Evidence of a Novel *Vitivirus* in Grapevine cv Riesling

Humberto Debat[1Ω*], Diego Zavallo[2Ω], Sebastian Gomez-Talquenca[3*] and Sebastian Asurmendi[24]

[1] Instituto de Patología Vegetal, Centro de Investigaciones Agropecuarias, Instituto Nacional de Tecnología Agropecuaria (IPAVE-CIAP-INTA), 11 de setiembre 4755, Córdoba, Argentina, X5020ICA.

[2] Instituto de Biotecnología, Centro de Investigación en Ciencias Veterinarias y Agronómicas (IB-CICVyA-INTA), Nicolas Repetto y de los Reseros, Buenos Aires, Argentina, 1686.

[3] Estación Experimental Agropecuaria Mendoza, Instituto Nacional de Tecnología Agropecuaria (EEA-Mendoza-INTA), San Martín 3853, Luján de Cuyo, Mendoza, Argentina, 5534.

[4] CONICET, Argentina.

[Ω] These authors contributed equally to this work

* Correspondence:

Humberto Debat, debat.humberto@inta.gob.ar

Sebastian Gomez-Talquenca, gomez.talquenca@inta.gob.ar

Abstract

Vitivirus is a genus within the family *Betaflexiviridae* (*Tymovirales*) of ssRNA(+) viruses. There are currently ten ICTV recognized virus species in the genus; nevertheless, the extended use of NGS technologies is rapidly expanding their diversity and six more have been proposed recently. Here, we present the *in silico* characterization of a novel virus detected in grapevine (cv Riesling) which fits the genomic architecture and evolutionary constraints to be classifiable within the *Vitivirus* genus. The detected virus sequence is 7,607 nt long, including a typical genome organization of ORFs encoding a replicase (RP), a 22 kDa protein, a movement protein, a coat protein (CP) and a nucleic acid binding protein. Phylogenetic insights based on the predicted RP and CP, which share a 70.2% and 78.2% identity with its closest neighbour's grapevine virus E proteins, unequivocally clusters the identified virus within the *Vitivirus* genus. Multiple independent RNAseq data confirmed the presence of the detected virus in berries at diverse developmental stages. We tentatively propose the name grapevine virus L (GVL) to the detected putative *Vitivirus*.

Keywords

Vitivirus, Grapevine, cv Riesling, virus discovery, *Betaflexiviridae*

Accession number

Grapevine virus L sequence (GVL-rs) has been deposited in GenBank under accession number MH248020

Report

Vitiviruses present flexuous, non-enveloped, filamentous virus particles of 725-785 nm and 12 nm length and diameter. Their nucleocapsid is cross-banded and striated diagonally. They have a linear ssRNA(+) genome (~7.3-7.6 kb), with a methylated nucleotide cap at the 5' end and a 3' poly (A) tail [1]. There are ten species of vitiviruses recognized by the ICTV, nevertheless, just in the last six months, six new species have been proposed, five of them infecting grapevine [2-6]. Grapevine is their most prevalent natural host but they have also been found to infect several crops such as Mint (*Mentha x glaciaris*), arracacha (*Arracacia xanthorrhiza*) blue agave (*Agave tequilana*) and kiwi (*Actinidia chinense*) [7-9]. Vitiviruses appear to be latent in *Vitis vinífera* cultivars, and so far, only *Grapevine virus A* and *Grapevine virus B* had been consistently associated to grapevine diseases of the rugose wood complex (Grapevine vitiviruses) or Shiraz disease (reviewed by Minafra [10]). The etiological role of the recently described vitiviruses should be assessed in order to establish its relationships with known or unknown viral diseases. The synergistic effects between vitiviruses and other grapevine viruses appears to be significant [11]. The availability of complete sequences of these viruses could allow the development of full-length infectious clones, and fulfillment of the Koch's postulates [12].

In order to explore the eventual grapevine viral landscape on NGS publicly available libraries, we downloaded the raw RNA data from several SRA accessions finding known virus in the vast majority of tested samples. The raw data from these files was processed in the following form: trimmed and filtered with the Trimmomatic tool as implemented in http://www.usadellab.org/cms/?page=trimmomatic, and the resulting reads of each library were assembled *de novo* with Trinity v2.6.6 release with standard parameters [14]. The obtained transcripts 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 hits were explored thoroughly by hand. Interestingly, one dataset, specifically NCBI Bioproject PRJNA400621, SRX3144921-SRX3144956 [13] showed a 6,155 nt long transcript from the assembled transcriptome of the SRX3144956 library obtained a highly significant hit (E-value = 0; sequence identity 69%) with the replicase protein of *Grapevine virus E* (GVE, YP_002117775.1, [15]). Iterative read mapping allowed to extend and polish the assembled contig into a 7,607 nt long sequence, supported by 25,112 reads and a mean coverage of 161.7X, sharing the genome organization of vitiviruses (**Figure 1.A**; **Table 1**). The assembled virus sequence has a pairwise identity with GVE of 68.2% at the genomic RNA level. The predicted structure of the detected virus is conformed by a 67 nt 5´UTR, followed by five ORFs predicted by ORFfinder https://www.ncbi.nlm.nih.gov/orffinder/ and a 66 nt 3´UTR, excluding the poly (A) tail of at least three adenosines, and unknown length. Both predicted UTRs are highly similar in length and size in relation to other *Vitivirus*, more precisely GVE and the proposed GVI and GVG (**Supp. Figure 1**). ORF1 (67-5,158 nt coordinates) encodes a replicase protein (RP) of 1,696 aa (192.1 kDa), sharing a 70.2% with GVE replicase (NC_011106). We employed the NCBI conserved domain search tool as implemented in https://www.ncbi.nlm.nih.gov/Structure/cdd to annotate the RP, which presents a characteristic domain architecture of *Vitivirus*. The 5' region of RP presents a viral methyltransferase domain (E-value = 1.76e-56; Pfam = pfam01660; 46-337 aa coordinates) which has been involved in

mRNA capping, followed by a DEXDc type viral RNA helicase (E-value = 1.47e-12; Pfam = pfam01443; 750-1047 aa coordinates). Interestingly, an Alkylated DNA repair dioxygenase domain (AlkB), of the2OG-Fe(II) oxygenase superfamily, was identified within the helicase domain (E-value = 2.34e-06; COG = COG3145; 917-1032 aa coordinates) as previously described for an American isolate of GVE [16]. Finally, as expected, an RNA dependent RNA polymerase domain was found at the 3' region of the RP (RdRP_2; E-value = 3.47e-27; Pfam = pfam00978; 1313-1637 aa coordinates).

