

A novel *Waikavirus* (the family *Secoviridae*) genome sequence identified in rapeseed (*Brassica napus*)

D. PARK, Y. HAHN*

Department of Life Science, Chung-Ang University, Seoul 06974, Republic of Korea

Received September 7, 2018; accepted October 23, 2018

Summary. – The genome sequence of a novel species of the genus *Waikavirus* (the family *Secoviridae*), which we named *Brassica napus* RNA virus 1 (BnRV1), was identified in a rapeseed (*Brassica napus*) transcriptome dataset. The BnRV1 genome was 12,293 nucleotides long followed by a poly(A) tail. Two open reading frames (ORFs), called ORF1 and ORFX, were predicted. The larger ORF, ORF1, encodes a polyprotein of 3,471 amino acids and the smaller ORF, ORFX, overlaps ORF1 and encodes an 87 aa long protein of unknown function. The BnRV1 ORF1 polyprotein was predicted to undergo proteolytic processing to yield seven mature proteins, including an RNA-dependent RNA polymerase and three distinct coat proteins. The ORF1 and ORFX proteins share sequence similarities with the respective proteins of viruses in the genus *Waikavirus*, including the bell-flower vein chlorosis virus, rice tungro spherical virus, and maize chlorotic dwarf virus. A phylogenetic tree inferred from a conserved segment of the polyproteins of several *Secoviridae* viruses confirmed that BnRV1 is a novel species of the genus *Waikavirus*. The BnRV1 genome sequence identified in this study may be useful for the study of waikavirus biology and waikavirus-derived diseases.

Keywords: *Brassica napus* RNA virus 1; *Waikavirus*; *Secoviridae*; rapeseed

Introduction

Viruses of the genus *Waikavirus* (the family *Secoviridae* of the order *Picornavirales*) have a positive-sense single-stranded RNA genome (Sanfaçon *et al.*, 2009; Thompson *et al.*, 2017). There are three known species in the genus *Waikavirus*: rice tungro spherical virus (RTSV), maize chlorotic dwarf virus (MCDV), and bellflower vein chlorosis virus (BVCV), which are all plant pathogens (Reddick *et al.*, 1997; Sailaja *et al.*, 2013; Seo *et al.*, 2015). RTSV and MCDV are agriculturally important plant pathogens that cause severe production losses in rice and corn, respectively (Reddick *et al.*, 1997; Sailaja *et al.*, 2013).

*Corresponding author. E-mail: hahny@cau.ac.kr; phone: +82-2-820-5812.

Abbreviations: BnRV1 = *Brassica napus* RNA virus 1; BVCV = bellflower vein chlorosis virus; CP = coat protein; MCDV = maize chlorotic dwarf virus; NCR = non-coding region; NTP = nucleoside triphosphate-binding protein; ORF = open reading frame; RdRp = RNA-dependent RNA polymerase; RTSV = rice tungro spherical virus

Waikavirus genomes are composed of a mono-segmented single-stranded RNA of about 12 kilobases (kb) with a poly(A) tail, which is encapsulated in an icosahedral particle (Sanfaçon *et al.*, 2009; Thompson *et al.*, 2017). Waikviruses are known to have at least two well-conserved open reading frames (ORFs) (Firth and Atkins, 2008; Sanfaçon *et al.*, 2009; Thompson *et al.*, 2017). The large ORF, ORF1, encodes a polyprotein that is subsequently processed into seven mature proteins, a hypothetical protein, three distinct coat proteins, a nucleoside triphosphate-binding protein, a 3C-like proteinase, and an RNA-dependent RNA polymerase. The second smaller ORF, ORFX, overlaps ORF1 and encodes an 87 aa protein of unknown function (Firth and Atkins, 2008). Possible additional small ORFs have been proposed near the 3'-end of the genome of other waikviruses, although their existence has not been confirmed (Shen *et al.*, 1993; Thole and Hull, 1996; Reddick *et al.*, 1997; Isogai *et al.*, 2000; Verma and Dasgupta, 2007; Sanfaçon *et al.*, 2009).

Rapeseed (*Brassica napus*), also known as oilseed rape, is a bright-yellow flowering herbaceous plant of the family *Brassicaceae* (also known as *Cruciferae*). Rapeseed has

two subgenomes, one derived from *B. rapa* and the other from *B. oleracea*, indicating that it is a hybrid species that originated from a recent hybridization event (Mason and Snowdon, 2016). Rapeseed is mainly cultivated to produce edible seed oil, and rapeseed oil is a rich source of natural bioactive components, including a range of antioxidants (Szydłowska-Czerniak, 2013).

High-throughput RNA sequencing (RNA-seq) is a powerful, cost-effective tool for RNA virus discovery in plants, as well as viral diagnostics and virus evolution research (Park and Hahn, 2017; Park *et al.*, 2017, 2018; Roossinck, 2017). In this paper, a novel RNA virus genome sequence was identified in a transcriptome dataset obtained from rapeseed tissues (Liu *et al.*, 2017). Sequence comparison and phylogenetic analyses showed that the novel RNA virus is a distinctive species of the genus *Waikavirus* in the family *Secoviridae*.

Materials and Methods

Data collection and sequence assembly. RNA-seq data obtained from the young floral buds of rapeseed plants (Acc. Nos. SRR2052475, SRR2052499, SRR2052502, and SRR2052505) (Liu *et al.*, 2017) were downloaded from the Sequence Read Archive (SRA) of National Center for Biotechnology Information (NCBI). The dataset contains about 19.95 gigabases from 239.7 million reads.

Raw sequence data were trimmed to collect high-quality reads using the Sickle program (version 1.33; <https://github.com/najoshi/sickle>). High-quality reads were *de novo* assembled into contigs using the SPAdes Genome Assembler (version 3.10.1; <http://spades.bioinf.spbau.ru>) (Bankevich *et al.*, 2012). Each of the four RNA-seq runs was independently assembled.

Viral data collection. A BLAST-searchable database containing representative viral RNA-dependent RNA polymerase (RdRp) motif sequences was prepared from the Pfam database (release 31.0; <http://pfam.xfam.org>). A total of 394 viral RdRp motif sequences were obtained from 19 families (PF00602, PF00603, PF00604, PF00680, PF00946, PF00972, PF00978, PF00998, PF02123, PF03431, PF04196, PF04197, PF05788, PF05919, PF07925, PF08467, PF08716, PF08717, and PF12426). A BLASTX search was performed against the representative RdRp motif database using the assembled rapeseed transcriptome contigs as queries to identify potential virus-derived sequences.

RNA sequence analysis. The rapeseed RNA-seq reads were mapped to a viral contig using the BWA program (version 0.7.17-r1194; <https://github.com/lh3/bwa>) (Li and Durbin, 2009) to collect virus-derived reads and to examine sequencing depth. The SAMtools and BCFtools programs (version 1.6; <http://www.htslib.org>) (Li *et al.*, 2009) were used to identify single nucleotide polymorphisms (SNPs). Integrative Genomics Viewer was used to visualize sequencing coverage and depth (Robinson *et al.*, 2011).

ORF and protein characterization. The getorf program (version 6.6.0.0; <http://www.bioinformatics.nl/cgi-bin/emboss/getorf>) of the EMBOSS package was used to predict ORFs and their encoded protein sequences. Sequence similarity searches of the putative viral contig against all known nucleotide and protein sequences were conducted using the BLAST programs at the NCBI website (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). The Pfam database (release 31.0; <http://pfam.xfam.org>) was utilized to characterize the functional domains in the viral genome.

Phylogenetic analysis. Multiple sequence alignments were generated using the MUSCLE program (version 3.8.31; <https://www.drive5.com/muscle>) (Edgar, 2004). A phylogenetic tree was inferred by the neighbor-joining method using MEGA (version X; <https://www.megasoftware.net>) (Kumar *et al.*, 2018).

Results and Discussion

One of the contig sequences assembled from the rape-seed (*Brassica napus*) flower bud RNA-seq set SRR2052475 contains a putative protein coding region showing high similarity with an RdRp motif (Pfam Acc. No. PF00680) of the RTSV ORF1 polyprotein (UniProt Acc. No. Q83034). RTSV is the prototype species of the genus *Waikavirus* of the family *Secoviridae* (Shen *et al.*, 1993). The contig was thought to be a genome sequence derived from a novel RNA virus related to the genus *Waikavirus* and was named *Brassica napus* RNA virus 1 (BnRV1). The BnRV1 genome sequence with annotation information has been deposited in the NCBI nucleotide database under Acc. No. MH844554.

