In-depth study of tomato and weed viromes reveals undiscovered plant virus diversity in an agroecosystem

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SUPPLEMENTARY INFORMATION

Additional File 06

Supplementary Table 10. Model selection and other parameters used for phylogenetic analyses. **Supplementary Figure 4.** Phylogenetic trees up to the virus family or order level showing relationships of identified known and novel virus species in this study.

Supplementary Table 10. Model selection and other parameters used for maximum likelihood phylogenetic analyses.

Abbreviations used: aa (amino acid), nt (nucleotide). RdRp (RNA-dependent RNA polymerase), Mtr (methyl transferase), Hel (helicase), CP (coat protein), RT (reverse transcriptase). Abbreviations of substitution models used are indicated in the succeeding paragraph.

Notes: *Substitution model was chosen based on the lowest Bayesian Information Criteria (BIC) value. a subtree of a larger but identical tree indicated in parentheses.

Virus family / families (or virus order)	Figure(s) where tree is shown (trees in supplementary figures are numbered consecutively)	Gene or genome segment used	Number of sequences in the dataset	Alignment and trimming programs used	Number of positions in final alignment	Substitution model selected*
Tospoviridae and Fimoviridae (Bunyavirales)	(01) Supplementary Figure 4-01	- RdRp	65	ClustalW, MAFFT (after manual trimming), trimAl	376 aa	LG+G+l+F
	Figure 4i a,(01)					
Aspiviridae (Serpentovirales)	(02) Supplementary Figure 4-02	- RdRp	29	ClustalW, MAFFT (after manual trimming), trimAl	447 aa	LG+G+l
	Figure 4k a,(02)					
Rhabdoviridae, Lispiviridae (Mononegavirales)	(03) Supplementary Figure 4-03	- RdRp	51	ClustalW, MAFFT (after manual trimming), trimAl	510 aa	LG+G+l+F
	Figure 5f b,(03)					
Kitaviridae (Martellivirales)	(04) Supplementary Figure 4-04	- RdRp	28	ClustalW, MAFFT (after manual trimming), trimAl	583 aa	LG+G+l+F
	Figure 6k b,(04)					
Bromoviridae (Martellivirales)	(05) Supplementary Figure 4-05	- RdRp	63	ClustalW, MAFFT (after manual trimming), trimAl	327 aa	LG+G+I
	Figure 6I b,(05)					
Closteroviridae (Martellivirales)	(06) Supplementary Figure 4-06	- RdRp	48	ClustalW, MAFFT (after manual trimming), trimAl	219 aa	LG+G+l
	Figure 6m b,(06)					
Endornaviridae (Martellivirales)	(07) Supplementary Figure 4-07	- RdRp	43	ClustalW, MAFFT (after manual trimming), trimAl	278 aa	LG+G+l
	Figure 6n b,(07)					

(08-A) Supplementary Figure 4-08-A	– Mtr, Hel	48	ClustalW, MAFFT (after manual trimming), trimAl	542 aa	LG+G+l
	— СР	48	ClustalW, MAFFT (after manual trimming), trimAl	68 aa	LG+G
Figure 7i	full genome nt sequence	41	MUSCLE (and manual trimming of ends)	6233 nt	GTR+G+
(09) Supplementary Figure 4-09	– RdRp	93	ClustalW, MAFFT (after manual trimming), trimAl	470 aa	LG+G+I
Figure 8g b,(09)					
(10) Supplementary Figure 4-10	– RdRp	108	ClustalW, MAFFT (after manual trimming), trimAl	291 aa	LG+G+I
Figure 9h b,(10)					
(11) Supplementary Figure 4-11	RdRp	57	ClustalW, MAFFT (after manual trimming), trimAl	326 aa	LG+G+I
(12) Supplementary Figure 4-12	replicase (C1 gene)	109	ClustalW, MAFFT (after manual trimming), trimAl	289 aa	LG+G
(13) Supplementary Figure 4-13	RdRp	71	ClustalW, MAFFT (after manual trimming), trimAl	363 aa	LG+G+I
(14) Supplementary Figure 4-14	RT	57	ClustalW, MAFFT (after manual trimming), trimAl	220 aa	LG+G
(15) Supplementary Figure 4-15	RdRp	29	ClustalW, MAFFT (after manual trimming), trimAl	460 aa	LG+G+l
(16) Supplementary Figure 4-16	RdRp	61	ClustalW, MAFFT (after manual trimming), trimAl	307 aa	LG+G
	(09) Supplementary Figure 4-09 Figure 8g b.(09) (10) Supplementary Figure 4-10 Figure 9h b.(10) (11) Supplementary Figure 4-11 (12) Supplementary Figure 4-12 (13) Supplementary Figure 4-13 (14) Supplementary Figure 4-14 (15) Supplementary Figure 4-15	Figure 7h b,(08-A) (left tree) (08-B) Supplementary Figure 4-08-B Figure 7h b,(08-B) (right tree) Figure 7i full genome nt sequence (09) Supplementary Figure 4-09 Figure 8g b,(09) (10) Supplementary Figure 4-10 Figure 9h b,(10) (11) Supplementary Figure 4-11 RdRp (12) Supplementary Figure 4-12 replicase (C1 gene) (13) Supplementary Figure 4-13 RdRp (14) Supplementary Figure 4-14 RT (15) Supplementary Figure 4-15 RdRp	Figure 7h b,(08-A) (left tree) (08-B) Supplementary Figure 4-08-B Figure 7h b,(08-B) (right tree) Figure 7i (09) Supplementary Figure 4-09 Figure 8g b,(09) (10) Supplementary Figure 4-10 Figure 9h b,(10) (11) Supplementary Figure 4-11 RdRp 93 (12) Supplementary Figure 4-12 (13) Supplementary Figure 4-13 RdRp 71 (14) Supplementary Figure 4-14 RT 57 (15) Supplementary Figure 4-15 RdRp 29	Figure 7h b.(08-A) (left tree) (08-B) Supplementary Figure 4-08-B Figure 7h b.(08-B) (right tree) CP 48 ClustalW, MAFFT (after manual trimming), trimAl Figure 7i full genome nt sequence GuestalW, MAFFT (after manual trimming), trimAl (09) Supplementary Figure 4-09 Figure 8g b.(09) RdRp 93 ClustalW, MAFFT (after manual trimming), trimAl (10) Supplementary Figure 4-10 Figure 9h b.(10) RdRp 108 ClustalW, MAFFT (after manual trimming), trimAl (11) Supplementary Figure 4-11 RdRp 57 ClustalW, MAFFT (after manual trimming), trimAl (12) Supplementary Figure 4-12 replicase (C1 gene) 109 ClustalW, MAFFT (after manual trimming), trimAl (13) Supplementary Figure 4-13 RdRp 71 ClustalW, MAFFT (after manual trimming), trimAl (14) Supplementary Figure 4-14 RT 57 ClustalW, MAFFT (after manual trimming), trimAl (15) Supplementary Figure 4-15 RdRp 29 ClustalW, MAFFT (after manual trimming), trimAl (16) Supplementary Figure 4-15 RdRp 29 ClustalW, MAFFT (after manual trimming), trimAl	Figure 7h b.(08-A) (left tree) (08-B) Supplementary Figure 4-08-B Figure 7h b.(08-B) (right tree) CP 48 ClustalW, MAFFT (after manual trimming), trimAl 68 aa Figure 7i full genome nt sequence Figure 8g b.(09) RdRp Figure 8g b.(09) RdRp Figure 9h b.(10) RdRp Figure 9h b.(10) RdRp Tigure 9h b.(10) ClustalW, MAFFT (after manual trimming), trimAl Tigure 9h b.(10) RdRp Tigure 9h b.(10) ClustalW, MAFFT (after manual trimming), trimAl Tigure 9h b.(10) ClustalW, MAFFT (after manual trimming), trimAl Tigure 9h b.(10) ClustalW, MAFFT (after manual trimming), trimAl Tigure 9h b.(10) ClustalW, MAFFT (after manual trimming), trimAl Tigure 9h b.(10) ClustalW, MAFFT (after manual trimming), trimAl Tigure 9h b.(10) ClustalW, MAFFT (after manual trimming), trimAl Tigure 9h b.(10) ClustalW, MAFFT (after manual trimming), trimAl Tigure 9h b.(10) ClustalW, MAFFT (after manual trimming), trimAl ClustalW, MAFFT (after manual trimming), trimAl Tigure 9h b.(10) ClustalW, MAFFT (after manual trimming), trimAl ClustalW, MAFFT (after manual trimming), trimAl ClustalW, MAFFT (after manual trimming), trimAl Tigure 9h b.(10) ClustalW, MAFFT (after manual trimming), trimAl ClustalW, MAFFT (after manual trimming), trimAl

