV contains 14,486 nt (GenBank accession MG604920).

**Morogoro maize-associated virus (MMaV)**

MMaV was detected in maize (*Zea mays*) plants showing symptoms of lethal necrosis disease i.e. mosaic and stunting, in Morogoro, Tanzania (Read et al., 2019). Plants were co-infected with maize chlorotic mottle virus (MCMV), assigned to a species within genus *Machlomovirus*, family *Tombusvididae,* and maize yellow mosaic virus (MaYMV), assigned to a species within genus *Polerovirus*, family *Luteoviridae*. Therefore, symptoms of a single infection by MMaV in maize are not well defined yet. Three complete genome sequences of MMaV have been obtained, comprising 12,185 nt (GenBank accession numbers MK063878 and MK063879) and 12,187 nt long (MK112501). The average nucleotide sequence identity between each gene of the three MMaV genomes is higher than 95%. There is no information available about the viral vector (Read et al., 2019).

**Physostegia chlorotic mottle virus (PhCMoV)**

PhCMoV isolates have been identified from *Physostegia* sp. (family Lamiaceae) and tomato (*Solanum lycopersicum*) infected plants. An infected physostegia plant, collected in Austria, showed severe chlorotic mottle symptoms and leaf deformation (Gaafar et al., 2017). A diseased tomato plant, collected in Hesse, Germany, exhibited marbling and discolored fruits, but not obvious symptoms in its leaves. Upon mechanical transmission, PhCMoV infects *Nicotiana benthamiana*, *N. clevelandii*, and *Chenopodium quinoa* inducing yellowing and leaf deformation.

The three known complete genomes of PhCMoV share >95% nt sequence identity. PhCMoV isolate PV-1182, from *Physostegia* sp. (accession KX636164) comprises 13,320 nt, whereas isolates PV-0831 (accession KY706238) and HZ15-192 (accession KY859866) comprise 13,321 nt. Although PhCMoV shares 71-72% nt identity with EMDV, the bullet-shaped particles of this virus do not react with the antiserum JKI-1073 for EMDV in immunosorbent electron microscopy assays. Putative viral vectors have not been identified yet (Gaafar et al., 2017).

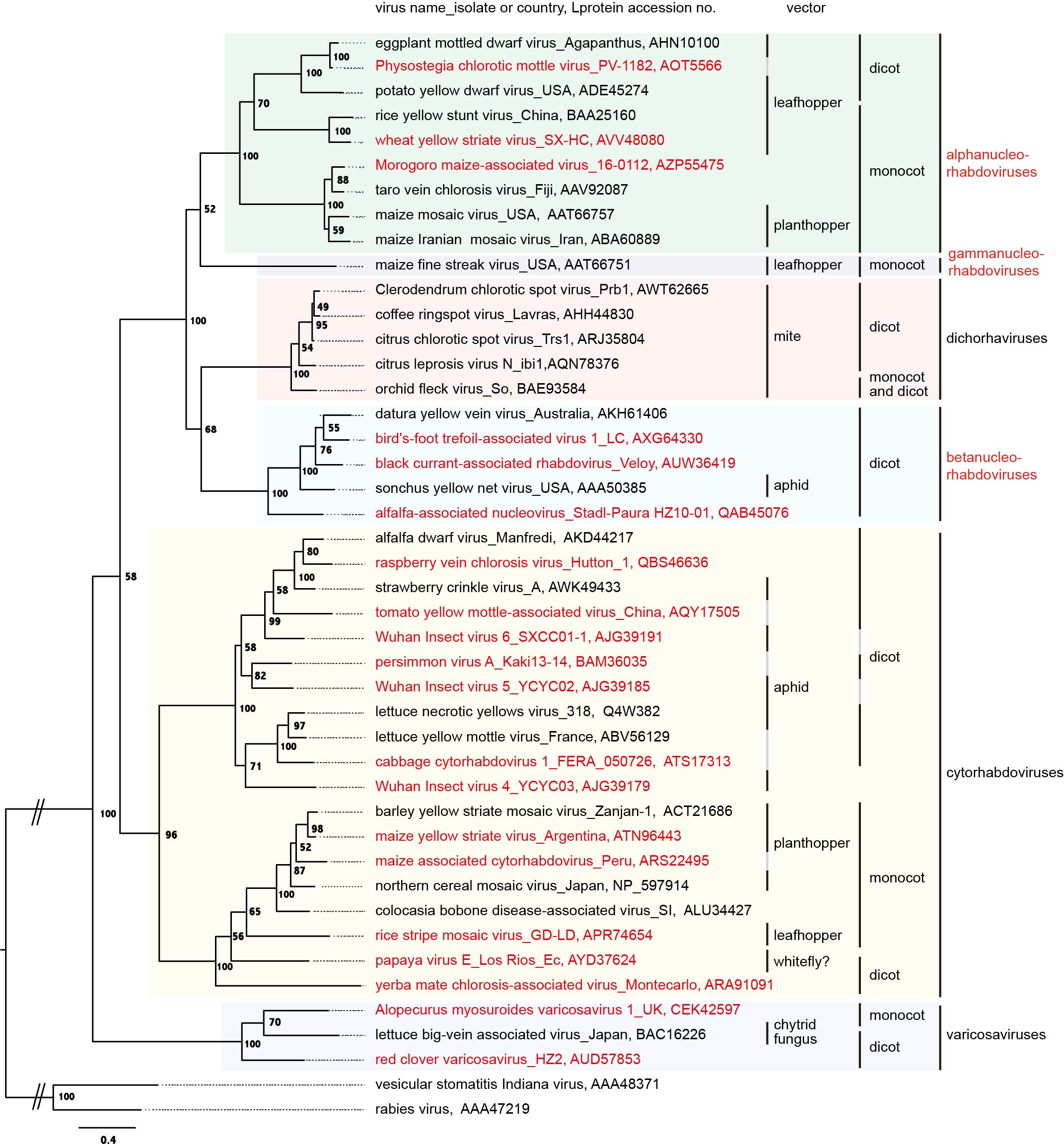
Based on the nucleotide and deduced amino acid sequence information compiled in Tables 1 to 5, we propose to classify WYSV, MMaV and PhCMoV to the new species *Wheat yellow striate alphanucleorhabdovirus, Morogoro maize-associated alphanucleorhabdovirus* and *Physostegia chlorotic mottle alphanucleorhabdovirus* in the genus *Alphanucleorhabdovirus*, family *Rhabdoviridae*.