The BnRV1 genome contig, which was assembled from 58,986 RNA-seq reads, is 12,293 nucleotides (nt) long followed by a poly(A) tail. A single nt sequence variation (G or T) was found at position 9660, which resulted in a methionine/isoleucine amino acid (aa) polymorphism in the ORF1 polyprotein. This indicated that the assembled BnRV1 genome was derived from a highly homogeneous population and may be descendant of a single clone.

Sequence comparison and ORF prediction revealed two well-conserved ORFs in the BnRV1 genome (Fig. 1), the larger ORF1 and the overlapping smaller ORF2, which were 10,416 nt (position 631–11046) and 264 nt (position 923–1186) long, respectively. The 5' non-coding region (NCR) was 630 nt long, and the 3' NCR was 1,247 nt long and was followed by a poly(A) tail. None of the other small ORFs previously suggested in other viruses of the genus *Waikavirus* were found (Shen *et al.*, 1993; Reddick *et al.*, 1997; Sanfaçon *et al.*, 2009).

BnRV1 ORF1 encoded a polyprotein of 3,471 aa. Pfam analysis showed that the polyprotein contained five conserved domains: a waikavirus capsid protein 1 (Pfam Acc. No. PF12264; aa positions 711–907), picornavirus capsid protein (PF00073; 895–1061), RNA helicase (PF00910; 1800–1906),

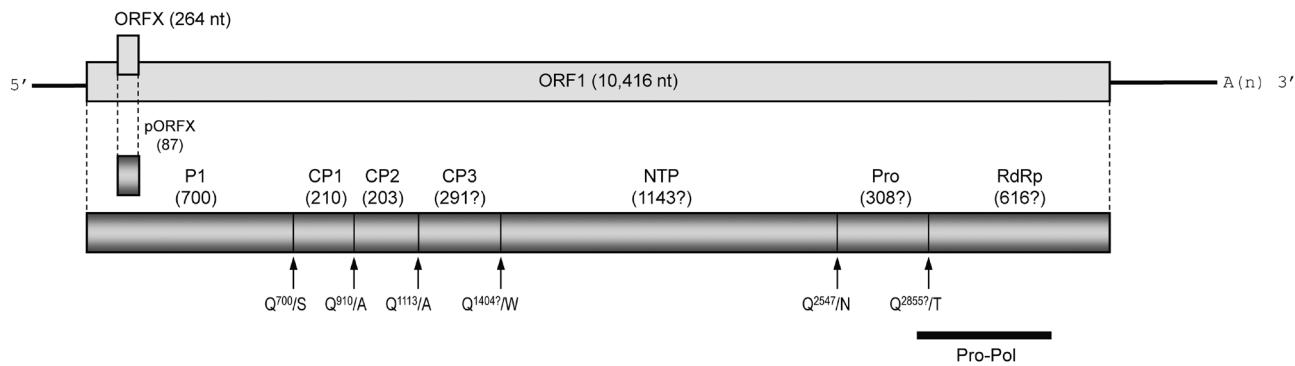


Fig. 1

Schematic representation of the BnRV1 genome

The BnRV1 genome contains two ORFs: the larger ORF1 (10,416 nt) and the overlapping smaller ORFX (264 nt), which are marked as boxes (top). ORF1 encodes a polyprotein (3,417 aa) that undergoes proteolytic cleavage to generate a hypothetical protein (P1), three coat proteins (CP1, CP2, and CP3), a nucleoside triphosphate-binding protein (NTP), a 3C-like proteinase (Pro), and an RdRp (middle). ORFX produces a small protein (pORFX, 87 aa) of unknown function. The aa residues and positions of the putative cleavage sites are marked by arrows below the polyprotein. The seven mature proteins are indicated with their respective sizes. Poorly supported sizes or positions are indicated by question marks. The "Pro-Pol" segment, which was used for the phylogenetic analysis, is marked with a line (bottom).

tungro spherical virus-type peptidase (PF12381; 2634–2864), and RdRp (PF00680; 2903–3413).

A BLASTP search was performed against the NCBI non-redundant protein database using the BnRV1 polyprotein sequence as a query. The BnRV1 polyprotein showed strong sequence similarity with the polyproteins of BVCV (Seo *et al.*, 2015), RTSV, and MCDV (Reddick *et al.*, 1997). These three viruses are species of the genus *Waikavirus*. The BnRV1 polyprotein also showed significant sequence similarity with the polyproteins of carrot necrotic dieback virus (Menzel and Vetten, 2008) and parsnip yellow fleck virus (Turnbull-Ross *et al.*, 1992). These two viruses are members of the genus *Sequivirus*, a sister taxon of the genus *Waikavirus*.

The virus with the highest similarity to BnRV1 was BVCV, and the ORF1 polyproteins showed 51% sequence identity, with almost full coverage. A BLASTN search against known viral genomes using the BnRV1 genome sequence as a query also revealed that BVCV was the closest to BnRV1, with 65% nt sequence identity and 44% coverage. Strong sequence similarity among the polyproteins of BnRV1 and other waikaviruses, including BVCV, RTSV, and MCDV, was observed in the multiple sequence alignment (Supplementary Fig. S1), indicating that BnRV1 is a novel member of the genus *Waikavirus*.

The BnRV1 polyprotein is predicted to be cleaved into seven mature proteins: a hypothetical protein (P1), three distinct coat proteins (CP1, CP2, and CP3), a nucleoside triphosphate-binding protein (NTP), a 3C-like proteinase (Pro), and an RdRp. The consensus sequence for cleavage sites is "Q/X," where "Q" is a glutamine residue and "X" is any aa residue (Shen *et al.*, 1993; Reddick *et al.*, 1997; Sanfaçon *et al.*, 2009; Seo *et al.*, 2015). Putative cleavage sites in the

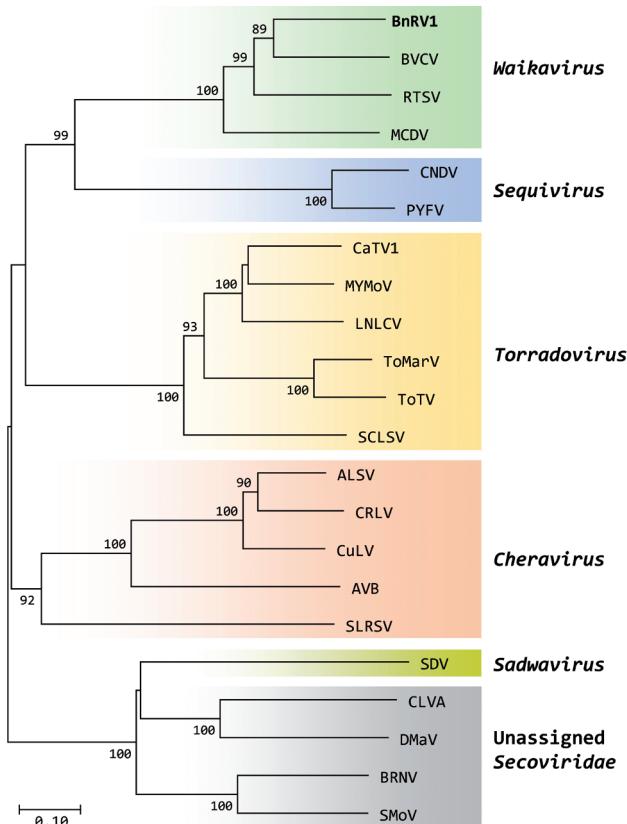


Fig. 2

Phylogenetic tree of BnRV1 and other Secoviridae viruses

A phylogenetic tree was inferred based on the "Pro-Pol" segment sequences of BnRV1 and other representatives of Secoviridae viruses using the neighbor-joining method. The bootstrap percentages calculated from 1,000 replicates are shown at the nodes (bootstrap support values of 80% or less are not marked). See Table 1 for the full names and Acc. Nos. of the viruses.