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Secoviridae (Picornavirales)	(17) Supplementary Figure 4-17	RdRp	63	ClustalW, MAFFT (after manual trimming), trimAl	364 aa	LG+G
Solemoviridae (Sobelivirales)	(18) Supplementary Figure 4-18	RdRp	47	ClustalW, MAFFT (after manual trimming), trimAl	191 aa	LG+G
Tymovirales	(19) Supplementary Figure 4-19	RdRp	75	ClustalW, MAFFT (after manual trimming), trimAl	293 aa	LG+G+I
Satellite viruses (Albetovirus, Aumaivirus)	(20) Supplementary Figure 4-20	СР	10	ClustalW, MAFFT (after manual trimming), trimAl	109 aa	LG+G

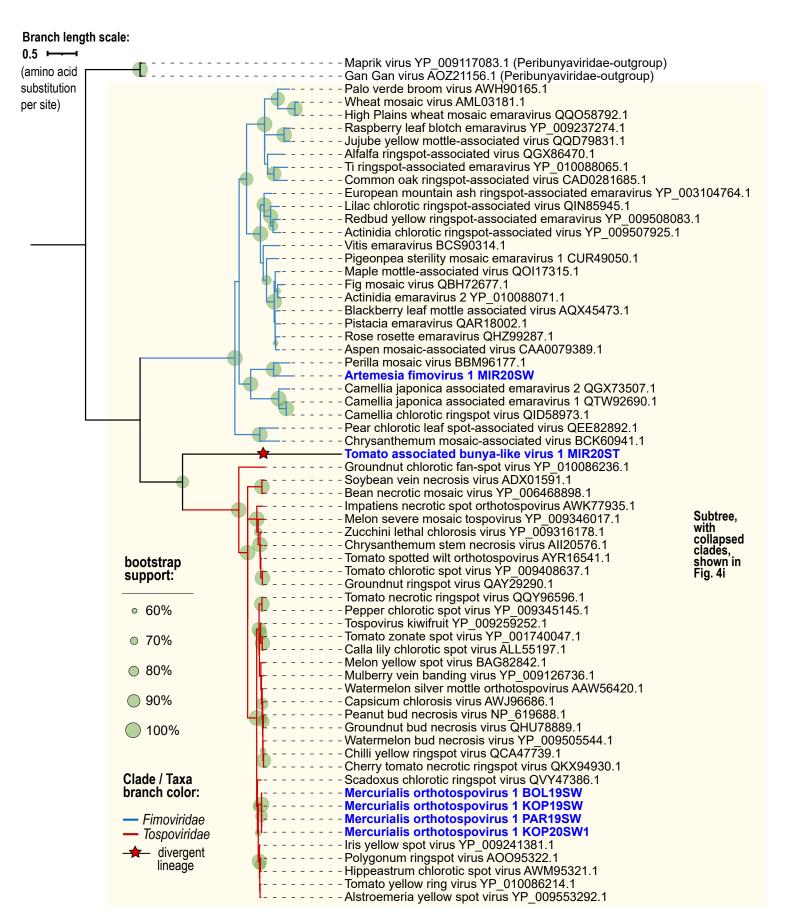
Details of multiple sequence alignments and phylogenetic tree construction:

Representative viral RefSeg genomes of ICTV-recognized species, which were translated to amino acid seguences, and selected best hit in BLASTp searches (E-val<10-4) of the viruses of interest, were gathered for the phylogenetic analyses. When applicable, outgroup virus sequences were chosen based on known phylogenetic relationships to the closest related taxa (i.e. species of a closely related family), and with reference to phylogenetic trees published in peer-reviewed articles. Multiple sequence alignments were initially done using ClustalW (Thompson et al., 1994), and after manual trimming of unaligned ends, MAFFT alignment was implemented (Katoh and Standley, 2013). Thereafter, further trimming of the alignments with trimAl (Capella-Gutiérrez et al., 2009) was performed. Highly divergent sequences (i.e. <30%) based on overall pairwise amino acid similarity, examined in SDT v. 1.2 (Muhire et al., 2014), were removed from the final alignment prior to further analyses. In the case of tobamoviruses, a nucleotide-based phylogenetic analyses was done on full genomes. Genomes of different isolates of turnip vein-clearing virus, ribgrass mosaic virus, wasabi mosaic virus, youcai mosaic virus and Plantago tobamovirus 1, were gathered from GenBank and viral RefSeq database and aligned using MUSCLE (Edgar, 2004). The unaligned ends were trimmed, then checked for possible recombinants before proceeding with the phylogenetic analyses with the alignments free of recombinants. Substitution model selection was done on the final alignments, with either amino acid or nucleotide alignments. The best model was chosen, based on the lowest Bayesian Information Criteria (BIC) value (Schwarz, 1978). Selected amino acid substitution models indicated in the table above are as follows: Le Gascuel 2008 model (Le and Gascuel, 2008) with discrete Gamma distribution with 5 rate categories (LG+G), which may also assume that a certain fraction of sites are evolutionarily invariable (LG+G+I) and may account for amino acid frequencies (LG+G+I+F). For the tobamovirus dataset, a General Time Reversible (GTR) model was selected, with discrete gamma distribution (+G) and evolutionarily invariable sites (+I). The final phylogenetic trees after the analyses have the highest log likelihood value inferred with 1000 bootstrap replicates (Felsenstein, 1985). In the succeeding phylogenetic trees shown in **Supplementary Figure 4**, the percentage of replicate trees in which the associated taxa clustered together in the bootstrap test is shown next to the branches, with only >0.6 (>60%) support shown. The trees are drawn to scale for easier visualization, with branch lengths (scale

bar is shown) measured in the number of amino acid (or nucleotide) substitutions per site. ClustalW alignment, manual trimming and pairwise similarity analysis were done in CLC Genomics Workbench v. 20.0 (Qiagen). MAFFT was executed in EMBL-EBI website (www.ebi.ac.uk/Tools/msa/mafft/), and trimAI was downloaded from (www.trimal.cgenomics.org), and was executed in command line, with 'automated1' option. Substitution model testing and phylogenetic tree construction were done in MEGA X (Kumar et al., 2018), and the final phylogenetic trees were annotated in Interactive Tree of Life (iToL) (Letunic and Bork, 2021).