Although vitiviruses utilize a series of sub-genomic (sgRNA) RNAs as general strategy for expression of ORF2-5, it is worth mentioning that ORF2 (5,155-5,766 nt) of the detected virus overlaps in the tetranucleotide AUGA with ORF1. This overlapping cue might probably function as a termination–reinitiation strategy signal, which was determined for caliciviruses and victoriviruses for coupled start-stop translation [17]. This putative AUGA signal between ORF1-ORF2, although not mentioned, is present in the genome sequence of the recently proposed grapevine virus J [4]. The predicted ORF2 encodes a leucine rich (15.3% L) 22.8 kDa protein, of unknown function, which is the most highly divergent protein of the identified virus (44% similarity and 98% coverage to the ORF2 encoded hypothetical protein of GVE, AHB08905). ORF3 (5,795-6,607 nt) encodes a putative movement protein (MP) of 270 aa (29.7 kDa) with a viral movement protein domain (E-value = 6.61e-07; Pfam = pfam01107) between the 26-195 aa coordinates. ORF4 (6,522-7,121 nt), partially overlapping with ORF3, encodes a coat protein (CP) 199 aa long (21.4 kDa) presenting a *Trichovirus* coat protein-like domain (Tricho_coat; E-value = 4.26e-45; Pfam = pfam05892; 37-199 aa coordinates). The predicted CP of the identified virus shares 78.4% similarity with GVE-CP. Lastly, ORF5 (7,139-7,540 nt) encodes a 133 aa putative nucleic acid binding protein (15 kDa) with a nucleic acid binding domain (Viral_NABP; E-value = 1.40e-06; Pfam = pfam05515) between the 5-76 aa coordinates. This region shares significant similarity with the CTV_P23 domain (E-value =3.79e-06) of *Citrus tristeza virus* (*Closteroviridae*) P23 protein which is involved in asymmetrical RNA Accumulation [18].

The criteria for species demarcation in family *Betaflexiviridae*, suggested by ICTV, is less than ca. 72% overall nucleotide identity and below 80% amino acid identity of CP or replicase predicted proteins [19]. Given that the detected virus shares with GVE a 68.2% similarity at the genomic RNA level, and a 70.2% and 78.2% identity with the respective RP and CP proteins (**Supp. Figure 2**), we tentatively suggest that the detected virus sequence corresponds to a new species, for which we propose the name grapevine virus L (GVL). In order to entertain this hypothesis, based on genetic criteria, we gathered evolutionary insights of the putative GVL in the context of the *Betaflexiviridae* family. In this direction we generated phylogenetic trees based on RP protein multiple alignments corresponding to the 93 refseq *Betaflexiviridae* RP sequences available at NCBI. The trees were generated by multiple amino acid alignments (BLOSUM62 scoring matrix) using as best-fit algorithm E-INS-I which drives local alignment with generalized affine gap costs, and thus is applicable for RNA polymerases where diverse domains are dispersed among several highly divergent regions, by MAFTT v7.394 as implemented in https://mafft.cbrc.jp/alignment/software/. The aligned proteins were subsequently used as input for FastTree 2.1.5 http://www.microbesonline.org/fasttree/ maximum likelihood phylogenetic trees (best-fit model = JTT-Jones-Taylor-Thorton with single rate of evolution for each site = CAT) computing local support values with the Shimodaira-Hasegawa test (SH) and 1,000 resamples. Unequivocally, the putative

GVL RP clustered within the *Vitivirus* genus (**Supp. Figure 3**). To provide local tree topology and additional phylogenetic insights we generated maximum likelihood phylogenetic trees of both the RP and CP protein of accepted and proposed members of the *Vitivirus* genus. In both cases, putative GVL clustered within one of the two major sub-clades, conformed by GVE and the proposed species GVG, GVI, and branching more distantly, the agave tequilana leaf virus (**Figure 1.B-C**). These results support the tentative assignment of the detected virus as a probable member of the *Vitivirus* genus.

To validate the putative GVL detection in SRX3144956 library, we took advantage of the massive collection of data generated from Chen et al [13]. In this direction, we mapped the 1,014,833,524 raw reads available at the PRJNA400621 project, using as threshold two nt mismatches and a 22 nt seed and a cut-off value >10 reads hits per library with the Bowtie2 tool available at http://bowtie-bio.sourceforge.net/bowtie2/. None of the 18 RNA libraries of cv Cabernet sauvignon presented the putative GVL. Interestingly, we were able to detect the virus in six additional cv Riesling libraries of the same study, obtained from independent biological samples conformed by grapeberries sampled in three distinct developmental stages (E-L 35, E-L 36 and E-L 38) (**Supp. Table 1**). Moreover, while analyzing the intrinsic variability of the detected GVL sequences, we noticed that two of the samples presented 24 highly supported SNPs at frequencies >0.5 as detected by the FreeBayes v0.9.18S tool with standard parameters implemented in https://github.com/ekg/freebayes (**Supp. Table 2**). Given the low p-values of the variants and that 70% of the detected SNPs denote a silent effect, it is tempting to suggest that this SNPs are in fact biological and not artifactual. This plausible scenario, suggesting a natural variation of the virus, could imply that the putative GVL positive individuals could have acquired the detected virus independently. Nevertheless, these light speculations should be addressed by the generation of new data, oriented to investigate the prevalence and diversity of this putative virus. Future studies should focus on the emerging biological properties of GVL. Given that the samples harboring GVL also presented *Grapevine rupestris stem pitting-associated virus* (*Foveavirus*), and *Grapevine yellow speckle viroid* 1 & 2 (*Apscaviroid*), and *Hop stunt viroid* (*Hostuviroid*), future studies should unravel whether GLV presence is associated to any specific symptoms in grapevine.

