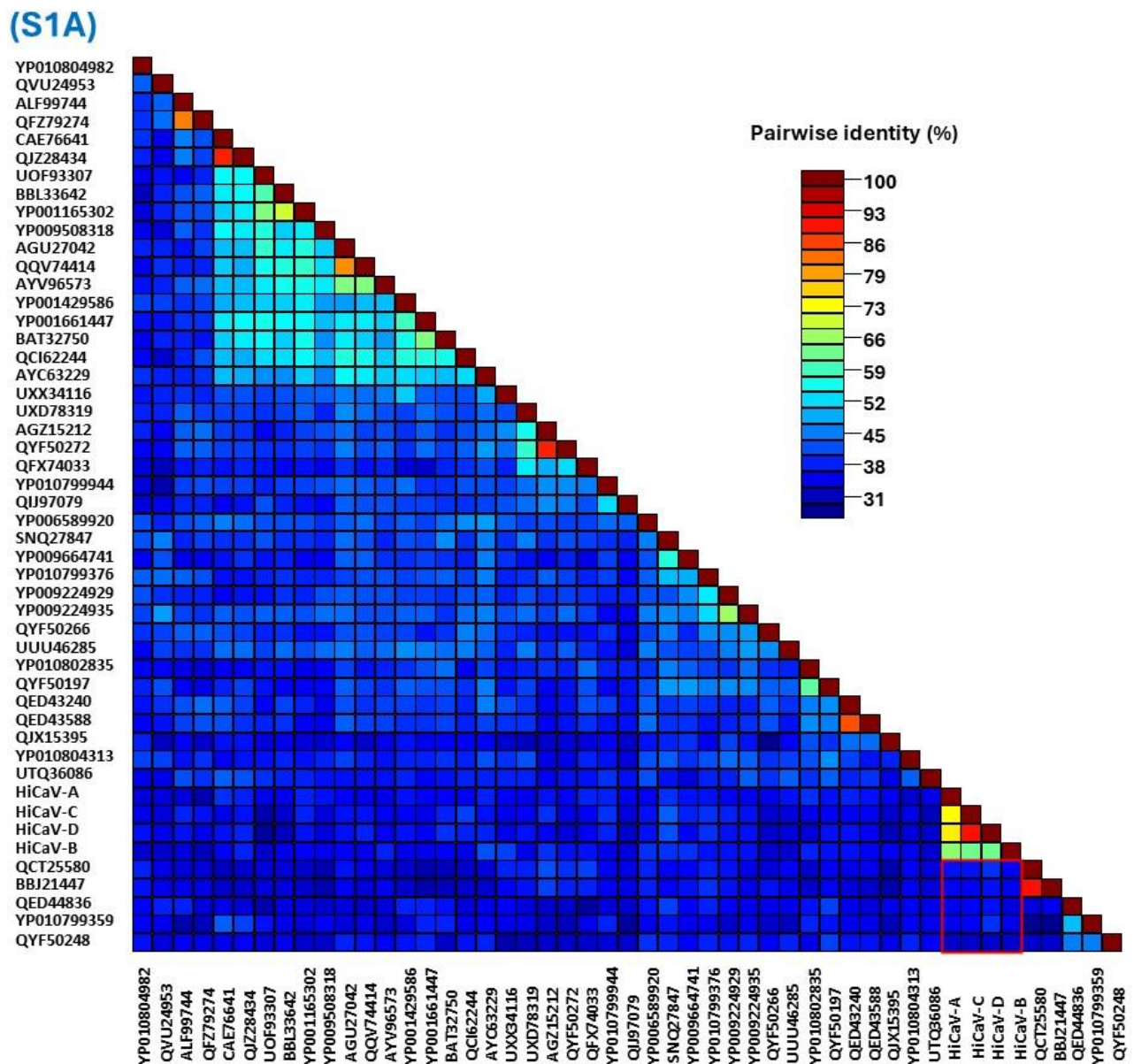
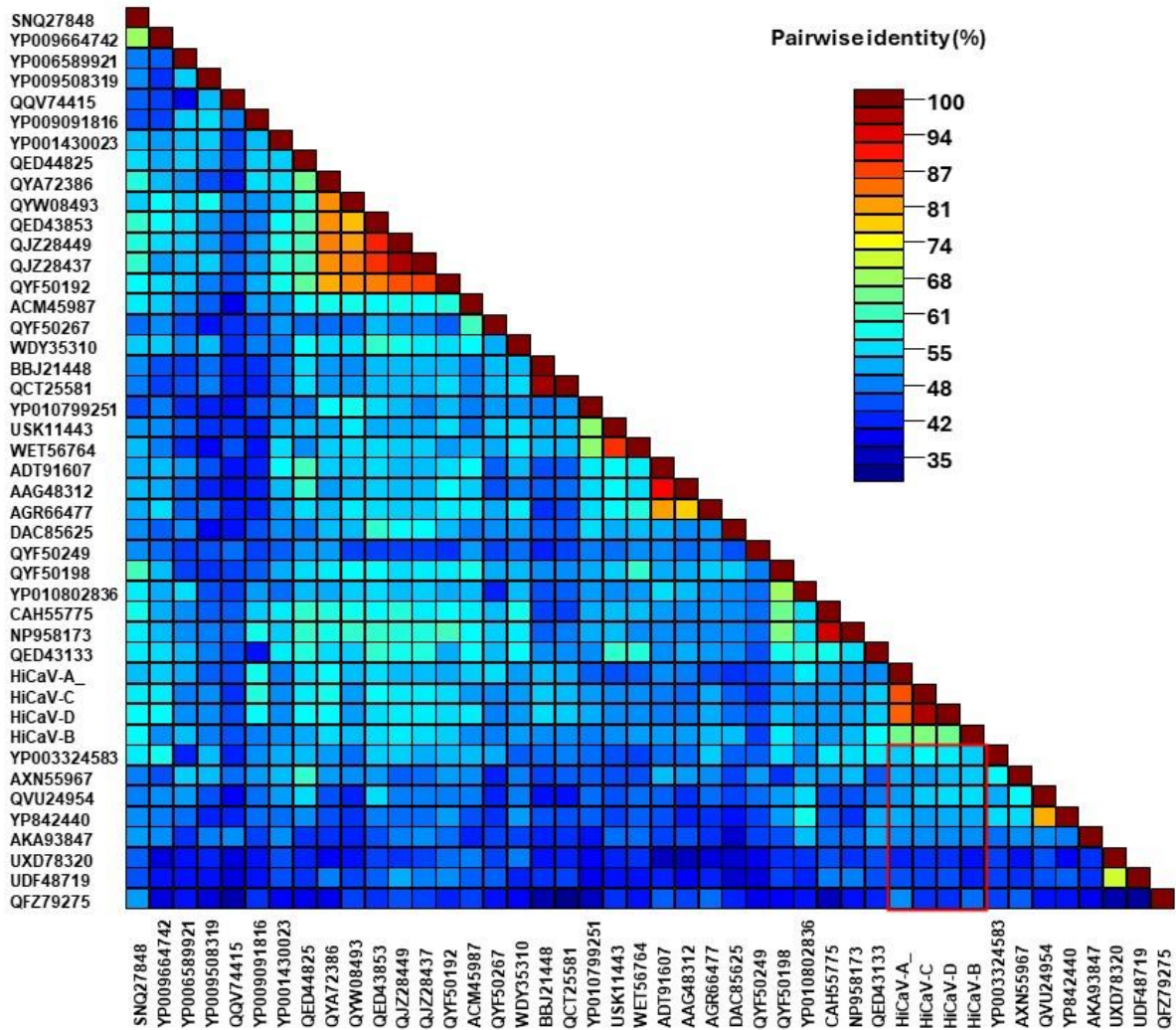


