Illuminating the plant rhabdovirus landscape through metatranscriptomics data

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Abstract

Rhabdoviruses infect a large number of plant species and cause significant crop diseases. They have a negative-sense, single-stranded unsegmented or bisegmented RNA genome. The number of plant-associated rhabdovirid sequences has grown in the last few years in concert with the extensive use of high-throughput sequencing platforms. Here we report the discovery of 26 novel rhabdovirus genomes associated with 24 different host plant species and one insect, which were hidden in public databases. These viral sequences were identified through homology searches in more than 3,000 plant and insect transcriptomes from the NCBI Sequence Read Archive (SRA) using known plant rhabdovirus sequences as query. Identification, assembly and curation of raw SRA reads resulted in sixteen viral genome sequences with full-length coding regions and ten partial genomes. Highlights of the obtained sequences include viruses with unique and novel genome organizations among known plant rhabdoviruses. Phylogenetic analysis showed that thirteen of the novel viruses were related to cytorhabdoviruses, one to alphanucleorhabdoviruses, five to betanucleorhabdoviruses, one to dichorhaviruses, and six to varicosaviruses. These findings resulted in the most complete phylogeny of plant rhabdoviruses to date and shed new light on the phylogenetic relationships and evolutionary landscape of this group of plant viruses. Furthermore, this study provides additional evidence for the complexity and diversity of plant rhabdovirus genomes and demonstrates that analyzing SRA public data provides an invaluable tool to accelerate virus discovery, gain evolutionary insights and refine virus taxonomy.

Keywords: plant rhabdovirus; evolution; taxonomy; metatranscriptomics

Introduction

The costs for high-throughput sequencing (HTS) have been significantly reduced each year due to advances in sequencing technologies; therefore, the number of genome and transcriptome sequencing projects has been steadily increasing, resulting in a massive number of nucleotides deposited in the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI). Over 16,000 petabases (10^{15} bases) have been deposited in the SRA, with over 6,000 petabases available as open-access data (Gilbert et al., 2019). Thus, this large amount of data has provided significant challenges for data storage, bioinformatic analysis and management. This impressive and potentially useful amount of data concomitantly raised two issues: (*i*) high logistical costs of data management, and (*ii*) large

amounts of neglected and unused data, awaiting secondary analysis and repurposing. In the specific case of large plant sequencing project datasets, virome studies are scarce.

Abundant novel viruses, many of them not known to induce any apparent symptoms in their host or without a known host, have been identified from diverse environments using metagenomic approaches. This has highlighted our limited knowledge about the richness of a continuously expanding plant virosphere, that appears highly diverse in every potential host assessed so far (Bejerman et al., 2020a; Dolja et al., 2020; Lefeuvre et al., 2019; Roosinck et al., 2015). Furthermore, the great number of newly discovered viruses by HTS, a miniscule portion of the virosphere, allowed a first glimpse of the path to a comprehensive megataxonomy of the virus world (Koonin et al., 2020).

The scientific interest of the submitters of transcriptome datasets is often limited to a narrow objective within their specific field of study, which leaves a large amount of potentially valuable data not analyzed (Bejerman et al., 2020b). In such transcriptome datasets, viral sequences may be hidden in plain sight, thus their analysis has become a valuable tool for the discovery of novel viral sequences (Debat and Bejerman, 2019; Goh et al., 2020; Jiang et al., 2019; Kim et al., 2018; Lauber et al., 2019; Longdon et al., 2015; Mushegian et al., 2016; Nibert et al., 2018: Sidharthan and Baranwal, 2021). In a recent consensus statement report, Simmonds and colleagues (2017) contend that viruses that are known only from metagenomic data can, should, and have been incorporated into the official classification scheme overseen by the International Committee on Taxonomy of Viruses (ICTV). Consequently, the analysis of public sequence databases constitutes a valuable resource for the discovery of novel plant viruses, which allows the reliable identification and characterization of new viruses in hosts with no previous record of virus infections (Debat and Bejerman, 2019). This approach to virus discovery is inexpensive as it does not require the acquisition of samples and subsequent sequencing, but on secondary analyses of publicly available data to address novel research questions and objectives. At the same time, it is more wide-ranging and comprehensive than any other current approach due to the millions of datasets from a large variety of potential host species available from the NCBI-SRA (Lauber et al., 2019).

Plant rhabdoviruses have negative-sense, single-stranded RNA genomes and are taxonomically classified in six genera: *Cytorhabdovirus*, *Alphanucleorhabdovirus*, *Betanucleorhabdovirus* and *Gammanucleorhabdovirus* for viruses which have an unsegmented genome, and *Dichorhavirus* and *Varicosavirus*, for viruses which have a bi-segmented genome, and infect both monocot and dicot plants (Dietzgen et al., 2020). These six genera were recently assigned to the subfamily *Betarhabdovirinae*

within the family *Rhabdoviridae* (Walker et al., 2020). Viruses classified in five of these genera are transmitted persistently by arthropods in which they also replicate (Dietzgen et al., 2017; 2020), whereas varicosaviruses are transmitted by soil-borne chytrid fungi (Dietzgen et al., 2020). Cyto- and nucleorhabdovirus genomes have six conserved canonical genes encoding in the order 3' - nucleocapsid protein (N) - phosphoprotein (P) – putative movement protein (P3) - matrix protein (M) - glycoprotein (G) – large polymerase (L) - 5'; the L gene of dichorhaviruses is located on RNA2 (Walker et al., 2018). Up to three accessory genes with unknown functions have been identified among cyto- and nucleorhabdovirus genomes leading to diverse genome organizations (Walker et al., 2011; 2018). Conserved gene junction sequences separate each gene and the overall coding region is flanked by 3´ leader and 5´ trailer sequences that feature partially complementary ends that may form a panhandle structure during replication (Dietzgen et al., 2017). Varicosavirus RNA 1 has 1 to 2 genes, with one of those encoding the RNA-dependent RNA polymerase L, while RNA 2 has 3-5 genes with the first open reading frame (ORF) encoding a coat protein (Walker et al., 2018; Dietzgen et al. 2020). The 3′- and 5′-terminal sequences of the two varicosavirus genome segments are similar but do not exhibit inverse complementarities (Walker et al., 2018).

In this study we queried the publicly available plant transcriptome datasets in the transcriptome shotgun assembly (TSA) database hosted at NCBI and identified 26 novel plant rhabdoviruses from 24 plant and one insect species, showing structural, functional and evolutionary cues to be classified in the family *Rhabdoviridae*, subfamily *Betarhabdovirinae*, genera *Cytorhabdovirus*, *Alphanucleorhabdovirus*, *Betanucleorhabdovirus*, *Dichorhavirus* and *Varicosavirus*.

Materials and methods

Identification of plant rhabdovirus sequences from public plant RNA-seq datasets

The detection of plant rhabdovirus sequences was done as described by Longdon and colleagues (2015). Briefly, the amino acid sequences corresponding to the nucleocapsid and polymerase proteins of several known cyto- and nucleorhabdoviruses were used as query in tBlastn searches with parameters word size = 6, expected threshold = 10, and scoring matrix = BLOSUM62, against the Viridiplantae (taxid:33090) and Hemiptera (taxid:7524) TSA databases. The obtained hits were explored by hand eye and based on percentage identity, query coverage and E-value (>1e-5), shortlisted as likely corresponding to novel virus transcripts, which were then further analyzed. Given the redundant nature of many retrieved hits, a

step of contig clustering was implemented using the CD-hit suite with standard parameters available at http://weizhongli-lab.org/cdhit_suite/cgi-bin/index.cgi?cmd=cd-hit. In addition, the raw sequence data corresponding to several SRA experiments associated with different NCBI Bioprojects (Table 1) was retrieved for further analyses.

Sequence assembly and identification

The nucleotide (nt) raw sequence reads from each analyzed SRA experiment linked to the TSA projects returning virus-like hits were downloaded and pre-processed by trimming and filtering with the Trimmomatic tool as implemented in http://www.usadellab.org/cms/?page=trimmomatic, and the resulting reads were assembled *de novo* with Trinity v2.6.6 using standard parameters. The transcripts obtained from *de novo* transcriptome assembly were subjected to bulk local BLASTX searches (E-value < $1e^{-5}$) against a Refseq virus protein database available at ftp://ftp.ncbi.nlm.nih.gov/refseq/release/viral/viral.1.protein.faa.gz. The resulting viral sequence hits of each bioproject were visually explored. Tentative virus contigs were extended by iterative mapping of each SRA library's raw reads. This strategy used BLAST/nhmmer to extract a subset of reads related to the query contig, used the retrieved reads to extend the contig and then repeated the process iteratively using as query the extended sequence. The extended and polished transcripts, now presenting overlapping regions, were reassembled using Geneious v8.1.9 (Biomatters Ltd.) alignment tool.

Bioinformatics tools and analyses

Sequence analyses

ORFs were predicted with ORFfinder (https://www.ncbi.nlm.nih.gov/orffinder/), functional domains and architecture of translated gene products were determined using InterPro (https://www.ebi.ac.uk/interpro/search/sequence-search) and the NCBI Conserved domain database v3.16 (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). Further, HHPred and HHBlits as implemented in https://toolkit.tuebingen.mpg.de/#/tools/ were used to complement annotation of divergent predicted proteins by hidden Markov models. Importin-α dependent nuclear localization signals were predicted using cNLS Mapper available at http://nls-mapper.iab.keio.ac.jp/, nuclear export signals were predicted using NetNES 1.1 available at www.cbs.dtu.dk/services/NetNES/, and transmembrane domains were predicted using the TMHMM version 2.0 tool (http://www.cbs.dtu.dk/services/TMHMM/).

Pairwise sequence identity

Percentage amino acid (aa) sequence identities of the predicted ORFs of each plant-associated rhabdovirid identified in this study based on available plant-associated rhabdovirus genome sequences were calculated using https://www.ebi.ac.uk/Tools/psa/emboss_needle/.

Phylogenetic analysis

Phylogenetic analysis based on the predicted nucleocapsid protein of all plant rhabdovirids, listed in Table S1, was done using MAFFT 7 https://mafft.cbrc.jp/alignment/software with multiple aa sequence alignments using FFT-NS-i as the best-fit model. The aligned aa sequences were used as input in MegaX software (Kumar et al., 2018) to generate phylogenetic trees by the maximum-likelihood method (best-fit model=WAG+G+F). Local support values were computed using bootstraps with 1000 replicates.

Results

Summary of discovered rhabdovirid sequences

The complete coding regions of 16 novel rhabdoviruses were identified; in addition, partial genomic sequences for 10 novel viruses were assembled. These viruses were associated with 24 plant host species and one insect species (Table 1). Bioinformatic and source data of each of the 26 viral sequences, as well as the GenBank accession number and proposed classification are listed in Table 1. Based on phylogenetic relatedness, genome organization and sequence identity, the novel viruses were assigned to the established plant rhabdovirus genera *Alphanucleorhabdovirus*, *Betanucleorhabdovirus*, *Cytorhabdovirus*, *Dichoravirus* and *Varicosavirus*. Most of the tentative plant hosts of the novel viruses are herbaceous dicots (16/24), six are herbaceous monocots, one a woody dicot and one a gymnosperm (Table 1). The characteristics of deduced proteins encoded by each rhabdovirid sequence were determined by predictive algorithms, and are shown in Table S2. Genomic architecture and evolutionary placement of the 26 discovered viruses are described below, grouped by affinity to members of the diverse genera within the *Betarhabdovirinae*.