The genomes of the proposed alphanucleorhabdoviruses contain six or seven ORF in the typical 3´ N-P-P3-M-G-L 5´ (or N-P-P3-M-P6-G-L, when the extra ORF of unknown function is present) genome organization including highly conserved intergenic regions and complementarity between the leader and trailer sequences.

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.

EMDV, MIMV, MMV, PYDV, RYSV, TaVCV, WYSV, and MMaV fulfill species demarcation criteria A and B, while PhCMoV fulfills all species demarcation criteria.

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**Figure 1.** A Maximum Likelihood phylogenetic tree of plant-adapted rhabdovirus L polymerase protein sequences. Amino acid sequences were aligned using MAFFT version 7 (Katoh et al., 2017) and then the alignment was filtered using Gblocks 0.91b (Talavera and Castresana 2007). The resulting alignment (783 amino acid positions remaining) was subjected to generate a phylogenetic tree using PhyML 3.0 (Guindon et al., 2010) with the best-fit model LG + G + I +F. For each genus, viruses belonging to either the proposed species or currently unassigned, related viruses are indicated by red color. The tree is rooted to two animal rhabdoviruses as outgroup. Numbers at the nodes indicate bootstrap support (100 replicates).

Table 1. Complete genome RNAalphanucleorhabdoviruses. Percent Identity Matrix - created in Clustal 2.1