BnRV1 polyprotein were predicted based on comparison to the reported cleavage sites of other waikavirus polyproteins (Fig. 1). Examination of a multiple sequence alignment of waikavirus polyproteins revealed that the cleavage sites for P1/CP1, CP1/CP2, CP2/CP3, and NTP/Pro were well aligned (Supplementary Fig. S1). For these four sites, the aligned glutamine residues of the BnRV1 polyprotein were predicted to be cleavage sites. In contrast, the sites for CP3/NTP and Pro/RdRp were poorly aligned. For these two sites, nearby glutamine residues were chosen as putative cleavage sites. The six predicted cleavage sites of the BnRV1 polyprotein are as follows (the positions of the glutamine residues are given in parentheses): Q(700)/S for P1/CP1, Q(910)/A for CP1/CP2, Q(1113)/A for CP2/CP3, Q(1404)/W for CP3/NTP, Q(2547)/N for NTP/Pro, and Q(2855)/T for Pro/RdRp.

A conserved segment of the polyprotein, which is known as the “Pro-Pol” region, has been used as a taxonomical indicator for viruses of the order *Picornavirales*, including the genus *Waikavirus* (Sanfaçon *et al.*, 2009; Seo *et al.*, 2015; Thompson *et al.*, 2017). The “Pro-Pol” segment spans from the “CG” motif of the Pro to the “GDD” motif of the RdRp (aa positions 2815–3269 in the BnRV1 polyprotein). Comparison of the BnRV1 “Pro-Pol” sequence with those of representative *Secoviridae* viruses revealed a close relationship (Table 1). The BnRV1 “Pro-Pol” sequence showed 29–67% identity with those of known *Secoviridae*. Among

them, three *Waikavirus* species – BVCV, RTSV, and MCDV – showed 67%, 61%, and 57% identity, respectively. One of the criteria for species demarcation within the family *Secoviridae* by International Committee on Taxonomy of Viruses is that the “Pro-Pol” sequence shows less than 80% identity (<http://www.ictv.global/report/secoviridae>) (Thompson *et al.*, 2017). According to this criterion, BnRV1 is considered to be a novel species in the genus *Waikavirus*.

A multiple sequence alignment of the collected “Pro-Pol” sequences of representative *Secoviridae* viruses and BnRV1 was generated (Supplementary Fig. S2). A phylogenetic tree based on this alignment confirmed the classification of BnRV1 in the genus *Waikavirus* (Fig. 2).

The BnRV1 genome was predicted to have a second ORF, called ORFX, that overlaps the N-terminal region of the ORF1 polyprotein and encodes an 87 aa protein (Fig. 1). The BnRV1 ORFX protein sequence showed high sequence similarity with other waikavirus ORFX proteins (Supplementary Fig. S3). Interestingly, the sequence conservation for the ORFX proteins was higher than that for the overlapping regions of the ORF1 polyproteins. For example, the ORFX proteins of BnRV1 and BVCV showed 74% aa sequence identity, while the overlapping ORF1 polyprotein regions showed 53% identity. The ORFX proteins have only been found in the four *Waikavirus* species. No homologous proteins have been found in other viruses or other cellular

Table 1. Sequence comparison of the “Pro-Pol” segments of BnRV1 and representatives of *Secoviridae* viruses

Genus	Virus	Acronym	NCBI Acc. No.	Sequence identity ^a
<i>Waikavirus</i>	bellflower vein chlorosis virus	BVCV	NC_027915	306/455 (67%)
	rice tungro spherical virus	RTSV	NC_001632	282/463 (61%)
	maize chlorotic dwarf virus	MCDV	NC_003626	262/458 (57%)
<i>Sequivirus</i>	carrot necrotic dieback virus	CNDV	EU980442	166/487 (34%)
	parsnip yellow fleck virus	PYFV	NC_003628	161/488 (33%)
<i>Torradovirus</i>	carrot torradovirus 1	CaTV1	NC_025479	149/460 (32%)
	motherwort yellow mottle virus	MYMoV	NC_035218	142/462 (31%)
	lettuce necrotic leaf curl virus	LNLCV	NC_035214	147/462 (32%)
	tomato marchitez virus	ToMarV	NC_010987	121/385 (31%)
	tomato torrado virus	ToTV	NC_009013	120/401 (30%)
	squash chlorotic leaf spot virus	SCLSV	NC_035221	144/476 (30%)
	apple latent spherical virus	ALSV	NC_003787	144/476 (30%)
<i>Cheravirus</i>	cherry rasp leaf virus	CRLV	NC_006271	141/457 (31%)
	currant latent virus	CuLV	NC_029038	140/457 (31%)
	Arracacha virus B	AVB	NC_020898	144/487 (30%)
	strawberry latent ringspot virus	SLRSV	NC_006964	114/386 (30%)
	satsuma dwarf virus	SDV	NC_003785	111/371 (30%)
<i>Unassigned</i>	chocolate lily virus A	CLVA	NC_016443	116/374 (31%)
<i>Secoviridae</i>	Dioscorea mosaic associated virus	DMaV	NC_031766	137/473 (29%)
	black raspberry necrosis virus	BRNV	NC_008182	141/470 (30%)
	strawberry mottle virus	SMoV	NC_003445	140/472 (30%)

^aAmino acid sequence identities are reported as “identical residues/aligned length (% identity).”

organisms. The strong conservation of the ORFX protein sequences suggests that they may play an important role in the biology of waikaviruses.

In conclusion, a genome sequence of a novel RNA virus, BnRV1, was identified in rapeseed. Analysis of the genomic features and phylogeny indicated that BnRV1 is a novel fourth member of the genus *Waikavirus* in the family *Secoviridae*. Three plant viruses are known to infect rapeseed – Brassica yellow virus, turnip crinkle virus, and turnip yellow virus (retrieved from the Virus-Host database at <https://www.genome.jp/virushostdb>) (Mihara *et al.*, 2016). BnRV1 is the fourth virus that is associated with rapeseed. Considering the economic value of rapeseed and the potential pathogenicity of waikaviruses, the BnRV1 genome sequence reported in this study may be useful for the study of waikavirus biology and the control of waikavirus-derived diseases.

Acknowledgment. This research was supported by the National Research Foundation of Korea funded by the Government of Korea [grant Nos. NRF-2017R1A1B4005866 and NRF-2018R1A5A1025077].

Supplementary information is available in the online version of the paper.

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Supplementary information

A novel *Waikavirus* (the family Secoviridae) genome sequence identified in rapeseed (*Brassica napus*)

D. PARK, Y. HAHN*

Department of Life Science, Chung-Ang University, Seoul 06974, Republic of Korea

Received September 7, 2018; accepted October 23, 2018

Fig. S1. Multiple sequence alignment of the ORF1 polyprotein sequences of BnRV1 and other waikavirus.

*Corresponding author. E-mail: hahny@cau.ac.kr; phone: +82-2-820-5812.