Alphanucleorhabdovirus

The complete coding region of a novel putative alphanucleorhabdovirus, tentatively named *Agave tequilana* virus 1 (ATV1), with the genome organization 3'-N- P–P3-M-G-L-5' (Fig. 1A) was assembled from Blue agave transcriptome data (Table 1). A nuclear localization signal (NLS) was predicted in every ATV1-encoded protein (Table S2). According to the NLS scores, the N protein is predicted to be located exclusively in the nucleus, whereas P and L proteins have a partial nuclear localization, while the P3, M

and G proteins are localized to both the nucleus and the cytoplasm. Leucine-rich nuclear export signals (NES) were predicted in the N, P and L proteins (Table S2). A transmembrane domain motif was detected in the C-terminus of the G protein, and a signal peptide was predicted in its N-terminus (Table S2). The consensus gene junction sequence 3'-AUUCUUUUU GGG UUG-5' of ATV1 genome is similar to that of the alphanucleorhabdoviruses maize mosaic virus (MMV), maize Iranian mosaic virus (MIMV), Morogoro maize-associated virus (MMaV) and taro vein chlorosis virus (TaVCV) (Table 2).

Pairwise aa sequence identities between the ATV1-encoded proteins and those from other alphanucleorhabdoviruses showed low sequence identities of 10.9 - 35.0 % (Table 3A). Nucleotide sequence identity for the complete genome sequence of ATV1 and other alphanucleorhabdoviruses ranged from 47 – 49.3 % (Table 3A).

Phylogenetic analysis based on N protein aa sequences showed that ATV1 clustered with the monocot-infecting alphanucleorhabdoviruses MMV, MIMV, MMaV and TaVCV (Fig. 2), which are also the most similar in pairwise sequence identity values, share equivalent genome organizations and have similar conserved gene junction sequences (Table 2).

Betanucleorhabdoviruses

The complete coding regions of three putative betanucleorhabdoviruses, tentatively named *Asclepias syriaca* virus 2 (AscSyV2), *Plectranthus aromaticus* virus 1 (PleArV1) and, *Rhododendron delavayi* virus 1 (RhoDeV1), as well as the partial genomes of *Cuscuta reflexa* virus 1 (CusReV1) and *Persicaria minor* virus 1 (PerMiV1), were assembled in this study (Table 1). The genome organization of these five viruses is 3'-N- P–P3-M-G-L-5' (Fig. 1A). A NLS was predicted in every protein encoded by AscSyV2, CusReV1, PleArV1 and RhoDeV1 (Table S2). According to the NLS scores, CusReV1 and PleArV1 N protein, RhoDeV1 P protein, CusReV1 M protein, and PleArV1 and RhoDeV1 L proteins are expected to be exclusively nuclear, whereas RhoDeV1 M protein is predicted to have a partial nuclear localization. AscSyV2 and RhoDeV1 N proteins, AscSyV2, CusReV1 and PleArV1 P proteins, AscSyV2, CusReV1, PleArV1 and RhoDeV1 P3 proteins, AscSyV2 and PleArV1 M proteins, AscSyV2, CusReV1, PleArV1 and RhoDeV1 G proteins, and AscSyV2 and CusReV1 L proteins are predicted to localize to both the nucleus and the cytoplasm. Leucine-rich NES were predicted in the AscSyV2, CusReV1 and PleArV1 N proteins, the CusReV1 and PleArV1 P proteins, the CusReV1 and RhoDeV1 P3 proteins, the PleArV1 M protein, and the PleArV1 and RhoDeV1 L proteins (Table S2). A transmembrane domain was detected in

the C-terminal sequence of the AscSyV2, CusReV1, PleArV1 and RhoDeV1 G proteins. A signal peptide was detected in the G protein N-terminus of AscSyV2, CusReV1 and RhoDeV1 (Table S2).

The deduced AscSyV2 M protein, PleArV1 G protein and RhoDeV1 L protein are the smallest such proteins reported so far among betanucleorhabdoviruses (Table S3).

Interestingly, the N proteins of AscSyV2, PleArV1 and RhoDeV1 are basic, whereas the CusReV1 N protein is acidic. Moreover, the CusReV1 and PleArV1 M proteins are basic, while the AscSyV2 and RhoDeV1 M proteins are acidic (Table S2).

The consensus gene junction sequence 3'-AUUCUUUUU GG UUG-5' of all five novel viral genomes is identical, and the same as in the genome of every betanucleorhabdovirus described thus far: alfalfa-associated nucleorhabdovirus (AaNV), apple rootstock virus A (ApRVA), Bacopa monnieri virus 2 (BmV2), bird´s-foot trefoil associated virus 1(BFTaV1), black currant-associated rhabdovirus (BCaRV), cardamom vein clearing virus 1 (CdVCV1), datura yellow vein virus (DYVV), green Sichuan pepper nucleorhabdovirus (GSPNuV), sonchus yellow net virus (SYNV) and sowthistle yellow vein virus (SYVV) (Table 2).

Pairwise aa sequence identity values between each encoded protein of the five novel viruses and those from other betanucleorhabdoviruses vary significantly (Table 3B). The highest nucleotide sequence identity for the genome sequence between AscSyV2, PleArV1 and RhoDeV1 and other betanucleorhabdoviruses ranged from 51.4 - 62.1% (Table 3B).

Phylogenetic analysis based on the deduced N protein aa sequences showed that CusReV1, RhoDeV1, PerMiV1 and PleArV1 clustered with the betanucleorhabdoviruses BFTaV1, BCaRV, BmV2, CdVCV1, DYVV, GSPNuV, SYNV and SYVV (Fig. 2), which are also most similar in pairwise sequence identity values of their cognate proteins; furthermore, all these viruses have a similar genome organization. Interestingly, AscSyV2 clustered closely with the betanucleorhabdoviruses AaNV and ApRVA, and had similar pairwise sequence identity values for their cognate proteins. However, the genome organization of AaNV and ApRVA is different in that it includes an accessory gene between the M and G genes.

Cytorhabdoviruses

The complete coding region of eight novel cytorhabdoviruses, tentatively named *Anthurium amnicola* virus 1 (AntAmV1), *Asclepias syriaca* virus 1 (AscSyV1), *Bemicia tabaci*-associated virus 1 (BeTaV1), *Glehnia littoralis* virus 1 (GlLV1), *Gymnadenia densiflora* virus 1 (GymDenV1), *Nymphaea alba* virus 1 (NymAV1), *Tagetes erecta* virus 1 (TaEV1) and *Trachyspermum ammi* virus 1 (TrAV1), as well as the

partial genomes of five other cytorhabdoviruses, named *Allium chinense* virus 1 (AChV1), *Dioscorea composite* virus 1 (DiCoV1), *Lotus corniculatus* virus 1 (LotCorV1), *Pelargonium radula* virus 1 (PelRaV1) and *Suaeda salsa* virus 1 (SuSV1), were assembled in this study (Table 1). The genome organization of these thirteen novel cytorhabdoviruses is highly diverse. AntAmnV1 and likely DiCoV1 lack additional accessory genes and have the genome organization 3'-N-P–P3-M-G-L-5' (Fig. 1B). AscSyV1, GlLV1, LotCorV1, NymAV1, PelRaV1 and likely AChV1 and SuSV1 have an accessory ORF between G and L genes, with the genome organization 3'-N-P–P3-M-G-P6-L-5' (Fig. 1B), while BeTaV1 have an accessory ORF between P3 and M genes, with the genome organization 3'-N-P–P3-P4- M-G-L-5' (Fig. 1B). The genome organization of GmyDenV1, TaEV1 and TrAV1 differs from that of all other known plant rhabdoviruses: GymDenV1 genome appears to only have four genes in the order 3'-N-P–M-L-5' (Fig. 1B); TaEV1 and TrAV1 genomes have five genes in the order 3'-N-P–P3-M-L-5' but do not encode a glycoprotein (Fig. 1B). An overlapping ORF within the P encoding ORF, named P´, is present in the AChV1, AscSyV1, GilV1, LotCorV1, NymAV1, PelRaV1 and SuSV1 genomes (Fig. 1B; Table 1). The predicted TaEV1 N protein is the largest described so far among cytorhabdoviruses, whereas the AntAmV1 L protein is the smallest reported so far among cytorhabdoviruses (Table S2).

Interestingly, the predicted N protein of almost every cytorhabdovirus identified in our study is acidic, but the PelRaV1 N protein is neutral and the BeTaV1 N protein is basic. The P protein of almost every cytorhabdovirus identified in our study is acidic, but the TrAV1 P protein is basic. Moreover, the M protein of almost every cytorhabdovirus identified in our study is basic, except GymDenV1 and TrAV1 M proteins are acidic. Furthermore, the predicted G proteins of AscSyV1, DiCoV1, GlLV1, NymAV1 and PelRaV1 are acidic, except LotCorV1 G protein is neutral, and AntAmV1and BeTaV1 G proteins are basic (Table S2).

A putative transmembrane domain was identified in the G protein C-terminal sequence of AChV1, AntAmV1, AscSyV1, BeTaV1, DiCoV1, GlLV1, LotCorV1, NymAV1 and PelRaV1; two additional transmembrane domains were identified in the G protein N-terminus of AntAmV1 and BeTaV1, and another one in the N-terminus of PelRaV1 G protein. A transmembrane domain was also predicted in every P6 protein of these viruses (Table S2). Moreover, one transmembrane domain was predicted in the SuSV1 P´ protein, and two in the AChV1, GlLV1, LotCorV1 and PelRaV1 P´ proteins, while three were predicted in the AscSyV1 and NymAV1 P´ protein (Table S2). A signal peptide sequence was identified in the G protein N-terminus of AChV1, AscSyV1, GlLV1, LotCorV1, NymAV1 and PelRaV1 (Table

S2). Interestingly, a leucine-rich NES was predicted in the AChV1, AntAmV1, AscSyV1, BeTaV1, DiCoV1, GlLV1, GymDenV1, LotCorV1, PelRaV1, SuSV1, TaEV1 and TrAV1 N proteins, the BeTaV1, GymDenV1, LotCorV1, NymAV1, PelRaV1, SuSV1 and TrAV1 P proteins, the AChV1, AntAmV1, AscSyV1, DiCoV1, GymDenV1, LotCorV1, NymAV1, PelRaV1, SuSV1 and TrAV1 M proteins and the PleArV1 and RhoDeV1 L proteins (Table S2).

The consensus gene junction sequences of the novel cytorhabdoviruses identified in our study are diverse (Table 2), but similar to those previously reported for phylogenetically-related cytorhabdoviruses. Interestingly, the intergenic sequence of the GymDenV1 and TrAV1 gene junctions starts with an adenine instead of a guanine residue like in every other plant rhabdovirus (Table 2).

Pairwise aa sequence identity values between each encoded protein of the thirteen novel viruses and those from known cytorhabdoviruses vary significantly (Table 3C). The highest nucleotide sequence identity for the genome sequence between AntAmV1, AscSyV1, BeTaV1, GlLV1, GymDenV1, NymAV1, TaEV1 and TrAV1and other cytorhabdoviruses ranged between 50.3-65.6% (Table 3C).