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Virus | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
| 1 | PhCMoV\_PV-1182\_RNA\_KX636164.1 | 100 |  |  |  |  |  |  |  |  |  |  |  |  |
| 2 | PhCMoV\_PV-0831\_RNA\_KY706238.1 | 96.92 | 100 |  |  |  |  |  |  |  |  |  |  |  |
| 3 | PhCMoV\_HZ15-192\_RNA\_KY859866.1 | 96.91 | 99.76 | 100 |  |  |  |  |  |  |  |  |  |  |
| 4 | EMDV\_Agapanthus\_RNA\_KJ082087.1 | 71.8 | 71.85 | 71.83 | 100 |  |  |  |  |  |  |  |  |  |
| 5 | PYDV\_SYDV\_RNA\_GU734660.1 | 54.88 | 54.74 | 54.71 | 53.73 | 100 |  |  |  |  |  |  |  |  |
| 6 | MMaV\_16-0112\_RNA\_MK063878.2 | 42.37 | 42.36 | 42.33 | 42.23 | 42.46 | 100 |  |  |  |  |  |  |  |
| 7 | MMaV\_16-0114\_RNA\_MK063879.2 | 42.18 | 42.16 | 42.16 | 42.19 | 42.37 | 96.68 | 100 |  |  |  |  |  |  |
| 8 | MMaV\_16-0121\_RNA\_MK112501.2 | 42.23 | 42.2 | 42.2 | 42.22 | 42.39 | 96.64 | 99.57 | 100 |  |  |  |  |  |
| 9 | TaVCV\_RNA\_AY674964.1 | 41.83 | 41.7 | 41.69 | 41.35 | 41.36 | 66.13 | 66.06 | 66.11 | 100 |  |  |  |  |
| 10 | MIMV\_RNA\_MF102281.1 | 42.19 | 41.99 | 41.97 | 42.23 | 41.9 | 60.38 | 60.19 | 60.2 | 59.17 | 100 |  |  |  |
| 11 | MMV\_RNA\_AY618418.1 | 41.9 | 41.9 | 41.89 | 41.72 | 41.65 | 59.96 | 60.06 | 60.14 | 58.9 | 58.87 | 100 |  |  |
| 12 | WYSV\_RNA\_MG604920.1 | 41.52 | 41.67 | 41.58 | 41.72 | 41.45 | 40.99 | 41.01 | 41.06 | 40.6 | 40.98 | 40.28 | 100 |  |
| 13 | RYSV\_RNA\_AB011257.1 | 40.97 | 41.16 | 41.12 | 41.49 | 41.7 | 40.86 | 40.89 | 40.9 | 40.71 | 41.3 | 40.63 | 60.37 | 100 |

Table 2. N ORF alphanucleorhabdoviruses. Percent Identity Matrix - created in Clustal 2.1

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Virus | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
| 1 | PhCMoV\_PV-1182\_N\_KX636164.1 | 100 |  |  |  |  |  |  |  |  |  |  |  |  |
| 2 | PhCMoV\_PV-0831\_N\_KY706238.1 | 96.44 | 100 |  |  |  |  |  |  |  |  |  |  |  |
| 3 | PhCMoV\_HZ15-192\_N\_KY859866.1 | 96.44 | 99.58 | 100 |  |  |  |  |  |  |  |  |  |  |
| 4 | EMDV\_Agapanthus\_N\_KJ082087.1 | 73.07 | 72.93 | 72.93 | 100 |  |  |  |  |  |  |  |  |  |
| 5 | PYDV\_SYDV\_N\_GU734660.1 | 54.46 | 54.31 | 54.24 | 54.51 | 100 |  |  |  |  |  |  |  |  |
| 6 | MMaV\_16-0112\_N\_MK063878.2 | 42.55 | 42.47 | 42.55 | 41.63 | 42.5 | 100 |  |  |  |  |  |  |  |
| 7 | MMaV\_16-0114\_N\_MK063879.2 | 42.4 | 42.63 | 42.7 | 41.48 | 42.27 | 96.71 | 100 |  |  |  |  |  |  |
| 8 | MMaV\_16-0121\_N\_MK112501.2 | 42.32 | 42.55 | 42.63 | 39.87 | 42.27 | 96.56 | 99.85 | 100 |  |  |  |  |  |
| 9 | TaVCV\_N\_AY674964.1 | 40.16 | 40.09 | 40.09 | 39.77 | 40.96 | 71.79 | 71.71 | 71.57 | 100 |  |  |  |  |
| 10 | MMV\_N\_AY618418.1 | 40.87 | 40.64 | 40.56 | 40.73 | 41.52 | 63.85 | 63.85 | 63.77 | 63.06 | 100 |  |  |  |
| 11 | MIMV\_N\_MF102281.1 | 40.58 | 40.1 | 40.95 | 41.46 | 41.25 | 62.33 | 62.26 | 62.19 | 61.35 | 60.71 | 100 |  |  |
| 12 | WYSV\_N\_MG604920.1 | 43.16 | 43.61 | 43.46 | 42.19 | 42.57 | 42.35 | 42.12 | 42.2 | 42.69 | 40.54 | 40.68 | 100 |  |
| 13 | RYSV\_N\_AB011257.1 | 41.18 | 41.32 | 41.25 | 40.63 | 42.56 | 41.75 | 42.05 | 42.05 | 42.09 | 43.34 | 42.29 | 61.55 | 100 |