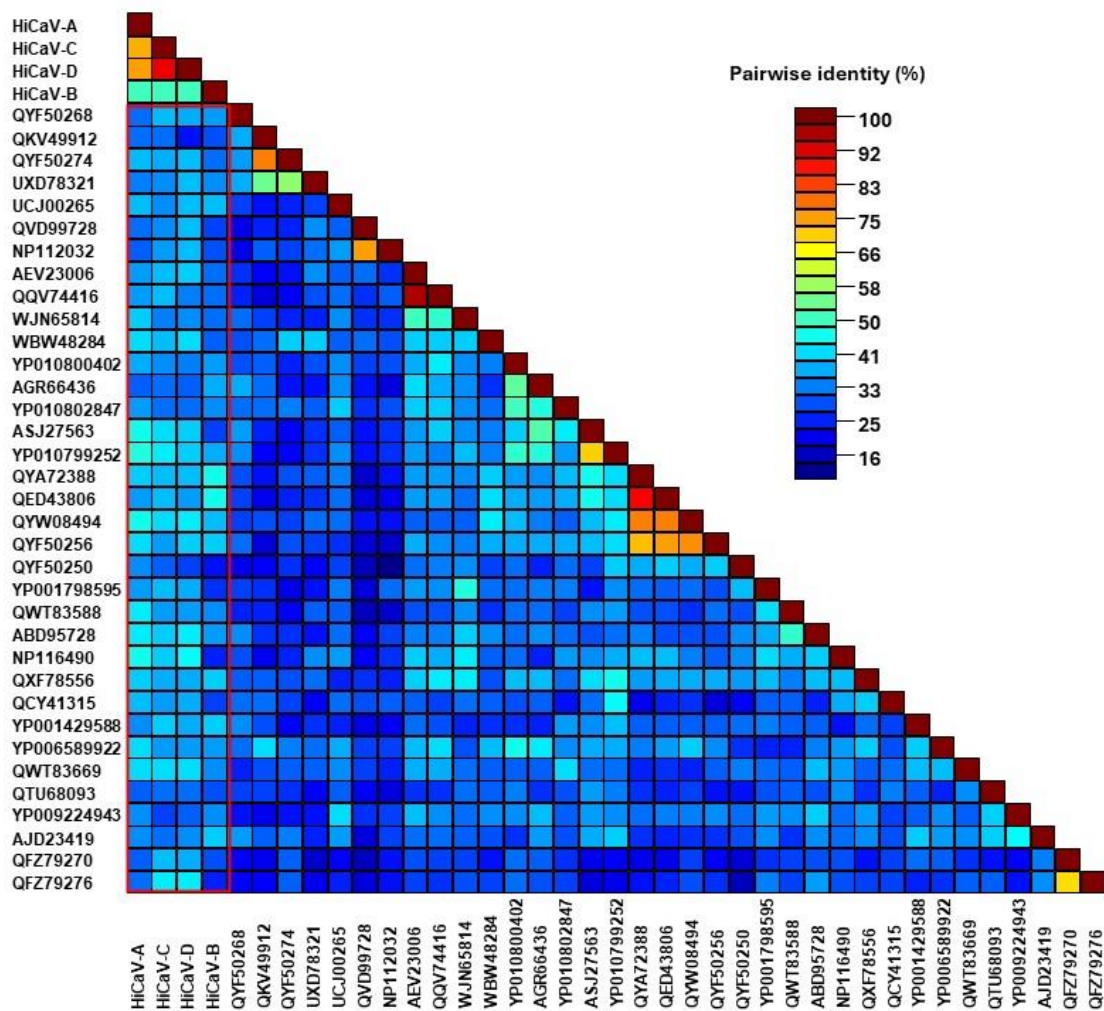
Supplementary Figure S1: Heatmap analysis of the pairwise amino acid identity matrixes of hibiscus carlaviruses (HiCaV-A, HiCaV-B, HiCaV-C, and HiCaV-D) infecting the RsTHr hibiscus sample. Collectively, 45, 40, and 35 carlavirus species Triple Gene Block (TGB) 1 (S1A), TGB2 (S1B), and TGB3 (S1C), amino acid sequences, respectively, were included from the NCBI database. Different shades of blue triangle for TGB1 to TGB3 inside the red-bordered rectangles refer to less than 55% amino acid identity of HiCaVs with any carlavirus sequences included in this analysis. Phylogenetic relationships of the hibiscus carlaviruses (HiCaV-A, HiCaV-B, HiCaV-C, and HiCaV-D) identified in the RsTHr high-throughput sequencing library with the TGB1 (S1D), TGB2 (S1E), and TGB3 (S1F) complete amino acid sequences of 35 to 45 *Betaflexiviridae* family members using the Multiple Alignment using Fast Fourier Transform (MAFFT, v7.520) program with bootstrap values of 1000 replicates. The names corresponding to the accession numbers in the heatmap analysis are displayed in the phylogenetic trees created using the TGB-1, -2, and -3 amino acid sequences.