Phylogenetic analysis based on the N protein aa sequence showed that the thirteen novel viruses grouped with known cytorhabdoviruses. GlLV1 and NymAV1 clustered with Kenyan potato cytorhabdovirus (KePCyV), Trifolium pratense virus A (TpVA), strawberry virus 1 (StrV1) and tomato yellow mottle-associated virus (TYMaV) with whom they share a similar genomic organization. AChV1 and SuSV1 formed a clade with alfalfa dwarf virus (ADV), chrysanthemum yellow dwarf-associated virus (ChYDaV), raspberry vein chlorosis virus (RVCV) and strawberry crinkle virus (SCV). AscSyV1, LotCorV1 and PelRaV1 clustered with Bacopa monnieri virus 1 (BmV1), Wuhan insect virus 4 (WhIV4), and all these viruses have a similar genomic organization. BeTaV1 clustered with cucurbit cytorhabdovirus 1 (CuCV1), and these viruses share a similar genomic organization. AntAmV1 clustered with Paper mulberry mosaic-associated virus (PMuMaV), with which it shares a similar genomic organization. TaEV1 clustered with a virus cluster formed by BeTaV1, CuCV1 and yerba mate chlorosis-associated virus (YmCaV), but these viruses have a dissimilar genomic organization. DiCoV1 clustered with Colocasia bobone disease-associated virus (CBDaV), but these viruses have a dissimilar genomic organization, while GymDenV1 and TrAV1 formed a monophyletic sister clade to the aphid-transmitted cytorhabdoviruses (Fig. 2).

Dichorha-like viruses

The near-complete RNA 1 and RNA 2 sequences of a dichorha-like virus, tentatively named *Viola verecunda* virus 1 (VVeV1) were identified in this study. We assembled three non-overlapping fragments corresponding to RNA 1 containing the complete N, P3 and G genes, and the near-complete RNA 2, which encodes the L protein (Table 1). Thus, the likely genome organization of VVeV1 is 3'-N-P-P3-M-G-5' for RNA 1 and 3'-L-5' for RNA 2 (Fig.1D). A NLS was predicted in every deduced VVe1 protein (Table S2). According to the NLS scores, the N and L proteins should be exclusively localized to the nucleus, whereas the P3 and G proteins are predicted to localize to both the nucleus and the cytoplasm. Leucine-rich NESs were predicted in the N and L protein sequences (Table S2). A transmembrane domain was identified in the C-terminus of the G protein (Table S2). We were not able to identify a consensus gene junction sequence in the genome fragments of VVeV1 (Table 2). Pairwise aa sequence identities between the VVeV1-encoded proteins and those from known dichorhaviruses showed low sequence identities of 20% or less in the P3 and G proteins, and 20.5 - 23.1% in the N protein (Table 3D). Phylogenetic analysis based on N protein aa sequences showed that VVeV1 clusters with the dichorhaviruses, but is placed by itself in a sister clade (Fig. 2).

Varicosa-like viruses

The complete coding sequences of four varicosa-like viruses, tentatively named *Brassica rapa* virus 1 (BrRV1), *Lolium perenne* virus 1 (LoPV1), *Melampyrum roseum* virus 1 (MelRoV1) and *Pinus flexilis* virus 1 (PiFleV1), as well as partial genomes of two others, named *Phlox pilosa* virus 1 (PhPiV1) and spinach virus 1 (SpV1), were assembled in this study (Table 1). The genomes of BrRV1, LoPV1, MelRoV1, PhPiV1 and SpV1 consist of two RNA segments. RNA 1 of BrRV1, LoPV1, MelRoV1 and SpV1 encode only the L protein, whereas RNA 1 of PhPiV1 likely carries an additional gene 3' to the L gene (Fig. 1C). RNA 2 of BrV1, LoPV1 and SpV1 has three genes in the order 3´-N-P2-P3-5´, RNA 2 of MelRoV1 has four genes in the order 3´-N-P2-P3-P4 -5´ and RNA 2 of PhPiV1 likely has five genes in the order 3´-N-P2-P3-P4-P5-5´ (Fig. 1C). Based on our assemblies, PiFleV1 genome does not appear to be segmented like the genomes of other varicosaviruses and has five ORFs in the order 3´-N-P2-P3-P4-L-5´ (Fig. 1C), suggesting that varicosa-like viruses may also have unsegmented genomes.

The predicted LoPV1 N protein is the longest varicosavirus N protein described so far, whereas MelRoV1 L protein is the smallest L protein described so far among varicosaviruses (Table S3). Interestingly, the PiFLeV1 N protein is basic, whereas the N proteins of BrRV1, LoPV1, MelRoV1, PhPiV1 and SpV1 are acidic. Moreover, BrR1 P2 is basic, while P2s of LoPV1, MelRoV1, PiFleV1 and SpV1 are acidic.

Furthermore, P3s of MelRoV1, PhPiV1 and PiFleV1 are basic, whereas the BrRV1 and LoPV1 P3s are acidic (Table S3).

Based on NLS scores, LoPV1 N and P2, and MelRoV1 L protein are expected to be exclusively located to the nucleus. BrRV1 N protein, BrRv1, PiFleV1 and SpV1 P2, PiFleV1 P3, and the BrRv1, LoPV1 and SpV1 L proteins are predicted to localize to both the nucleus and the cytoplasm. Leucine-rich NESs are predicted in the BrRV1, MelRoV1, PhPiV1, PiFleV1 and SpV1 N protein, the BrRV1, LoPV1, MelRoV1, PiFleV1 and SpV1 P2, the BrRV1, LoPV1, MelRoV1, PhPiV1, PiFleV1 and SpV1 P3, and the MelRoV1, PhPiV1 and PiFleV1 P4 (Table S2). No transmembrane domain or signal peptide was detected in any of the proteins encoded by BrRV1, LoPV1, MelRoV1, PhPiV1, PiFleV1 and SpV1.

Consensus gene junction sequences 3' AU$(N)_5$UUUUU G CUCU 5' of LoPV1, MelRoV1, PhP1V1 and SpV1 are identical and the same as in the genomes of the known varicosaviruses *Alopecurus myosuroides* varicosavirus 1 (AMVV1), lettuce big-vein associated virus (LBVaV), and red clover-associated varicosavirus (RCaVV). The gene junction sequences of BrRV1 and PiFleV1 differ slightly: BrRV1 5´end sequence is CUCA instead of CUCU, while PiFleV1 3´end sequence is GU$(N)_5$UUUUU instead of AU$(N)_5$UUUUU (Table 2).

Pairwise aa sequence identities between the cognate encoded protein of BrRV1, LoPV1, MelRoV1, PhPiV1, PiFleV1 and SpV1 and those from other varicosaviruses vary significantly (Table 3E).

Phylogenetic analysis based on the deduced N protein aa sequences placed all six novel varicosa-like viruses into a clade with the known varicosaviruses. BrRV1 and SpV1 clustered with RCaVV and these viruses have a similar genomic organization with three ORFs on RNA 2. LoPV1 clustered with AMVV1, and both infect monocots. PhPV1 clustered with LBVaV, while the unique PiFleV1 clustered with MelRoV1 and formed a sister clade to the clade composed of PhPV1 and LBVaV, and LoPV1 and AMVV1 clusters (Fig. 2).

Discussion

In the last few years several novel plant rhabdoviruses, which may be asymptomatic, have been reported in HTS studies (Baek et al., 2019; Bejerman et al., 2020c; Bulos et al., 2021; Ding et al., 2019; Koloniuk et al., 2018; Orfanidou et al., 2020; Read et al., 2019; Wu et al., 2018). Thus, it is tempting to speculate that some plant rhabdovirus-like sequences may be hidden in published plant transcriptome databases, generated with diverse objectives beyond virus research, where viral RNA was inadvertently co-purified

with endogenous plant RNA and sequenced. To prove this point, we and others have previously identified the sequences of a small number of plant rhabdoviruses contained in public transcriptome databases (Debat and Bejerman, 2019; Goh et al., 2020; Sidharthan and Balanwan, 2021); however an extensive search has not been conducted to date. Therefore, we queried the publicly available plant transcriptome datasets at the transcriptome shotgun assembly (TSA) database hosted at NCBI which resulted in the identification of 26 novel plant rhabdoviruses.

Discovery of novel plant rhabdoviruses expands the diversity and evolutionary history of rhabdovirids

The coding-complete or complete genomic sequences of 64 plant rhabdoviruses have been reported by early 2021. Thirty-two of them are cytorhabdoviruses, thirteen alphanucleorhabdoviruses, ten betanucleorhabdoviruses, five dichorhaviruses, three varicosaviruses and one gammanucleorhabdovirus. Similarly, half of the novel sequences reported in this study are those of putative cytorhabdoviruses, indicating the extensive diversification of this group of viruses. The novel 26 viruses discovered in this study, all appear to be members of new species, and account for nearly half of the plant rhabdovirus species reported so far. In addition, five new putative betanucleorhabdoviruses shed more light on the diversity and evolution of this recently created genus. One newly identified putative alphanucleorhabdovirus and one novel putative dichorhavirus expand the diversity of their respective genera. Finally, we detected six novel varicosa-like viruses, which doubles the number of known varicosaviruses, providing new information about the genomic diversity and evolution of these little-studied fungi-transmitted viruses. Thus, our study provides the most complete insight to-date about the genomic diversity and evolution of plant rhabdoviruses, complementing the status quo of these viruses with additional data on genomic organization and highlighting their apparent genetic flexibility. Overall, sequence identity between the newly discovered viruses and those plant rhabdoviruses already described was low, a common feature among these taxa characterized by a high level of diversity in both genome sequence and organization (Dietzgen et al., 2017). Low sequence identity of the novel viruses with the closest previously described virus may indicate that there is still a significant amount of virus ‘dark matter’ within the plant rhabdovirus space worth exploring that potentially contains a significant number of yet to be discovered plant rhabdoviruses. Future work should be focused on the analysis of additional RNA datasets of diverse potential hosts of the partial virus genomes identified in this study.

Viruses assigned to different species within the genera *Alphanucleorhabdovirus*, *Betanucleorhabdovirus* and *Cytorhabdovirus* have a nucleotide sequence identity lower than 75% in the complete genome sequence and occupy different ecological niches as evidenced by differences in host species and/or arthropod vectors (https://talk.ictvonline.org/ictv-reports/ictv_online_report/negative-sense-rna-viruses/w/rhabdoviridae). Cytorhabdoviruses may also have an aa sequence identity of less than 80% in all cognate ORFs. Viruses assigned to different species in the genus *Dichorhavirus* have less than 80% nucleotide sequence identity in the L gene. Based on these species demarcation criteria, ATV1 should be classified in a new *Alphanucleorhabdovirus* species, and AscSyV2, PleArV1 and RhoDeV1 in new species in the genus *Betanucleorhabdovirus*; due to incomplete sequences, PerMiV1 and CusReV1 cannot be classified based on these criteria. AntAmnV1, AscSyV1, BeTaV1, GlLV1, GymDenV1, LotCorV1, NymAV1, TaEV and TrAV1 should be classified in new species in the genus *Cytorhabdovirus*, whereas AChV1, DiCoV1, PelRaV1 and SuSV1 due to incomplete genome sequence data cannot be classified. The dichorha-like VVeV1 also awaits at least coding-complete sequence data before it can be formally classified in this genus. Species demarcation criteria recently proposed for the genus *Varicosavirus* by the ICTV *Rhabdoviridae* Study Group include a minimum nucleotide sequence divergence of 50% in the L protein. Based on this genetic distance, the varicosa-like viruses BrRV1, LoPV1, MelRoV1, PiFleV1 and SpV1 would not be considered different species, and PhPiV1 cannot be classified due to incomplete sequence data. Considering the diverse host plant species these viruses were identified in and their phylogenetic relationships, these viruses should likely be classified in different species. Thus, we propose a nucleotide sequence identity of 75% across the genome and in the N gene as thresholds for species demarcation in the *Varicosavirus* genus, in line with the species demarcation criteria for the other genera of plant rhabdoviruses.