Table 3. N protein alphanucleorhabdoviruses. Percent Identity Matrix - created in Clustal 2.1

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Virus | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
| 1 | PhCMoV\_N\_AOT55656.1 | 100 |  |  |  |  |  |  |  |  |  |  |  |  |
| 2 | PhCMoV\_N\_AUY61878.1 | 99.37 | 100 |  |  |  |  |  |  |  |  |  |  |  |
| 3 | PhCMoV\_N\_ARU76996.1 | 99.37 | 99.16 | 100 |  |  |  |  |  |  |  |  |  |  |
| 4 | EMDV\_N\_AHN10094.1 | 82.98 | 82.98 | 82.87 | 100 |  |  |  |  |  |  |  |  |  |
| 5 | PYDV\_N\_ADE45268.1 | 46.98 | 46.98 | 46.77 | 48.06 | 100 |  |  |  |  |  |  |  |  |
| 6 | MMaV\_N\_AZP55470.1 | 23.48 | 23.48 | 23.25 | 22.8 | 21.3 | 100 |  |  |  |  |  |  |  |
| 7 | MMaV\_N\_AZP55476.1 | 23.25 | 25.25 | 23.02 | 23.02 | 21.3 | 98.9 | 100 |  |  |  |  |  |  |
| 8 | MMaV\_N\_AZP55482.1 | 23.25 | 25.25 | 23.02 | 23.02 | 21.3 | 98.68 | 99.78 | 100 |  |  |  |  |  |
| 9 | TaVCV\_N\_AAV92082.1 | 22.17 | 22.17 | 21.96 | 22.83 | 21.05 | 74.95 | 75.16 | 75.16 | 100 |  |  |  |  |
| 10 | MMV\_N\_AAT66752.1 | 24.42 | 24.19 | 23.73 | 25.12 | 22.88 | 68.78 | 68.78 | 68.55 | 64.43 | 100 |  |  |  |
| 11 | MIMV\_N\_ATY38954.1 | 22.3 | 22.3 | 22.07 | 23.68 | 21.97 | 61.24 | 61.24 | 61.01 | 59.68 | 62.73 | 100 |  |  |
| 12 | WYSV\_N\_AVV48075.1 | 26.04 | 26.04 | 26.04 | 25.82 | 28.51 | 24.55 | 25 | 25 | 22.25 | 25.51 | 21.97 | 100 |  |
| 13 | RYSV\_N\_BAA25154.1 | 23.87 | 23.87 | 23.66 | 25.16 | 25.54 | 26.73 | 26.73 | 26.73 | 24.9 | 29.61 | 25.63 | 58.46 | 100 |

Table 4. L ORF alphanucleorhabdoviruses. Percent Identity Matrix - created in Clustal 2.1

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Virus | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
| 1 | PhCMoV\_PV-1182\_L\_KX636164.1 | 100 |  |  |  |  |  |  |  |  |  |  |  |  |
| 2 | PhCMoV\_PV-0831\_L\_KY706238.1 | 97.41 | 100 |  |  |  |  |  |  |  |  |  |  |  |
| 3 | PhCMoV\_HZ15-192\_L\_KY859866.1 | 97.38 | 99.79 | 100 |  |  |  |  |  |  |  |  |  |  |
| 4 | EMDV\_Agapanthus\_L\_KJ082087.1 | 73.87 | 73.93 | 73.86 | 100 |  |  |  |  |  |  |  |  |  |
| 5 | PYDV\_SYDV\_L\_GU734660.1 | 57.74 | 57.81 | 57.77 | 47.21 | 100 |  |  |  |  |  |  |  |  |
| 6 | WYSV\_L\_MG604920.1 | 46.87 | 46.92 | 46.85 | 46.67 | 46.33 | 100 |  |  |  |  |  |  |  |
| 7 | RYSV\_L\_AB011257.1 | 46.61 | 46.89 | 46.85 | 46.39 | 46.85 | 63.1 | 100 |  |  |  |  |  |  |
| 8 | MMaV\_16-0112\_L\_MK063878.2 | 45.02 | 45.02 | 44.93 | 45.4 | 45.29 | 45.62 | 44.21 | 100 |  |  |  |  |  |
| 9 | MMaV\_16-0114\_L\_MK063879.2 | 44.84 | 44.75 | 44.7 | 45.15 | 45.27 | 45.56 | 44.03 | 96.88 | 100 |  |  |  |  |
| 10 | MMaV\_16-0121\_L\_MK112501.2 | 44.82 | 44.73 | 44.67 | 45.16 | 45.27 | 45.61 | 44.21 | 96.87 | 99.69 | 100 |  |  |  |
| 11 | TaVCV\_L\_AY674964.1 | 45.71 | 45.96 | 45.85 | 45.27 | 44.46 | 43.87 | 43.62 | 67.34 | 66.96 | 66.93 | 100 |  |  |
| 12 | MIMV\_L\_MF102281.1 | 45.12 | 45.12 | 45.05 | 44.94 | 45.12 | 44.39 | 44.54 | 62.96 | 62.61 | 62.63 | 61.73 | 100 |  |
| 13 | MMV\_L\_AY618418.1 | 44.54 | 44.69 | 44.67 | 45.17 | 44.47 | 44.21 | 44.13 | 62.61 | 62.39 | 62.44 | 61.91 | 62.12 | 100 |