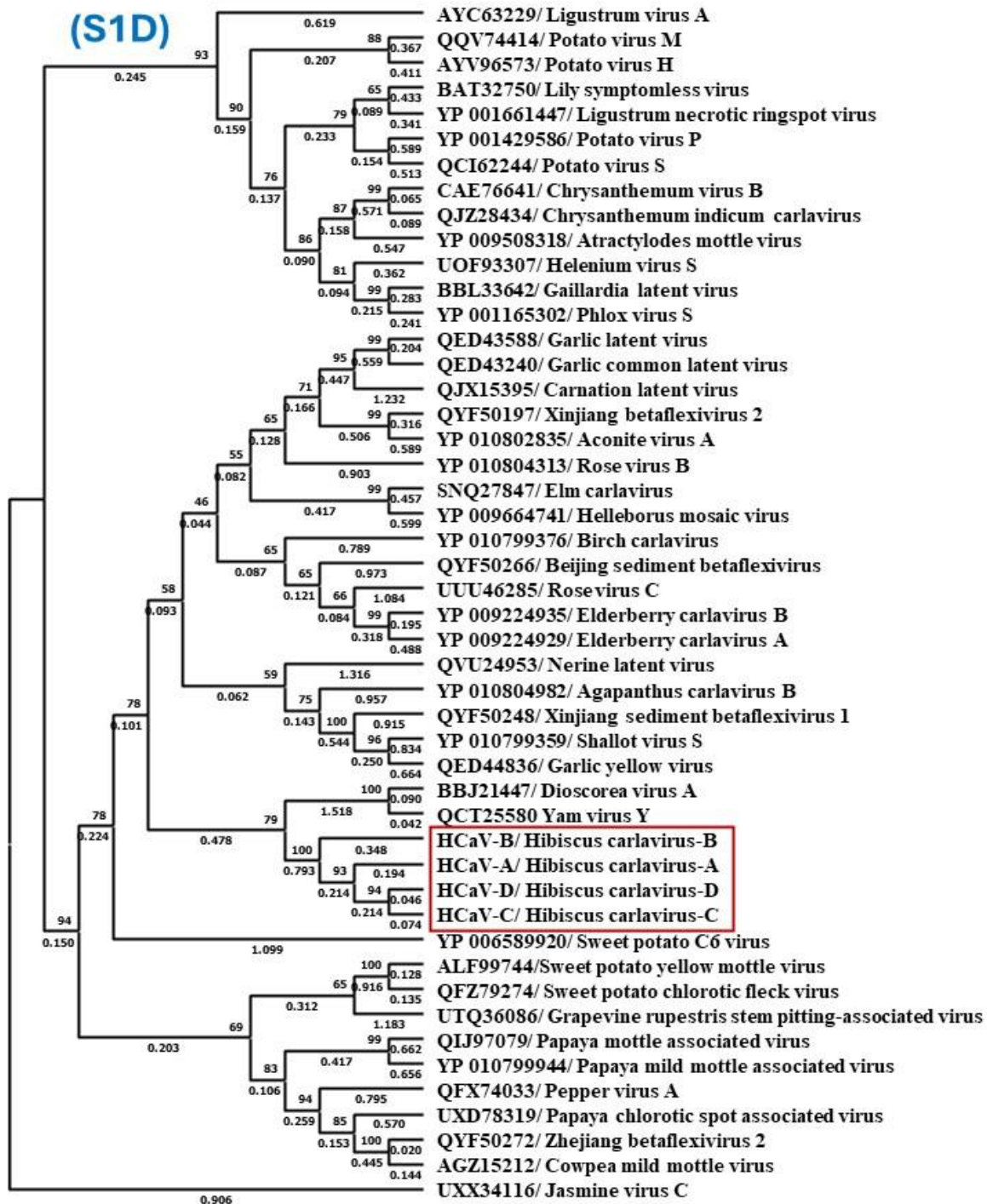


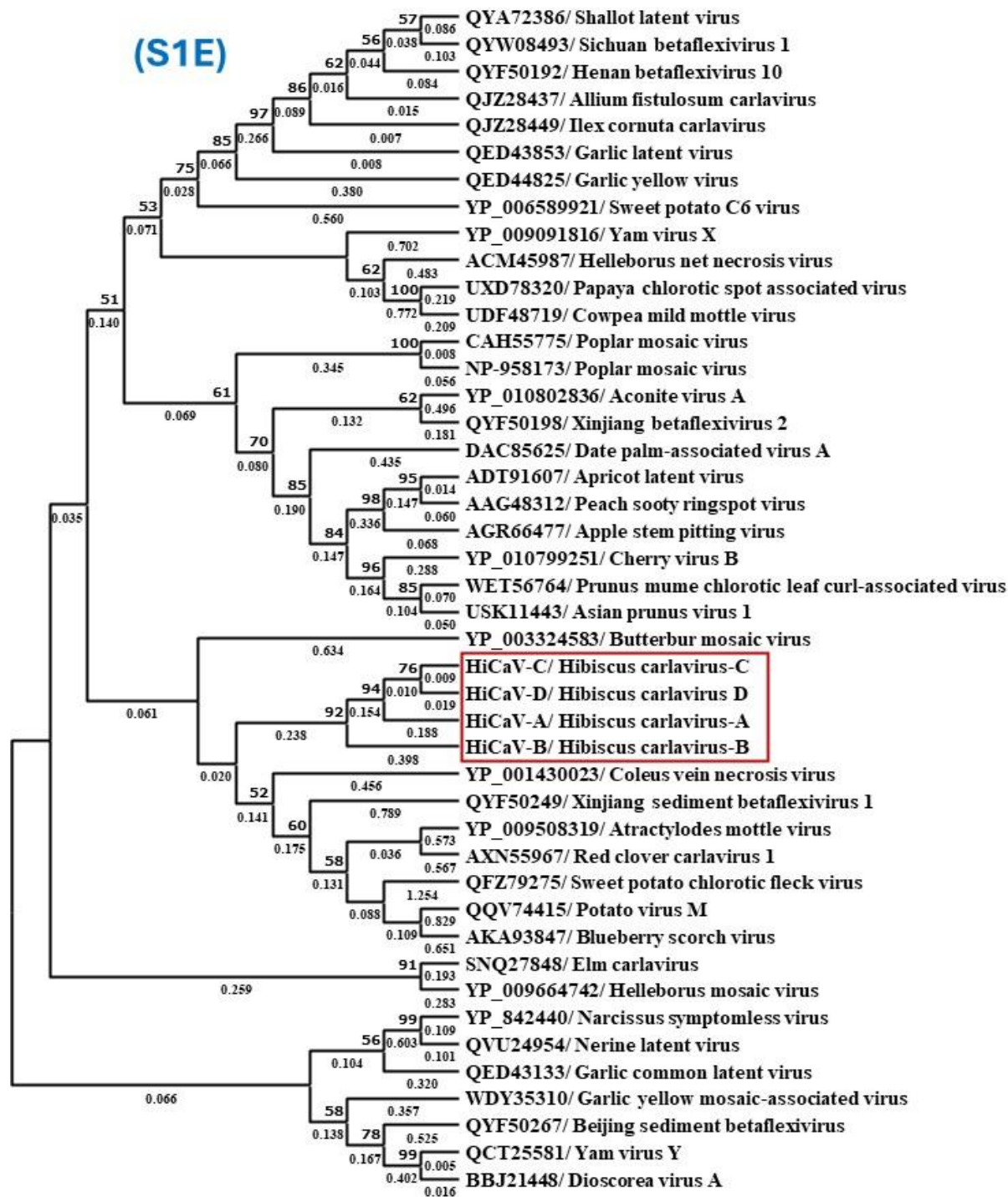
(S1B)