BnRV1	LELDDSGDVSKLAYYRRRNILVERDERIPRDESNPNTAGLVFTVLGYHIVDER-----SGRAFGIKETWEEPLRSIDTSQWRFERPVYPVFL	1997
BVCV	LELDDTGDVVTKLAYYRRRNVLVEVRNFDIPRDESDPDKLGVFTVLGYNVTVGN-----NDRVNFGVKDVWDESLFRDVTNDWRFERVSYKTFL	1954
RTSV	LNLDDTGVDVTKMAYYRRRNVLVVERDPDKPNEANTEPLGFTVLFVGLHDQNC-----QGDPQFVVKENWDPEFLREVDTEGWRFERVEYRTFL	1968
MCDV	THLDDTGVDVTKMAYYRRRNVLVVERDLAMPMSPEDPASGLFTIGDIHENGRNVSVVESRLLNNGRVPFRAGD-----LRNMSYNYFM	1960
	: : * ****: * ; * :	
BnRV1	RFLCVYTDAYMESQENLLTGT--CYRPDMDEIPTLDIEFP--VVAQAGGA-----LCTLGDVIEKFDFKRGLSGRD	2064
BVCV	RFLCTYTDAYMSQEKLSSGK--NFKCN---PFEDDG---VVAQSGTSVESVGREIVSV-----FKNYDTITLGEAIAKDFERMWPVPT	2032
RTSV	RFLCMYTDAYMSQVQLQGK--TFKMN---PFAPEPEF---AQAQNGEEAECEIVEEMQEVPGPEAPQEAKELVKIETAPNMDELVEAFNKLRVTPGH	2059
MCDV	EVRFYIATYIMENOQOLVAKLSDGYDVESSSSFPENELEFDLAQAHNGV-----YLTIIEEEVAKFESMFSGKQ	2031
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BnRV1	LCASFQKRGSKYFPPDAWGT--KSMSFNKLILTCECSQDGQCNFDIMMSQIRELSVQKDVKRIVKAIRVHKLKADPLETEMVNSNGVEGL--FEDV	2160
BVCV	FASFF--KGTRFMAPEKWQSR--KVASFSTLLANLCGCGSTGNHCFDLYFKHLIEVCKAKGVH--QNIFVLGQLSLVPEETLVIKKA--DF--FSDLR	2120
RTSV	LNDIL--RDGSGCYIDEWIAGPRLSFLHELLPTCGCHHTRVCFDNIVYNNMCKAVRSQSVD--FKYRANQAIKYAYTHKLHSQCRCYSDFEKLRRECN	2154
MCDV	LNAEI--EKFERIGVDGWRTN--KALSFNDLVKRCFCGCLGDDCNFDHYRTLKFVLIENKQIPAYKCMVLHVNPDRMKTQIKMVNGYLET-MFKTLN	2126
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BnRV1	ALTTFSMLACWYRWSL-PGYMCV-YRINQQQSAQRFAEKKRDEVENEDRIPNCVEIKVVEDGNSFVWDKAMKFFPSCLSAIGCVPFLDGCEYYLASTLSM	2258
BVCV	DIDIFVALASYYRFSF-RSDLCF-FHFISPKNSKAVSHVGALDLDFFERLP-LESLDHPNGDAFWVPSVRLVFPTLIDTICVPLCVNGKFVFLVEKVDS	2217
RTSV	PLDVFVCLVSKYT---ADDHSFERRCPKKMMVVRMRPPVFEKLMPRSSDVSVEDEQGQRFIEWPHLYIFL----RYRAIEFKDDKGSLTVREDAGA	2245
MCDV	PLTFLYLVFLVKCGISADNVCLSYQLFAMNDAEQVE---FEIEDSLRLD-EQVQIQGQSCYVWPSVSGKVFYPEILAKRGCIAVNDGTTFYIFVSSSQI	2220
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BnRV1	QRL--EERRSKIWEIWANGVEISCDPLSLL-PRRERLTIAGIVNGISSLGTAEEPSGEVKVQVQSIKENYGLNIYLTIIILVAQAHSDRVKEMEMTKN	2355
BVCV	KFV--PVPSCKWKDWWSKGFQEQNVLPLSLL-POAERDIINCSLNICAFGSDPEQSVKEAIDQIQRSLYEGGGVLYTFVLLAQNENARRAFFSRENE	2314
RTSV	DVC---PNFELKLPWLDDQDQ---KSVL-PAHLRHMVQARLEQEIMEENGNGYSGEMRNAIAEIKEYLDQDHQNVAAVLVACA---VKERRMTH	2332
MCDV	DKIHPPEAWSMDLQVGVRGGVDI---LSIAGPTKTFKLHVESCYETLKSPEDWAKCKEYYESIS---LYEYLLMAGVSGRAGIETQRMSK	2308
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BnRV1	NAKKVKVFSAYEKLQRY---EKSVEGISGKMKVFLSIAGGIAATGTIVGLFLVKK--LFSPKSEEVGAEEIEVEDAEAEAMSGAHESGMFKTAHIKTRS	2450
BVCV	FQTKRNAFRASETTFKEY---EKAAGNLNSTKALIALAGAGITASGILIGLGVFLKSL---LFNL---IKGDDDEEDNAEKAESGAHESDMFQTNFVKTQR	2405
RTSV	DKLHRKSFNALDKLDAW---YTTAPAKTSKSKMILLAIAGASVAVAGVAVGAVILQLQNTLFGS---KEDEIEEGEEGETQASGAHESDGIVTQHQLK-RD	2424
MCDV	YQARKNKIRMPEVLEKYIEVEKATIGKLSPAKTCLAIAGAGVAIFGVLAGLGVLYKL-ITHFSKTDSEEDNDIEIDDLVPEMSGAHASDENVTYAVRQQ	2407
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BnRV1	F--PVRVSR-----AKAEMMGHAESGA---FRTEHVRRIQRPYVQKAQAHVRGGVHYDEVSELKRLVIEKRKKKRNLAIAKS	2524
BVCV	MKPQIRIVN-----LEPQTSQGAHESDQ---FQTSYMKKQRAOPRVRQLQAEKGGLGTSYQGDSDDLNCLYVERRSKRSKVAQAVR	2481
RTSV	IRPKMRVTY-----TDHHVAEEAHEEKDAEKPRSKGNPRTKSYLGLSPGFAERGMGVTYEEHTPLKDALLDESNKVFRRKIVASVE	2504
MCDV	V-PKVRLAKQFKVRSSPSPSDNEQPKVDILVPEMTGCHASD---LHTKHTKRRVTKRKGAVKESHIVTYDENTPHVRLI---RNLLRRTLRAIK	2498
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BnRV1	QAVKKDKRSVTRDSDKA-IDLWQNSLIQKGMLASNG-EMEDVEKSLIERKIL-REEKHSAEQSYITGDPFRVHYEIVEELEKTRKAVLRRSKELIENG	2621
BVCV	HKGNSKKVV--PDVVG-IDKWDQDKVQLGILPGES-ESKGPLSSIAKGNMV-AGESQAAAGELLVGDGPFLRNSEVQDMLSKMICIDTDEFSALIQDG	2576
RTSV	SAVKQGGKAS--KDTVLSQLIGWDQDKVATGVIARQLEASGSLKIHNLNSR-RTSSHVMGP-LVHVDAFERSDEVDAELHRITIDEVKSCPCKMIKG	2600
MCDV	QMAQLGEL---PDTLSE-IQWQYQVYVVDKGIRPAEHTTDFRLFSAIADQEQEPEEINMASGETMKFDEN--KYNEIVQVVKGQSPKSD-IVTMTTKG	2590
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BnRV1	MHVTVEKQSTIGSF-----GMRDENMVRLRSHVSKMSCTILIKRGSFYQSFMLVRLKGTMLVSPAHYEEIEEKDEMYFICPKHVV	2704
BVCV	TQNVVTQOAHVGDY-----GLTRDNMVVELQTHVSKMSCTILRWNEDKCVSYGLRKGTWVLAFAHYYEEFSEEDKLYFVCPNPKV	2659
RTSV	VSTLSVKKASVGLMALQKAESQLSPPFTSRAVQDRLSMTNLIDTHMAGMSCIISELGNNVFRFTGVLRGCTVCPMAHYLDEITSEHTLYFVCPSPKIT	2700
MCDV	AHHTAIKQVRIGYK-----SLDKDPNMVSIISLNQLQTKISCVILNVTPTGRTAYLNVMLRCGTFVCPAHYLEAEEEDDTIVFISFSVCI	2673
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BnRV1	RJQLEMRSVALVSAHQLDVLWGLKSAPPSSIDYMSHIPTRKDWEARYRPCSGALAFTEYTQDMTLQMVSLDTIEMTTNEVPTAEEYMLESTHTVILG	2804
BVCV	QIPFV PANVLSVLSDIQDVLWVNLGNTVPPSVDFTCIPTTEDWKHFRKCSGALSTKYNQKMALQVTHALDTIELTSADVEVPTGSYAMYGSTHAVIMG	2759
RTSV	QIQLERHRVCLVNGQFQETVWVLGDPVPPSRNYIDFIANADDWKNYKATSGALVMSKYSVDSMLQCVHFLDSELTEANVSVPSTSYYEANGGIHTISGL	2800
MCDV	KLRFPDRVTLVNTHQDLVWVLGNSVPPAIDVLSMPIVTADWDKFQDGPAGFVTQYKRNARYPTNYINTLDMIERIRADTQNPTGIYKMLNSDHTITGL	2773
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BnRV1	RYRVHCMVGFCGAAVVRADAKAIRKTIQGMHVAGNVSRGVGYAEMLIKETIQAIDALSK-----VVQKAMDEPAMKV---CEKQCATIEGKGNIGQIGM	2896
BVCV	RYRVHCMPGFCGSAIVRADTRAIRKVGIMHVAGHKQKGVYAEITLSEPILEAKRVCPT---VVERSPVNGIEK---CEKQCVVLEGKGNLGLILGR	2851
RTSV	RYRVHCMPGFCGRAIMRADATCFRKIIQGMHVGSRLRNKCMGYAETLTQEHLMQAIETLKETGLKHIPKGAGIAGAGEEKLPEHSKQKSLSLEGKGNLGVQ	2900
MCDV	RYQMSLESGFCGCGLLRACTMRVLRIVGLHVAASANHAMGYAECLVQEDLKHAINKLSPD--ARSLIIGHLNPKVET---ATQCGIVRSLGSLGCHGK	2867
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BnRV1	IEDELLPKMPSRTTICKSLIHGLIGDVVSEPSILSKWDRRLGEKR-GTWDPEVEDAKVKGIMVVPFPREEVKEVEEHLTRVFAKRHSLSNKRREVNTLEIG	2995
BVCV	VVQSAVPNVPKTTIKAISLISLHGLIGEVRTEPSILSKWDRRLGEKR-GWDPVMEAVKVGATVPPFPNEIQLQEVETHLCGVQFQNFENS.RKREVNDVEVG	2950
RTSV	LTAQLVPTSVTTICKSRSIYKLGMLIHLGEIKEKTEPVSLSAWDRRLPFPP--GEWDPMKDAVQKSYLIPFTEEQEVENFLIKFRKRSNRSRTRNVNSLEV	2999
MCDV	VTSEDVAMTATKTTIRKSRSIYKLGDIKTEPSILHADHPRLPEDQIGKWDVPEFAALKYGTREPIPEEVELEHDLSIILKGMNDTLLKRNVNNELEVG	2967
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BnRV1	INGIDQTPFHSPIMKTSAGYPVYLRTPSGASGKWLKEVGQYPSGRARYEMEDPGLIASYDEMGLQIKRGVAPTFITVHPKDERRKLKKIYEVPATR	3095
BVCV	INGIDRSDFWSPIMKTSAGYPVYLRKPPGATGKWLFLNSVCGYESGREKFEMKEEQFKTSFEAMRTQILNGVVPNIMTMECPKDERRKLGIYDNP	3050
RTSV	INGIDGSDFWSPIMKTSAGYPVYLRKPSGAQGKYLFEELLEPVPSGRPKYAMKDPELTENYERIKEEVTSVGVKPSIMTECLDKERLAKIYEKPATR	3099
MCDV	INGIDQSDYWLQIETNTSPGWPTKRPKGAEGKWLKEVGQYPSGKPILEMEDSGLIESYNKMLRDAQGVAPIVTVCEPKDERRKLSKIYEQPATR	3067
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BnRV1	TFTILPPEVNILFRQYFGDAAMVMNRFDTSFQVGINSPELEWSLMSLMMKKGKRGFGADYAKFDGIGSPEIYHSIVSVNAWYDDGEENALARHALI	3195
BVCV	TFTILPPEVNILFRMFQFGDAAMVMTRFDFHSQVGINPESLMSLMMKKGKRGFGADYAKFDGIGSPEIYHSIVSVNAWYDDGEENALARHALI	3150
RTSV	TFTILSPEVNILFRQYFGDAAMVMSTRREHFSQVGINSPEMEWSDSLINSLLRVTNGKFGADYSKFDGIGSPEIYHSIVSVNAWYDDGEENALARHSLI	3199
MCDV	TFTILPPEVNILFRQYFGDAAMIMTRNSRSLFCQVGINPENMEWSDSLHFLHKSHTGFGDYSKFDGIGDPOQYHSTQVNNWYDDGEENALARHALI	3167

