

Hepeliviruses of two waterbodies in Berlin, Germany

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Supplementary Material

Legends to Supplementary Figures

Supplementary Figure 1: Genome organisation of Havel hepe-like viruses (HHLV) and Teltowkanal hepe-like viruses (TkHLV).

Conserved domains and sequence motifs were identified by searching the NCBI Conserved Domain Database (CDD, <https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>). Green boxes represent open reading frames (ORFs) without detectable conserved domains. **Abbreviations:** UTR, untranslated region; VMethTr, viral methyltransferase; Hel, helicase; RdRP, polymerase; FtsJ, FtsJ-like methyltransferase; Macro, macrodomain with similarity to the X-domain; PeptA6, putative capsid protein with similarity to peptidase A6 family; rhv, putative capsid protein with similarity to the rhinovirus capsid protein with jelly roll fold and drug-binding pocket; Ubox, U-box protein with modified RING finger domain that lacks some zinc binding residues.

Supplementary Figure 2: Phylogenetic analysis of 105 viral N7-methyltransferase sequences (A), 107 helicase sequences (B), and 130 RdRP sequences (C) of hepeviruses.

Sequences of Havel hepe-like viruses (HHLV; printed in red), Teltowkanal hepe-like viruses (TkHLV; printed in green), unclassified hepe-like viruses (printed in black) and reference strains of the families *Alphatetraviridae*, *Benyviridae*, *Hepeviridae*, *Matonaviridae*, and *Astroviridae* (printed in blue) were aligned with Mega and used for tree inference with IQ-TREE 2. Optimal substitution models: TVM+F+R6 (A) and (B), and TVMe+R7 (C). The trees in (A) and (B) were arbitrarily rooted with the *Matonaviridae*, in (C) with *Astroviridae* and astro-like viruses. Blue boxes indicate reference virus families; yellow, brown and green boxes denote unclassified bastroviruses, 'bastro-like' viruses and a clade of hepe-like viruses with permuted RdRP. Scale bars indicate substitutions per site. Teltowkanal hepe-like viruses which were detected in consecutive samples of 2016, 2017 and 2018 are labelled with a diamond (◆). Triangles (▲) and dots (●) indicate viruses of this study with almost complete and partial genomes, respectively. Given are GenBank accession numbers, genus names (printed in italics) of classified reference viruses, virus names and strain designations (in brackets) where available. Numbers at nodes indicate bootstrap values obtained after 50,000 ultrafast bootstrap replications. The bar indicates the substitutions per site.

Supplementary Figure 3: Phylogenetic analysis of capsid protein sequences of astroviruses, bastroviruses and 'bastro-like' viruses.

The blue box indicates reference viruses; yellow and brown boxes denote unclassified bastroviruses and 'bastro-like' viruses. Sequences of Havel hepe-like viruses (HHLV; printed in red), bastroviruses, bastro-like and astro-like viruses (all printed in black) and reference strains of the family *Astroviridae* (printed in blue) were aligned with Mega and used for tree inference with IQ-TREE 2. Optimal substitution model: TVM+F+R4. The scale bar indicates substitutions per site. The triangle (▲) and dots (●) indicate HHLVs with complete and partial genomes, respectively. Note that Guangdong fish caecilians hepevirus, an animal bastrovirus, clusters with astroviruses.

Supplementary Figure 4: Phylogenetic analysis of viral capsid proteins with similarity to peptidases A6 and A21.

Capsid protein sequences of representative members of the families *Alphatetraviridae* and *Permutotetraviridae* (printed in blue) and unclassified viruses (printed in black) including Havel hepe-like viruses and Teltowkanal hepe-like viruses (printed in red) were used for tree inference with IQTree (optimal substitution model: TVM+F+G4). Prior to phylogenetic analysis, similarity to peptidases A6 or A21 was verified by a CDD search. Numbers at nodes indicate bootstrap support obtained after 50,000 ultrafast bootstrap replications. The bar indicates substitutions per site. The green and ochre boxes indicate viruses with capsid proteins with similarity to peptidase A6 and A21, respectively. Note the

