

Species Prototype Virus		Premotif A	Motif A	Motif B	Motif C	Motif D	Motif E
		*****	*****	*****	*****	*****	*****
Bujaru virus	:	LFKKQHGGREIYVNGABERIVSVVETIARQIGEFPSDTLCNPNKTIPE	TSDDARKWN	PWIGKATFLKRTGMMQGIHHTSSLLHT	MQGSDSSMLI	IYPSERSTPNT	ENSEF
Chandiru virus	:	LFKKQHGGREIYVNGABERIVSVVETIARQIGEFPSDTLCNPNKTIPE	TSDDARKWN	PWIGATFLKRTGMMQGIHHTSSLLHT	MQGSDSSMLI	IYPSERSTPNT	ENSEF
Frijoles virus	:	LFKKQHGGREIYVNGABERIVSVVETIARQIGEFPSDTLCNPNKTIPE	TSDDARKWN	PWIGRTYLGRTGMMQGIHHTSSLLHT	MQGSDSSMLV	IYPSERSTPNT	ENSEF
Mukawa virus	:	LFKKQHGGREIYVNGABERIVSVVETIARQIGEFPSDTLCNPNKTIPE	TSDDARKWN	PWIGCSYLETETGMMQGIHHTSSLLHT	MQGSDSSAMI	IYPSERSTPNT	ENSEF
Punta Toro virus	:	LFKKQHGGREIYVNGABERIVSVVETIARQIGEFPSDTLCNPNKTIPE	TSDDARKWN	PWIGATFIKRTGMMQGIHHTSSLLHT	MQGSDSSMLL	IYPSERSTPNT	ENSEF
Rift Valley fever virus	:	LFKKQHGGREIYVNGABERIVSVVETIARQIGEFPSDTLCNPNKTIPE	TSDDARKWN	PWIGATYLETETGMMQGIHHTSSLLHT	MQGSDSSMLI	IYPSERSTPNT	ENSEF
Salehabad virus	:	LFKKQHGGREIYVNGABERIVSVVETIARQIGEFPSDTLCNPNKTIPE	TSDDARKWN	PWIGCTYLGRTGMMQGIHHTSSLLHT	MQGSDSSMLI	IYPSERSTPNT	ENSEF
Sandfly fever Naples virus	:	LFKKQHGGREIYVNGABERIVSVVETIARQIGEFPSDTLCNPNKTIPE	TSDDARKWN	PWIGCTYLGRTGMMQGIHHTSSLLHS	MQGSDSSMLI	IYPSERSTPNT	ENSEF
Ukuniemi virus	:	LFKKQHGGREIYVNGABERIVSVVETIARQIGEFPSDTLCNPNKTIPE	TSDDARKWN	PWIGGAFVQRTGMMQGIHHTSSLLHT	LQSDSSAMI	IYPSERSTPNT	ENSEF
HEDV1867-2	:	LFKKQHGGREIYVNGABERIVSVVETIARQIGEFPSDTLCNPNKTIPE	TSDDARKWN	PWIGRTYLGRTGMMQGIHHTSSLLHT	MQGSDSSMLI	IYPSERSTPNT	ENSEF

B

Species Prototype Virus		*	20	*	40	*	60	*	80	*	100	*	120
		*		*		*		*		*		*	PD
Bujaru virus	:	MDAIIILNQPEL-RRGFNRRAIENYRDTLMAMELDFDSLEKIPGALKIELSLDLPNSTVGSILRDNPAIIVESDSLSLIINVTVGILASHIDKMFSSVFPIKN---	DGFGDHTPDMLI										
Chandiru virus	:	MBEILNKQIPEGVGLFRPEIKQYDSDIMDEIPEFHITKCDGYMKIDLDLNGVDYSTVGSLLI--STIEVPDKSLPNLVIVVFSLASTDVRFSVFQVYA---	DSYDHLSPDMLI										
Frijoles virus	:	MDIISKQVEL-ADGFNRRLNLEHYNEILLDTPIPEFSVSKESGSIISILVGLDQDRSTVGSLLK--PEKVQSAKLPNVFVDFTFGLSGSTDRAFISEPSPVD---	DGFNFMSPDMLI										
Mukawa virus	:	MEPILSRIG-N-FQGQKPVQVHHEADLFTALPSFQVSNKAGLSLDVNEEDLDRESTVGSITIT--KDQVIMDQILNFVDFTFGLTKSTDRFSEAPLIG---	DGFGDHTPDMLI										
Punta Toro virus	:	MESLRRKQITN-NEGFFKPELRHSDHDLNADLPFLVERSSNKIVIDLNLDPNPNSTVGSLLT--SVSIEIPEKNLMNMVDFTFGLIADSTDVKLSKSGFIVG---	DGYDHLSPDMLI										
Rift Valley fever virus	:	MDSILSKQLVD-KTGFYRVPKHFCDTMLTLALPTFDVSKMVDRIITDFNLDDIQGASEIGSTLL--PMSIDVEDMANFVDFTFGLADKTDLRLMREFPMMN---	DGFDHLSPDMLI										
Salehabad virus	:	INELLAKQAHL-RQGFSSGALMHYDDETLHLPIDPFFVRRSLEGILIDLSDLQDSSSTVGSLLM--PNVDVKATSIIPNFIDNFTFGLSSSTDQPFSSKFPVIN---	DGFDLTPDMLI										
Sandfly fever Naples virus	:	MERILKKQAP-VRALTIHPLRRYESSYDTPIPAYVIKHSSDGVTIDIASLADL-QGSGSTIQ--PFESVPAQNLTLEKIDFTFGLADTDDKKFVEVGVLENRADDSDQSPDMLI											
Ukuniemi virus	:	MLLATCSRTIR-QQGLNCPPAVTFSSHMRPPIPSFLWLTEGSDVLMDFDLDTIPAGSVTGSIG--PKFKIKTQAASSFVDFTFGLWCDASDMLRDHAPLVN---	DTFDHWTDFIS										
HEDV1867-2	:	INDILLKQPEL-RPGFTKRELRYHDETLFGVGLPEFSISRVKQIDLEIAVETLDDRSTVGSILLREPLIRIDPKNSLNFVDFTFGLCNSTDORRFSTVFPIMN---	DGFDNLSPDMLI										