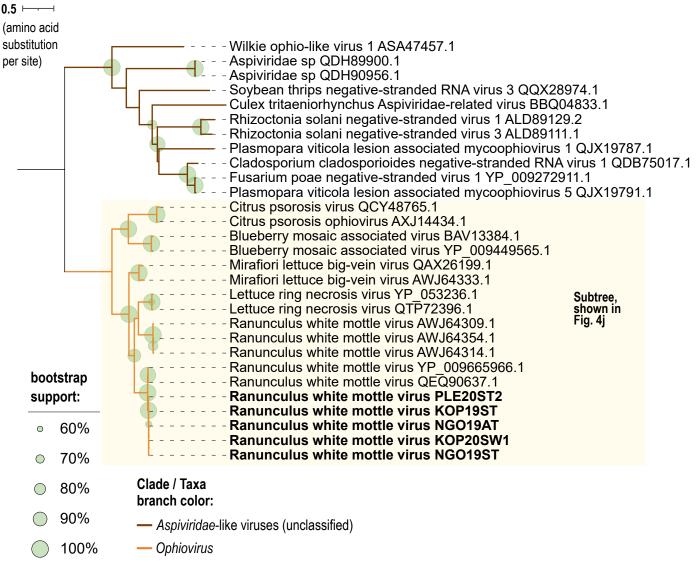
References for substitution models, online tools and programs used in the phylogenetic analyses:

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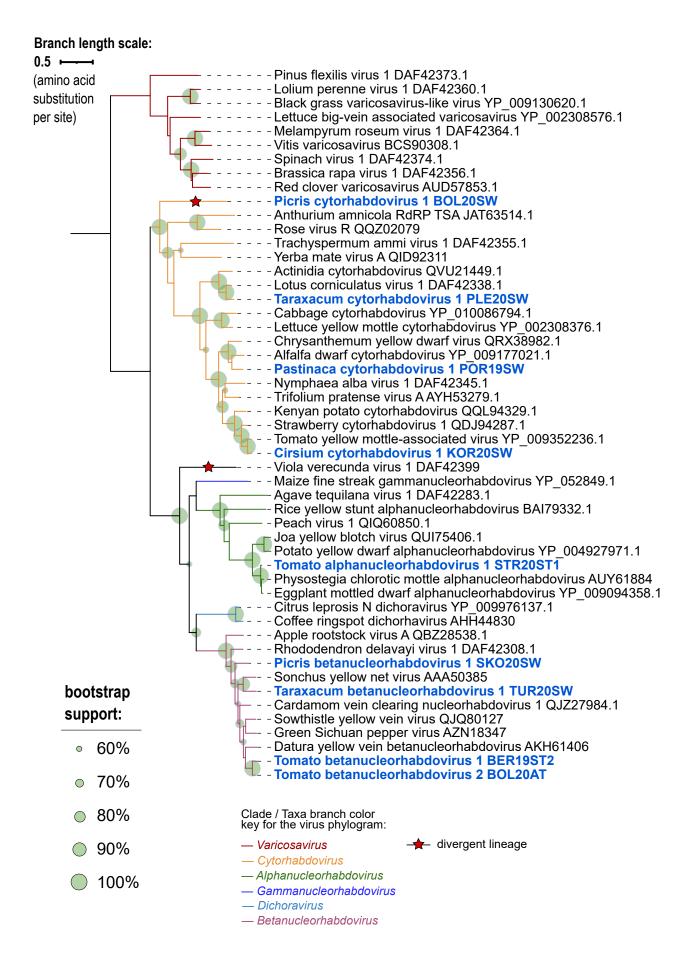


Supplementary Figure 4-01. Maximum likelihood phylogenetic tree based on the alignment of conserved RNA-dependent RNA polymerase amino acid sequence of virus species under *Tospoviridae* and *Fimoviridae* (*Bunyavirales*).

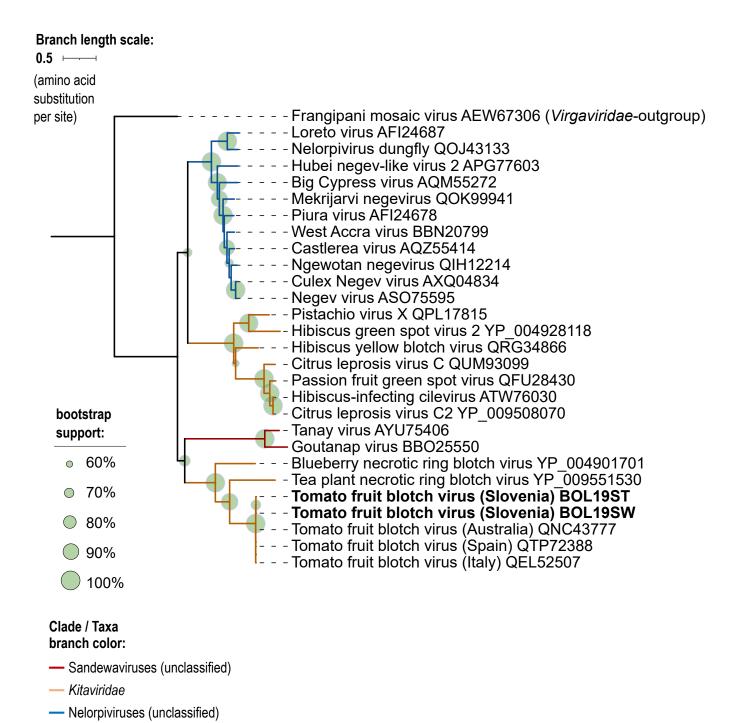
Branch length scale:



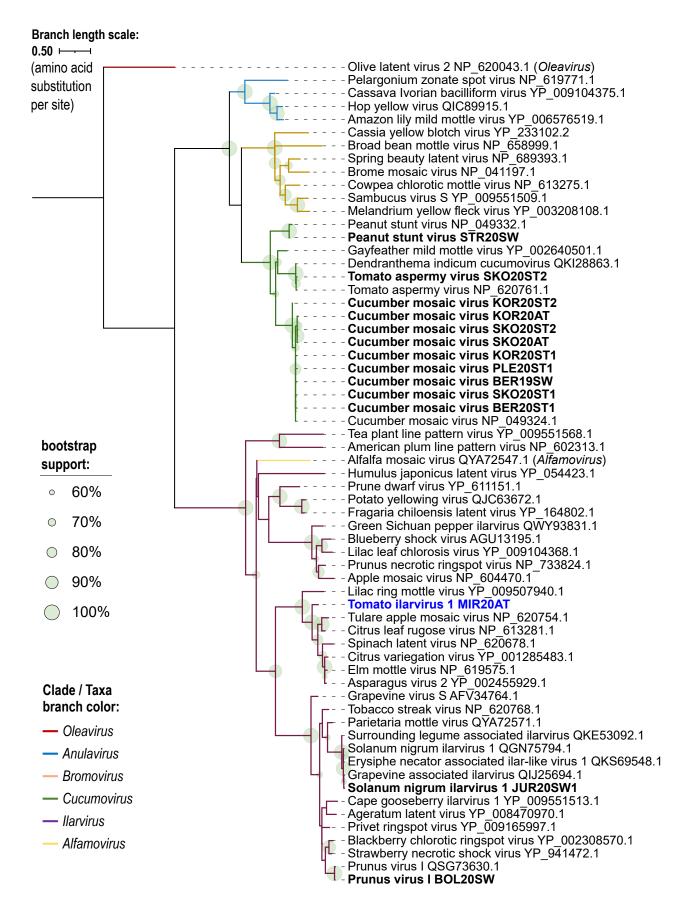
Supplementary Figure 4-02. Maximum likelihood phylogenetic tree based on the alignment of conserved RNA-dependent RNA polymerase amino acid sequence of virus species under family *Aspiviridae* (*Serpentovirales*).