Table 5. L protein alphanucleorhabdoviruses. Percent Identity Matrix - created in Clustal 2.1

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Virus | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
| 1 | PhCMoV\_L\_AOT55662.1 | 100 |  |  |  |  |  |  |  |  |  |  |  |  |
| 2 | PhCMoV\_L\_AUY61884.1 | 99.43 | 100 |  |  |  |  |  |  |  |  |  |  |  |
| 3 | PhCMoV\_L\_ARU77002.1 | 99.43 | 99.69 | 100 |  |  |  |  |  |  |  |  |  |  |
| 4 | EMDV\_L\_AHN10100.1 | 83.92 | 84.17 | 83.97 | 100 |  |  |  |  |  |  |  |  |  |
| 5 | PYDV\_L\_ADE45274.1 | 53.13 | 53.02 | 52.97 | 53.13 | 100 |  |  |  |  |  |  |  |  |
| 6 | WYSV\_L\_AVV48080.1 | 33.6 | 33.49 | 33.54 | 34.02 | 35.09 | 100 |  |  |  |  |  |  |  |
| 7 | RYSV\_L\_BAA25160.1 | 33.77 | 33.67 | 33.61 | 34.08 | 34.31 | 62.95 | 100 |  |  |  |  |  |  |
| 8 | MMaV\_L\_AZP55475.1 | 32.89 | 32.84 | 32.79 | 33 | 32.62 | 31.43 | 31.29 | 100 |  |  |  |  |  |
| 9 | MMaV\_L\_AZP55481.1 | 32.68 | 32.63 | 32.58 | 32.84 | 32.41 | 31.16 | 31.13 | 99.01 | 100 |  |  |  |  |
| 10 | MMaV\_L\_AZP55487.1 | 32.66 | 32.61 | 32.56 | 32.82 | 32.39 | 31.14 | 31.11 | 99.06 | 99.84 | 100 |  |  |  |
| 11 | TaVCV\_L\_AAV92087.1 | 32.84 | 32.79 | 32.73 | 32.63 | 35.65 | 30.23 | 30.48 | 74.21 | 74.21 | 74.17 | 100 |  |  |
| 12 | MMV\_L\_AAT66757.1 | 32.28 | 33.22 | 32.22 | 32.96 | 31.24 | 31.3 | 31 | 64.88 | 64.98 | 64.98 | 64.65 | 100 |  |
| 13 | MIMV\_L\_ATY38959.1 | 33.6 | 33.49 | 33.49 | 34.13 | 32.39 | 31.67 | 31.78 | 65.3 | 65.4 | 65.35 | 63.56 | 64.03 | 100 |