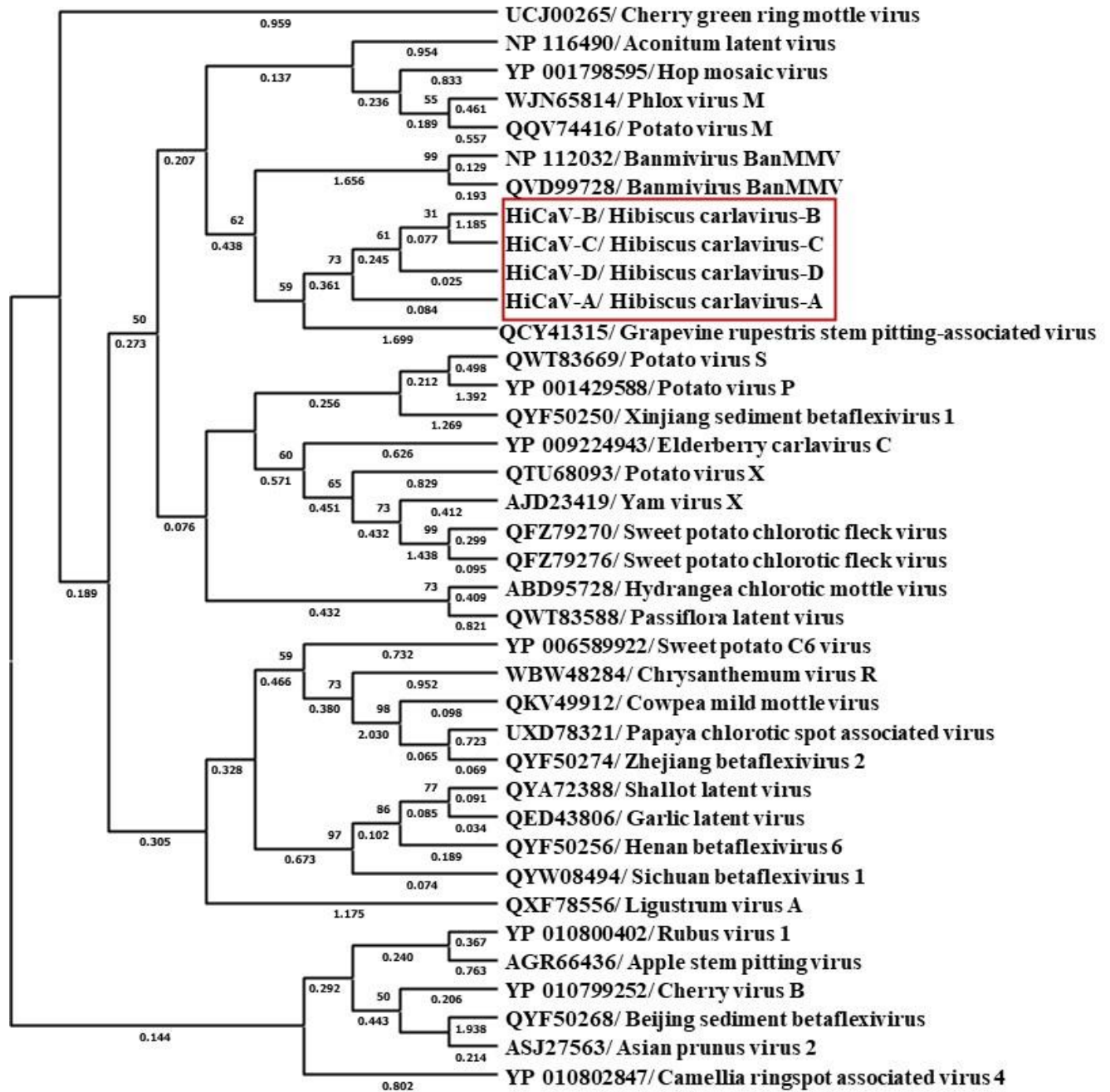
(S1C)