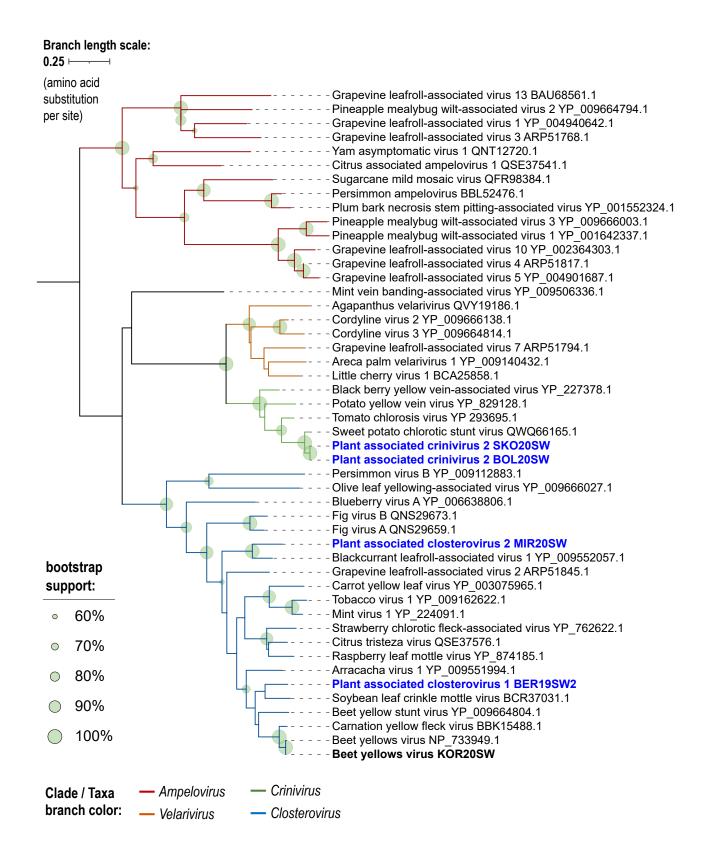
Supplementary Figure 4-03. Maximum likelihood phylogenetic tree based on the alignment of conserved RNA-dependent RNA polymerase amino acid sequence of virus species under family *Rhabdoviridae* (*Mononegavirales*).



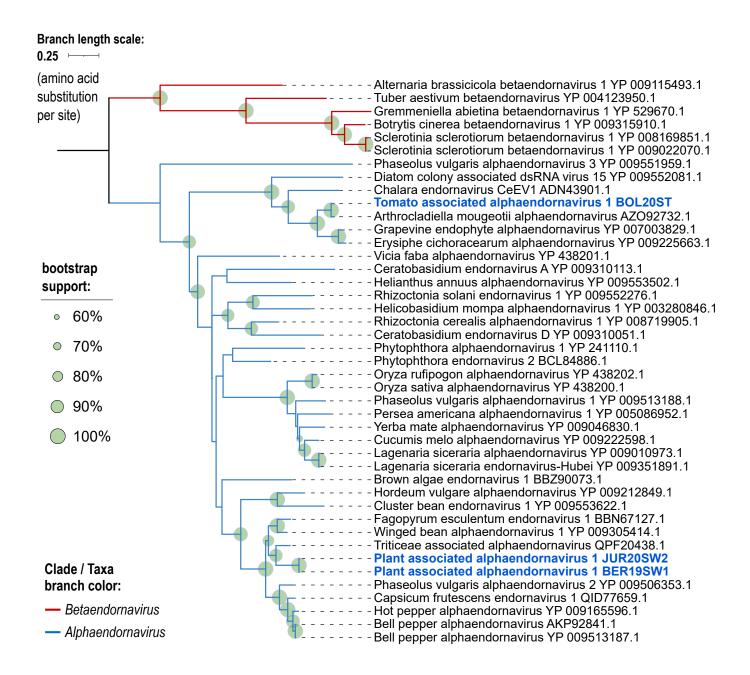
Supplementary Figure 4-04. Maximum likelihood phylogenetic tree based on the alignment of conserved RNA-dependent RNA polymerase amino acid sequence of virus species under family *Kitaviridae* (*Martellivirales*).



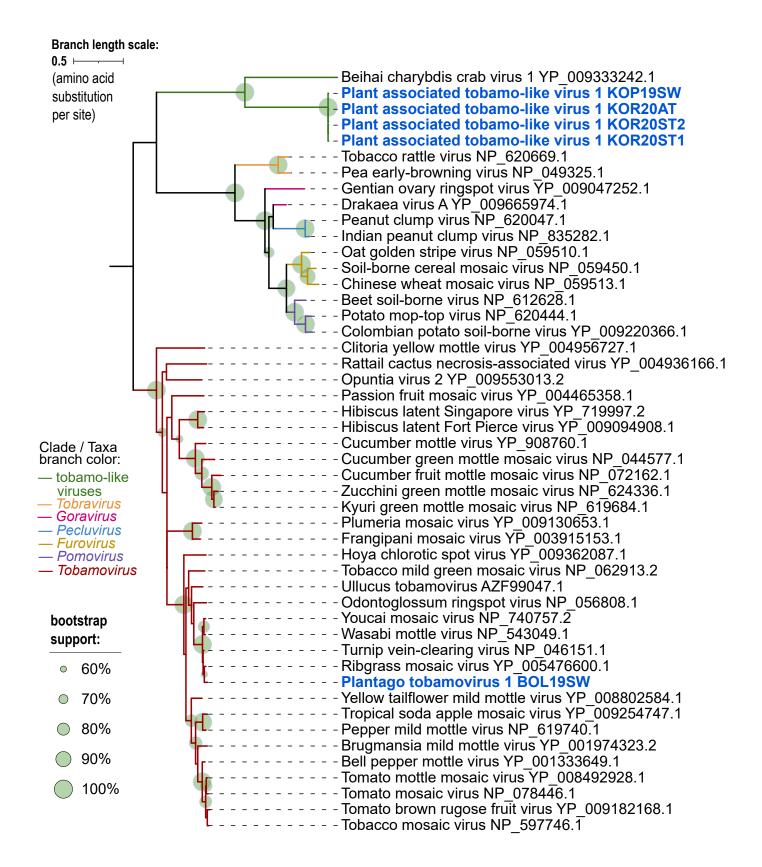
Supplementary Figure 4-05. Maximum likelihood phylogenetic tree based on the alignment of conserved RNA-dependent RNA polymerase amino acid sequence of virus species under family *Bromoviridae* (*Martellivirales*).