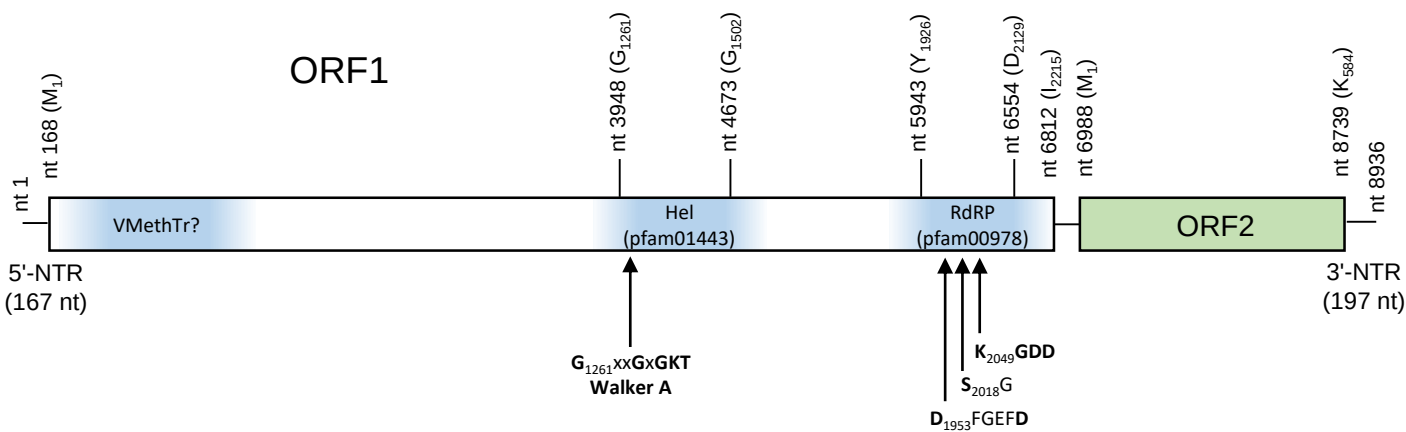
unexpected clustering of Teltowkanal hepe-like virus 14 (TkHLV-14) which exhibits a capsid protein with similarity to peptidase A6.

Supplementary Figure 5: Partial alignment of 50 hepelivirus RdRP sequences.

Representative sequences of the four hepelivirus families (printed in blue), unclassified hepeliviruses (printed in black) and a clade of 11 hepeliviruses with permuted RdRP palm motifs (printed in red) were aligned with Mega. Hyphens indicate gaps or missing data. Three conserved palm domain motifs of the RdRP, named A, B and C, are highlighted in green. Highly conserved amino acids of the RdRP active site are highlighted in yellow, i.e., DxxxxD in motif A, SGxxxTxxxN in motif B and GDD in motif C.

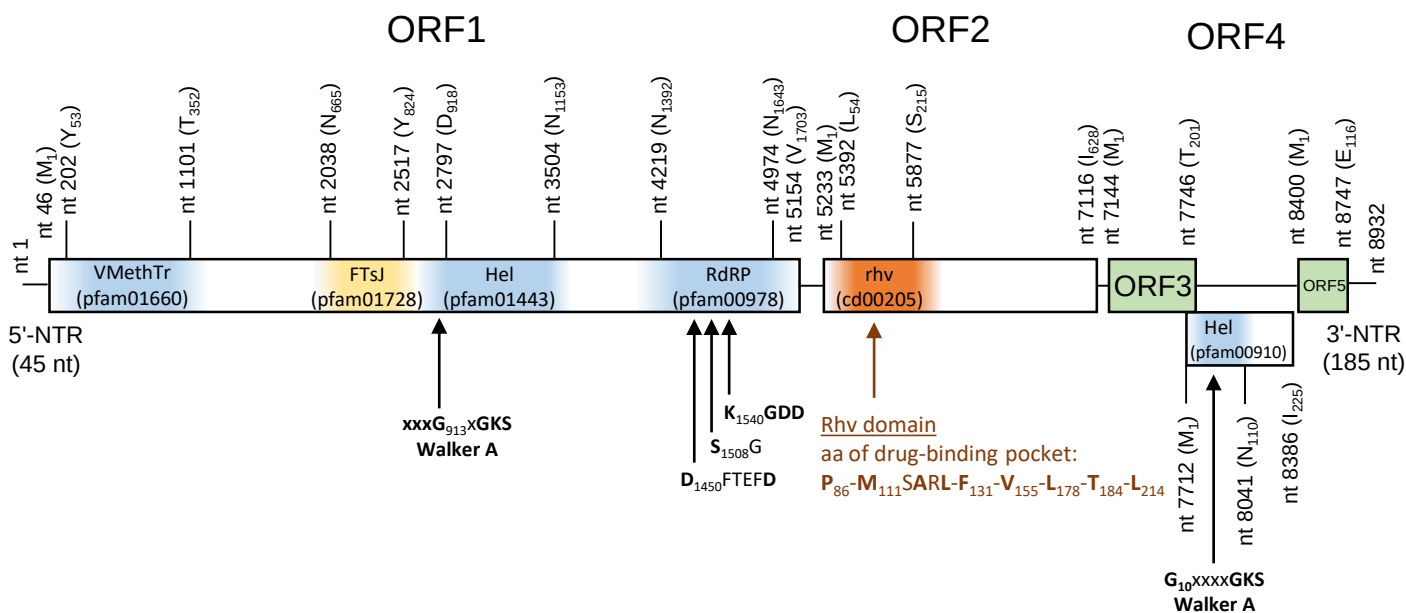
A

Havel hepe-like virus 1 (HHLV-1 strain MR644-18E/91, OP699055, 8936nt)