Host range of the novel plant rhabdoviruses

Most of the plant hosts in which the novel viruses of this study were identified are dicots; nevertheless, AChV1 which was detected in Chinese onion is likely the first monocot-infecting cytorhabdovirus that belongs to a clade of aphid-transmitted viruses. Furthermore, most of the source hosts in which the novel viruses were identified are herbaceous plants, which overall are the most common hosts of plant rhabdoviruses (Dietzgen et al., 2020). However, one novel putative varicosavirus, *Pinus flexilis* virus 1, was associated with a gymnosperm host. Few viruses were identified previously from gymnosperms

(Alvarez-Quinto et al., 2020; Han et al., 2002; Koh et al., 2015; Lauber et al., 2019; Nibert et al., 2014). Interestingly, a putative RdRp sequence was identified in the gymnosperm *Sciadopitys verticillata* and proposed to belong to a varicosavirus (Mushegian et al., 2016). Therefore, to our knowledge, this study is the first to report the complete coding sequence of a plant rhabdovirus associated with a gymnosperm host, and presenting a unique unsegmented genome redefining the genome architecture of varicosaviruses. Future virus discovery studies should focus on gymnosperm viromes, which have been understudied and likely hide a rich and diverse number of novel viruses.

Diverse genome organizations of plant rhabdoviruses

Interestingly, the genome sequence of four novel viruses discovered in this study, GymDenV1 (only four predicted genes), TaEV and TrAV1 (both with only five predicted genes), and PiFleV1 (a unsegmented varicosa-like virus), have a unique genome architecture among plant rhabdoviruses, which differs from the consensus genome organization reported for previously-known cytorhabdoviruses and varicosaviruses (Walker et al., 2018). The genomes of the cytorhabdoviruses GymDenV1, TaEV1 and TrAV1 lack a glycoprotein ORF which for rhabdoviruses is not essential for replication and systemic movement as demonstrated using an infectious clone of Sonchus yellow net virus (Wang et al., 2015). Furthermore, isolates of citrus-associated rhabdovirus (CiaRV) were recently shown to have an impaired G ORF (Zhang et al., 2021).

Five canonical structural protein genes 3'-N-P-M-G-L-5' are thought to be conserved among all rhabdoviruses (Dietzgen et al., 2017), while plant rhabdovirus genomes contain at least six ORFs (Walker et al., 2018). However, only four genes were predicted in the genome of the varicosavirus RCaVV (Koloniuk et al., 2018). Four genes were also identified in some of the varicosa-like virus genomes assembled in this study, suggesting that a minimal set of four genes may be sufficient for varicosaviruses to replicate in a plant host. This hypothetical minimal requirement appears to also apply to cytorhabdoviruses. For example, GymDenV1 encodes only the nucleocapsid core (NC) proteins N, P and L that are essential for virus replication and transcription, and the M protein that is required for condensation of the core during virion assembly (Dietzgen et al., 2017). Thus, how GymDenV1 moves from cell to cell remains to be unraveled. Interestingly, no cell-to-cell movement protein has been identified so far in the varicosaviruses.

The discovery of diverse novel rhabdo-like viruses in metagenomics studies of arthropods (Li et al., 2015) supports the assumption that arthropods have been fundamental to rhabdovirus evolution (Whitfield et al., 2018), and the G protein was found to be essential for virus attachment to predicted cellular receptors in the midgut of arthropod vectors that facilitate virus uptake (Dietzgen et al., 2017). This agrees with the hypothesis that cytorhabdoviruses, nucleorhabdoviruses and dichorhaviruses evolved from viruses of plant-feeding arthropods that acquired movement proteins and assorted RNAi suppressors through recombination with preexisting plant viruses (Dolja et al., 2020). Viruses in these three genera appear to be the least plant-specialized among negative-sense RNA viruses. However, the evolution of the non-enveloped negative-sense RNA plant viruses, such as the fungal-transmitted varicosaviruses, which likely do not encode a G protein, clearly reflects the adaptation to a plant-specific lifestyle, raising the possibility that their origin is via *trans*-kingdom horizontal transfer between fungi and plants (Dolja et al., 2020). A G protein-defective genome was recently identified in citrus isolates of CiaRV, and the authors speculated that the eventual "simplification" of viral genomes to adapt to plants without requiring an arthropod vector could provide an evolutionary advantage, especially in fruit trees that are propagated artificially by asexual modes, such as cutting and grafting (Zhang et al., 2021). Nevertheless, the tentative hosts of GymDenV1, TaEV1 and TrAV1 are herbaceous plants, thus an evolutionary advantage linked to the lack of a G protein is not obvious for these viruses. Strikingly, TaEV1 is phylogenetically related to arthropod-transmitted cytorhabdoviruses. Thus, further studies should focus on the potential vector and the mode of transmission of GymDenV1, TaEV1 and TrAV1 to complement their peculiar minimalist genome organization and evolutionary links with biological data.

Phylogenetic relationships among plant rhabdoviruses as predictors for vector types

Among all plant rhabdoviruses studied so far, there is a strong correlation between phylogenetic relationships and vector types (Dietzgen et al., 2017, 2020). We therefore predict that the novel betanucleorhabdoviruses are likely aphid-transmitted, while the putative dichorhavirus VVeV1 is likely transmitted by *Brevipalpus* mites, and the varicosa-like viruses may be transmitted by chytrid fungi. Based on its phylogenetic clustering, the alphanucleorhabdovirus ATV1 is likely transmitted by a planthopper. Among the novel cytorhabdoviruses identified in this study, AChV1, AscSyV1, GlLV1, LotCorV1, NymAV1, PelRaV1 and SuSV1 are likely aphid-transmitted, while vectors for AntAmV1, DiCoV1, GymDenV1, TaEV1 and TrAV1 cannot be predicted. BeTaV1 genome was assembled from

whitefly transcriptome data and therefore is likely a whitefly-transmitted cytorhabdovirus. The L protein-like TSA sequences included in the assembly of BeTaV1 genome were previously reported to be 95-98% identical to soybean blotchy mosaic virus partial L gene sequences (Dietzgen et al., 2020), thus it is tempting to speculate that the plant host of BeTaV1 may be soybean.

At least one transmembrane domain was identified in each P´ protein predicted in the genomes of a group of cytorhabdoviruses discovered in this study, consistent with a previous analysis of other cytorhabdoviruses which also identified at least one transmembrane domain in every P´ protein (Franova et al., 2019). Interestingly, this overprinted accessory protein is encoded by every cytorhabdovirus that appears to be aphid-transmitted. On the other hand, P´ protein is not encoded in the genomes of aphid-transmitted betanucleorhabdoviruses. Therefore, the potential function of the P´ protein is unlikely to be directly associated with vector specificity.

Cytorhabdoviruses

Two of the cytorhabdoviruses, AChV1 and SuSV1, whose genomes were only partially assembled, clustered phylogenetically in a monophyletic clade with ADV, ChYDaV, RVCV and SCV, all viruses that contain a P6 accessory ORF between G and L genes (Bejerman et al., 2015; Jones et al., 2019; Koloniuk et al., 2018; Liu et al., 2021). Given this clustering, it is likely that AChV1 and SuSV1 will have a similar genomic organization. At least one transmembrane domain has been predicted in every cytorhabdovirus with an accessory P6 ORF. Transmembrane domains were also predicted in the accessory ORF between G and L genes of other cytorhabdoviruses (Ding et al., 2019; Franova et al., 2019; Yang et al., 2017). Thus, the protein encoded by this small ORF may have membrane-associated functions similar to viroporins in vertebrate rhabdoviruses (Joubert et al. 2014).

The phylogenetic relationships of the now expanded number of known cytorhabdoviruses provide some support for splitting the genus *Cytorhabdovirus* to establish three genera that represent different evolutionary lineages: *i*) *Alphacytohabdovirus* would include species for all aphid-transmitted cytorhabdoviruses, *ii*) *Betacytorhabdovirus* would include species for all those cytorhabdoviruses likely transmitted by leafhoppers, planthoppers and whiteflies, as well as AntAmnV1, DiCoV1 and TaEV1; *iii*) *Gammacytorhabdovirus* would include species for GymDenV1 and TrAV1 (Fig. 2).

Nucleorhabdoviruses

The ATV1 genome that was assembled from transcriptome data of the monocot tequila agave does not encode any accessory ORFs, like most of the alphanucleorhabdoviruses, except the group of PYDV-like viruses that encode an X ORF of unknown function (Dietzgen et al., 2020). ATV1, while branching in a sister clade, appears to have a close evolutionary relationship with the cluster of planthopper-transmitted monocot-infecting alphanucleorhabdoviruses MIMV, MMV, MMaV and TVCV. It is tempting to speculate that this clade evolved from a common ancestor that adapted to infect monocots and to be transmitted by planthoppers. Furthermore, the isoelectric point (IEP) of ATV1 predicted proteins is similar to that of MIMV, MMV, MMaV and TVCV predicted proteins, thus supporting a link between these viruses and ATV1.

All the betanucleorhabdoviruses identified in this study, AscSyV2, CusReV1, PerMiV1, PleArV1 and RhoDeV1, appear to be associated with dicot hosts, which is consistent with every betanucleorhabdovirus reported so far (Dietzgen et al., 2020). The dicot host range of the betanucleorhabdoviruses is likely linked to their insect vector, given that every betanucleorhabdovirus where a vector has been experimentally determined is transmitted by aphids (Dietzgen et al., 2020). Although aphids can colonize both dicots and monocots (Doring, 2014), it has been suggested that these insects more successfully feed on dicots (van Bell and Will, 2016). Regarding their genome organization, the five novel betanucleorhabdoviruses identified in this study encode six plant rhabdovirus ORFs in the conserved order 3'-N-P-P3-M-G-L-5' without any accessory ORFs, like the majority of previously known betanucleorhabdoviruses such as the well-studied SYNV. CusReV1, PerMiV1, PleArV1 and RhoDeV1 likely belong to the same evolutionary lineage as BFTaV1, BCaRV, CdVCV1, DYVV, GSPNuV, SYNV and SYVV, which may represent the ancestral clade within the betanucleorhabdoviruses. However, AscSyV2's closest evolutionary relationship is with the betanucleorhabdoviruses ApRVA and AaNV, which differ in their genome organization by having an accessory ORF located between M and G genes (Baek et al., 2019; Gaafar et al., 2019). Interestingly, the IEP of AscSyV2, CusReV1, PerMiV1, PleArV1 and RhoDeV1 N protein is different to the IEP of the cognate proteins encoded by the phylogenetically related betanucleorhabdoviruses. It is unknown if this difference in the IEP may have a biologically effect in terms of RNA or protein binding. Moreover, the RhoDeV1 M protein is acidic, while the BFTaV1, BCaRV, BmV2, CdVCV1, CusReV1, DYVV, GSPNuV, PleArV1, SYNV and SYVV N proteins are basic. It has been suggested that charge differences in the M protein may be associated with differences in their abilities to interact with the negatively charged lipids of the membrane (Sun et al., 2018). NLS and

NES were predicted for most of the AscSyV2, ATV1, CusReV1, PerMiV1, PleArV1 and RhoDeV1 encoded proteins, which can be expected since nucleorhabdoviruses replicate in the nucleus of the infected cells (Dietzgen et al., 2017).