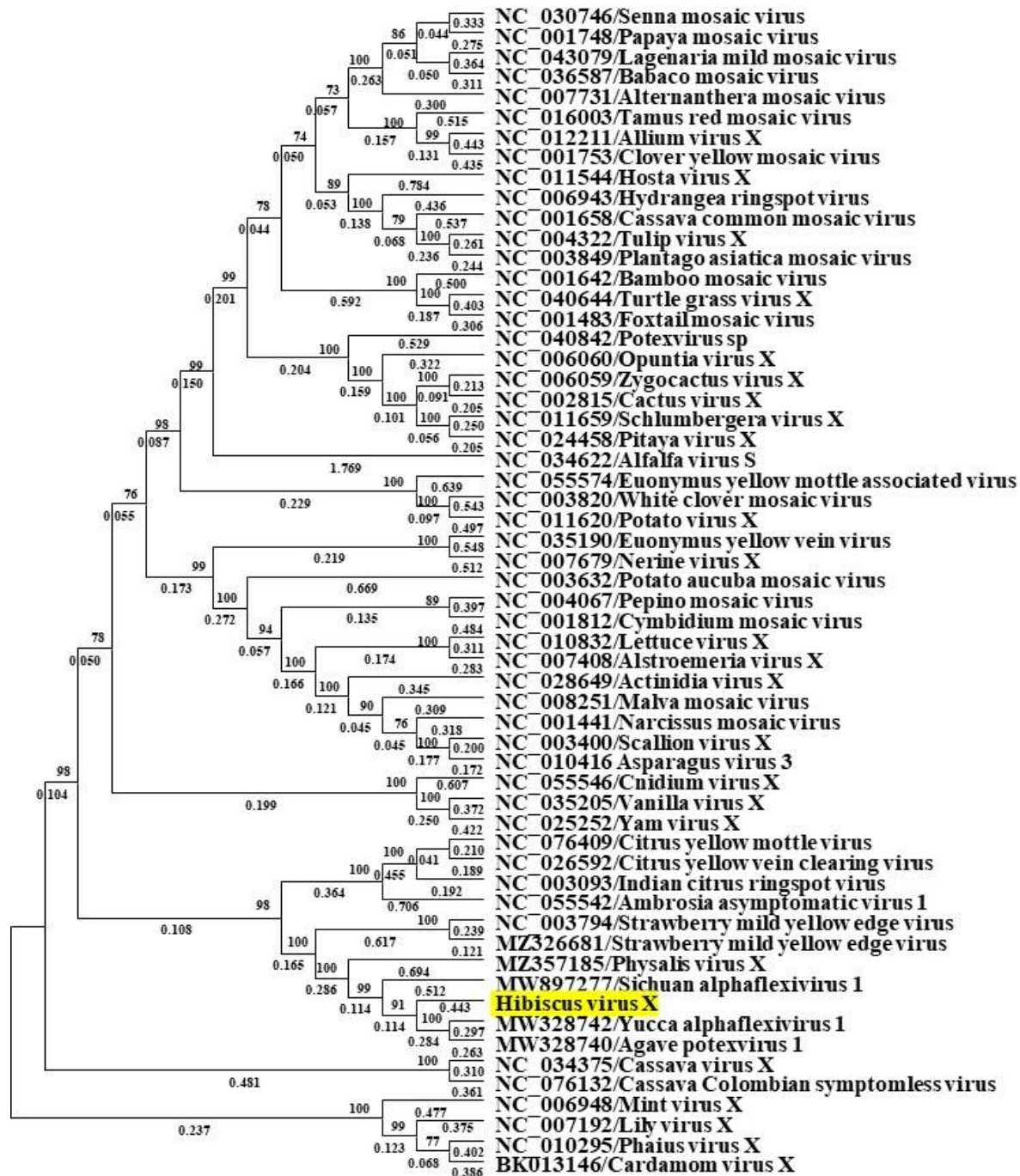




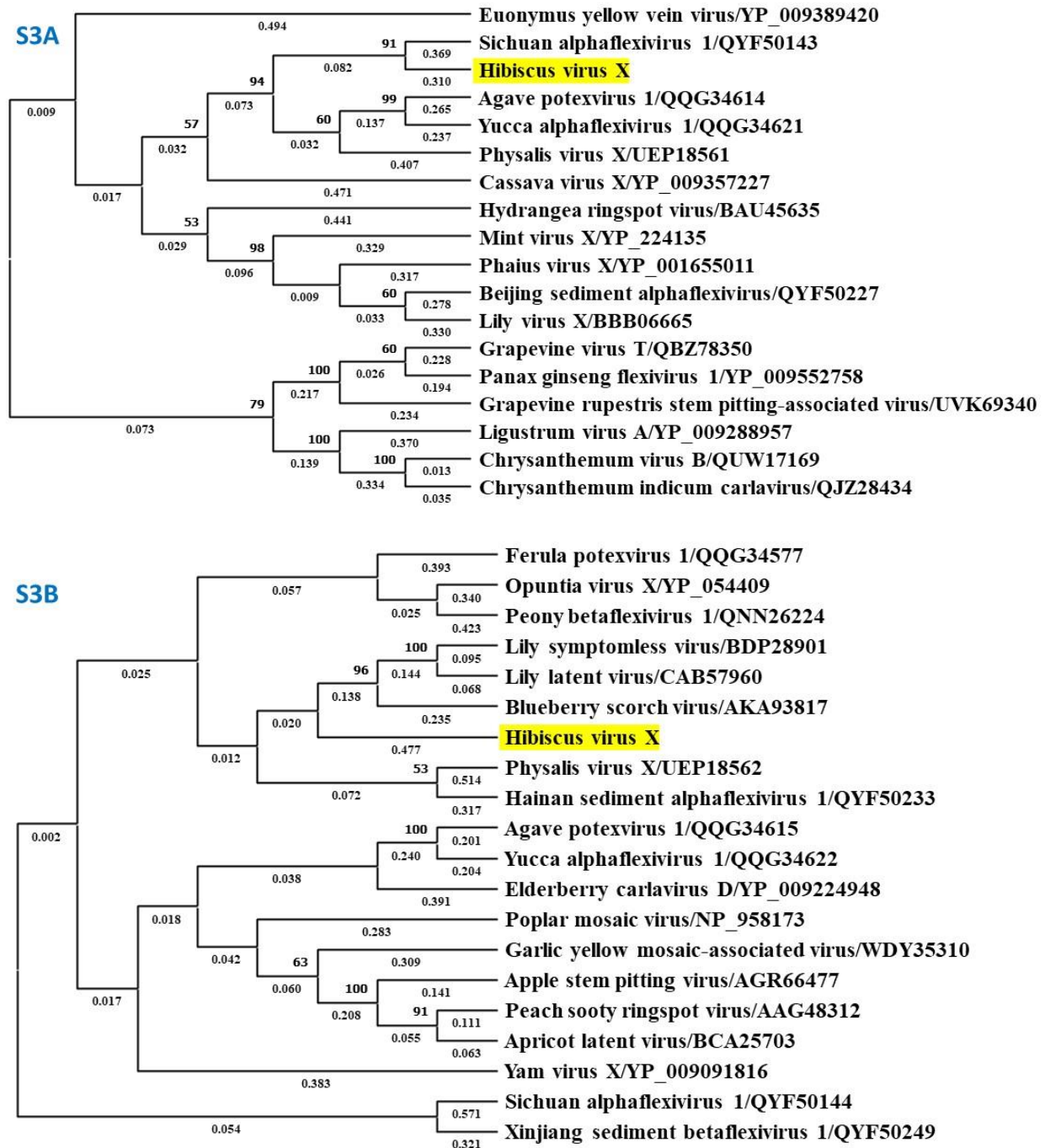
(S1F)



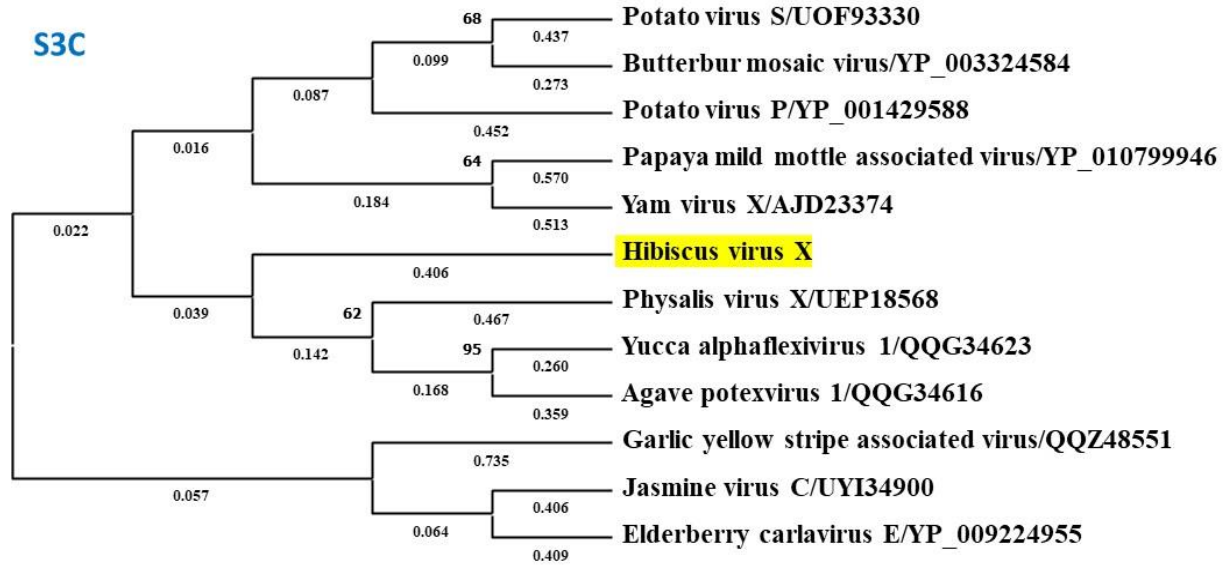
Supplementary Figure S2: Phylogenetic relationships of the newly discovered hibiscus potexvirus (HiVX) in the RsTHr high-throughput sequencing library with 57 potexvirus species genome sequences (at nucleotide level) using the Multiple Alignment using Fast Fourier Transform (MAFFT, v7.520) program with bootstrap values of 1000 replicates.