Species Prototype Virus		*	140	*	160	*	180	*	200	*	220	*	240
		E *T		K **	**			*	*	*		*	*
Bujaru virus	:	QTTAGAYYVDFTFRGSEEGAA-QAALTMLAKVEIPCMNRSDGETLSLSVSVHRSVGVSALASEDDVNLVFRFLVAIFYEAM--KVQPELNDDSELKAEKEVLGTISMISMDIE											
Chandiru virus	:	KTAAGSHHVDFTFNRGGEGAF-QACKDRSRYIIPCNRAVGGRVSLFVSVYVRGVWSNLDLTDDEVNLVFRFLVAIMEEAK--RYYPELDESEMTKQEREILGIVSSIQMDIK											
Frijoles virus	:	KTTSGSIYVDFTFRGGEDGAK-QAIGKIGKVEACENRSRGRKLCISLVSVHRHGVSNLLEEQVNLVFRFLVAISIFEDIK--ILLPEVSTIDEELTKSERETGLASSLTIND											
Mukawa virus	:	ITPGQRVYVDFTFNRGNERQAE-QAAYTKAKVEIACENRSSIMPIMLCVISVHRDGVWSNPLTQAEVDNLVFRFLVAIFGEIR--EMSPDALKDDPELSKTEREVLGISQIPLDIE											
Punta Toro virus	:	ETTSGSYIVDFTFRGSERQCL-NAKDKRAKYIACENRSRNAPVSLYIVAVHRDGLWTNMTMQEEVNLVFRFLVAISIHESR--KIQPELTDDSDLSKSERELLGTLSSIDLDD											
Rift Valley fever virus	:	KTTSGMYNVDFTFRGDERGAF-QAMTKLAKVEVPCENRSRQRTVVIYVVSAYRHGVWSNLEDESEAFIMVYRRLALSVMDLR--TLFPELSTDEELGKTERELLAMVSSIQINDS											
Salehabad virus	:	QSTAGSYHVDFTFRGREGAR-QAASLKLAKVEQACNRVSGRNVGLYIAVHRGGVWTNIMSKEDVDLCYRMLAEALFADIQ--VVGPEYRMADEEATKLEREMMGIVASIGMDIS											
Sandfly fever Naples virus	:	ETETGHVYVDFTFMGDANSAD-LAARNKLAKVEIACLNRSIAIKPISLYIIVAVHFGVTSNLDLSDEEVNLVFRFLVARDIFFEEL--EINPALFDSDETSRLREVNVSMAIQIDDD											
Ukuniemi virus	:	QRLDGSKVYVDFTFNRSDQEQSLISAFNRVQKVEVALINRSTSSILFGVVVSETTVVTLNALKQEEVDLCFRFLVRAVHLEMTTKMIIPEYDDEDED--KRSREVKAAFHSVQPDN											
HEDV1867-2	:	QTTAGGHVYVDFTFRGTEKGAR-NAANLRFKVEVACENRAGQAGLALHVIISTHISGVWSNLDLEEEANMIFRFLVAISIFEELK--IMPELRADDELGQERAVLGVVSSIIMDD											

Figure S1. Sequence alignment of the conserved polymerase motifs (A) and endonuclease domain (B) of representative strains of *Phlebovirus* and HEDV1867-2. The conserved residues in the domain are identified by asterisks. The highly conserved endonuclease residues are shown in bold.