The phylogenetic relationships of nucleorhabdoviruses, including the novel alpha- and betanucleorhabdoviruses discovered in our analysis, clearly support the recent split of the previous genus *Nucleorhabdovirus* into the three genera *Alphanucleorhabdovirus*, *Betanucleorhabdovirus*, and *Gammanucleorhabdovirus* (Dietzgen et al., 2020).

Varicosaviruses

Currently there are only three recognized varicosaviruses, LBVaV, AMVV1 and RCaVV, and the available information regarding their gene functions is generally scarce. The functional roles of varicosavirus P2, P3, P4, P5 and P6 proteins remain to be elucidated. No transmembrane domain was predicted in any of the proteins encoded by the varicosavirus genomes identified in this study as well as in those already described, thus it appears that no varicosavirus- encoded protein has a membrane-associated function. LoPV1 N and P2 proteins, and AMVV1 and MelRoV1 L proteins are predicted to be exclusively located in the nucleus, whereas NES are predicted in most N, P2 and P3 proteins, and some P4 proteins (Table S2). This suggests a potential role of these proteins in the cell nucleus.

Interestingly, the predicted N protein of the unsegmented PiFLeV1 is basic, whereas the N proteins of AMVV1, BrRV1, LBVaV, LoPV1, MelRoV1, PhPiV1, RCaVV and SpV1 are acidic. This difference in the IEP may be associated with a different replication mechanism of the unsegmented PiFLeV1 compared to the segmented varicosaviruses. Moreover, BrRV1 P2 is basic, while the P2s of AMVV1, LBVaV, LoPV1, MelRoV1, PiFleV1, RCaVV and SpV1 are neutral or acidic. The biological significance of this difference is unknown because the functional role of P2 still needs to be unraveled.

LoPV1 and AMVV1 are phylogenetically closely related, their N and P3 aa sequences are >50% identical, and both are associated with grasses in the family *Poaceae*. However, LoPV1 RNA1 has one ORF while AMVV1 RNA1 has two (Sabaddin et al., 2017). PhPiV1 clustered with LBVaV, thus it is likely that its genome that was partially assembled, has a similar genomic organization as LBVaV with two ORFs encoded in RNA1 and five in RNA2 (Sasaya et al., 2004). Interestingly, RNA2 of three of the novel varicosa-like viruses identified in this study has three ORFs, similar to RCaVV and AMVV1 RNA2 (Koloniuk et al., 2018; Sabaddin et al., 2017). On the other hand, RNA2 of one novel varicosa-like virus

identified in our study has four ORFs. Thus, the number of ORFs identified in RNA2 of every novel varicosa-like virus reported in this study, except for PhPiV1, is different from the first identified varicosavirus, LBVaV, which has five ORFs (Sasaya et al., 2004). The RNA1 segments of BrRV1, LoPV1, SpV1 and MelRoV1 are similar to RNA1 of RCaVV in that they only encode the L protein, whereas a small ORF before the L gene was identified in PhPiV1 and previously in AMVV1 and LBVaV (Koloniuk et al., 2018).

Among the varicosa-like viruses identified in this study, PiFleV1 is unique in terms of genome organization, since its genome is unsegmented, a characteristic that differs from the currently known bi-segmented genome architecture of varicosaviruses (Walker et al., 2018). This may be an adaptation to its gymnosperm host. Unfortunately, we were not able to assemble the complete genome of the varicosavirus associated with the gymnosperm *Sciadopitys verticillata* previously reported by Mushegian et al., (2016) to support this hypothesis. Based on genome organization and phylogenetic placement, PiFleV1 appears to be the first example where rhabdoviruses with segmented and unsegmented genomes are closely related and may be classified in the same genus.

Phylogenetically the newly described and previously reported varicosaviruses form two sister clades that could be classified as species in two new genera, tentatively named *Alphavaricosavirus* and *Betavaricosavirus*. The alphavaricosaviruses would include AMVV1, LBVaV, LoPV1, MelRoV1, PhPiV1 and PiFleV1, while the betavaricosaviruses would include RCaVV, BrRV1 and SpV1 (Fig. 2).

Conclusion remarks

In summary, this study illustrates the complexity and diversity of plant rhabdoviruses genomes and demonstrates that analyzing SRA public data is a valuable tool not only to accelerate the discovery of novel viruses but also to gain insight into their evolution and to refine virus taxonomy.

Data availability

The sequences have been deposited in GenBank with accession number BK014297-014366.

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Figure 1. Diagrams depicting the genomic organization of each (A) alpha- and betanucleorhabdovirus, (B) cytorhabdovirus, (C) varicosavirus and (D) dichorhavirus sequence assembled in this study. Abbreviations: N, nucleoprotein coding sequence (CDS); P, phosphoprotein CDS; P3, putative cell-to-cell movement protein CDS; M, matrix protein CDS; G, glycoprotein CDS; L, RNA dependent RNA polymerase CDS.

Figure 2. A Maximum Likelihood phylogenetic based on a multiple amino acid alignment of the N protein sequences of plant rhabdoviruses constructed with the WAG+G+F model. Bootstrap values following 1000 replicates are given at the nodes, but only values above 50% are shown. The scale bar indicates the number of substitutions per site. Viruses identified in this study are noted with a green rectangles. Accession numbers of every virus used to construct the ML tree are listed in supplementary Table S1.

Table 1. Summary of rhabdovirus contigs identified from plant RNA-seq data available in the NCBI database

Genus	Plant/insect host; monocot/dicot	Virus name	Abbrevi-ation	Bioproject ID	Data Citation	Segment number	Length (nt)	Accession number	Protein ID	Length (aa)
Alphanucleorhabdovirus	Blue agave (*Agave tequilana*); monocot	*Agave tequilana* virus 1	ATV1	PRJNA193469	Gross et al., (2013)	1	13,166	BK014297	N P P3 M G L	452 335 287 257 597 1937
Betanucleorhabdovirus	Common milkweed (*Asclepias syriaca*); dicot	*Asclepias syriaca* virus 2	AscSyV2	PRJNA210776	Weitemier et al., (2019)	1	12,940	BK014299	N P P3 M G L	459 337 343 250 643 2023
	Giant dodder (*Cuscuta reflexa*); dicot	*Cuscuta reflexa* virus 1	CusReV1	PRJNA290291	Olsen et al., (2016)	1	8,869*	BK014340; BK014341; BK014342; BK014343; BK014344; BK014345	N P P3 M G L	457 336 325 269* 550* 740*
	Small water-pepper (*Persicaria minor*); dicot	*Persicaria minor* virus 1	PerMiV1	PRJNA208436	Loke et al., (2017)	1	4,793*	BK014346; BK014347; BK014348; BK014349; BK014350; BK014351; BK014352; BK014353; BK014354	N P P3 M G L	372* 115* 172* 227* 260* 430*

	Cuban oregano (*Plectranthus aromaticus*); dicot	*Plectranthus aromaticus* virus 1	PleArV1	PRJNA491230	Ab Rahim, M.H.; University Malaysia Pahang, Malaysia; unpublished	1	12,994	BK014300	N P P3 M G L	450 332 321 286 582 2085
	Red rhododendron (*Rhododendron delavayi*); dicot	*Rhododendron delavayi* virus 1	RhoDeV1	PRJNA358123	Zhang et al., (2017)	1	13,719	BK014301	N P P3 M G L	464 338 326 279 636 2016
Cytorhabdovirus	Chinese onion (*Allium chinense*); monocot	*Allium chinense* virus 1	AChV1	PRJNA310810	Zhu et al., (2017)	1	7,981*	BK014319; BK014320; BK014321; BK014322; BK014323; BK014324	N P P´ P3 M G P6 L	457 316 128 231 165 542* ? 531*
	Flamingo lily (*Anthurium amnicola*); monocot	*Anthurium amnícola* virus 1	AntAmV1	PRJNA288827	Susuki et al., (2017)	1	12,480	BK014302	N P P3 M G L	409 314 207 176 565 2047
	A. syriaca	*Asclepias syriaca* virus 1	AscSyV1	PRJNA210776	Weitemier et al., (2019)	1	13,392	BK014298	N P P´ P3 M G P6 L	445 304 87 334 176 551 86 2101

Cytorhabdovirus	Silverleaf whitefly *(Bemisia tabaci*)	*Bemisia tabaci* - associated virus 1	BeTaV1	PRJNA237273	Upadhyay et al., (2015)	1	13,025	BK014303	N P P3 P4 M G L	447 326 187 48 206 579 2102
	Yam (*Dioscorea composita*)	*Dioscorea composita* virus 1	DiCoV1	PRJNA253902	Wang et al., (2015)	1	9,959*	BK014355; BK014356; BK014357; BK014358	N P P3 M G P6 L	441 N/A N/A 201 586 72 1546*
	Beach silvertop (*Glehnia littoralis*); dicot	*Glehnia littoralis* virus 1	GlLV1	PRJNA248158	Li et al., (2018)	1	12,193	BK014304	N P P´ P3 M G P6 L	412 324 84 201 167 551 66 2072
	Marsh fragrant orchid (*Gymnadenia densiflora*); monocot	*Gymnadenia densiflora* virus 1	GymDenV1	PRJNA504609	Piñeiro-Fernandez et al., (2019)	1	9,887	BK014305	N P M L	444 310 189 2068
	Bird's foot trefoil (*Lotus corniculatus*); dicot	*Lotus corniculatus* virus 1	LotCorV1	PRJNA77207	Wang et al., (2013)	1	12,599*	BK014306	N P P´ P3 M G P6 L	479 311 98 362 179 558 56 1985*
	European white	*Nymphaea alba*	NymAV1	PRJNA472003	Unlu E.S.,	1	12,886	BK014307	N	421

	waterlily (*Nymphaea alba*); dicot	virus 1			and Yildiz, G.G; Abant Izzet Baysal University, Turkey; unpublished				P P´ P3 M G P6 L	308 85 228 195 560 69 2076
	Crowfoot geranium (*Pelargonium radula*), dicot	*Pelargonium radula* virus 1	PelRaV1	PRJNA491235	Ab Rahim, M.H.; University Malaysia Pahang, Malaysia; unpublished	1	11,130*	BK014325; BK014326; BK014327; BK014328	N P P´ P3 M G P6 L	483 309 69 354 177 578* 54 1391*
Cytorhabdovirus	Seepweed (*Suaeda salsa*); dicot	*Suaeda salsa* virus 1	SuSV1	PRJNA395283	Xu et al., (2017)	1	6,156*	BK014359; BK014360; BK014361; BK014362; BK014363; BK014364; BK014365; BK014366	N P P´ P3 M G P6 L	332* 294* 66* 218 165 70* ? 711*
	African marigold (*Tagetes erecta*); dicot	*Tagetes erecta* virus 1	TaEV1	PRJNA431782	Feng et al., (2018)	1	11,707	BK014308	N P P3 M L	520 505 200 187 2079
	Ajwain (*Trachyspermum ammi*); dicot	*Trachyspermum ammi* virus 1	TrAV1	PRJNA359623	Soltani Howyzeh et al., (2018)	1	10,920	BK014309	N P P3 M L	455 343 237 200 2069
Dichorhavirus	Hidden violet (*Viola verecunda*); dicot	*Viola verecunda* virus 1	VVeV1	PRJNA345302	Park et al., (2017)	1	5,304*	BK014329; BK014330;	N P P3	494 N/A 323