Acknowledgements

This work was supported by project PE1131022 of the Instituto Nacional de Tecnología Agropecuaria (INTA) and by ANPCyT PICT 2016-0429. The funders had no role in study design and analysis, decision to publish, or preparation of the manuscript.

Author contributions

HD, SGT and SA designed the study. HD analyzed the data together with DZ. HD wrote the initial draft of the manuscript and DZ, SGT and SA revised the manuscript. All authors approved the final version.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors. Therefore, informed consent was not required for this work.

References

1. Du Preez J, Stephan D, Mawassi M, Burger JT (2011) The grapevine-infecting vitiviruses, with particular reference to grapevine virus A. Arch Virol. 156: 1495.

2. Blouin AG, Chooi KM, Warren B, Napier KR, Barrero RA, MacDiarmid RM (2018) Grapevine virus I, a putative new vitivirus detected in co-infection with grapevine virus G in New Zealand. Arch Virol. 163: 1371-1374.

3. Blouin AG, Keenan S, Napier KR, Barrero RA, MacDiarmid RM (2018) Identification of a novel vitivirus from grapevines in New Zealand. Arch Virol.163: 281–284.

4. Diaz-Lara A, Golino D, Al Rwahnih M (2018) Genomic characterization of grapevine virus J, a novel virus identified in grapevine. Arch Virol. https://doi.org/10.1007/s00705-018-3793-y

5. Candresse T, Theil S, Faure C, Marais A (2018) Determination of the complete genomic sequence of grapevine virus H, a novel vitivirus infecting grapevine. Arch Virol. 163: 277–280.

6. Jo Y, Song MK, Choi H, Park JS, Lee JW, Lian S, Cho WK (2017) Genome Sequence of Grapevine Virus K, a Novel Vitivirus Infecting Grapevine. Genome Announc. 5: e00994-17.

7. Tzanetakis IE, Postman JD, Martin RR (2007) Identification, detection and transmission of a new vitivirus from Mentha. Arch Virol.152: 2027–2033.

8. Blouin AG, Chavan RR, Pearson MN, Macdiarmid RM, Cohen D (2012) Detection and characterisation of two novel vitiviruses infecting Actinidia. Arch Virol. 157: 713–722.

9. Oliveira LM, Orílio AF, Inoue-Nagata AK, Nagata T, Blawid R (2017) A novel vitivirus-like sequence found in Arracacia xanthorrhiza plants by high throughput sequencing. Arch Virol. 162: 2141-2144.

10. Minafra A, Mawassi M, Goszczynski D, Saldarelli P (2017) Grapevine Vitiviruses. In: Grapevine Viruses. Meng B, Martelli GP, Golino DA, Fuchs M. eds. Springer International Publishing AG.

11. Rowhani A, Daubert S, Arnold K, Al Rwahnih M, Klaassen V, Golino D, Uyemoto JK (2018) Synergy between grapevine vitiviruses and grapevine leafroll viruses. Eur J Plant Pathol. https://doi.org/10.1007/s10658-018-1426-7

12. Goszczynski DE (2015) Brief report of the construction of infectious DNA clones of South African genetic variants of Grapevine virus A and Grapevine virus B. Springer Plus. 4: 739.

13. Chen WK, Bai XJ, Cao MM, Cheng G, Cao XJ, Guo RR, Duan CQ (2017) Dissecting the Variations of Ripening Progression and Flavonoid Metabolism in Grape Berries Grown under Double Cropping System. Front Plant Sci. 8: 1912.

14. Haas B J, Papanicolaou A, Yassour M, Grabherr M, Blood PD, Bowden J, MacManes MD (2013) De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. Nat Protoc. 8: 1494.

15. Nakaune R, Toda S, Mochizuki M, Nakano M (2008) Identification and characterization of a new vitivirus from grapevine. Arch Virol. 153: 1827.

16. Alabi OJ, Poojari S, Sarver K, Martin RR, Naidu RA (2013) Complete genome sequence analysis of an American isolate of Grapevine virus E. Virus genes. 46: 563-566.

17. Li H, Havens WM, Nibert ML, Ghabrial SA (2011) RNA sequence determinants of a coupled termination-reinitiation strategy for downstream open reading frame translation in Helminthosporium victoriae virus 190S and other victoriviruses (Family Totiviridae). J Virol. 85: 7343-7352.

18. Satyanarayana T, Gowda S, Ayllón MA, Albiach-Martí MR, Rabindran S, Dawson WO (2002) The p23 protein of Citrus tristeza virus controls asymmetrical RNA accumulation. J Virol. 76: 473-483.

19. Adams MJ, Candresse T, Hammond J, Kreuze JF, Martelli GP, Namba S, Pearson MN, Ryu KH, Saldarelli P, Yoshikawa N (2012) Family *Betaflexiviridae*. In: Virus Taxonomy – Ninth Report on the International Committee on Taxonomy of Viruses. AMQ King, MJ Adams, EB Carstens, EJ Lefkowitz, eds. Elsevier Academic Press, London, pp 920-941

Figure and Table Legends

Figure 1. (A) Molecular characterization of grapevine virus L (GVL). Mapping pattern of virus RNA reads from library SRX3144956 to the consensus putative GVL assembly indicating coverage per position. The genome architecture of GVL is characterized by a monosegmented ssRNA(+) virus encoding for five ORFs arranged as 5'-UTR-RP-HP-MP-CP-NABP-UTR-3'. The predicted product of each ORF is depicted including the associated domains determined by NCBI-CDD. Abbreviations: RP, replicase protein; 22kDa, 22k Dalton hypothetical protein; MP, movement protein; CP, coat protein; NABP, nucleic acid binding protein. See the text for additional domain information. (**B**) Phylogenetic insights of putative grapevine virus L (GVL) in relation to accepted and proposed members of the genus *Vitivirus* based on MAFFT alignments (BLOSUM62 scoring matrix using as best-fit algorithm E-INS-I) of the predicted replicase and capsid protein (**C**) followed by maximum likelihood trees generated by FastTree (best-fit model = JTT-Jones-Taylor-Thorton with single rate of evolution for each site = CAT) computing local support values with the Shimodaira-Hasegawa test (SH) and 1,000 resamples). Accession numbers of the corresponding sequences: actinidia virus A (JN427014), actinidia virus B (NC_016404), mint virus 2 (AY913795), grapevine virus B (NC_003602), grapevine virus H (MF521889), grapevine virus F (NC_018458), grapevine virus A (NC_003604), grapevine virus K (NC_035202), arracacha virus V (NC_034264), grapevine virus E (NC_011106), agave tequilana leaf virus (NC_034833), grapevine virus D (KX828708), grapevine virus G (MF405923), grapevine virus I (MF927925), heracleum latent virus (X79270), and grapevine Pinot gris virus (*Trichovirus*, NC_015782) used as outgroup. Scale bar represents substitutions per site. Node labels are FastTree support values. Asterisk indicates GVL.