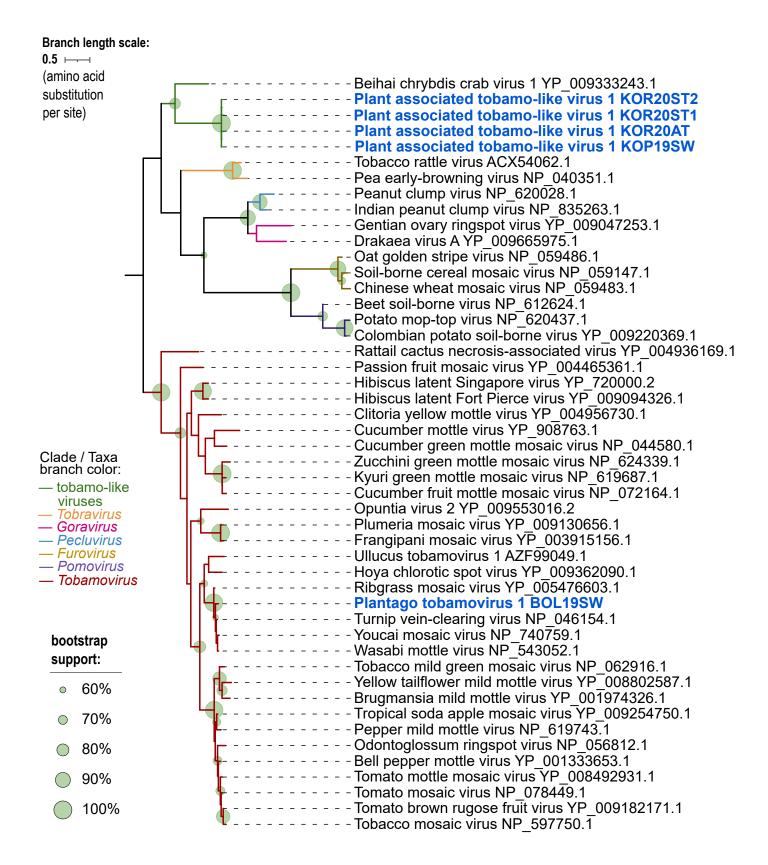
Supplementary Figure 4-06. Maximum likelihood phylogenetic tree based on the alignment of conserved RNA-dependent RNA polymerase amino acid sequence of virus species under family *Closteroviridae* (*Martellivirales*).



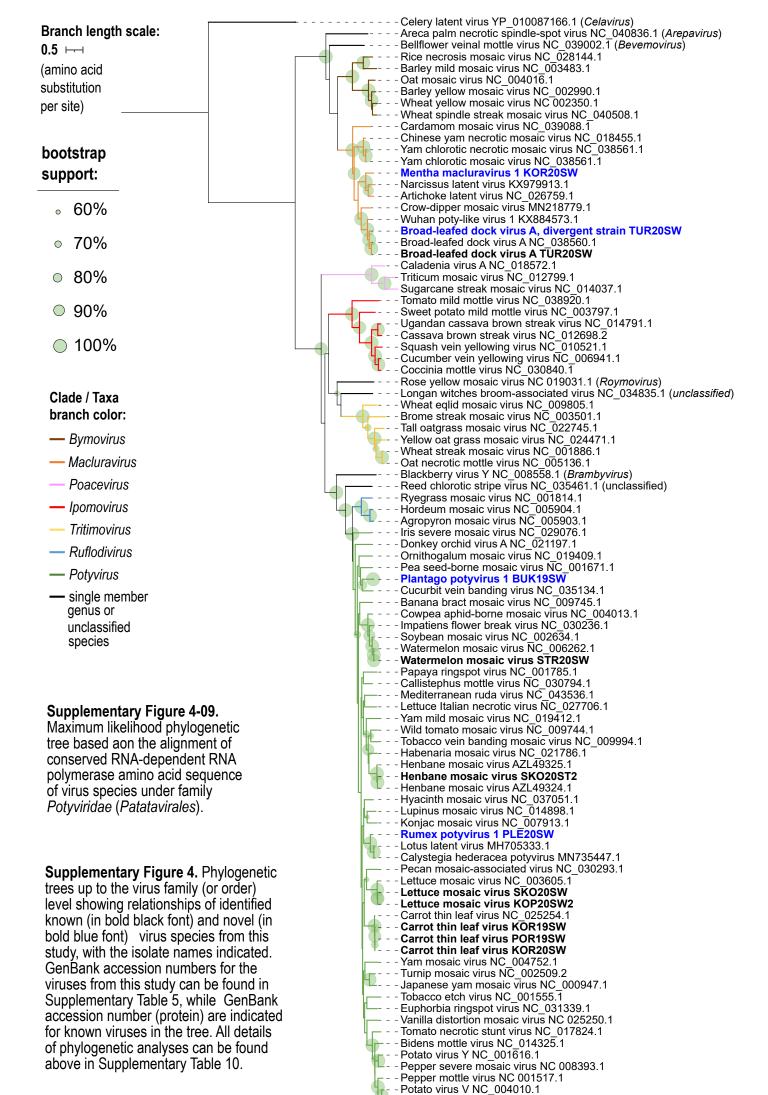
Supplementary Figure 4-07. Maximum likelihood phylogenetic tree based on the alignment of conserved RNA-dependent RNA polymerase amino acid sequence of virus species under family *Endornaviridae* (*Martellivirales*).



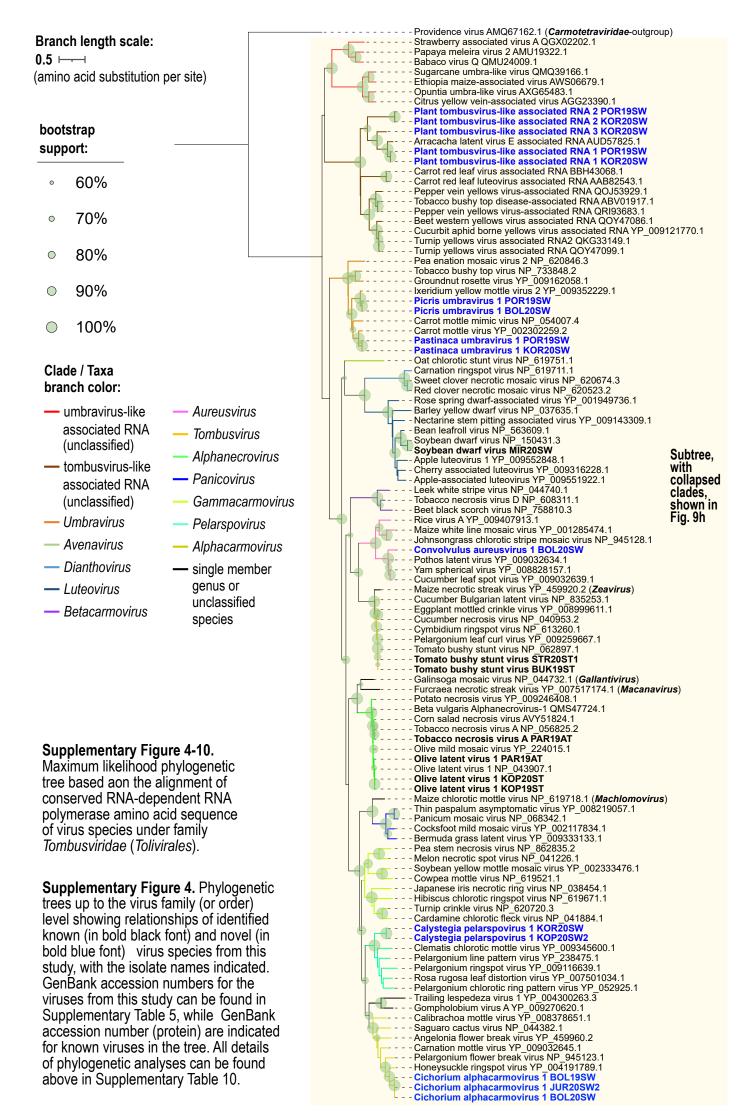
Supplementary Figure 4-08-A. Maximum likelihood phylogenetic tree based on the alignment of conserved methyl transferase-helicase amino acid sequence of virus species under family *Virgaviridae* (*Martellivirales*).