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| --- |

**Genus *Betanucleorhabdovirus***

When genomes of viruses assigned to the genus *Dichorhavirus* were sequenced, it became clear that the nucleorhabdoviruses did not form a monophyletic clade upon complete L protein sequence alignments. Members assigned to the species *Datura yellow vein nucleorhabdovirus* and *Sonchus yellow net nucleorhabdovirus* appeared phylogenetically closer to members of the genus *Dichorhavirus* than to those assigned to the other nucleorhabdovirus species (Ramos-González et al., 2017; Chabi-Jesus et al., 2018; Dietzgen et al., 2018; Freitas-Astúa et al., 2018; Liu et al., 2018; Fig. 1). Therefore, we propose that these members should be reclassified to the species *Datura yellow vein betanucleorhabdovirus* and *Sonchus yellow net betanucleorhabdovirus*. We propose the latter as the type species of the genus, since members of this species have been studied in detail and more biological and molecular data are available about them (Ganesan et al. 2013).The genome of sowthistle yellow vein virus (SYVV) is being sequenced and unpublished data indicate that it also groups with DYVV and SYNV. Therefore, the members assigned to the species *Sowthistle yellow vein nucleorhabdovirus* should be reclassified to the species *Sowthistle yellow vein betanucleorhabdovirus*.

In addition to members of these three species, other viruses will be assigned to three new species within this new genus, totaling six species of betanucleorhabdovirus. Some characteristics of the new viruses are as follows:

**Black currant-associated rhabdovirus (BCaRV)**

High-throughput sequencing (HTS) of genomic material from an asymptomatic blackcurrant (*Ribes nigrum* L.) accession (cultivar Veloy) yielded a large contig with sequence similarities to betanucleorhabdoviruses (Wu et al., 2018). The complete negative-sense RNA genome of BCaRV is 14,432 nucleotides (nt) (GenBank accession MF543022). There is currently no information on any insect vector species.

**Bird´s-foot trefoil-associated virus 1 (BFTV-1)**

*In silico* analysis of transcriptome data of bird´s-foot trefoil (*Lotus corniculatus* L.) tissues from Shaanxi Province, China, led to the identification of the 13,626 nt genome of a new rhabdovirus (GenBank accession MH614262.1) that is closely related to BCaRV, DYVV and SYNV (Debat and Bejerman, 2019). Since the BFTV-1 genome was obtained from transcriptome analysis data, there is currently no information available on symptoms or vectors.

**Alfalfa-associated nucleorhabdovirus (AaNV)**

Transmission electron microscopy (TEM) analyses and HTS of an alfalfa (*Medicago sativa*) plant showing virus-like symptoms collected in Stadl-Paura, Austria, revealed the presence of a new rhabdovirus with a complete genome comprising 13,875 nt (GenBank accession MG948563; Gaafar et al., 2019). Bacilliform-shaped virus particles were observed in TEM, some of them budding from the inner nuclear envelope in the perinuclear space (Gaafar et al., 2019). An antiserum produced against AaNV did not react with at least two nucleorhabdoviruses. The virus was mechanically transmitted to *Nicotiana benthamiana, Pisum sativum* and *Vicia faba,* but no arthropod vector was identified (Gaafar et al., 2019).

The genomes of DYVV, SYNV, BCaRV and BFTV-1 contain six open reading frames (ORF) in the order 3’N-P-P3-M-G-L 5’, whereas AaNV seems to harbor an extra ORF of unknown function (U) between M and G. Highly conserved regulatory regions separating the genes were identified in all of the betanucleorhabdoviruses, and all deduced viral protein sequences contain predicted mono- or bipartite nuclear localization signals, indicative of at least partial nuclear localization (Wu et al., 2018; Debat and Bejerman, 2010; Gaafar et al., 2019).

Phylogenetic analysis based on L protein amino acid sequences also identified DYVV and SYNV as closest relatives of BCaRV, BFTV-1 and AaNV, and these five viruses grouped in a distinct cluster within the family *Rhabdoviridae* (Fig. 1).

Based on the nucleotide and deduced amino acid sequence information compiled in Tables 6 to 10, we therefore propose to classify BCaRV, BFTV-1 and AaNV in the new species *Blackcurrant betanucleorhabdovirus, Trefoil betanucleorhabdovirus* and *Alfalfa betanucleorhabdovirus,* 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.

DYVV, SYNV, BCaRV and BFTV-1 fulfill species demarcation criteria A and B, while SYVV fulfills species demarcation criteria B and C, and AaNV fulfills all species demarcation criteria.