						2	5212*	BK014331 BK014332; BK014333	M G L	N/A 661 1668*
Varicosavirus	Bok choy (*Brassica rapa subsp. chinensis*): dicot	*Brassica rapa* virus 1	BrRV1	PRJNA396268	Chen et al., (2017)	1 2	6397 4068	BK014310 BK014311	L N 2 3	2019 435 411 180
	Perrenial ryegrass (*Lolium perenne*); monocot	*Lolium perenne* virus 1	LoPV1	PRJNA222646	Farrell et al., (2014)	1 2	6302 4167	BK014312 BK014313	L N 2 3	2029 533 379 161
	Asian cow-wheat (*Melampyrum roseum*); dicot	*Melampyrum roseum* virus 1	MelRoV1	PRJDB5395	Kado and Innan (2018)	1 2	6365 4635	BK014314 BK014315	L N 2 3 4	2003 443 350 304 193
	Downy phlox (*Phlox pilosa*); dicot	*Phlox pilosa* virus 1	PhPiV1	PRJNA360978	Roda et al., (2017)	1 2	4896* 3201*	BK014334; BK014335; BK014336; BK014337 BK014338; BK014339	L N 2 3 4	1589* 378 81* 289 110*
	Limber pine (*Pinus flexilis*); gymnosperm	*Pinus flexilis* virus 1	PiFleV1	PRJNA315892	Liu et al., (2016)	1	11,740	BK014316	N 2 3 4 L	405 447 318 219 2049
	Spinach (*Spinacia oleracea*); dicot	Spinach virus 1	SpV1	PRJDB3392	Takahata et al., (2016)	1 2	6,151* 3,750*	BK014317 BK014318	L N 2 3	2010* 439 450 136*

*partial sequence
N/A: not available

Table 2. Consensus plant rhabdovirus gene junction sequences

Genus	Virus*	3´end mRNA	intergenic spacer	5´end mRNA
Alphanucleorhabdovirus	ATV1	AUUCUUUUU	GGG	UUG
	CYDV	AUUAUUUUU	GGG	UUG
	EMDV	AUUAUUUUU	GGG	UUG
	JYBaV	AUUAUUUUU	GGG	UUG
	MIMV	AUUCUUUUU	GGG	UUG
	MMV	AUUCUUUUU	GGG	UUG
	MMaV	AUUCUUUUU	GGG	UUG
	PeV1	AUUU(A/C)UUUU	G(N)n	UUG
	PhCMoV	AUUAUUUUU	GGG	UUG
	PYDV	AUUAUUUUU	GGG	UUG
	RYSV	AUUAUUUUU	GGG	UUG
	TaVCV	AUUCUUUUU	GGG	UUG
	WYSV	UAAAUUUUU	GGGG	UUG
Betanucleorhabdovirus	AscSyV2	AUUCUUUUU	GG	UUG
	CusReV1	AUUCUUUUU	GG	UUG
	PerMiV1	AUUCUUUUU	GG	UUG
	PleArV1	AUUCUUUUU	GG	UUG
	RhoDeV1	AUUCUUUUU	GG	UUG
	AaNV	AUUCUUUUU	GG	UUG
	ApRVA	AUUCUUUUU	GG	UUG
	BFTaV	AUUCUUUUU	GG	UUG
	BCaRV	AUUCUUUUU	GG	UUG
	BmV2	AUUCUUUUU	GG	UUG
	CdVCV1	AUUCUUUUU	GG	UUG
	DYVV	AUUCUUUUU	GG	UUG
	GSPNuV	AUUCUUUUU	GG	UUG
	SYNV	AUUCUUUUU	GG	UUG

	SYVV	AUUCUUUUU	GG	UUG
Gammanucleorhabdovirus	MFSV	AUUUAUUUU	GUAG	UUG
Dichorhavirus	VVeV1	-	-	-
	CiCSV	AUUUAUUUU	GUAG	UU
	CiLV-N	AUUUAUUUU	GUAG	UU
	ClCSV	AUUUAUUUU	GUAG	UU
	CoRSV	AUUUAUUUU	GUAG	UU
	OFV	AUUUAUUUU	GUUG	UU
Cytorhabdovirus	AChV1	AAUUAUUUU	GAU	CUA
	AntAmV1	AUUAUUUUU	GCU	CUU
	AscSyV1	AAUUAUUUU	GNU	CNN
	BeTaV1	UUAUUUUUU	GA	CUC
	DiCoV1	AUAUUUUUU	$GG(N)_n$	CNN
	GlLV1	AAUUAUUUU	GAU	CUU
	GymDenV1	AAUCUUUUU	$A(N)_n$	CNN
	LotCorV1	AAUUAUUUU	$GGU(N)_n$	CUG
	NymAV1	AUUAAUUUU	GAU	CUN
	PelRaV1	AAUUAUUUU	$GGU(N)_n$	CUG
	SuSV1	AAUUAUUUU	GAU	CUU
	TaEV1	AUUCUUUUU	$GG(N)_n$	CUN
	TrAV1	AUUCUUUUU	$A(N)_n$	CNU
	ADV	AAUUAUUUU	GAU	CUU
	BmV1	AAUUAUUUU	GAN	CUG
	BYSMV	AUUAUUUUU	GA	CUC
	CCyV1	AAUUCUUUU	$G(N)_n$	CUU
	CBDaV	AUUCUUUUU	GG	CUC
	ChYDaV	AAUUAUUUU	GAU	CUN
	CuCV1	AUUAUUUUU	GA	CUC
	KePCyV	AAUUAUUUU	GAU	CUU
	LNYV	AAUUCUUUU	$G(N)_n$	CUU
	LYMoV	AAUUCUUUU	$G(N)_n$	CUN
	MaCyV	AUUCUUUUU	GA	CUC

	MYSV	AUUAUUUUU	GA	CUC
	NCMV	AUUCUUUUU	GA	CUC
	PMuMaV	AUUAUUUUU	$G(N)_n$	CUA
	PpVE	AUUCUUUUU	GAC	CCU
	PeVA	AAUUAUUUU	$G(N)_n$	CUN
	RVR	AUUUAUUUU	GACU	CUA
	RSMV	AUUCUUUUU	GCU	CUG
	RVCV	AUUUAUUUU	GAU	CUU
	SCV	AAUUAUUUU	GAU	CUU
	StrV1	AAUUAUUUU	GAU	CUU
	TpVA	AAUUAUUUU	GAU	CUU
	TpVB	AAUUCUUUU	$G(N)_n$	CUN
	TrARV1	AAUUAUUUU	GAU	CUU
	TYMaV	AAUUAUUUU	GAU	CUU
	WhIV4	AAUUAUUUU	GNU	CUU
	WhIV5	AAUUAUUUU	GAU	CNN
	WhIV6	AAUUAUUUU	GAU	CUN
	YmCaV	UUAUUUUUU	GA	CUC
	YmVA	AUUCUUUUU	GGU	CCU
Varicosavirus	BrRV1	$AU(N)_5UUUUU$	G	CUCA
	LoPV1	$AU(N)_5UUUUU$	G	CUCU
	MelRoV1	$AU(N)_5UUUUU$	G	CUCU
	PhPiV1	$AU(N)_5UUUUU$	G	CUCU
	PiFleV1	$GU(N)_5UUUUU$	G	CUCU
	SpV1	$AU(N)_5UUUUU$	G	CUCU
	AMVV1	$AU(N)_5UUUUU$	G	CUCU
	LBVaV	$AU(N)_5UUUUU$	G	CUCU
	RCaVV	$AU(N)_5UUUUU$	G	CUCU

The consensus gene junction sequences of the viruses identified in this study are highlighted in light grey. * Names and abbreviations of newly identified viruses are listed in Table 1; while the names and abbreviations of known viruses are listed in Supp Table 1.

Table 3A. Pairwise identity percentages between ATV1 and selected alphanucleorhabdoviruses

		genome[a]	N[b]	P[b]	P3[b]	M[b]	G[b]	L[b]
ATV1 vs	MMaV	47.0	32.4	16.7	17.5	17.5	29.3	35.0
	MMV	49.3	29.1	13.7	20.5	16.4	29.1	34.5
	PeV1	49.1	26.9	18.8	20.1	14.3	28.7	32.9
	PYDV	48.8	24.1	10.9	18.2	13.6	23.5	31.8

Table 3B. Pairwise identity percentages between AscSyV2, CusReV1, PleArV1 and RhoDeV1 and selected betanucleorhabdoviruses

		genome[a]	N[b]	P[b]	P3[b]	M[b]	G[b]	L[b]
AscSyV2 vs	ApRVA	51.3	47.5	24.3	26.3	23.6	33.2	44.2
	BCaRV	50.4	40.5	23.4	25.0	22.8	30.3	38.5
	CusReV1	N/C	40.9	23.0	18.1	N/C	N/C	N/C
	DYVV	50.6	42.3	19.5	22.2	20.5	27.0	38.1
	GSPNuV	46.3	42.7	21.7	19.9	20.1	29.0	38.7
	PleArV1	49.3	41.1	19.6	19.3	19.1	26.4	37.2
	RhoDeV1	51.4	40.7	18.1	25.5	19.3	27.1	39.9
CusReV1 vs	ApRVA	N/C	42.5	20.8	19.6	N/C	N/C	N/C
	AscSyV2	N/C	40.9	23.0	18.1	N/C	N/C	N/C
	BCaRV	N/C	48.3	27.6	28.4	N/C	N/C	N/C
	DYVV	N/C	59.0	32.6	41.7	N/C	N/C	N/C
	GSPNuV	N/C	59.0	30.8	44.0	N/C	N/C	N/C
	PleArV1	N/C	60.5	31.6	40.8	N/C	N/C	N/C
	RhoDeV1	N/C	38.6	24.8	23.0	N/C	N/C	N/C
PleArV1 vs	ApRVA	49.8	44.0	22.2	20.6	16.7	27.2	37.4
	AscSyV2	49.3	41.1	19.6	19.3	19.1	26.4	37.2
	BCaRV	52.1	49.9	30.1	26.5	31.6	36.4	47.3
	CusReV1	N/C	60.5	31.6	40.8	N/C	N/C	N/C
	DYVV	62.1	70.7	45.6	63.2	48.1	61.0	62.2
	GSPNuV	45.8	64.5	34.8	43.7	41.0	48.4	55.4
	RhoDeV1	50.9	42.5	22.7	29.8	25.4	31.9	42.8
RhoDeV1 vs	ApRVA	51.7	40.4	23.2	22.4	22.2	30.2	40.8
	AscSyV2	51.4	40.7	18.1	25.5	19.3	27.1	39.9
	BCaRV	53.1	42.0	27.2	34.5	27.4	33.6	45.0
	CusReV1	N/C	38.6	24.8	23.0	N/C	N/C	N/C
	DYVV	51.3	42.2	23.2	27.5	26.5	34.4	43.7
	GSPNuV	46.3	41.1	22.4	25.4	28.6	32.8	43.3