Table 1. Diverse structural highlights of putative grapevine virus L in the context of proposed and ICTV recognized (in italics) *Vitivirus* species. *Grapevine Pinot gris virus* (genus *Trichovirus*) is included as outgroup for phylogenetic analyses. Accession #: NCBI Genbank accession number; Genome: Genome size (nt), 5´U: 5´UTR length (nt); RP: replicase protein length (aa); HP: hypothetical protein length (aa); MP: movement protein length (aa); CP: coat protein length (aa); NABP: nucleic acid binding protein length (aa); 3´UTR: 3´UTR length excluding the poly A tail (nt).

Supplementary Figure 1. ClustalW alignment of 5'-UTR (**A**) and 3'-UTR (**B**) of GVL, GVE and the proposed GVI and GVG. GVL 5'-UTR terminal 20 nt are identical to that of GVI. At the 3'-UTR in an equidistal position of 3' terminus a ten nucleotides monomer "GGCGAAAUAA" is conserved among the four virus sequences.

Supplementary Figure 2. Genetic distances expressed as percentage values and heat-maps of predicted RP (A) and CP (B) of accepted and proposed members of the genus *Vitivirus* and GVL, based on MAFFT alignments (BLOSUM62 scoring matrix using as best-fit algorithm E-INS-I). See Table 1 for abbreviations.

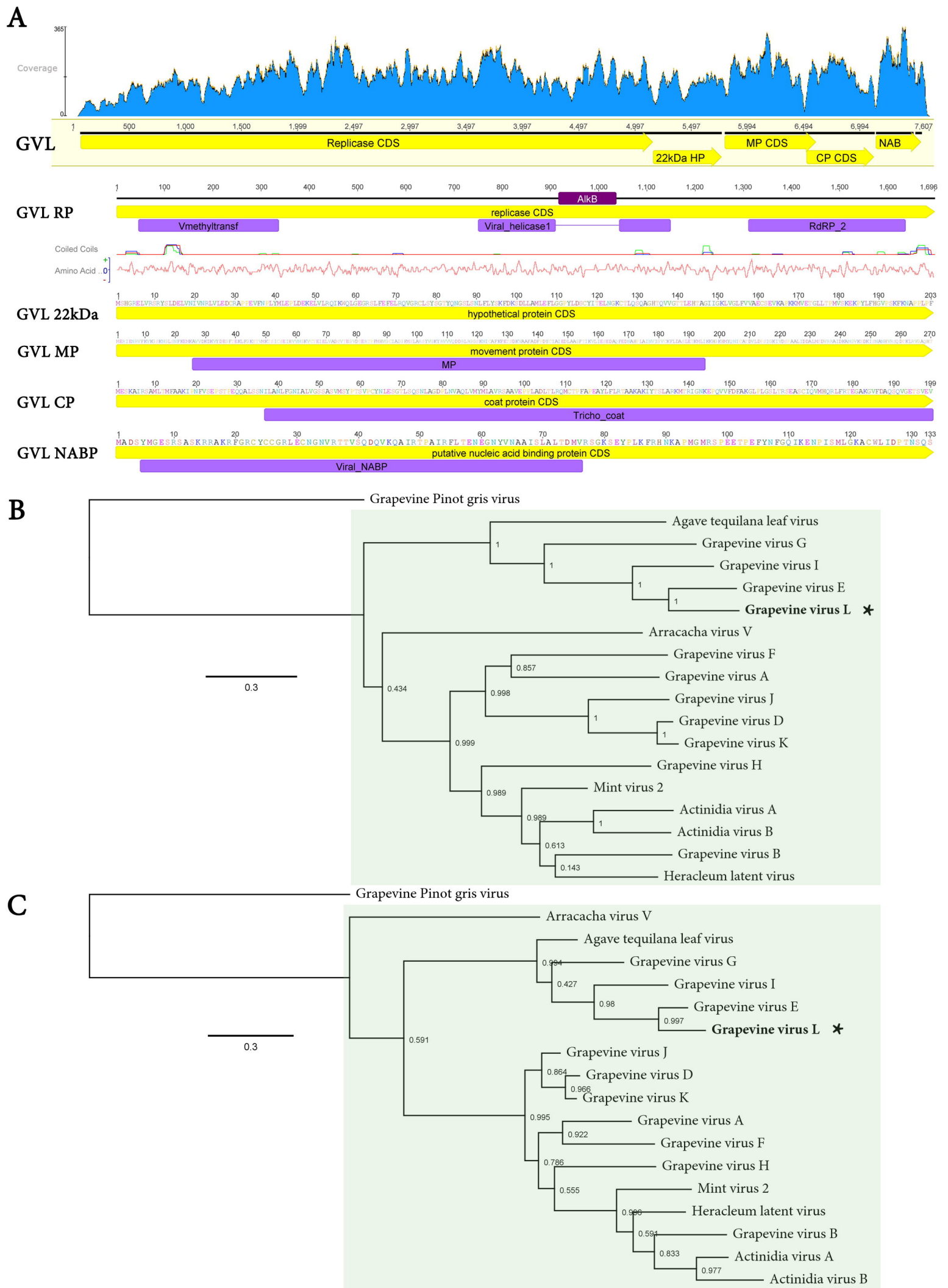
Supplementary Figure 3. Phylogenetic insights of putative grapevine virus L (GVL) in the context of family *Betaflexiviridae*, based on MAFFT alignments (BLOSUM62 scoring matrix using as best-fit algorithm E-INS-I) of NCBI refseq replicase proteins of *Betaflexiviridae* members, followed by maximum likelihood radial trees generated by FastTree (best-fit model = JTT-Jones-Taylor-Thorton with single rate of evolution for each site = CAT) computing local support values with the Shimodaira-Hasegawa test (SH) and 1,000 resamples). Scale bar represents substitutions per site. *Betaflexiviridae* family is depicted in blue and the monophyletic clade of vitiviruses is shown in red. GVL is indicated with an asterisk.