Table 6. Complete genome RNAbetanucleorhabdoviruses. Percent Identity Matrix - created in Clustal 2.1

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Virus | 1 | 2 | 3 | 4 | 5 |
| 1 | DYVV\_RNA\_KM823531.1 | 100 |  |  |  |  |
| 2 | BFTV-1\_RNA\_MH614262.1 | 54.92 | 100 |  |  |  |
| 3 | SYNV\_RNA\_L32603.1 | 52.59 | 50.6 | 100 |  |  |
| 4 | BCaRV\_RNA\_MF543022.1 | 52.63 | 52.17 | 51.6 | 100 |  |
| 5 | AaNV\_RNA\_MG948563.1 | 46.44 | 46.54 | 46.21 | 46.24 | 100 |

Table 7. N ORF­ betanucleorhabdoviruses. Percent Identity Matrix - created in Clustal 2.1

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Virus | 1 | 2 | 3 | 4 | 5 |
| 1 | DYVV\_N\_KM823531.1 | 100 |  |  |  |  |
| 2 | BFTV-1\_N\_MH614262.1 | 64.33 | 100 |  |  |  |
| 3 | BCaRV\_N\_MF543022.1 | 58.36 | 57.87 | 100 |  |  |
| 4 | SYNV\_N\_L32603.1 | 56 | 55.01 | 56.02 | 100 |  |
| 5 | AaNV\_N\_MG948563.1 | 49.65 | 49.66 | 49.65 | 49.66 | 100 |

Table 8. N protein betanucleorhabdoviruses. Percent Identity Matrix - created in Clustal 2.1

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Virus | 1 | 2 | 3 | 4 | 5 |
| 1 | DYVV\_N\_AKH61401.1 | 100 |  |  |  |  |
| 2 | BFTV-1\_N\_AXG64325.1 | 60.36 | 100 |  |  |  |
| 3 | BCaRV\_N\_AUW36414.1 | 51.12 | 50.98 | 100 |  |  |
| 4 | SYNV\_N\_AAA50380.1 | 50.78 | 48.68 | 48.8 | 100 |  |
| 5 | AaNV\_N\_QAB45070.1 | 38.71 | 36.78 | 36.32 | 37.47 | 100 |

Table 9. L ORF betanucleorhabdoviruses. Percent Identity Matrix - created in Clustal 2.1

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Virus | 1 | 2 | 3 | 4 | 5 |
| 1 | DYVV\_L\_ KM823531.1 | 100 |  |  |  |  |
| 2 | BFTV-1\_L\_ MH614262.1 | 56.41 | 100 |  |  |  |
| 3 | SYNV\_L\_L32603.1 | 53.53 | 53.68 | 100 |  |  |
| 4 | AaNV\_L\_ MG948563 | 50.38 | 50.2 | 49.79 | 100 |  |
| 5 | BCaRV\_L\_ MF543022.1 | 56.04 | 55.44 | 54.72 | 52.01 | 100 |

Table 10. L proteinbetanucleorhabdoviruses. Percent Identity Matrix - created in Clustal 2.1

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Virus | 1 | 2 | 3 | 4 | 5 |
| 1 | DYVV\_L\_AKH61406.1 | 100 |  |  |  |  |
| 2 | BFTV-1\_L\_AXG64330.1 | 50.94 | 100 |  |  |  |
| 3 | BCaRV\_L\_AUW36419.1 | 49.76 | 50 | 100 |  |  |
| 4 | SYNV\_L\_AAA50385.1 | 44.47 | 44.57 | 45.59 | 100 |  |
| 5 | AaNV\_L\_QAB45076.1 | 39.17 | 37.89 | 38.11 | 38.59 | 100 |

**Genus *Gammanucleorhabdovirus***

Although maize fine streak virus (MFSV) is phylogenetically closer to alphanucleorhabdoviruses, nucleotide and deduced amino acid sequence comparisons between them, as well as between MFSV and other plant rhabdoviruses, are extremely low (Tables 11, 12 and 13). Additionally, phylogenetic analyses of L protein sequences indicate that bootstrap support for its inclusion in that genus is very weak. Therefore, we propose the creation of genus *Gammanucleorhabdovirus* with *Maize fine streak gammanucleorhabdovirus* as its only and type species. Therefore, *Maize fine streak nucleorhabdovirus,* currently assigned within the genus *Nucleorhabdovirus*, shall be reassigned as *Maize fine streak gammanucleorhabdovirus.*

**Maize fine streak virus (MFSV)**

The full-length negative-sense RNA genome of MFSV contains 13,782 nt (GenBank accession AY618417) distributed in seven ORF in the order 3’ N-P-3-4-M-G-L 5’ (Tsai et al., 2005).