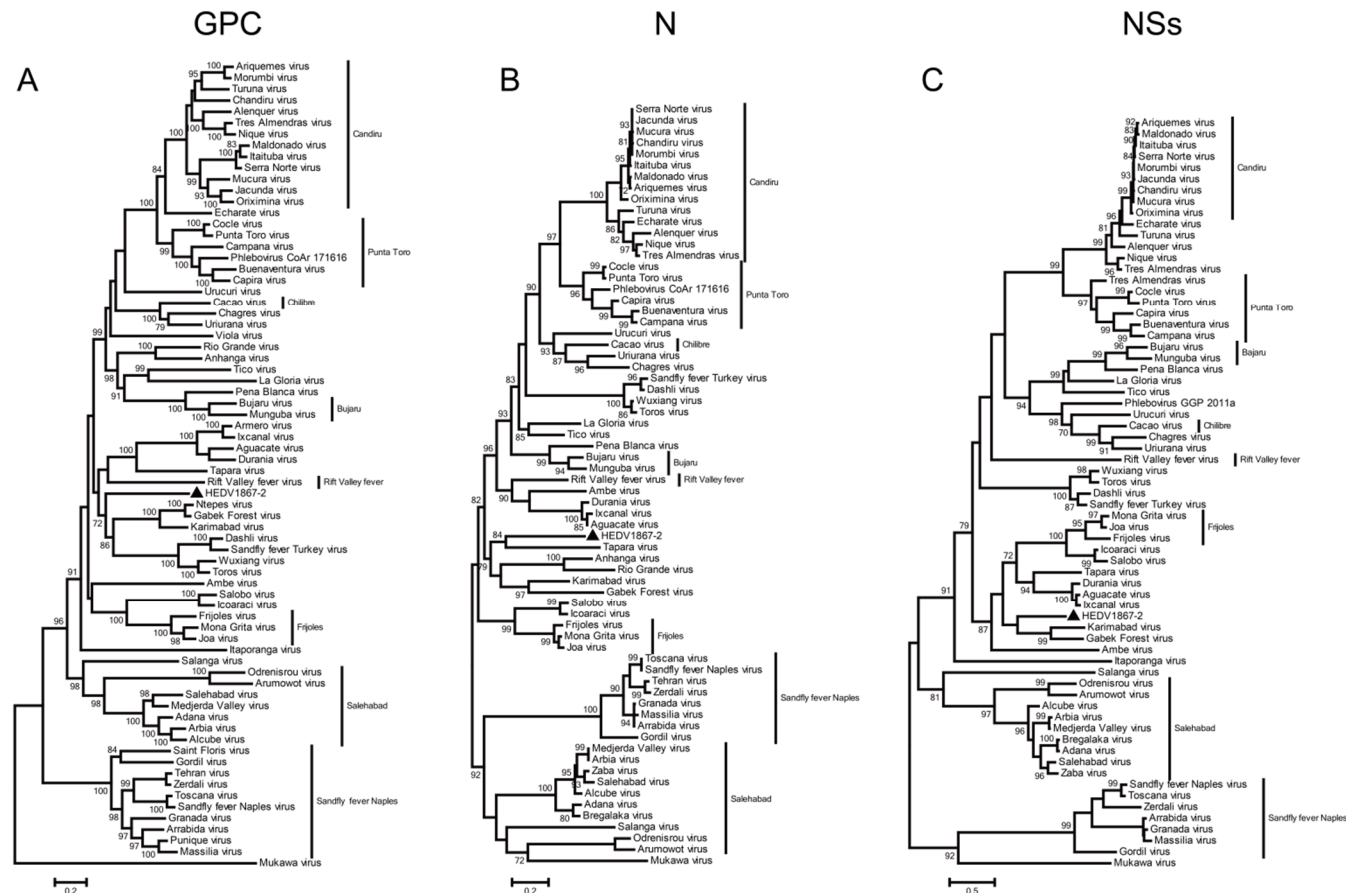


Figure S2. The evolutionary relationships of (A) GPC proteins, (B) N proteins, and (C) NSs proteins of HEDV1867-2 and 59 other phleboviruses based on maximum likelihood phylogenetic analysis. The HEDV1867-2 virus identified in this study was marked with black triangles.

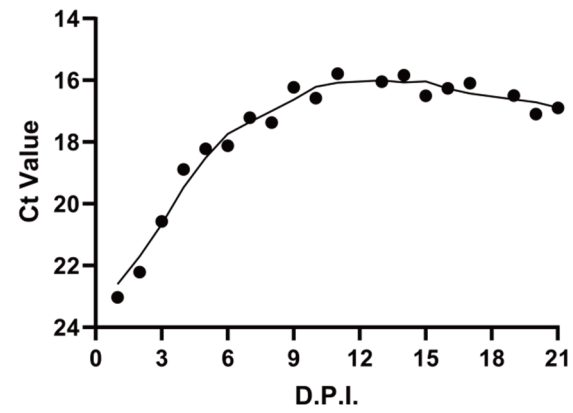


Figure S3. Proliferation curve of HEDV1867-2-infected BHK-21 cells.