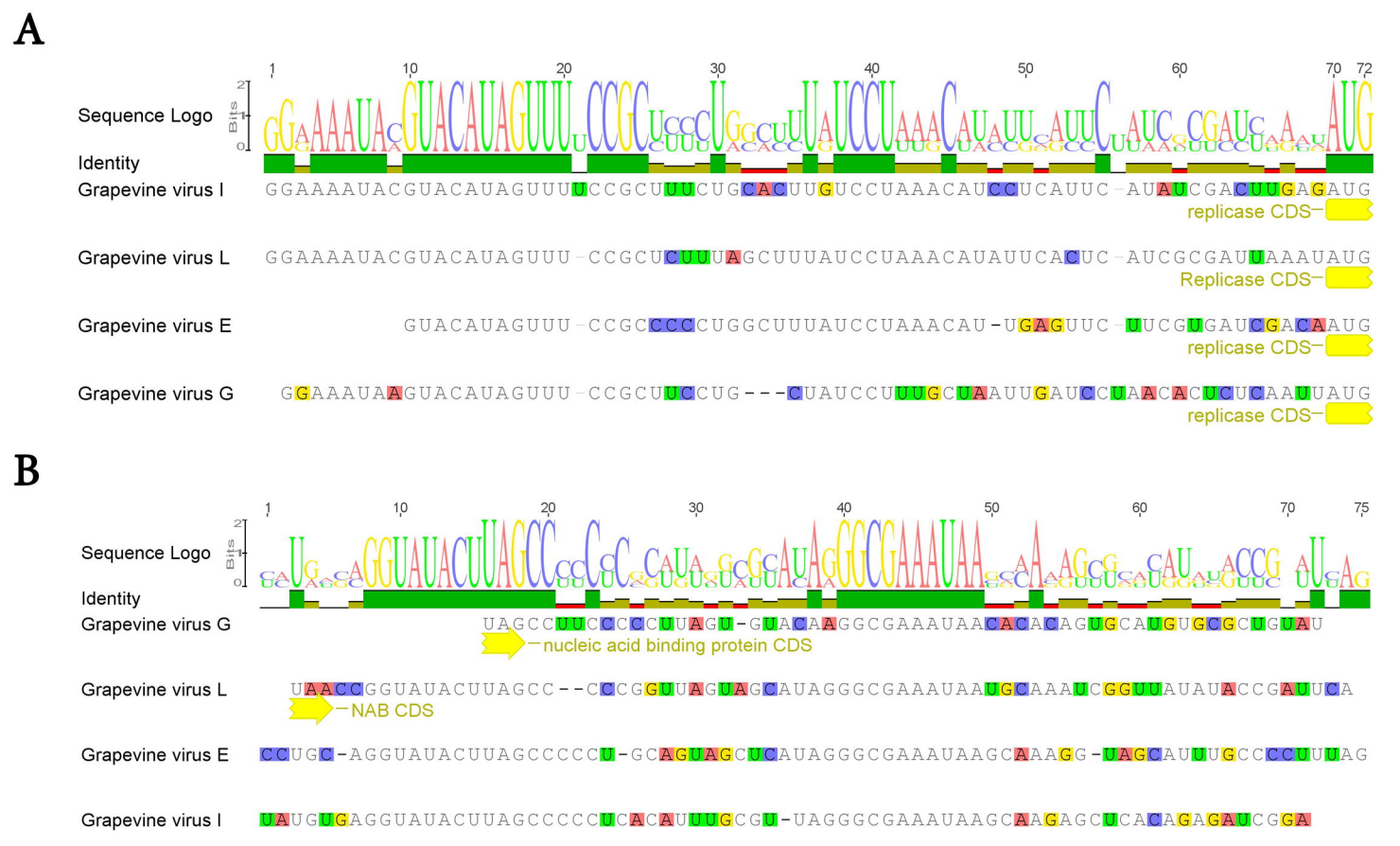
Supplementary Table 1. NGS RNA libraries used in this study generated by Chen et al [13]. Stage: sampled grapeberries developmental stage; Total reads: Total raw 50 nt reads generated for each library; GVL virus reads: viral reads detected in each sample; GVL RPM: virus reads per million total library reads.

Supplementary Table 2. Variable sites of grapevine virus L identified in the virus RNA reads detected in SRX3144939- SRX3144940 (E-L 35 cv Riesling grapeberries). Polymorphism are indicated in relation with the consensus sequence of the E-L-38 cv Riesling strain determined for SRX3144956. Only variable sites with a frequency >0.5 of virus reads are shown.

Virus	Abbreviation	Accession #	Genome	5'UTR	RP	HP	MP	CP	NABP	3'UTR
Actinidia virus A	AcVA	JN427014	7566	NA	1714	219	297	198	105	137
Actinidia virus B	AcVB	NC_016404	7488	70	1707	231	290	198	106	83
Agave tequilana leaf virus	ATLV	NC_034833	6958	52	1539	151	267	199	126	86
Arracacha virus V	AVV	NC_034264	7398	51	1705	NA	293	192	110	73
Grapevine virus A	GVA	NC_003604	7351	86	1707	177	278	198	90	68
Grapevine virus B	GVB	NC_003602	7599	48	1707	179	322	197	123	148
Grapevine virus D	GVD	MF774336	7479	79	1698	161	273	197	91	87
Grapevine virus E	GVE	NC_011106	7564	57	1698	191	265	199	108	134
Grapevine virus F	GVF	NC_018458	7551	90	1727	159	271	198	109	82
Grapevine virus G	GVG	MF405923	7496	64	1703	154	286	201	117	53
Grapevine virus H	GVH	MF521889	7446	97	1717	162	265	197	106	82
Grapevine virus I	GVI	NC_037058	7507	68	1696	167	264	199	121	104
Grapevine virus J	GVJ	MG637048	7390	95	1702	164	273	197	103	69
Grapevine virus K	GVK	NC_035202	7476	80	1700	161	273	197	91	77
Heracleum latent virus	HLV	X79270	3006	NA	253	NA	343	197	115	56
Mint virus 2	MV-2	AY913795	3897	NA	500	209	329	209	111	85
Grapevine virus L	GVL	MH248020	7607	67	1696	203	270	199	133	67
Vitivirus x□		NA	7438	72	1692	177	286	198	108	89
Grapevine Pinot gris virus	GPGV	NC_015782	7275	94	1845	NA	369	195	NA	99