Table 11. Comparison between the L ORF from MFSV (gammanucleorhabdovirus) and the type members of the plant rhabdoviruses: SYNV (betanucleorhabdovirus), PYDV (alphanucleorhabdovirus), LNYV (cytorhabdovirus), OFV (dichorhavirus) and LBVV (varicosavirus). Percent Identity Matrix - created in Clustal 2.1

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | Virus | 1 | 2 | 3 | 4 | 5 | 6 |
| 1 | Maize\_fine\_streak\_virus\_L\_AY618417.1 | 100 |  |  |  |  |  |
| 2 | Lettuce\_necrotic\_yellows\_virus\_L\_AJ867584.2 | 47.4 | 100 |  |  |  |  |
| 3 | Sonchus\_yellow\_net-virus\_L\_ L32603.1 | 45.84 | 43.95 | 100 |  |  |  |
| 4 | Potato\_yellow\_dwarf\_virus\_SYDV\_L\_GU734660.1 | 44.19 | 43.4 | 44.42 | 100 |  |  |
| 5 | Orchid\_fleck\_virus\_L\_AB244418 | 45.43 | 42.54 | 45.18 | 43.47 | 100 |  |
| 6 | Lettuce\_big-vein\_virus\_L\_AB075039.1 | 42.26 | 41.51 | 41.63 | 39.41 | 40.43 | 100 |

Table 12. Comparison between the L protein from MFSV (gammanucleorhabdovirus) and the type members of the plant rhabdoviruses: SYNV (betanucleorhabdovirus), PYDV (alphanucleorhabdovirus), LNYV (cytorhabdovirus), OFV (dichorhavirus) and LBVV (varicosavirus). Percent Identity Matrix - created in Clustal 2.1

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | Virus | 1 | 2 | 3 | 4 | 5 | 6 |
| 1 | Maize\_fine\_streak\_virus\_L\_AAT66751.1 | 100 |  |  |  |  |  |
| 2 | Potato\_yellow\_dwarf\_virus\_L\_ADE45274.1 | 31.17 | 100 |  |  |  |  |
| 3 | Orchid\_fleck\_virus\_L\_BAE93584 | 32.87 | 31.8 | 100 |  |  |  |
| 4 | Sonchus\_yellow\_net\_virus\_L\_AAA50385.1 | 30.28 | 28.16 | 28.93 | 100 |  |  |
| 5 | Lettuce\_big-vein\_associated\_virus\_L\_BAC16226.1 | 24.45 | 22.63 | 23.82 | 23.75 | 100 |  |
| 6 | Lettuce\_necrotic\_yellows\_virus\_L\_CAI30426.1 | 24.29 | 24.96 | 23.73 | 24.95 | 24.64 | 100 |

Table 13. Comparison between MFSV (gammanucleorhabdovirus) and definitive and proposed members of the genus *Alphanucleorhabdovirus*. Percent Identity Matrix - created in Clustal 2.1

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| alphanucleorhabdoviruses | MFSV  (gammanucleorhabdovirus) | | | | |
| Genome | N ORF | | L ORF | |
| nt | aa | nt | aa |
| PhCMoV\_PV-1182\_RNA\_KX636164.1 | 40.36 | 41.4 | 23.74 | 42.43 | 28.07 |
| EMDV\_Agapanthus\_RNA\_KJ082087.1 | 39.64 | 39.69 | 22.6 | 41.75 | 28.08 |
| PYDV\_SYDV\_RNA\_GU734660.1 | 39.37 | 40.03 | 21.84 | 42.43 | 28.03 |
| MMaV\_16-0112\_RNA\_MK063878.2 | 39.73 | 41.77 | 25.54 | 41.8 | 28.03 |
| TaVCV\_RNA\_AY674964.1 | 39.43 | 41.82 | 23.83 | 42.37 | 27.23 |
| MIMV\_RNA\_MF102281.1 | 39.87 | 41.59 | 24.09 | 41.49 | 27.38 |
| MMV\_RNA\_AY618418.1 | 39.53 | 40.6 | 25.85 | 41.9 | 27.68 |
| WYSV\_RNA\_MG604920.1 | 38.97 | 38.53 | 22.66 | 43.68 | 26.77 |
| RYSV\_RNA\_AB011257.1 | 39.15 | 40.73 | 23.62 | 43.28 | 26.19 |

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| --- |
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