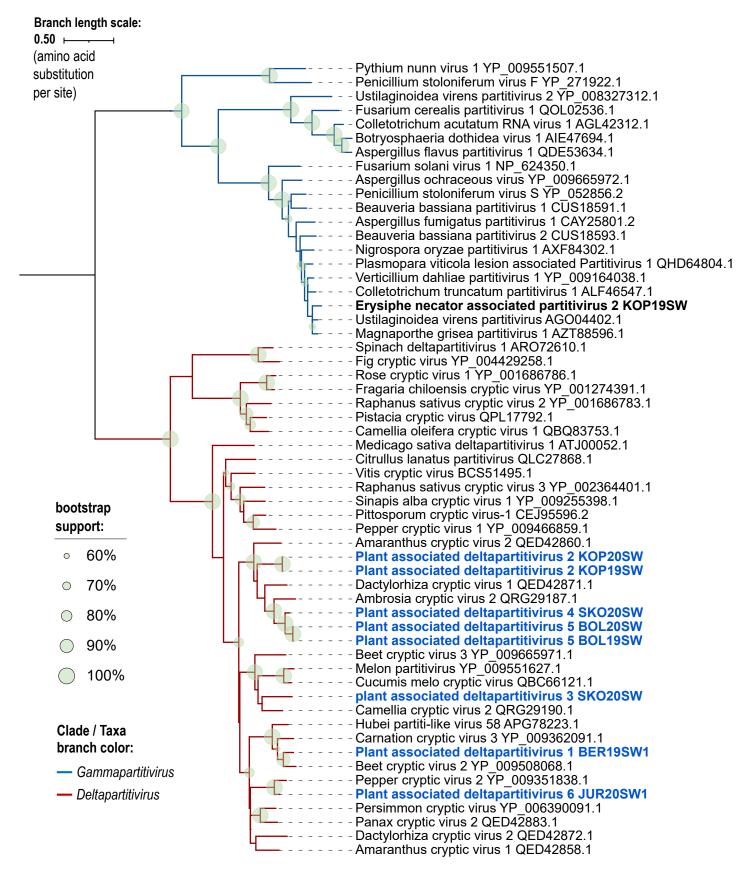


Supplementary Figure 4-08-A. Maximum likelihood phylogenetic tree based on the alignment of conserved coat protein amino acid sequence of virus species under family *Virgaviridae* (*Martellivirales*).