Fig. S2. Multiple sequence alignment of the “Pro-Pol” segments of BnRV1 and representative Secoviridae viruses

BnRV1	CG-----	AAVVRADAKAIR-----	KIIGMH-VAGNVSRGV-----	GYAEML--IKETIQTADALSKEVV-----	QKAMDEP
BVCV	CG-----	SAIVRADTRAIR-----	KVIGHM-VAGHKQKGV-----	GYAETL--SLEPILEAIKRCVPTVV-----	ERSPVN
RTSV	CG-----	RAIMRADATCFR-----	KIIGMH-VSGLRNCKM-----	GYAETL--TQEHLMQIAETLKETGLLKHIPKGAIAGAGEEK	
MCDV	CG-----	GLILRACTRMVR-----	KIVGLH-VAASANHAM-----	GYAECL--VQEDLKHAINKLSPDARSL-----	IIGHLNP
CNDV	CG-----	SCMVLSEKLDG-----	KVFML-VAGTYDNLTG-----	QVYSTYVPVTVNMMRKAISGTTLQAGD-----	CQGTICD
PYFV	CG-----	SCLVSTSDKLDG-----	KVFCNL-VAGTYDRVTC-----	KYVSTYVPITCDMIKKSISSLTGAEFSE-----	SQSSICD
CaTV1	CG-----		VH-CAGYTSMVKQGHVASY-----	ASVIYQEDLISMLPVALK-----	IPQAPCP
MYMoV	CG-----		IH-CAGAKAEYIRKGLKESC-----	ASAIIYREDLEELLPTPKL-----	EAQGP
LNLCV	CG-----		IH-CAGLKEKYVAQGSQKS-----	AAAVYREDLEALLPKQML-----	CSQVOCS
ToMarV	CGT-----	LLLLPSVQNQKP-----	VIVGIH-CASYDGVAERGFISSN-----	ATAIYREQLEDL-PTGPVK-----	AAMVRCD
ToTV	CGT-----	LLLLPNVQDKQP-----	CIVGIH-CASYDEEAAHKGFBASN-----	ATAIFRDQLEDL-PTGPVK-----	VAMVRCQ
SCLSV	CGS-----	AVLIPGAINGQP-----	EIVGIH-CAGFS DAMQRKGYRGTT-----	AALIFYEDIEKYLPSPVLDQ-----	EAQTNIP
ALSV	CGSLGSKPMPACYSYTFDTAGLCTSPLISMGGRCVLLGLH-----	VAGDKSKM-----GY-----	AQIVTLEDFS DINFSEK-----	VGQGP-----	
CRLV	CGSLGSKQMPACYSYVFETYAGLCTSPLIAQEGR CILGLH-----	VVGDRSKM-----GY-----	AQIVT LDDFS DVALSDK-----	VGQGP-----	
CuLV	CGSLGSLRMPPCYSYTFDTYPGLCTSPLICMSGGRCILLGLH-----	VVGNSMKT-----GF-----	SQIVT LDDFS EVPQSGLT-----	VGEGP-----	
AVB	CG-----	DVL LQCVSSGV-----	KILGMHTTAGKQSIW-----RY-----	ANTVTLQDIEGAYPSEW-----	VGEEDPL
SLRSV	CG-----	TPLVANYGKGKGLKIASIHVVHHFSATEKDTIAGSGSLLTKEEY LEASLLGDIKHPLETDRIQ-----		ASGCLSG	
SDV	CG-----	RLLCA-NLAGHW-----	RVIGMC-AGEGKNRA-----	GVTKAL-YADIPHEFLRADNLNAVQ-----	GAELDAA
CLVA	CG-----	RLLLVERHNTL-----	AIAGMH-----	TYGREY-PPLSGFADIPDFVTNPV-----	VAQGPSD
DMaV	CG-----	RLALV-EKNGTL-----	LIVGMH-----	NFGNA-RNCAFSDIPSEFKPADELQ-----	SEFSMTE
BRNV	CG-----	RLLLAHCPAGNL-----	MVVGLH-VSGG-----	GLNE-RATSIFGGIDGSYKDVEDG-----	KFYQQGD
SMoV	CG-----	RLLLARDEAKCL-----	KIVGIH-VAGVVIQE-----	KHISLSEINGMHKTAEMA-----	VQQGMEV
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BnRV1	AM-----	KVCEKQCAT-----	IEGKGNIGQIGMIEDKLKP KPM SRTTICKS LIHGLI-----	G-DVSEPSILSKWD RRLRGK	
BVCV	GI-----	EKCEKQC VV-----	LEGKGNL GILGRV VQSAV NVPTK TIAKSLI HGLI-----	G-EVRTEPSILSKWD KRLGDK	
RTSV	LP-----	EHSKKQ SLS-----	LEGKGNL GIVG QLTAQ LPTV S VT K TICKS M I HGLI-----	G-EIKTEPSVLS AWR DRLPFP	
MCDV	KV-----	ETATKQCGI-----	VRSLGSLGCHGKV TSE DVA M TAKT TIRK SRI YGLV-----	G-DIKTEPSILH AHD PDRPL PED	
CNDV	SPVSEVF STL KMDELLS STPTPSGNLGVFKPNDR MGLIEV VGRIFP NTPPKA ICRSTI IPSL I QRFM-----PR-----	KPLTEPA ILSP LDGRL GEC			
PYFV	SPISDTVA ETIKV DQLF SS KPGAS GKG FGV FGV NDTIGI IDV VGRTF PET PPKS ITK STI VPSL I QPYM-----PR-----	KPLTEPA ILDP RDV RLGEN			
CaTV1	LL-----	KQLR VELEN-----	PFQIKQV ALL QGQ VP NEL S VPHKT TL RQ SEL FHV L-----SEEIG-PHLTEPSL LIKG DR RTK E		
MYMoV	QL-----	RQLR QVDTN-----	PFEIKQV ALV GRM PPE LAV NVP HKT TL R KSE I FEEL-----ETHLG-PHLTEPSI LT IRD TRA AS		
LNLCV	VL-----	KKL RAST TN-----	PFEIKQV AFL GTV P QEL A I NVP HKT TL R KSE I FDAM-----TKV LG-PHSTE PSI LTH H D R P E V E		
ToMarV	IL-----	KSIRS RET Q-----	L FEEQ NQ YY LGT VP QEL A LAT VPH KTT LR KSQL FEA F-----G-PAETAPSIL T VHD KRG DG		
ToTV	LL-----	KDL RAR DAA-----	L FEEQ KVA FG T L P AE Q A T VPH KTT LR R S G L FEA F-----G-PAETAPS I SAS DR K RG EG		
SCLSV	AI-----	EEFF Q S-----	PFDT KQV F CLG KV PRE A D API HDTA L KLSIA H DIL-----TEVVG-PCTTEPSI L TS RDK R L SGK		
ALSV	-----	EQMY IPT AR-----	SECFG SV C LL GK-WTGP KPY FLE NS L TPS L I H DC I-----DI-----DMTTEPA ILSKK D PRL Q YT		
CRLV	-----	EEMY IPT KN-----	SECFG SV T KLG A-WTGP KPY FLE KTS LIP S L I ST S I-----DV-----ERTTEPA IL S QRD K RL K DS		
CuLV	-----	ECY CIPT AS-----	SEC YG S V T KLG K-WTGP A P Y FLE KTS L VPS M ISK D I-----DI-----EMTTEPA IL TT K DP RL A HS		
AVB	PI-----	SNT SR-----	DFCAGE VAR VGT-NEGLKPHF PT KTS L VPS I S S D L K EDD D NHIPS VMT PAIL H S RD PR IVE F		
SLRSV	DE-----		FFDA ETV PEG L L L P S Q A P R Q A T S S E I H K S S I S D G L-----EKL VGE K R K TEP A I S N R D T R L K D R		
SDV	IL-----	DR FS IPI SE CR-----	KELTPM T R L G Y-VVGQ Y P R A L R K T S I V P S I I H D N L-----WR-KP ETE P T I L G K I D R S P FP		
CLVA	D-----	AEFI YF DAE-----	EEIT PMV SKI G YV-----STAV P Q L S K S Q I E E S P I F D S L-----CQ KLG-DPK VAP T I L S K H D P R P P T		
DMaV	TPL-----		KQITEMV S QVG F L-----DAPV P Q L R T T Q I E K S P I F D E L-----EDL NG-SALTE P T I L S V T D P R P P E P		
BRNV	LD-----	VHM KELIE A-----	ERV T D M V D K I G R V D S S Q F R P S Q G T S I I K S E I H D D L-----WR-RAET CPT V L T R S D P R P R E V P		
SMoV	DI-----	LEL VEP-----	EVK T DM V K I G H V A Q G Q Q F R P A T K T S I V K S Q I H D T L-----WR-APETEPT V ISP V D P R P R V PYA		