Table 1 Diverse structural highlights of grapevine virus L in the context of proposed and ICTV recognized (in italics) *Vitivirus* species. Grapevine Pinot gris virus (genus Trichovirus) is included as outgroup for phylogenetic analyses. Accession #: NCBI Genbank accession number; Genome: Genome size (nt), 5´U: 5´UTR length (nt); RP: replicase protein length (aa); HP: hypothetical protein length (aa); MP: movement protein length (aa); CP: coat protein length (aa); NABP: nucleic acid binding protein length (aa); 3´UTR: 3´UTR length excluding the poly A tail (nt).


A
Coverage
GVL
Replicase CDS
22kDa HP
MP CDS
CP CDS
NAB
GVL RP
AlkB
replicase CDS
Vmethyltransf
Viral_helicase1
RdRP_2
Coiled Coils
Amino Acid ..
GVL 22kDa
hypothetical protein CDS
GVL MP
movement protein CDS
MP
GVL CP
coat protein CDS
Tricho_coat
GVL NABP
putative nucleic acid binding protein CDS
Viral_NABP
B
Grapevine Pinot gris virus
Agave tequilana leaf virus
Grapevine virus G
Grapevine virus I
Grapevine virus E
Grapevine virus L
Arracacha virus V
Grapevine virus F
Grapevine virus A
Grapevine virus J
Grapevine virus D
Grapevine virus K
Grapevine virus H
Mint virus 2
Actinidia virus A
Actinidia virus B
Grapevine virus B
Heracleum latent virus
0.3
C
Grapevine Pinot gris virus
Arracacha virus V
Agave tequilana leaf virus
Grapevine virus G
Grapevine virus I
Grapevine virus E
Grapevine virus L
Grapevine virus J
Grapevine virus D
Grapevine virus K
Grapevine virus A
Grapevine virus F
Grapevine virus H
Mint virus 2
Heracleum latent virus
Grapevine virus B
Actinidia virus A
Actinidia virus B
0.3