	PleArV1	50.9	42.5	22.7	29.8	25.4	31.9	42.8

Table 3C. Pairwise identity percentages between the cytorhabdoviruses assembled in this study and selected cytorhabdovirus

		genome[a]	N[b]	P[b]	P3[b]	M[b]	G[b]	P6[b]	L[b]
AChV1 vs	ADV	N/C	44.3	30.8	34.5	27.9	N/C	N/C	N/C
	ChYDaV	N/C	41.5	38.3	44.9	32.4	N/C	N/C	N/C
	RVCV	N/C	42.2	29.6	35.1	22.8	N/C	N/C	N/C
	SCV	N/C	48.4	29.9	38.2	26.9	N/C	N/C	N/C
	SuSV1	N/C	N/C	N/C	42.7	36.7	N/C	N/C	N/C
AntAmV1 vs	CBDaV	51.2	31.7	21.7	21.0	18.8	21.5	-	39.7
	MaCyV	50.8	28.3	19.3	17.9	16.4	22.1	-	40.2
	PMuMaV	51.8	41.0	25.6	29.3	35.3	36.4	-	45.5
AscSyV1 vs	BmV1	54.8	50.4	37.6	55.3	34.5	44.3	27.6	53.9
	PelRaV1	N/C	39.7	31.9	35.6	22.0	28.9	20.2	N/C
	WhIV4	53.2	48.7	35.3	54.2	30.8	41.8	22.8	50.1

		genome[a]	N[b]	P[b]	P3[b]	P4[b]	M[b]	G[b]	P6[b]	L[b]
BeTaV1 vs	CuCVi1	61.8	68.2	64.8	53.5	45.6	61.2	60.4	-	72.5
	DiCoV1	N/C	19.4	N/C	N/C	-	14.9	17.2	-	N/C
	YmCaV	51.5	30.6	20.3	31.7	19.9	21.2	30.8	-	42.4
DiCoV1 vs	MYSV	N/C	23.3	N/C	N/C	-	20.2	21.0	17.3	N/C
	CBDaV	N/C	25.2	N/C	N/C	-	23.8	22.5	-	N/C
	RVR	N/C	20.7	N/C	N/C	-	17.6	18.7	19.0	N/C
GlLV1 vs	NymAV1	49.7	40.0	23.8	27.3	-	24.0	37.6	24.1	50.0
	StrV1	49.9	38.7	25.0	37.0	-	26.5	42.1	22.4	50.5
	TpVA	65.6	67.8	60.9	78.1	-	66.7	71.6	75.8	76.1
	TYMaV	45.9	36.2	21.6	34.2	-	20.7	44.1	11.9	49.1
GymDenV1 vs	LYMoV	43.5	22.0	19.0	-	-	19.4	-	-	29.5
	SCV	40.0	23.5	17.2	-	-	18.7	-	-	30.1
	TrAV1	53.8	34.4	23.1	-	-	26.8	-	-	56.3
	WhIV5	44.4	19.9	15.9	-	-	10.7	-	-	30.6
LotCorV1 vs	AscSyV1	50.3	39.9	28.5	40.5	-	20.2	30.1	18.5	43.5
	PelRaV1	N/C	61.8	44.7	58.6	-	55.9	47.9	31.6	N/C

	WhIV4	52.0	40.9	29.7	39.7	-	20.8	30.7	12.5	43.8
NymAV1 vs	GlLV1	49.7	40.0	23.8	27.3	-	24.0	37.6	24.1	50.0
	KePCyV	52.9	39.3	23.6	32.9	-	23.7	40.7	19.4	47.9
	StrV1	51.2	38.6	22.6	24.5	-	19.8	40.5	18.0	48.0
	TpVA	53.8	39.2	24.9	30.8	-	23.6	37.7	17.0	50.1
PelRaV1 vs	AscSyV1	N/C	39.7	31.9	35.6	-	22.0	28.9	20.2	N/C
	LotCorV1	N/C	61.8	44.7	58.6	-	55.9	47.9	31.6	N/C
	WhIV4	N/C	40.5	28.5	39.6	-	20.4	27.5	11.7	N/C
SuSV1 vs	AChV1	N/C	N/C	N/C	42.7	-	36.7	N/C	N/C	N/C
	ADV	N/C	N/C	N/C	37.0	-	30.2	N/C	N/C	N/C
	ChYDaV	N/C	N/C	N/C	37.9	-	35.6	N/C	N/C	N/C
	RVCV	N/C	N/C	N/C	40.2	-	30.8	N/C	N/C	N/C
	SCV	N/C	N/C	N/C	36.8	-	26.5	N/C	N/C	N/C
TaEV1 vs	MYSV	50.3	23.3	15.2	16.2	-	15.0	-	-	41.7
	PpVE	47.4	21.3	17.6	15.1	-	14.1	-	-	34.9
	RSMV	48.7	25.0	15.7	17.8	-	16.3	-	-	38.4
	RVR	48.5	23.8	16.8	26.7	-	21.0	-	-	38.6
TrAV1 vs	CCyV1	46.3	23.0	16.0	18.7	-	16.3	-	-	30.2
	GymDenV1	53.8	34.4	23.1	-	-	26.8	-	-	56.3
	StrV1	44.7	24.6	16.7	16.1	-	15.9	-	-	30.0
	WhIV5	46.9	20.5	19.3	11.2	-	13.3	-	-	29.6

Table 3D. Pairwise identity percentages between VVeV1 and dichorhaviruses

		genome[a]	N[b]	P[b]	P3[b]	M[b]	G[b]	L[b]
VVeV1 vs	CiCSV	N/C	21.3	N/C	18.5	N/C	19.0	N/C
	CiLV-N	N/C	20.8	N/C	18.8	N/C	19.6	N/C
	ClCSV	N/C	21.1	N/C	18.2	N/C	19.2	N/C
	CoRSV	N/C	20.5	N/C	18.4	N/C	18.7	N/C
	OFV	N/C	23.1	N/C	19.1	N/C	20.1	N/C

Table 3E. Pairwise identity percentages between varicosaviruses assembled in this study and reported varicosaviruses

		N[b]	2[b]	3[b]	4[b]	L[a/b]
BrRV1 vs	AMVV1	22.5	10.9	18.3	-	52.8/36.9
	LBVaV	19.9	15.4	6.7	-	53.6/37.3
	LoPV1	21.5	21.0	19.0	-	55.2/37.5
	MelRoV1	23.0	16.4	7.8	-	55.8/42.0
	PhPiV1	18.8	N/C	6.9	-	N/C
	PiFleV1	22.9	17.5	6.6	-	52.3/32.2
	RCaVV	26.9	17.7	21.8	-	59.6/50.5
	SpV1	24.6	18.1	N/C	-	59.8/51.5
LoPV1 vs	AMVV1	52.0	32.7	50.9	-	67.6/71.5
	BrRV1	21.5	21.0	19.0	-	55.2/37.5
	LBVaV	19.1	15.9	11.3	-	54.8/37.0
	MelRoV1	19.4	16.6	5.9	-	55.1/37.8
	PhPiV1	21.2	N/C	6.3	-	N/C
	PiFleV1	17.1	17.1	8.8	-	52.5/32.1
	RCaVV	18.8	9.6	18.5	-	54.5/37.5
	SpV1	18.8	16.8	N/C	-	54.1/35.9
MelRoV1 vs	AMVV1	22.6	16.8	6.7	-	54.8/38.0
	BrRV1	23.0	16.4	7.9	-	55.8/42.0
	LBVaV	19.6	16.0	24.0	11.1	53.6/37.7
	LoPV1	19.4	16.6	5.9	-	55.1/37.8
	PhPiV1	20.8	N/C	29.1	N/C	N/C
	PiFleV1	19.7	17.4	27.7	14.2	50.9/31.5
	RCaVV	22.5	15.3	9.9	-	55.2/41.0
	SpV1	21.9	20.5	N/C	-	56.3/41.0
PhPiV1 vs	AMVV1	22.5	N/C	7.1	-	N/C
	BrRV1	18.8	N/C	6.9	-	N/C
	LBVaV	30.4	N/C	30.4	N/C	N/C
	LoPV1	21.2	N/C	6.3	-	N/C
	MelRoV1	20.8	N/C	29.1	N/C	N/C
	PiFleV1	26.8	N/C	25.5	N/C	N/C
	RCaVV	24.3	N/C	13.7	-	N/C
	SpV1	21.4	N/C	N/C	-	N/C
PiFleV1 vs	AMVV1	19.1	15.3	9.5	-	51.3/32.7

	BrRV1	22.9	17.5	6.6	-	52.3/32.2
	LBVaV	23.4	16.4	25.4	14.5	51.6/32.4
	LoPV1	17.1	17.1	8.8	-	52.5/32.1
	MelRoV1	19.7	17.4	27.7	14.2	50.9/31.5
	PhPiV1	26.8	N/C	25.5	N/C	N/C
	RCaVV	20.4	15.9	6.8	-	52.0/31.1
	SpV1	20.5	15.0	N/C	-	51.7/32.4
SpV1 vs	AMVV1	18.3	16.6	N/C	-	53.1/35.5
	BrRV1	24.6	18.1	N/C	-	59.8/51.5
	LBVaV	18.8	16.7	N/C	-	53.5/36.1
	LoPV1	18.8	16.8	N/C	-	54.1/35.9
	MelRoV1	21.9	20.5	N/C	-	56.3/41.0
	PhPiV1	21.4	N/C	N/C	-	N/C
	PiFleV1	20.5	15.0	N/C	-	51.7/32.4
	RCaVV	27.7	18.5	N/C	-	60.8/54.8

[a]nucleotide percentages
[b]amino acid percentages
N/C: not complete; virus names are listed in Supp. Table 1 and Table 1