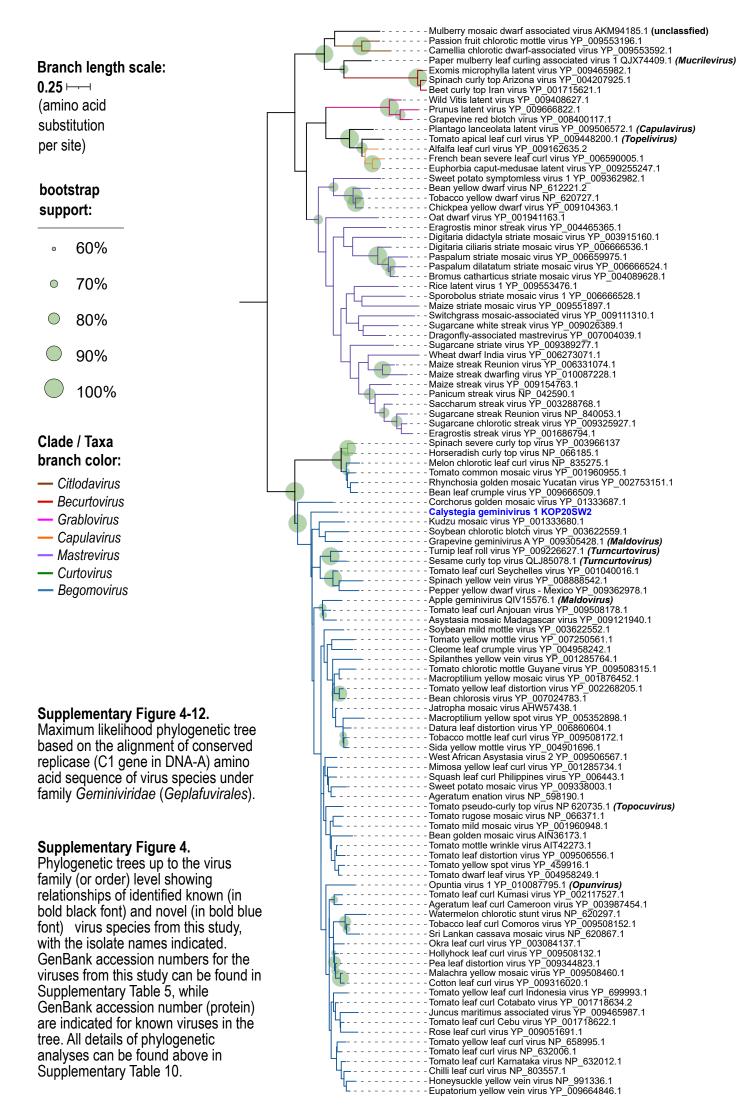


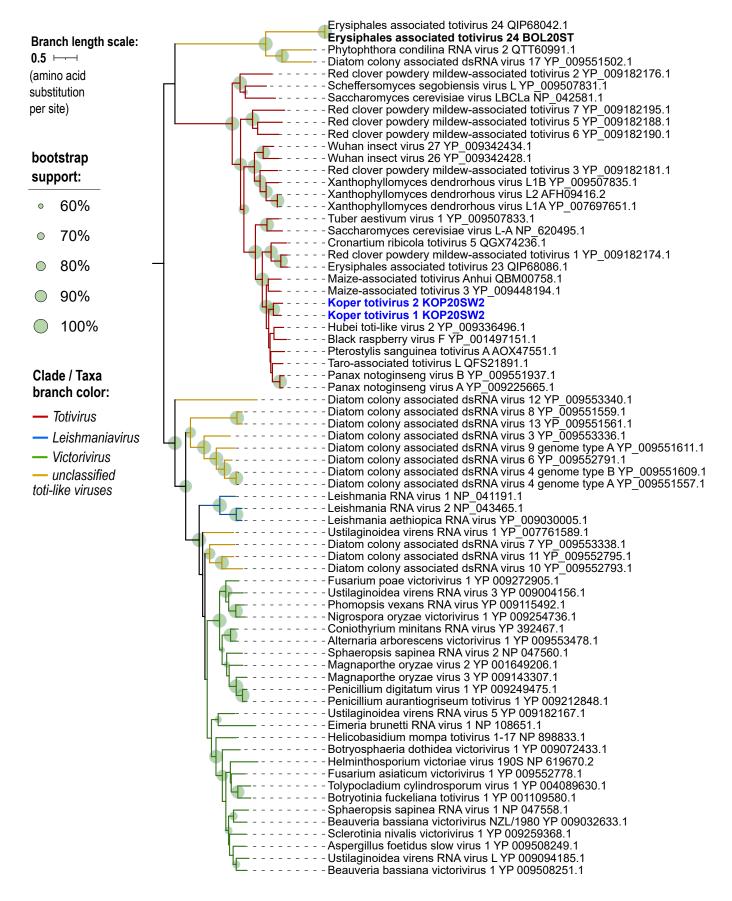
- Peru tomato mosaic virus NC_004573.1



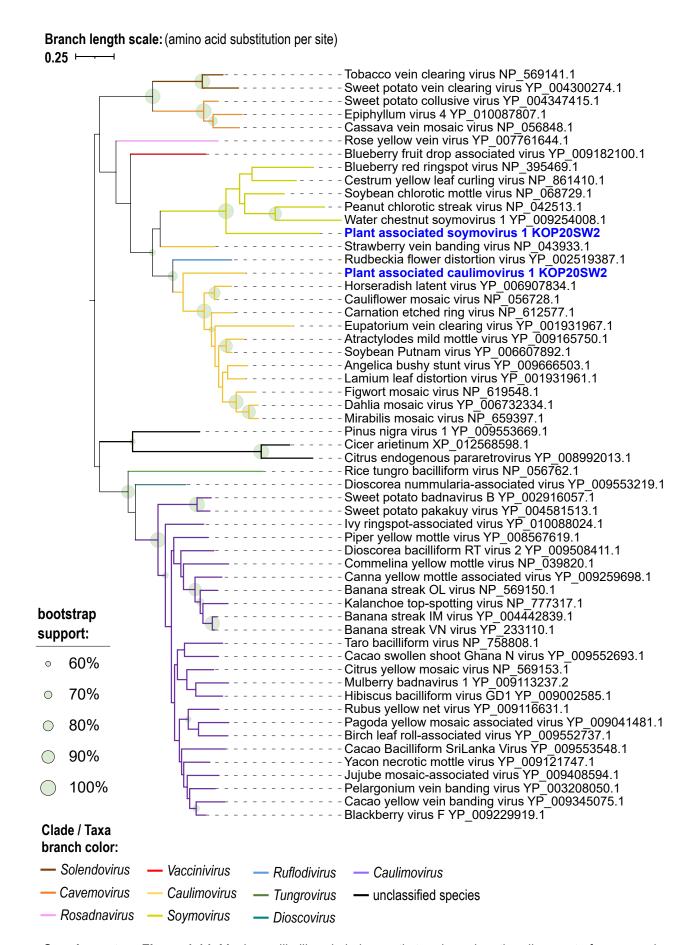


Supplementary Figure 4-11. Maximum likelihood phylogenetic tree based on the alignment of conserved RNA-dependent RNA polymerase amino acid sequence of virus species under family *Partitiviridae* (*Durnavirales*).





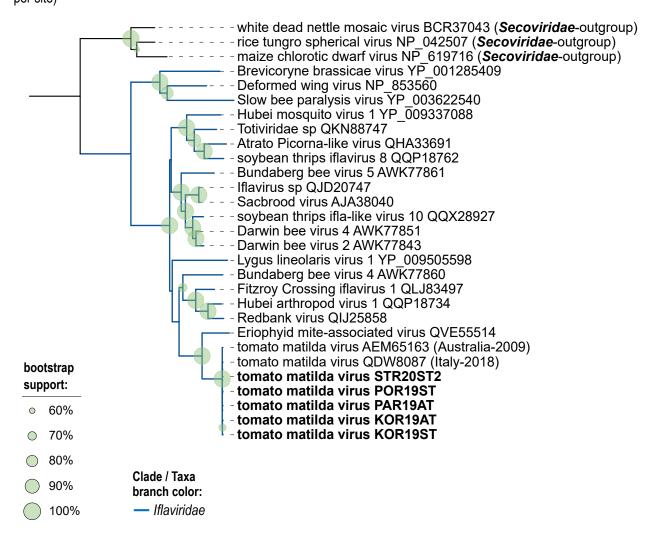
Supplementary Figure 4-13. Maximum likelihood phylogenetic tree based on the alignment of conserved RNA-dependent RNA polymerase amino acid sequence of virus species under family *Totiviridae* (*Ghabrivirales*).



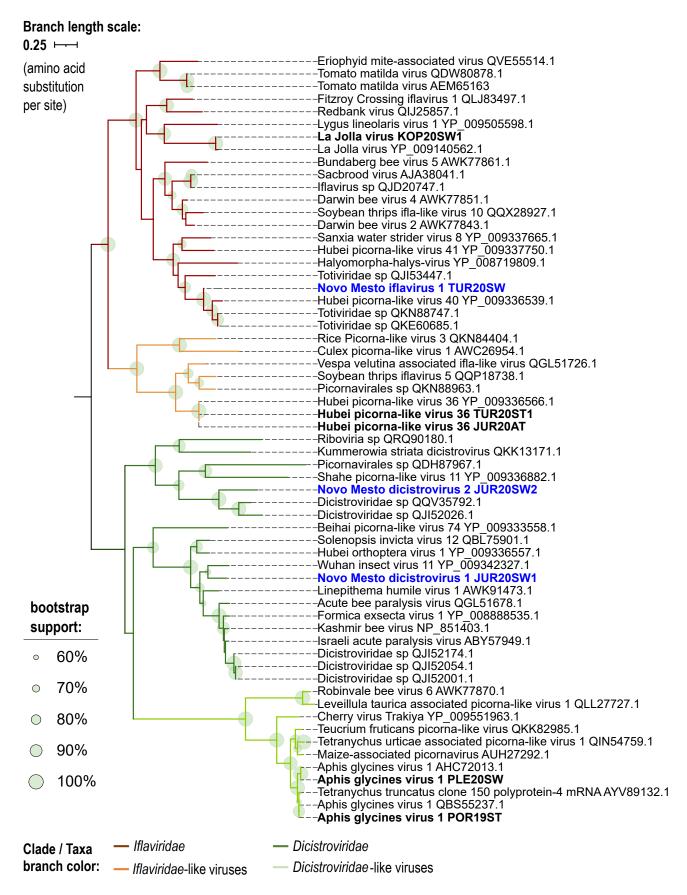
Supplementary Figure 4-14. Maximum likelihood phylogenetic tree based on the alignment of conserved reverse transcriptase (RT) amino acid sequence of virus species under family *Caulimoviridae* (*Ortelivirales*).

Branch length scale:

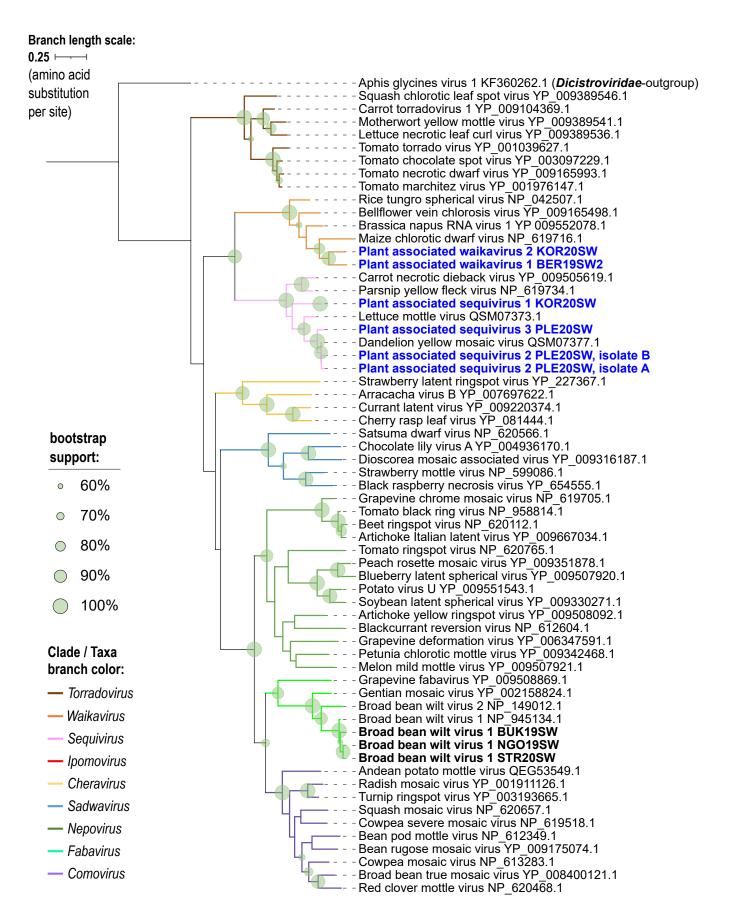
0.5 (amino acid substitution per site)