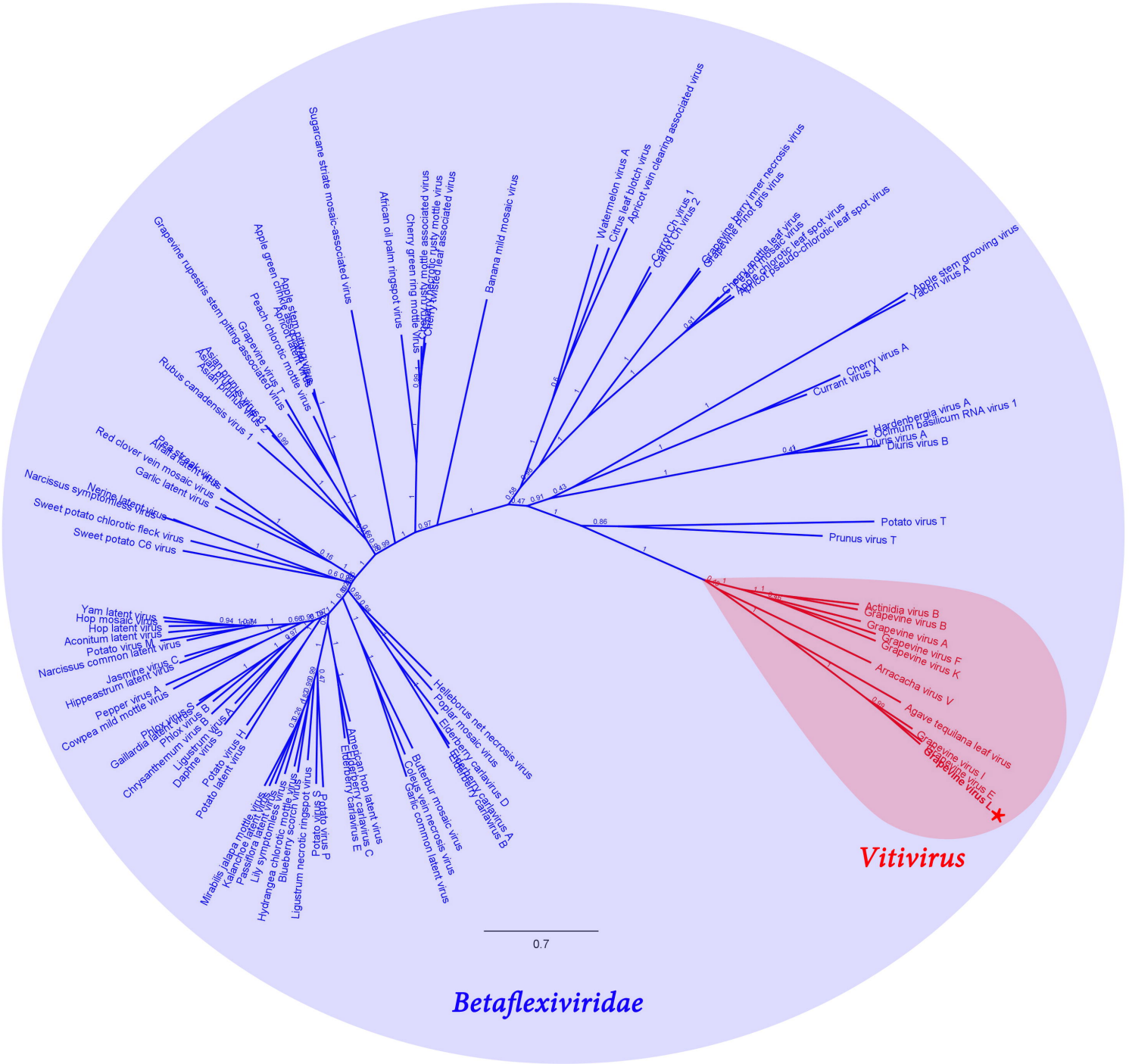

A
Sequence Logo
Identity
Grapevine virus I
GGAAAAUACGUACAUAGUUUUCCGCUUUCUGCACUUGUCCUAAACAUCCUCAUUC-AUAUCGACUUGAGAUG
replicase CDS
Grapevine virus L
GGAAAAUACGUACAUAGUUU-CCGCUCUUUAGCUUUAUCCUAAACAUAUUCACUC-AUCGCGAUUAAAUAUG
Replicase CDS
Grapevine virus E
GUACAUAGUUU-CCGCCCCCUGGCUUUAUCCUAAACAU-UGAGUUC-UUCGUGAUCGACAAUG
replicase CDS
Grapevine virus G
GGAAAUAAGUACAUAGUUU-CCGCUUCCUG---CUAUCCUUUGCUAAUUGAUCCUAACACUCUCAAUUAUG
replicase CDS
B
Sequence Logo
Identity
Grapevine virus G
UAGCCUUCCCCCUUAGU-GUACAAGGCGAAAUAACACACAGUGCAUGUGCGCUGUAU
nucleic acid binding protein CDS
Grapevine virus L
UAACCGGUAUACUUAGCC--CCCGGUUAGUAGCAUAGGGCGAAAUAAUGCAAAUCGGUUAUAUACCGAUUCA
NAB CDS
Grapevine virus E
CCUGC-AGGUAUACUUAGCCCCCU-GCAGUAGCUCAUAGGGCGAAAUAAGCAAAGG-UAGCAUUGCCCCUUUAG
Grapevine virus I
UAUGUGAGGUAUACUUAGCCCCCUCACAUUUGCGU-UAGGGCGAAAUAAGCAAGAGCUCACAGAGAUCGGA

A

	HLV	MV-2	AcVB	AcVA	GVB	GVH	GVF	GVA	GVD	GVK	GVJ	GVI	* GVL	GVE	GVG	ATLV	AVV
HLV		34.6%	9.8%	9.9%	9.5%	9.1%	9.1%	8.9%	9.0%	8.9%	9.2%	8.0%	8.2%	8.2%	7.6%	8.4%	7.2%
MV-2	34.6%		72.8%	73.2%	70.6%	67.3%	66.2%	66.2%	64.4%	63.6%	66.1%	54.8%	56.3%	56.0%	54.9%	56.0%	51.9%
AcVB	9.8%	72.8%		65.9%	56.0%	50.1%	46.7%	47.1%	45.8%	45.9%	46.2%	30.5%	31.1%	31.5%	30.3%	35.1%	37.4%
AcVA	9.9%	73.2%	65.9%		54.8%	50.7%	45.5%	46.8%	45.9%	46.0%	45.5%	30.2%	31.0%	31.2%	29.0%	33.9%	38.0%
GVB	9.5%	70.6%	56.0%	54.8%		48.1%	46.7%	47.7%	46.1%	46.4%	46.6%	31.7%	31.4%	32.0%	30.6%	34.4%	37.6%
GVH	9.1%	67.3%	50.1%	50.7%	48.1%		45.8%	46.5%	45.6%	45.5%	44.9%	30.4%	30.9%	31.7%	30.3%	35.0%	37.8%
GVF	9.1%	66.2%	46.7%	45.5%	46.7%	45.8%		49.0%	48.5%	48.6%	48.6%	29.7%	30.2%	30.5%	30.8%	35.0%	36.4%
GVA	8.9%	66.2%	47.1%	46.8%	47.7%	46.5%	49.0%		50.2%	50.0%	50.1%	30.3%	30.7%	30.3%	30.7%	34.1%	37.2%
GVD	9.0%	64.4%	45.8%	45.9%	46.1%	45.6%	48.5%	50.2%		88.9%	65.6%	30.0%	30.6%	30.6%	29.3%	35.4%	37.9%
GVK	8.9%	63.6%	45.9%	46.0%	46.4%	45.5%	48.6%	50.0%	88.9%		65.1%	30.2%	30.6%	30.7%	29.1%	35.1%	37.4%
GVJ	9.2%	66.1%	46.2%	45.5%	46.6%	44.9%	48.6%	50.1%	65.6%	65.1%		29.9%	31.2%	31.0%	30.1%	35.1%	38.4%
GVI	8.0%	54.8%	30.5%	30.2%	31.7%	30.4%	29.7%	30.3%	30.0%	30.2%	29.9%		64.7%	64.2%	51.6%	41.7%	28.3%
* GVL	8.2%	56.3%	31.1%	31.0%	31.4%	30.9%	30.2%	30.7%	30.6%	30.6%	31.2%	64.7%		70.2%	51.0%	41.5%	28.8%
GVE	8.2%	56.0%	31.5%	31.2%	32.0%	31.7%	30.5%	30.3%	30.6%	30.7%	31.0%	64.2%	70.2%		50.7%	40.8%	28.9%
GVG	7.6%	54.9%	30.3%	29.0%	30.6%	30.3%	30.8%	30.7%	29.3%	29.1%	30.1%	51.6%	51.0%	50.7%		41.5%	27.3%
ATLV	8.4%	56.0%	35.1%	33.9%	34.4%	35.0%	35.0%	34.1%	35.4%	35.1%	35.1%	41.7%	41.5%	40.8%	41.5%		33.6%
AVV	7.2%	51.9%	37.4%	38.0%	37.6%	37.8%	36.4%	37.2%	37.9%	37.4%	38.4%	28.3%	28.8%	28.9%	27.3%	33.6%	