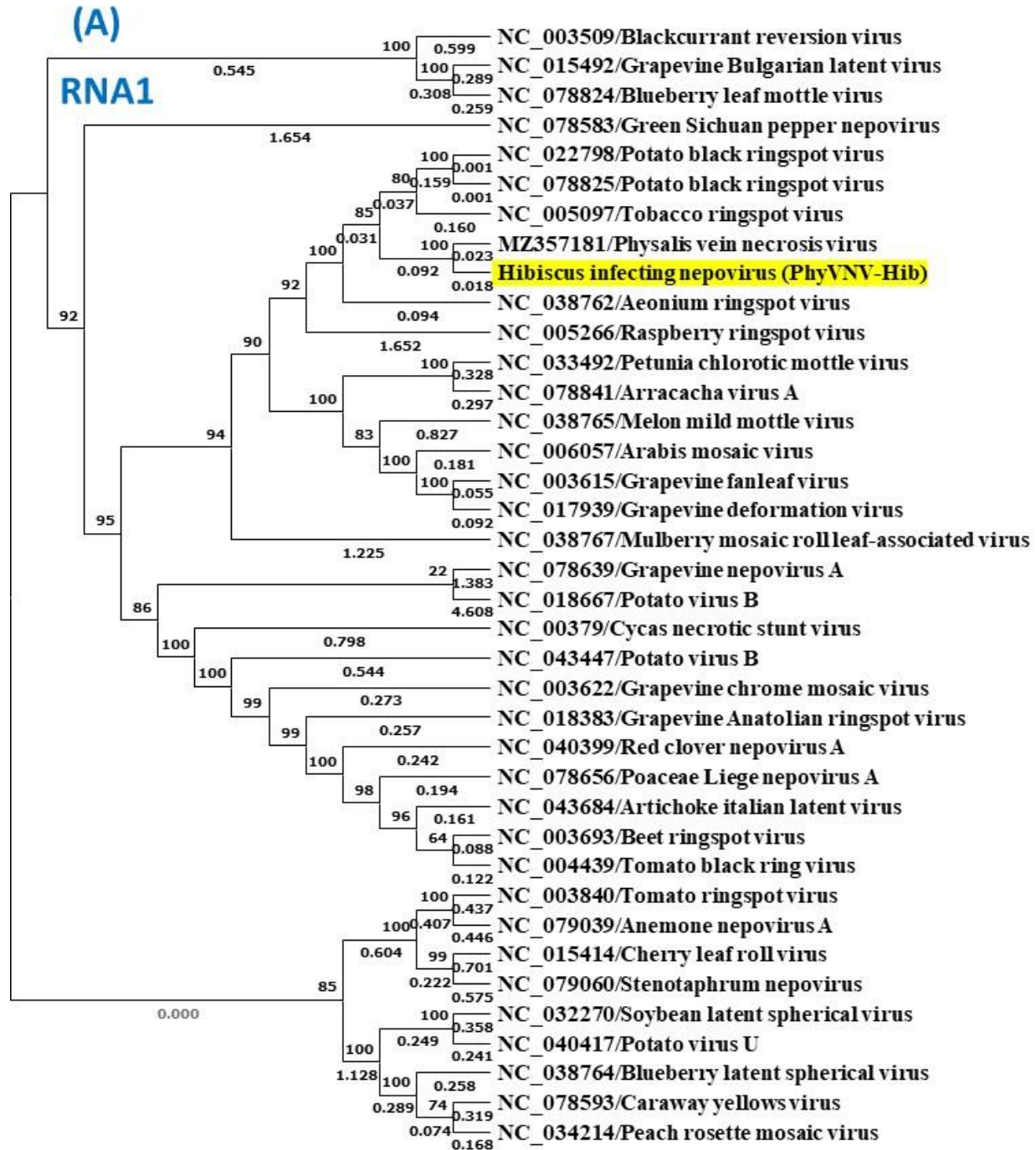
Supplementary Figure S3: Phylogenetic relationships of the newly discovered hibiscus potexvirus (HiVX) in the RsTHr high-throughput sequencing library with the most closely related potexvirus TGB1 (S3A), TGB2 (S3B) and TGB3 (S3C) amino acid sequences available in GenBank utilizing the Multiple Alignment using Fast Fourier Transform (MAFFT, v7.520) program with bootstrap values of 1000 replicates.

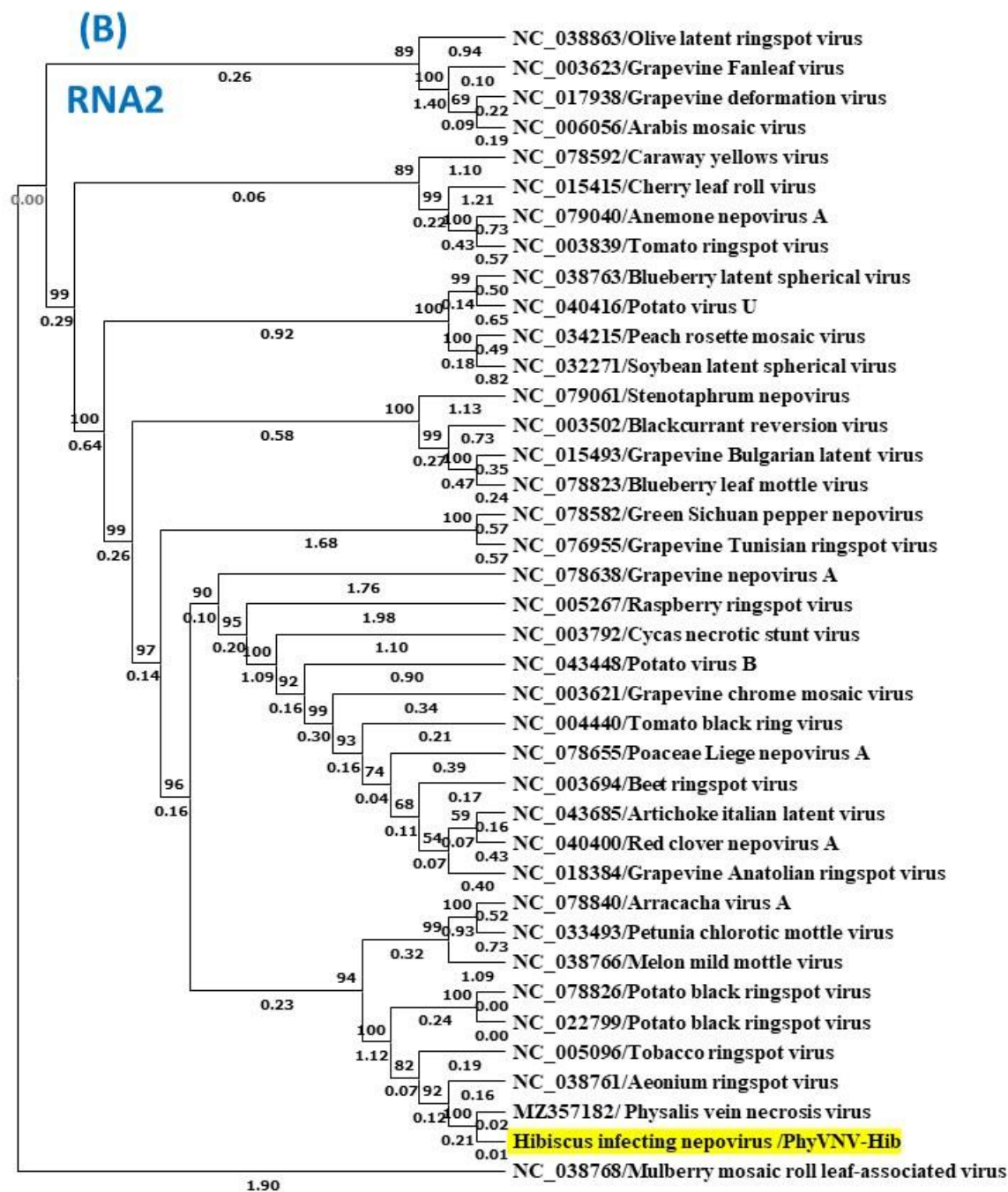


S3C



Supplementary Figure S4: Phylogenetic relationships of the hibiscus variant of physalis vein necrosis virus (PhyVNV) identified in the RsTHr high-throughput sequencing library with the 37 RNA1 (S4A) and 38 RNA2 (S4B) nepovirus species sequences (nucleotide level) utilizing the Multiple Alignment using Fast Fourier Transform (MAFFT, v7.520) program with bootstrap values of 1000 replicates





Supplementary Table S1: Published primer sequences of the citrus leprosis virus C2 and its hibiscus strain (CiLV-C2H), Hibiscus soymovirus (HSV) and Passion fruit green spot virus (PFGSV) are detailed with their citation.