BnRV1	R-GTWDPVEDAVKKYGINVVFPPR--EEVKEVEEHLTRVFAKRHNSLNK-----REVNTLEIINGIDQTPFHSPIMKTSAGYPVVLRTFS	:
BVCV	R-GEWDPVMEAVKKYGVATVPFPP--NEIQEVETHLCGVFQNFENS LRK-----REVNDVEVGINGIDRSDFWSPIEMKTSAGYPVVLKPP	:
RTSV	P-GEWDMKDAVKKYGSYILPFPT--EEIQEVENFLIKKFRRKENS RRT-----RNVNSLEVGINGIDGSDFWSPIEMKTSAGYPVYLKRP S	:
MCDV	QIGKWDPVFEAALKYGRTRIEPFPI--EEILEVEDHLSIILKGMDNLKK-----RNVNNSLEVGINGIDQSDYWLQIETNTSPGWPYTKRKP K	:
CNDV	R---YDPMIEGIKKYQDQAKPIRG--NWRKRRIIDSREQMEDWETFMVRENYD---TLDLPMHTIINGISGIEYYEPLNMSTSEGYPLILSRPS	:
PYFV	R---YDPMIDGIKKYEEQARPIKI--SWRNQIIIESMAAQMDWETFMVREGYM---TMDLPMSSVINGIDGVEYYEPLNMSTSEGYPLILNRPK	:
CaTV1	G---FDPYVAGVQKFNETACCFDM--RVAETVMESSMSDDLLSKLANIQVPGGK---PVRSEEQILNGIIPGEKYYDSMDFSTCGYPFMIM-GF	:
MYMoV	----FDPYVKGVEKFNETACNFN--EVAQIVMQHMKA SLLANLERISVPGGK---PIVRGEHEILNGIIPGEKFYDAMDMSTSCGYPFSL S-EF	:
LNLCV	G---FDPYVKGVEKFNETACCFDH--ETVEVALQHMSEDLLVELVVKDVPGGK---PTVRSELEI LNGIIPGEQFYDSDMDSTACGYPYTLS-EF	:
ToMarV	----FDPYVAGVMKYNETACGFDD--DIAKLAFENLKCSLLPIMRSQKIPGGR--PCERDEDVVNLNGIDCDDYYDGMLSTSCGYPFNKM-GM	:
ToTV	----FDPYVAGI QKYNETAQNFD E-DIARLAYEGLRQAIPVLPVLSQRVPFGK---PVTQNEDVVNLNGVDGFDFDGMELSTSCGYPVNKL-GM	:
SCLSV	A---FDPYLAGIAKFNETAHFSNM--HVAQEAEFYMKRRLRLHSKIPVPGEK---PEVRSEVALNGIIPGEEYYDPMDSLSTSSGWP FNKG-ER	:
ALSV	NNKDFDFPLSGMGKYAVEAH SFD--EDEELFEDALDRVFSEIPMFT-----CEDLSNDQVCNGIEDDDYAEGLVMQTAEGYPFCTMRPP	:
CRLV	INPEFDVFLGEMKKYAVEAHSLD--EDLEVFDALDRVFLEIPEHA-----CEDLTNDQVCNGIEDDDYAEGLVMQTAEGFPCTQRPA	:
CuLV	NDPDFNPFKAGMLKYAVEAHGFN--EDEEFFD ALDRVFSEIPDFN-----CQELADDEV CNGIEDDPYAEPLVMQTSEGYPFCTQRPA	:
AVB	GHEGFDPKDGMLKYTRAAGPFD EADEDFQDALDDVFLIDGDLRTEKEIEQNQS KAICRILNENDTLNGGIEQDFEDPIVSKTAE GYPFCQR P	:
SLRSV	N---LDIFKKGMMKYKAVAADMSPVTDEEKIWNWTDSIFDLPGGIQS K---CHLSEDENLNGVNGDNEYRGMVWSTSEGWP EVLNRKN	:
SDV	----YDPYATIGEKFVQE VPFDL--SVGSDASL V VANIGSSWKAVGKPQC---PTVLTWEVAINGDAAIPYCERLPLSTSEGYPD SIQRF	:
CLVA	----YDPYSAGIRKF DKEVGPFDW -SEESDLQFACAEILD TWTQKPEKFAI---ETVCSLDVAINGVHGLPFAENPFIGTSEGFPWLDRAR	:
DMaV	----FDPYLQGIHKFEKQAGPFD F--SEESDLQD LAQKNISSEWERP EQFDV---DTVTITLEVAIQGIDGLDYAESLPIATSEGFPYI LERRA	1
BRNV	----FDPYQAGIRKF EKEVGP LD F--IDEFTESTVLV DIAEELNNKKREVGFF-ELDTVLDNHAI NGVEGEVEA EPLVMGTSEGYPYVLERQP	:
SMoV	----FDPYTAGIMK F EKEVGP LD F--SDPDTPESTVIEDISEELLREKKS LGGF-ALDTVCSNEVAINGVDSV PYAERLVMSTSEGYPV LSRKA	:
	: * : : : * : : * : * : * : * : * : * :	
BnRV1	GASGKKWLF--KEVGQYPSGRARYEMEDPGLIASYDEMLGQIKRGVAP-TFITV EHPKDER RLK KIYEVPATRTFTILPPEVNI LFRQYFGDFA	:
BVCV	GATGKKWLF--NSVGCYESGREKFEMKEE QFKTSFEAMRTQI LNGVP-NIMTMECPKDERRLKGKIYDNPATRTFTILPPEVNI LFRMFFGDFA	:
RTSV	GAQGKKYLF--E ELEPPYPSGRPKYAMKDPEL IENYERIKEEVTS GVKP-SIMTMECLKDERRLKLAKIYEKPATRTFTILSPEVNI LFRQYFGDFA	:
MCDV	GAEGKKWLF--KEVGNYPSGKPILEMEDSGLIESYNKMLRAKQVAP-IVTVTECPDKERRLKLKIYEQPATRTFTILPPEVNI LFRQYFGDFA	:
CNDV	DAHGKEYLFEICEDGSRAIK-----SAKL NANYEAYGSSLSSGEFP-PLISIECPKDERRALDKIYD KPKTRL FSILPVEFNMHARRLFLDFN	:
PYFV	DAHGKEYLFETMESGERRIK-----SAK LEAHY ESYGH ALQSTEPF-PLISIECPKDERRALDKIYD KPKTRL FSILPVEFNMHARRLFLDFN	:
CaTV1	GKNKREFLD--GEPGYLLAR-----DKPVYEEYMA MDDAI SQGIVM-EMVTCECAKDERLPLEK IYEKP KTRL FTILPFHYNMLVR KYFLDFS	:
MYMoV	GKNKRGYLD--GEPGDYMLHR-----SRPVYEEFCRM DDEV RAGIVT-EMITCECAKDERLPLEK IYEKP KTRL FTILPFHYNMLVR KYFLDFS	:
LNLCV	GKSKRGYLD--GTPGEYVLHR-----ERPVFA DFIELDDESIRQGRIT-EIVSCECAKDERLPLEK IYEKP KTRL FTILPFHYNMLVR KYFLDFS	:
ToMarV	G MNKREFVQSTGEGERVELKR-----DTPVFAEWEELDVQIRKG IHV-DLVTQC A KDERLPLEK IYEKP KTRL FTILPFHYNMLVR KYFLDFS	:
ToTV	GTSKREFVEPSGDGDRVQLKR-----TTPIFDWEALDVEIRKG NFV-ELVTTQC A KDERLPLEK IYEKP KTRL FTILPFHYNMLVR KYFLDFS	:
SCLSV	GKSKRGHVV--EVEGVHLLDR-----TSEAYTAYIELLQSLADGEVP-VMVTECAKDERLPLEK IYEKP KTRL FTILPFHYNMLVR KYFLDFS	:
ALSV	GVTGKTWLF-AGSPGDWHIVP-----NSLLANELNVKELNLSKNIFE-PVIGIDFPKDEKV DSSKV YIKQKTRL FTILPFHYNMLVR KYFLFSV	:
CRLV	GASGKSWLF-AGAPGDWHIVP-----GSLLANEMHKKEVAPS RGLFE-PLIGIDFPKDEKV DSKV YIKP KTRL FTILPFHYNMLVR KYFLFSV	:
CuLV	GATGKSWLF-CGSPGDWHILP-----GSLLHNEMNK MERNL SQGIFE-PVIGIDFPKDEKV DSKV YIKP KTRL FTILPFHYNMLVR KYFLFSV	:
AVB	GKSGKGWLL-GGIPGDWSLKK-----EGPLNDAIEHLEDNL ANEVE-PLIGIDFPKDEKV MRAKVEV KPKVRL FTILPFHYNLV VRRFLDFV	:
SLRSV	GEAGKERFL-LGLPGCYTLNR-----NLPMYQRILD D-ALSATTIP-CIVGLDTAKDERLPLSKIYQDV KTRL FTILPFME NYLVR KYFGSFV	:
SDV	GEKGKKRFF--DLKGENVRVP-----TPALMEELEVLERE LQKEEVCLTCINTACAKDEKTAPKKV RVQPKTRIFEILPFQINIIIRRYLMFWM	:
CLVA	NESGKERFFEEVAPGHRVP-----RG SWVEDIAEIEAAYRGDY--LTTTITCAKDEKT KLDKVFVNPKTRIFELEMNFCLNLVIRKYFFFWM	:
DMaV	GDTGKERYF-EEINGK RIP-----KGDWQDIDQIERAAVAGNL--EIYT MACAKDEKT KLDKVFVNPKTRIFEILPF TFLVIRKYFLFW M	:
BRNV	GDVGKFRYF-SKNLYHWEL-----NEGPAKELDELEQSVAREDFDGKII ITIACAKDEKT KLIKV YEKPKTRIFEILPFHYNILVR KYFLFFM	:
SMoV	QDTGKFRFF-DKDGDRLWVA-----KDEV LDDLK ELEA IKSDDFKGGIITIACAKDEKT KMKRV REVPKTRIFEILPFHYNILVR KYFLFFM	:
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BnRV1	AMVMENRFDTFSQVGINPESLEWSELMSNLKKKGKRGFAGDYAKFDGIGSPEIYHSIVSVNAWYDD-----	GEENALARHALISSIVH
BVCV	AMVMTRTFDHFSQVGINAESMEWMSEMMNGLLAKGKRGFAGDYAKFDGVGSÆIYHSIVNVNSWYGD-----	GEVNARARHCLISSIVH
RTSV	AMVMSTRREHFSQVGINPESMEWSLDLINSLLRVNTKGFAGDYSKFDGIGSPAIYHSIVNVNNAWYND-----	GEVNARARHSLISSIVH
MCDV	AMIMTNRSKLFCQVGINPENMEWSLDLMHEFLHKSTHGFAGDYSKFDGIGDPQIYHSITQVNNNWYDD-----	GEENARTHALISSIIH
CNDV	VFVMANRHKRJGMVGINPHSREWSLDAISLASFSPYGFNGDFANFDGMFHPSFDSMVAELANIFY-----	GFFQSDERTSLTKALT TN
PYFV	VFVMANRHKHGIMVGINPHSREWSLDAISLASFSPYGFNGDFANFDGMFHPSFMSVSELANIFY-----	GNFLSTERDNLTRML TN
CaTV1	ATLMRAHNEIPCKVGVINPEGIEWTTLANGFVEKSVGSFADYSSFDGRAPVFIFQWFCDMVDKYYGDKP-----	GSENSLARHALLMMAS
MYMoV	ASLMRRAHGTIPCKVGVINPEGFWTALANSFLEVSQTGSFADYSSFDGRAPVFIFQWFCDMVDEYYGDHS-----	DSPASLARHALLQMSC
LNLCV	ASLMRRAHNTLPCKVGVINPGSLEWTVLAKNFSETADVGFSFADYSSFDGRAPVFIFQRCFDIVDKYYGDLP-----	GSENSLARHALLMMAS
ToMarV	ATLMALHNAIPCKVGVIDPTSEWTLANGFRAVDVGFADYSSFDGRAPVFAFQWFCDLVDEYYGSKP-----	GSPDSNARHALLMMAS
ToTV	ASLMASHNALPCKVGVINPGGIEWTLLANGFRAVDSTGFADYSSFDGRAPIFIFAQWFCDLVDDYYGSP-----	GSPDSNARHVLLMMAS
SCLSV	ASLMRSRHNDLPCVKVGIADGIEWTTLANQFLAVSDQGFADYSSFDGRAPIFIFAQWFCDLVSEYYGDL-----	QGESARIRRGLLMMAS
ALSV	SQLMALHNEVPTKVGIDPISNEWSILCHSLHSKGNTWNFGDYSRFDGITPRNVLQGIVKRISRRYANKSSFAITDPTLSINGDLARSLLMDMCST	
CRLV	SHIMTOQHNTIPVKVGIDCLSNEWSILYHQRLRSKGNTWNFGDYSRFDGITPRNVLQGIVKRINKFYNKNNSLAITDNLNSLNSDLARSLLTMAST	
CuLV	SQLMALHNETPTVKVGINPLSNEWGILQFALSAKGENWFNGDYSRFDGITPRNVLQGIVKRISRNRFVNKNSLAITDKTLSINGDLARSLLMDMAST	
AVB	ARLMQKHNETPTVKVLNTSLEWQHLYDQLSAVGFNWFNGDYSRFDGITPRCVLQGLAIRISKFYRQDEAQGLVSKK-KIRTSVAHLLMADMANS	
SLRSV	AELMKLHNICPTKVGINPLGYDWTILGKMHAKGNTWNFGDYSRFDGVTPRCLLIEISNIRITKLY-----	GDEHRERRLHLMLAATT
SDV	QLLMVAHDELPSKVGINVYSESWDRLLGRHTRLANH-FTGDYSGFDSTPRVLYVIAIDIKEINELADD-----	GEVNQRTTRNNIIRFVNL
CLVA	QWMMSLHSIDLPCVKVGINPFSYDWDIMAMKHSAYAH-FCGDYSGFDNTNVELVVRIGDMISMADD-----	GPRNRVIRRNLLRCVNL
DMaV	QWMMKHNHLNPCKVGLNVFGFWDEMFKFHGAYSHH-FCGDYSGFDNSTNVAMLDVADMISMDFARD-----	GARNRLIRRNLMHAAVT
BRNV	QFIMRMHNVLPCVKGLNPFSQDWDEMHAEHTRFEEH-FNGDYSGFDGTGPRQLLLKFADLISELAAD-----	GRRNKVIRRNLMQLAVD
SMoV	QFIMSLHDFLPKVKGLNVYKSWDTMHAHNRFAYH-FNGDYTGFDTATPRVLMKIAADMVSNLAD-----	GRENAIVRRNLMKMAVE
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BnRV1	RDGIAGAHLRLYSQGMPSGSFMTVIFNSFVNYYYMALAWMYIVSRSELSPQADLGSFDRFTKIV--YGDD	
BVCV	RDGLAGDVLLRLYSQGMPSGSFMTVIFNSFVNYYYMALSWINIIANSPLSPQADLVSFDYTYKIVV--YGDD	
RTSV	RDGICGDLILRLYSQGMPSGFAMTVIFNSFVNYYYMALAWMNLISHSPLSPQSTVRDFDNYCKVV--YGDD	
MCDV	REGIVKEYLFQYCOGMPSGSFAMTVIFNSFVNYYYLAMAIWMLISHSPLSPQSTVRDFDNYCKVV--YGDD	
CNDV	RFSLVKGGVLRLISGGGGPSGFPMTVIFNSFINLFYQSAWIMLASQNGRSDISCPNSNPFSPVRACV--YGDD	
PYFV	RFSLMKGAILRVPGGGGPSGFPMTVIFNSFINLFYQSAWIMLARFNGRQDISHPCNFPKYVRACV--YGDD	
CaTV1	HYTLCGDKLFRVVGGMPSGSFTLVLFNSLNLNEFYMRAYAFEILLRKPANAARTIGMSQTNFNELFVAIYGDD	
MYMoV	HFTLCEDKCYRVVGGMPGSFSLTVLFNSLNLNEFYMRAYAFEMLLRKPNQRARTLGMTQRNFEDLFVAIYGDD	
LNLCV	HTLTCGDKLFRVVGGMPSGSFTLVLFNSLNLNEFYMRAYAFERLLHPPRNITRTMGMTQRTFSELFIAIYGDD	
ToMarV	HTLTCEDKFRVLLVGGMPSGFALTIVFNSLNLNEFYMRAYAFISLRLRPHIAARAIGVKPSDFNQLFIAVYGD	
ToTV	HYTICENKFRVLLVGGMPSGFALTIVFNSLNLNEFYMRAYAFISLRLRPHIAAQAAIGCKPSDFNKLFVAVYGD	
SCLSV	HTLTCGDKLFSVKGGMPGSFTLTVFNSLNLNEFYMRAYAFGMLLRSIDIKARSIGVTMNDFDRIIFIAYVYGD	
ALSV	RYGLTNGDLWVYVTSGIPSGFPLTVIVNSLVSFFVHFAYMLKHSSEVHAKLYPLSFRTLVSYAV--YGDD	
CRLV	RYGLTNGDLWVYVTSGIPSGFPLTVIVNSLVSFFVHFAYMLKHSSEVHAKLYPLSFRTLVSYAV--YGDD	
CuLV	RYGLTQGDLWVYVNSGIPSGFPLTVIVNSLVSFFVHFAYMLKHSSEVHAKLYPLSFRTLVSYAV--YGDD	
AVB	RYGIAGLGVYKVSSGIPSGFPLTVIVNSLVSFFLHFAYRKLMQSLSKSHFW---FTKNAFAV--YGDD	
SLRSV	RLGVAGIGLYRVSGGIPPGFALTIVNSLVSFVHFVWSWEHMMASSL-----FTDCVELAV--VGDD	
SDV	RYLISDGVLYEIHGGTPSGFAPTVMINSSVNEFYLKWSWIGLLKEAGYANQATLYAFHEATEISL--YGDD	
CLVA	RQVIVGRDLYVKGGTPSGFALTVMINSSVNEIYLKMAWFGLSRK-YDPLLARDADLRHHVCMV--YGDD	
DMaV	RRMIVGRNIYKIIGGTPSGFALTVMVNSIINELYLKMAWFNLSKQ-HFPEISRDAADLSHNVHIST--YGDD	
BRNV	RRIILVLAELYHVRGGTPSGFALTIVIINSMVNQFYLMWAWRKIMSR-IPSPSMVTVRMRTHCTFSV--YGDD	
SMoV	RRIILVLRDLYQVKGGTPSGFALTIVIINSMVNQFYLMWAWRKIMSR-IDPGLVPYRVMRSHCTFSV--YGDD	
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Fig. S3. Multiple sequence alignments of the waikavirus ORFX proteins (a) and the overlapping region of the ORF1 polyproteins (b)
 Identical and similar residues in all sequences are shaded in black and gray, respectively. Note that the ORFX proteins are more well conserved than the ORF1 polyproteins.