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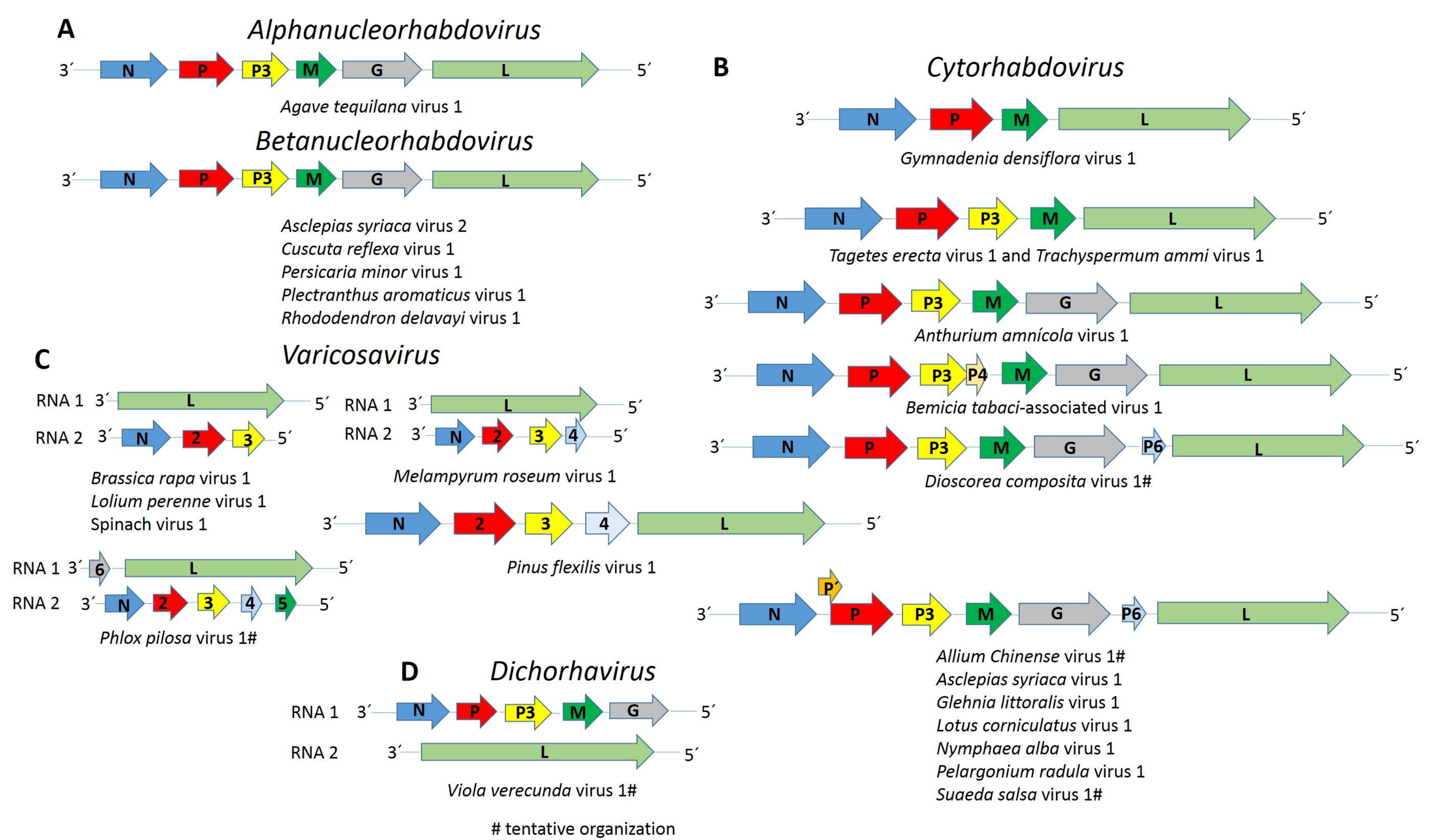
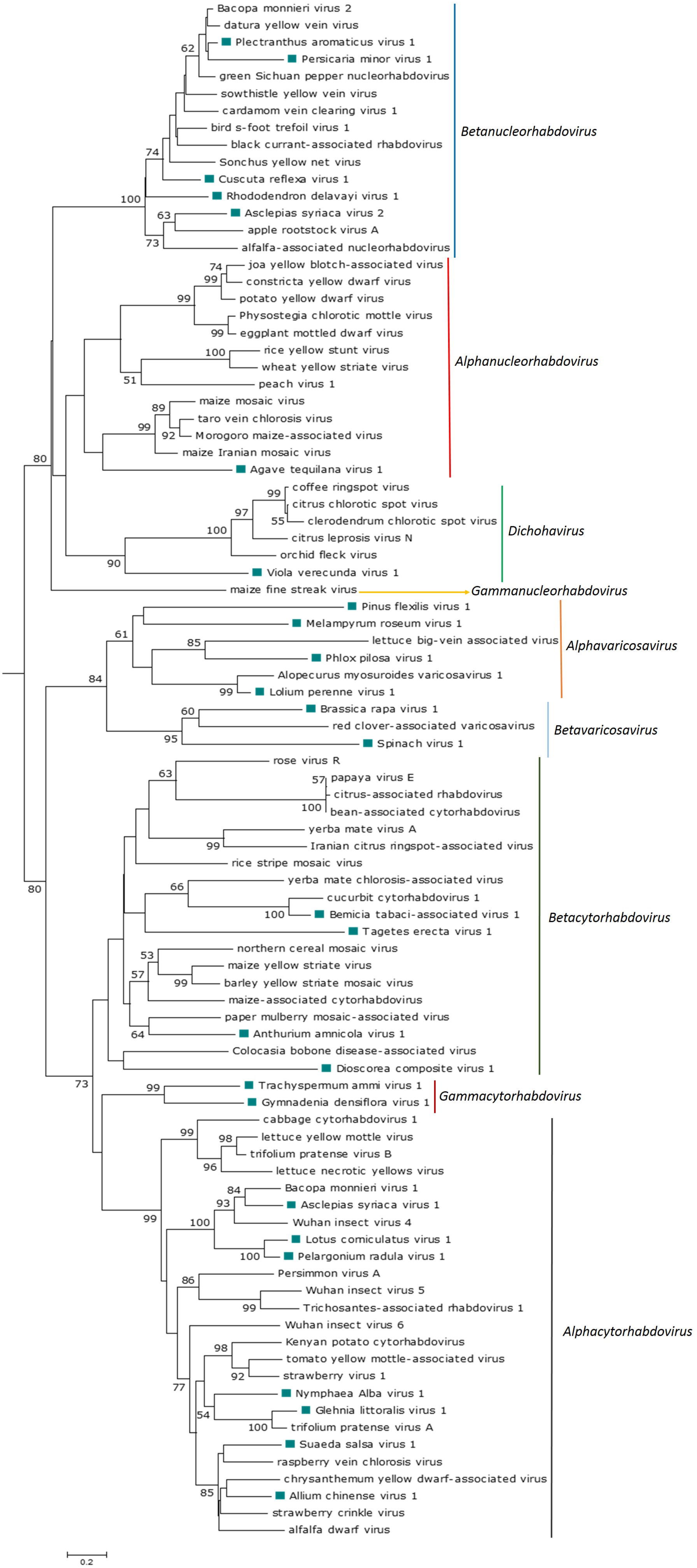

A
Alphanucleorhabdovirus
3′ N P P3 M G L 5′
Agave tequilana virus 1
Betanucleorhabdovirus
3′ N P P3 M G L 5′
Asclepias syriaca virus 2
Cuscuta reflexa virus 1
Persicaria minor virus 1
Plectranthus aromaticus virus 1
Rhododendron delavayi virus 1
B
Cytorhabdovirus
3′ N P M L 5′
Gymnadenia densiflora virus 1
3′ N P P3 M L 5′
Tagetes erecta virus 1 and Trachyspermum ammi virus 1
3′ N P P3 M G L 5′
Anthurium amnícola virus 1
3′ N P P3 P4 M G L 5′
Bemicia tabaci-associated virus 1
3′ N P P3 M G P6 L 5′
Dioscorea composita virus 1#
3′ N P′ P P3 M G P6 L 5′
Allium Chinense virus 1#
Asclepias syriaca virus 1
Glehnia littoralis virus 1
Lotus corniculatus virus 1
Nymphaea alba virus 1
Pelargonium radula virus 1
Suaeda salsa virus 1#
C
Varicosavirus
RNA 1 3′ L 5′
RNA 2 3′ N 2 3 5′
Brassica rapa virus 1
Lolium perenne virus 1
Spinach virus 1
RNA 1 3′ L 5′
RNA 2 3′ N 2 3 4 5′
Melampyrum roseum virus 1
3′ N 2 3 4 L 5′
Pinus flexilis virus 1
RNA 1 3′ 6 L 5′
RNA 2 3′ N 2 3 4 5 5′
Phlox pilosa virus 1#
D
Dichorhavirus
RNA 1 3′ N P P3 M G 5′
RNA 2 3′ L 5′
Viola verecunda virus 1#
tentative organization


Bacopa monnieri virus 2
datura yellow vein virus
Plectranthus aromaticus virus 1
Persicaria minor virus 1
green Sichuan pepper nucleorhabdovirus
sowthistle yellow vein virus
cardamom vein clearing virus 1
bird s-foot trefoil virus 1
black currant-associated rhabdovirus
Sonchus yellow net virus
Cuscuta reflexa virus 1
Rhododendron delavayi virus 1
Asclepias syriaca virus 2
apple rootstock virus A
alfalfa-associated nucleorhabdovirus
Betanucleorhabdovirus
joa yellow blotch-associated virus
constricta yellow dwarf virus
potato yellow dwarf virus
Physostegia chlorotic mottle virus
eggplant mottled dwarf virus
rice yellow stunt virus
wheat yellow striate virus
peach virus 1
maize mosaic virus
taro vein chlorosis virus
Morogoro maize-associated virus
maize Iranian mosaic virus
Agave tequilana virus 1
Alphanucleorhabdovirus
coffee ringspot virus
citrus chlorotic spot virus
clerodendrum chlorotic spot virus
citrus leprosis virus N
orchid fleck virus
Viola verecunda virus 1
Dichohavirus
maize fine streak virus
Gammanucleorhabdovirus
Pinus flexilis virus 1
Melampyrum roseum virus 1
lettuce big-vein associated virus
Phlox pilosa virus 1
Alopecurus myosuroides varicosavirus 1
Lolium perenne virus 1
Alphavaricosavirus
Brassica rapa virus 1
red clover-associated varicosavirus
Spinach virus 1
Betavaricosavirus
rose virus R
papaya virus E
citrus-associated rhabdovirus
bean-associated cytorhabdovirus
yerba mate virus A
Iranian citrus ringspot-associated virus
rice stripe mosaic virus
yerba mate chlorosis-associated virus
cucurbit cytorhabdovirus 1
Bemicia tabaci-associated virus 1
Tagetes erecta virus 1
northern cereal mosaic virus
maize yellow striate virus
barley yellow striate mosaic virus
maize-associated cytorhabdovirus
paper mulberry mosaic-associated virus
Anthurium amnicola virus 1
Colocasia bobone disease-associated virus
Dioscorea composite virus 1
Betacytorhabdovirus
Trachyspermum ammi virus 1
Gymnadenia densiflora virus 1
Gammacytorhabdovirus
cabbage cytorhabdovirus 1
lettuce yellow mottle virus
trifolium pratense virus B
lettuce necrotic yellows virus
Bacopa monnieri virus 1
Asclepias syriaca virus 1
Wuhan insect virus 4
Lotus corniculatus virus 1
Pelargonium radula virus 1
Persimmon virus A
Wuhan insect virus 5
Trichosantes-associated rhabdovirus 1
Wuhan insect virus 6
Kenyan potato cytorhabdovirus
tomato yellow mottle-associated virus
strawberry virus 1
Nymphaea Alba virus 1
Glehnia littoralis virus 1
trifolium pratense virus A
Suaeda salsa virus 1
raspberry vein chlorosis virus
chrysanthemum yellow dwarf-associated virus
Allium chinense virus 1
strawberry crinkle virus
alfalfa dwarf virus
Alphacytorhabdovirus
0.2