B

	AVV	GVB	AcVA	AcVB	HLV	MV-2	GVD	GVK	GVJ	GVA	GVH	GVF	ATLV	GVG	GVI	GVE	* GVL
AVV		44.6%	45.6%	43.5%	45.1%	43.0%	45.1%	44.0%	44.6%	41.2%	43.5%	39.2%	41.5%	39.5%	39.0%	38.0%	38.0%
GVB	44.6%		68.0%	60.9%	65.5%	62.4%	58.9%	57.9%	57.4%	55.1%	54.8%	54.0%	41.5%	36.6%	35.5%	37.5%	37.5%
AcVA	45.6%	68.0%		75.3%	67.5%	60.8%	58.4%	59.9%	57.9%	58.6%	56.9%	55.6%	41.0%	37.6%	37.0%	39.0%	37.5%
AcVB	43.5%	60.9%	75.3%		65.5%	57.4%	57.4%	58.4%	57.4%	54.5%	54.3%	56.6%	37.0%	34.7%	34.0%	36.0%	36.0%
HLV	45.1%	65.5%	67.5%	65.5%		66.5%	62.4%	59.4%	64.0%	55.6%	56.3%	57.6%	38.5%	38.6%	39.0%	38.5%	38.0%
MV-2	43.0%	62.4%	60.8%	57.4%	66.5%		58.4%	58.4%	59.4%	58.1%	53.8%	54.0%	38.0%	36.6%	35.5%	36.0%	36.5%
GVD	45.1%	58.9%	58.4%	57.4%	62.4%	58.4%		91.9%	84.3%	68.7%	62.9%	62.6%	43.5%	44.1%	39.5%	43.5%	43.0%
GVK	44.0%	57.9%	59.9%	58.4%	59.4%	58.4%	91.9%		84.3%	69.2%	63.5%	64.1%	43.0%	43.1%	38.5%	43.0%	42.5%
GVJ	44.6%	57.4%	57.9%	57.4%	64.0%	59.4%	84.3%	84.3%		69.2%	62.9%	66.2%	46.0%	44.6%	40.0%	44.5%	44.0%
GVA	41.2%	55.1%	58.6%	54.5%	55.6%	58.1%	68.7%	69.2%	69.2%		58.1%	61.6%	43.8%	39.4%	38.8%	41.8%	41.8%
GVH	43.5%	54.8%	56.9%	54.3%	56.3%	53.8%	62.9%	63.5%	62.9%	58.1%		58.6%	42.0%	41.1%	39.5%	39.0%	39.0%
GVF	39.2%	54.0%	55.6%	56.6%	57.6%	54.0%	62.6%	64.1%	66.2%	61.6%	58.6%		39.3%	39.9%	37.8%	38.8%	39.8%
ATLV	41.5%	41.5%	41.0%	37.0%	38.5%	38.0%	43.5%	43.0%	46.0%	43.8%	42.0%	39.3%		66.8%	65.3%	60.3%	56.8%
GVG	39.5%	36.6%	37.6%	34.7%	38.6%	36.6%	44.1%	43.1%	44.6%	39.4%	41.1%	39.9%	66.8%		61.8%	58.3%	54.8%
GVI	39.0%	35.5%	37.0%	34.0%	39.0%	35.5%	39.5%	38.5%	40.0%	38.8%	39.5%	37.8%	65.3%	61.8%		62.8%	62.3%
GVE	38.0%	37.5%	39.0%	36.0%	38.5%	36.0%	43.5%	43.0%	44.5%	41.8%	39.0%	38.8%	60.3%	58.3%	62.8%		78.4%
* GVL	38.0%	37.5%	37.5%	36.0%	38.0%	36.5%	43.0%	42.5%	44.0%	41.8%	39.0%	39.8%	56.8%	54.8%	62.3%	78.4%	


Sugarcane striate mosaic-associated virus
African oil palm ringspot virus
Cherry green ring mottle virus
Cherry rusty mottle associated virus
Cherry chlorotic rusty mottle virus
Cherry mottled leaf associated virus
Banana mild mosaic virus
Watermelon virus A
Citrus leaf blotch virus
Apricot vein clearing associated virus
Carrot Ch virus 1
Carrot Ch virus 2
Grapevine berry inner necrosis virus
Grapevine Pinot gris virus
Cherry mottle leaf virus
Apple mosaic virus
Apricot chlorotic leaf spot virus
Apricot pseudo-chlorotic leaf spot virus
Apple stem grooving virus
Yacon virus A
Cherry virus A
Currant virus A
Hardenbergia virus A
Ocimum basilicum RNA virus 1
Diuris virus A
Diuris virus B
Potato virus T
Prunus virus T
Actinidia virus B
Grapevine virus B
Grapevine virus A
Grapevine virus F
Grapevine virus K
Arracacha virus V
Agave tequilana leaf virus
Grapevine virus I
Grapevine virus E
Grapevine virus L
Vitivirus
Grapevine rupestris stem pitting-associated virus
Apple green crinkle virus
Apple stem pitting virus
Apricot latent virus
Peach chlorotic mottle virus
Grapevine virus T
Asian prunus virus 3
Asian prunus virus 2
Asian prunus virus 1
Rubus canadensis virus 1
Pea streak virus
Alfalfa latent virus
Red clover vein mosaic virus
Garlic latent virus
Narcissus symptomless virus
Nerine latent virus
Sweet potato chlorotic fleck virus
Sweet potato C6 virus
Yam latent virus
Hop mosaic virus
Hop latent virus
Aconitum latent virus
Potato virus M
Narcissus common latent virus
Jasmine virus C
Hippeastrum latent virus
Pepper virus A
Cowpea mild mottle virus
Phlox virus S
Gaillardia latent virus
Phlox virus B
Chrysanthemum virus B
Ligustrum virus A
Daphne virus S
Potato virus H
Potato latent virus
Mirabilis jalapa mottle virus
Kalanchoe latent virus
Passiflora latent virus
Lily symptomless virus
Hydrangea chlorotic virus
Blueberry scorch virus
Ligustrum necrotic ringspot virus
Potato virus S
Potato virus P
American hop latent virus
Elderberry carlavirus C
Elderberry carlavirus E
Hellebrus net necrosis virus
Poplar mosaic virus
Elderberry carlavirus D
Elderberry carlavirus A
Elderberry carlavirus B
Butterbur mosaic virus
Coleus vein necrosis virus
Garlic common latent virus
0.7
Betaflexiviridae