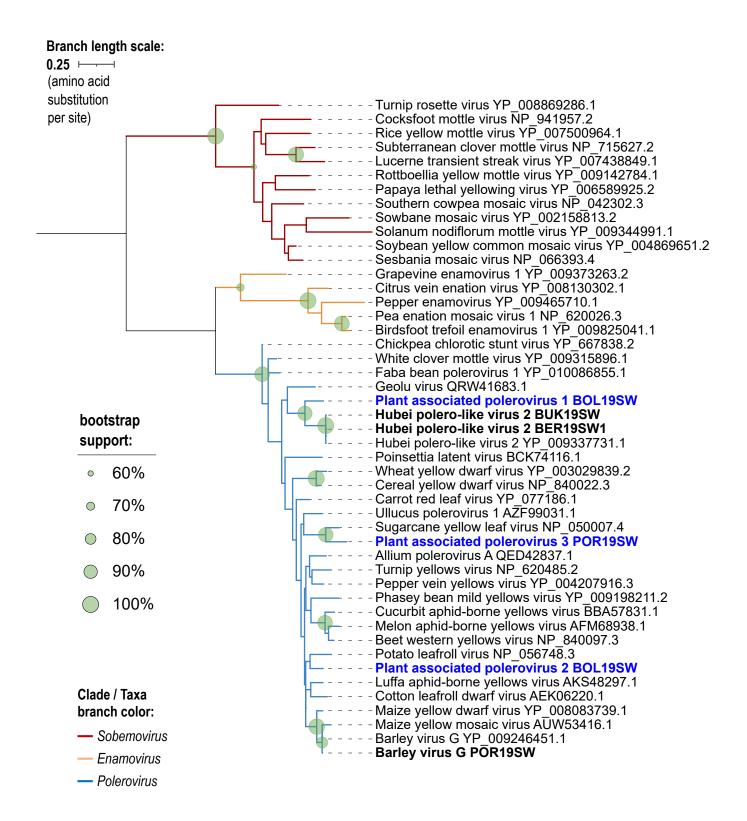
Supplementary Figure 4-15. Maximum likelihood phylogenetic tree based on the alignment of conserved RNA-dependent RNA polymerase amino acid sequence of virus species under *Iflaviridae* (*Picornavirales*), highlighting isolates of Tomato matilda virus from this study.



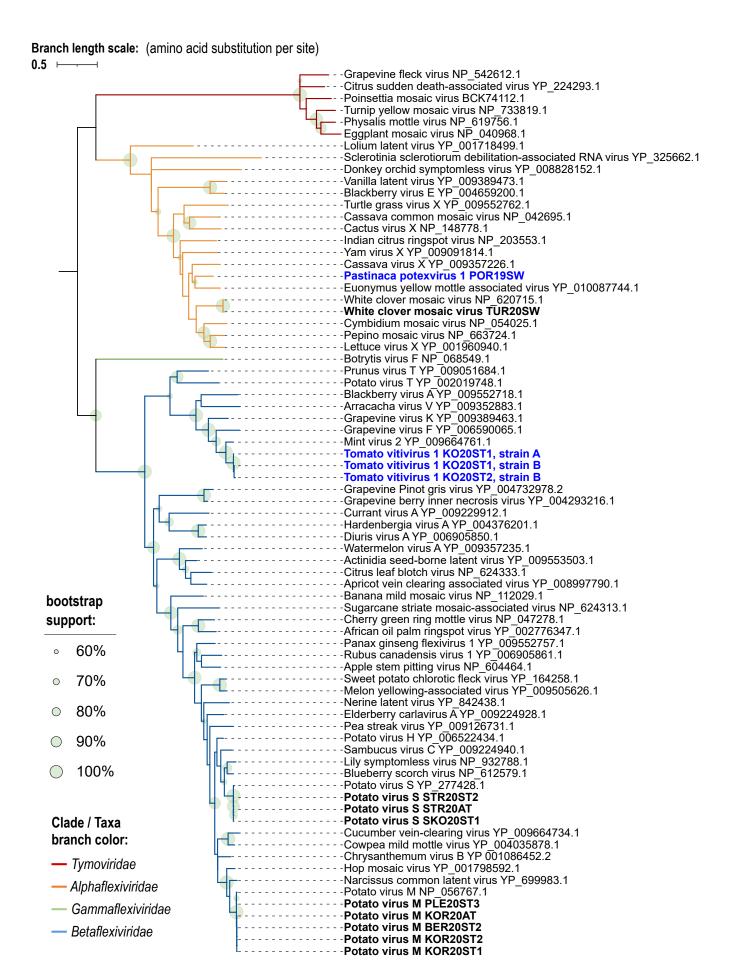
Supplementary Figure 4-16. Maximum likelihood phylogenetic tree based on the alignment of conserved RNA-dependent RNA polymerase amino acid sequence of virus and virus-like species classifiable under family *Dicistroviridae* and *Iflaviridae* (*Picornavirales*).



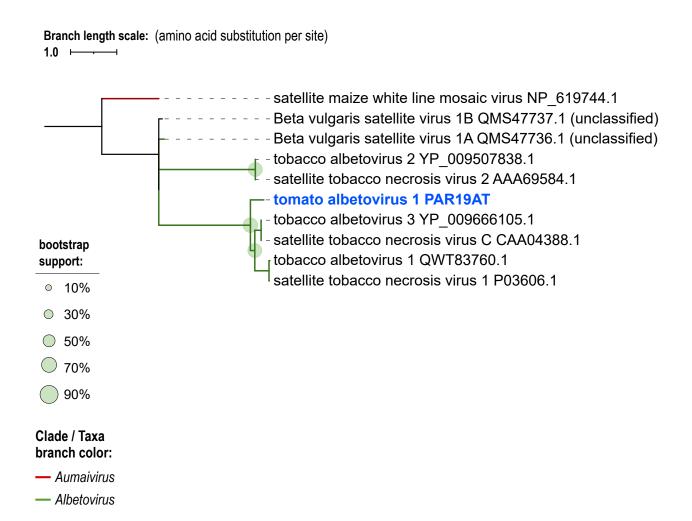
Supplementary Figure 4-17. Maximum likelihood phylogenetic tree based on the alignment of conserved RNA-dependent RNA polymerase amino acid sequence of virus species under family *Secoviridae* (*Picornavirales*).



Supplementary Figure 4-18. Maximum likelihood phylogenetic tree based on the alignment of conserved RNA-dependent RNA polymerase amino acid sequence of virus species under family *Solemoviridae* (*Sobelivirales*).



Supplementary Figure 4-19. Maximum likelihood phylogenetic tree based on the alignment of conserved RNA-dependent RNA polymerase amino acid sequence of virus species under order *Tymovirales*.



Supplementary Figure 4-20. Maximum likelihood phylogenetic tree based on the alignment of conserved coat protein amino acid sequence of satellite virus species with emphasis on genus *Albetovirus*.