Virus Name	Polarity	Primers		Amplified region	Amplicon size (Nts)	References
		Name	Sequence from 5' to 3'			
Citrus leprosis virus C2 (CiLV-C2)	sense	CiLV-C2-CPG-F	ATGAGTAACATTGTGTCGTTTTCTGTTGT	Coat protein	795	Roy et al., 2013 [49]
	antisense	CiLV-C2-CPG-R	TCACTCTTCTGTTCATCAACCTGTT			
Hibiscus strain of Citrus leprosis virus C2 (CiLV-C2H)	sense	R1CiLV-C2-GF5	TTGAAGTTAAAGTTCAGGTTCAACG	RdRp and Coat protein	1447	Roy et al., 2018 [6]
	antisense	R1CiLV-C2H-R5	GGATGTTTACTCTCCCTGCTCTTCTG			
Hibiscus Soymovirus (HSV)	sense	HSV-REP-F	TAA CAG CAT TCA GTT GTC CAC CTC A	Replicase	631	Roy et al., 2023 [8]
	antisense	HSV-REP-R	TGA AGG ATT GCT CCC CAA GAA TCT T			
	sense	HSV-CPG-F	TAG ATC ACC ATC TCC AGT ACC TCC A	Coat protein	401	
	antisense	HSV-CPG-R	CTG TTT CTT GAC CAG TTG TTC CCA T			
	sense	SVF1	AGGAAGATGGTCGTTTTGGG	Transactivation	430	Wang et al. 2023 [31]
	antisense	SVR1	ACTGGACTGCTGGTTGTATG			
Passion fruit green spot virus (PFGSV)	sense	C13R	ATTCATGCGTTTCACGGTTA	RNA1	321	Ramos-González et al. 2020 [48]
	antisense	C13F	CGAATGCCTCTGACACAAC			
	sense	C6F	CGATATTGATCAATCCGTT	RNA2	244	
	antisense	C6R	CACCTTAAAATTCGAGGGTT			
	sense	C8F	TTCATCGCAAGTTCGTATACCT		299	
	antisense	C8R	CTGTTGTGCCAATCATCAA			

Supplementary Table S2: Analysis of the complete genome nucleotide sequence and the translated amino acid gene sequence of each open reading frame of the newly discovered Hibiscus carlaviruses with nine or ten nearest carlavirus relatives; percentage sequence coverage is indicated in parentheses. NB. Some of the related viruses listed in the table have multiple sequences in GenBank, thus the sequence accession numbers with the highest amino acid sequence identity for different HiCaV proteins may represent distinct isolates of the same virus. NS = not significant.

Hibiscus Carlavirus	Nucleotide identity									
Full genome of HiCaVs	NC_018448	MW897314	LT898349	MW897296	MW371117	MZ173553	MK778784	MN059240	NC_077068	OM671312
	Sweet potato virus C6	Beijing sediment betaflexivirus	Elm carlavirus	Xinjiang sediment betaflexivirus 1	Cowpea mild mottle virus (MW201798)	Papaya chlorotic spot associated virus	Sweet potato chlorotic fleck virus	Garlic latent virus	Rose virus B	Ligustrum virus A
Hibiscus carlavirus A (8229 nt)	68.70 (20)	67.37 (20)	66.67(18)	70.06 (16)	69.33 (15)	69.44 (15)	71.98 (10)	66.26 (14)	67.70 (19)	68.40 (15)
Hibiscus carlavirus B (8202)	68.77 (22)	67.67 (18)	66.73 (17)	70.42 (11)	71.22 (15)	70.79 (13)	67.62 (18)	67.11 (18)	70.86 (12)	67.43 (18)
Hibiscus carlavirus C (8117nt)	68.01 (22)	68.28 (18)	66.72 (17)	68.59 (16)	69.85 (22)	68.41 (20)	69.18 (18)	68.29 (15)	66.90 (26)	69.57 (13)
HiCaV-D (Contig 836 nt)	64.16 (45)	68.80 (14)	NS	72.34 (22)	70.87 (35)	71.52 (28)	73.54 (26)	72.31 (20)	71.49 (70)	73.31 (70)
HiCaV-D (Contig 3009 nt)	100 (01)	NS	69.53 (04)	NS	73.85 (02)	NS	74.79 (03)	68.27 (03)	77.55 (01)	82.86 (03)
HiCaV-D (Contig 641 nt)	68.75 (92)	67.83 (997)	67.96 (56)	71.08 (88)	66.50 (92)	68.75 (56)	67.62 (92)	66.24 (84)	70.15 (68)	68.65 (57)
HiCaV-D (Contig 1899 nt)	69.66 (9)	75.44 (03)	NS	71.24 (08)	72.29 (12)	NS	70.92 (07)	69.30 (16)	70.59 (05)	65.35 (14)
HiCaVs-ORF encoding gene	Amino Acid identity									
RdRp protein of HiCaVs	YP_006589919	BAL03071.1	YP_009224952.1	WCC72446.1	WCR76308.1	SNQ27851.1	WBG54312.1	WAK43334.1	QXQ32728.1	
	Sweet potato virus C6	Helleborus net necrosis virus	Elderberry carlavirus E	Rose virus B	American hop latent virus	Elm carlavirus	Poplar mosaic virus	Ligustrum virus A	Helenium virus S	
HiCaV-A	52.83 (81)	52.24 (79)	49.08 (79)	48.46 (80)	42.81 (99)	50.14 (75)	43.62 (99)	51.46 (78)	41.18 (99)	
HiCaV-B	45.12 (100)	44.48 (99)	51.24 (79)	51.15 (78)	49.02 (80)	50.45 (79)	50.00 (80)	50.96 (78)	48.81 (79)	
HiCaV-C	51.62 (83)	51.65 (80)	43.47 (100)	49.33 (78)	42.19 (99)	50.00 (76)	43.94 (99)	49.82 (80)	41.04 (99)	
HiCaV-D (836 nt)	53.82 (98)	55.47 (98)	57.95 (58)	56.88 (99)	52.33 (98)	57.87 (90)	55.07 (99)	56.00 (98)	51.27 (98)	
HiCaV-D (3009 nt)	40.99 (59)	38.64 (59)	35.79 (62)	39.82 (44)	36.67 (60)	36.48 (63)	37.94 (62)	35.34 (61)	34.69 (61)	
HiCaV-D (641 nt)	69.95 (99)	72.77 (99)	66.20 (99)	64.79 (99)	65.26 (99)	67.61 (99)	69.95 (99)	65.73 (99)	66.20 (99)	
Coat protein of HiCaVs	YP_006589923	ACM45995.1	YP_009224950.1	YP_009224938.1	YP_009224932.1	YP_010802838.1	YP_003324585.1	WBG54313.1	QED43237.1	
	Sweet potato virus C6	Helleborus net necrosis virus	Elderberry carlavirus D	Elderberry carlavirus B	Elderberry carlavirus A	Aconite virus A	Butterbur mosaic virus	Poplar mosaic virus	Garlic common latent virus	
HiCaV-A	48.64 (93)	50.17 (89)	49.80 (79)	48.76 (78)	48.39 (80)	44.98 (93)	49.03 (84)	42.72 (98)	44.74 (86)	
HiCaV-B	52.14 (82)	50.52 (91)	49.39 (78)	47.76 (78)	46.77 (79)	46.69 (95)	49.23 (85)	48.57 (78)	47.64 (81)	
HiCaV-C	46.84 (96)	47.39 (99)	50.20 (79)	48.76 (78)	47.98 (80)	50.21 (78)	47.97 (87)	43.56 (98)	48.96 (78)	
HiCaV-D (last 444 nt of 1899 nt)	33.33 (68)	40.40 (66)	36.67 (60)	35.63 (58)	33.33 (62)	No similarity	37.96 (72)	31.29 (99)	42.53 (58)	
TGB1 protein of HiCaVs	QED43240.1	QED43588.1	SNQ27852.1	UZP17216.1	UDF48730.1	YP_010799376.1	YP_009224929.1	WMN13755.1	BAT32750	
	Garlic common latent virus	Garlic latent virus	Elm carlavirus	Chrysanthemum virus B	Cowpea mild mottle virus	Birch carlavirus	Elderberry carlavirus A	Aconite virus A	Lily symptomless virus	
HiCaV-A	37.93 (100)	37.99 (99)	38.82 (100)	38.98 (100)	36.91 (98)	37.45 (99)	36.48 (100)	41.10 (100)	34.32 (100)	
HiCaV-B	36.36 (100)	36.80 (100)	38.82 (100)	36.64 (100)	35.62 (98)	37.91 (88)	35.19 (100)	38.03 (100)	37.02 (100)	
HiCaV-C	38.96 (100)	35.93 (100)	40.08 (100)	36.97 (100)	37.55 (98)	38.14 (99)	37.71 (100)	37.92 (100)	37.61 (100)	
HiCaV-D	36.36 (100)	35.50 (100)	41.77 (100)	36.32 (100)	37.55 (98)	37.61 (88)	36.02 (100)	36.29 (100)	37.18 (100)	
TGB2 protein of HiCaVs	CAA92817.1	QED43133.1	BAZ96205.1	YP_003324583.1	YP_009664742.1	YP_006589921	AGR66477.1	SNQ27848.1	ACM45987.1	
	Garlic latent virus	Garlic common latent virus	Shallot latent virus	Butterbur mosaic virus	Helleborus mosaic virus	Sweet potato virus C6	Apple stem pitting virus	Elm carlavirus	Helleborus net necrosis virus	
HiCaV-A	55.45 (100)	49.53 (100)	53.64 (100)	55.14 (100)	52.25 (100)	53.92 (95)	44.34 (96)	50.91 (100)	52.34 (95)	
HiCaV-B	53.64 (100)	55.05 (100)	58.18 (100)	50.93 (100)	49.09 (100)	53.47 (94)	51.89 (96)	57.73 (89)	53.41 (82)	
HiCaV-C	56.88 (100)	51.85 (100)	55.05 (100)	55.14 (100)	53.64 (100)	49.02 (95)	49.53 (96)	57.00 (92)	56.70 (88)	
HiCaV-D	55.05 (100)	50.93 (100)	53.21 (100)	54.21 (100)	54.55 (100)	50.00 (95)	49.53 (96)	59.00 (92)	56.70 (88)	
TGB3 protein of HiCaVs	CAC83689.1	UCJ00265.1	QVD99728.1	QWT83588.1	ABD95728.1	QWT83669.1	YP_001429588.1	QTW21092.1	AAB81271.1	
	Garlic latent virus	Cherry green ring mottle virus	Banmivirus BanMMV	Passiflora latent virus	Hydrangea chlorotic mottle virus	Potato virus S	Potato virus P	Potato virus X	Potato virus M	
HiCaV-A	35.48 (93)	36.84 (86)	32.26 (93)	40.62 (93)	40.62 (93)	42.37 (89)	48.57 (53)	30.16 (95)	54.84 (56)	
HiCaV-B	45.59 (94)	38.81 (98)	30.88 (95)	32.76 (85)	42.86 (51)	40.38 (72)	38.81 (98)	42.59 (77)	33.33 (83)	
HiCaV-C	39.68 (98)	35.43 (95)	38.71 (93)	33.85 (100)	39.68 (98)	38.71 (96)	39.06 (100)	40.00 (92)	37.93 (90)	
HiCaV-D	40.32 (96)	35.70 (95)	39.39 (96)	34.92 (96)	40.62 (96)	38.71 (96)	48.57 (54)	40.35 (89)	58.06 (48)	