(a)

BnRV1	1	MQR-VLLIASLAINMAALFMQVLGLLLLKQPITILIVGICVIMLNIFLNVLALVTKPEEDFSQFLERASAGTPLARNAERRAPLPVRERR--	87
BVCV	1	MQR-VLLIISLAVNMIALFLFTLGLILKLPVILIVGVFVIMLNIFLSVLALVIKPENGSOFLERVTTGTPLARVAVPRAPLPRPVVR--	87
RTSV	1	MQRGFYLIICLSSLMAILFVLMIAUFRKPVLVMITLCVIMLSIFLNVLALVVRPEENFSELVARVGTGTPLARLAERRVDLPTRGRI--	88
MCDV	1	MQR-WLLIIGLAVNMLSCFFQMLGLVFRLPLLVIGLCVTMLNIFLSVLALVTRSEDTLDDLMTRMHIGTPRAQNVPVARERTLGPSSR	89

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(b)

BnRV1	99	AKSAINCKLGYKY-GCALYAGIGTSSQA[INYSDSNNMCDNAEHLFECFSLSQDQTRGRLFSPVGEVGWHTTCAKCGASCFAAGPREAL--	185
BVCV	99	AARVINNKFSSQY-DCSVSVHPGSNPEV[SHSDCWSVCNNAQHLFECVSFSQDQTRGEWFVPLGKSNNWHTTCSGCGASCFAATPREGV--	185
RTSV	99	AKGFLFDNLSIPFDACCPNAGTRLSQLGVSHDHFVCNYVEHLFECASFRETGGKFFRACSEGWHWNATCTTCGASCRCANPRENI--	186
MCDV	178	AEVVADNRVSCEY-AKLLLSNARVGVQVTPPACDWVCCNNVEHLFECFGISDAQRGHITGFNDENAYNASCAKCGACCQGANARSAPI	266

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