Supplementary Table S3: Nucleotide and amino acid % identities and sequence coverage (in parentheses) of each ORF of the newly discovered hibiscus virus X with the seven nearest alphaflexivirus relatives and lily symptomless carlavirus. NB: TGB3 and CPG sequences for Sichuan alphaflexivirus 1 are not available in GenBank. Open reading frame numbers with nucleotide positions in the HiVX genome and the name of the gene representing each ORF with their lengths (number of amino acids) are shown. NS = not significant; ND = not determined, as these sequences are not available.

Hibiscus Potexvirus	Nucleotide identity							
HiVX	Yucca alpha-flexivirus 1 (MW328742)	Agave potexvirus 1 (MW328740)	Physalis virus X (MZ357185)	Yam virus X (NC_025252)	Sichuan alpha-flexivirus 1 (MW897277)	Clover yellow mosaic virus (OK558678)	Strawberry mild yellow edge virus (MZ326676)	Lily symptom -less virus (LC720794)
ORF1 (94-4170)	65.80 (42)	65.06 (55)	68.44 (16)	70.36 (16)	66.53 (48)	68.29 (21)	64.85 (38)	NS
ORF2 (4205-4870)	NS	NS	NS	84.38 (4)	NS	NS	NS	NS
ORF3 (4860-5252)	NS	NS	NS	NS	NS	NS	NS	73.68 (24)
ORF4 (5122-5316)	NS	NS	NS	NS	ND	NS	NS	NS
ORF5 (5355-6275)	66.01 (32)	69.59 (18)	NS	67.61 (15)	ND	60.08 (19)	NS	NS
Full Genome	65.80 (31)	65.06 (38)	68.44 (10)	70.36 (12)	66.53 (30)	68.29 (12)	64.85 (24)	73.68 (1)
HiVX-ORF encoding gene	Amino Acid identity							
	Yucca alpha-flexivirus 1 (QQG34620-24)	Agave potexvirus 1 (QQG34613-17)	Physalis virus X (UEP18564-68)	Yam virus X (AJD3371-75)	Sichuan alpha-flexivirus 1 (QYF50142-44)	Clover yellow mosaic virus (UZP17132-36)	Strawberry mild yellow edge virus (UVT84557-61)	Lily symptomless virus (BAT32749-52)
RdRp(1358)	55.10 (95)	54.26 (96)	49.27 (99)	49.77 (95)	58.51 (95)	50.36 (90)	48.20 (94)	23.36 (77)
TGB1 (221)	44.20 (100)	45.29 (100)	39.01 (98)	34.21 (99)	50.45 (99)	35.40 (99)	35.14 (97)	31.88 (100)
TGB2 (130)	41.67 (100)	41.22 (100)	35.66 (98)	47.96 (73)	53.23 (47)	42.00 (73)	45.26 (70)	47.37 (70)
TGB3 (64)	47.37 (87)	38.18 (85)	43.64 (85)	43.33 (90)	ND	NS	NS	37.74 (78)
CPG (306)	54.50 (68)	52.61 (68)	35.12 (66)	32.88 (69)	ND	32.94 (81)	34.64 (50)	29.10 (60)