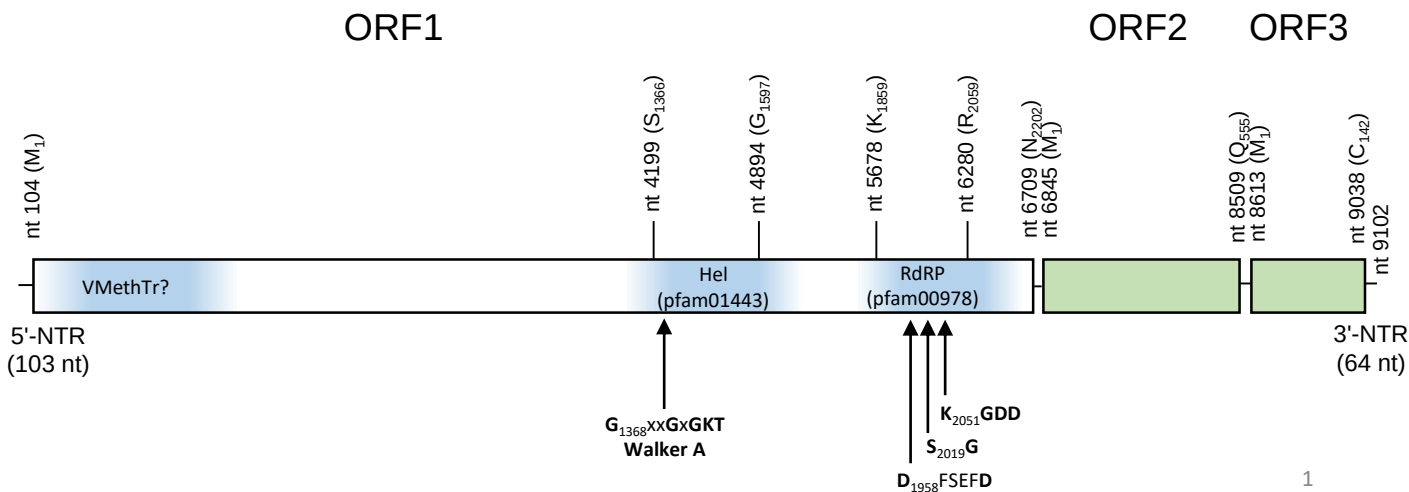
B

Havel hepe-like virus 2 (HHLV-2 strain MR644-18E/36, OP699056, 8932nt)



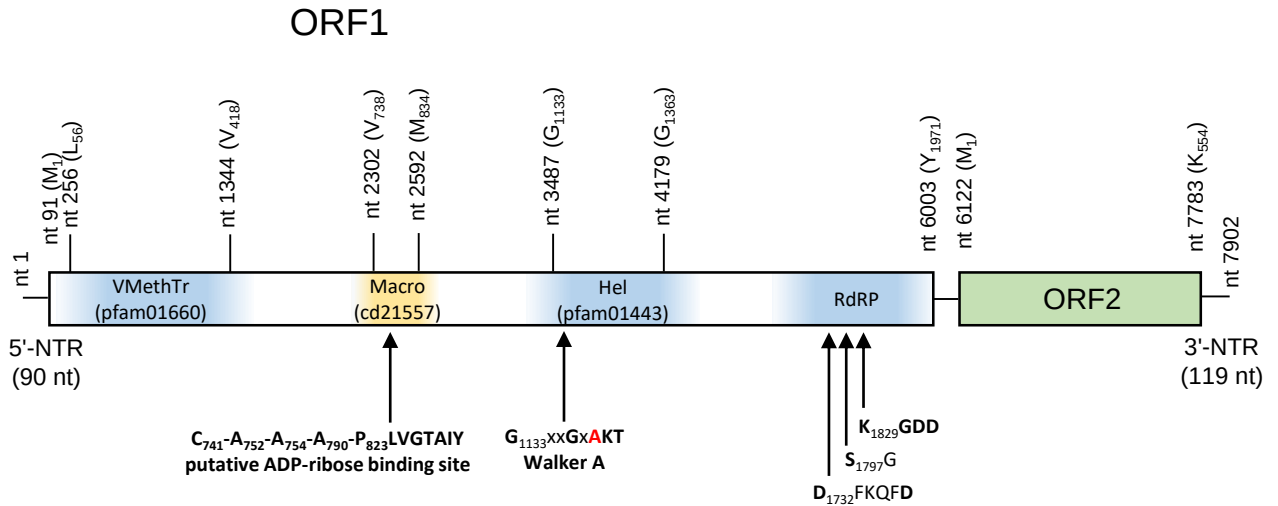
C

Havel hepe-like virus 3 (HHLV-3 strain MR644-18E/70, OP699057, 9102nt)



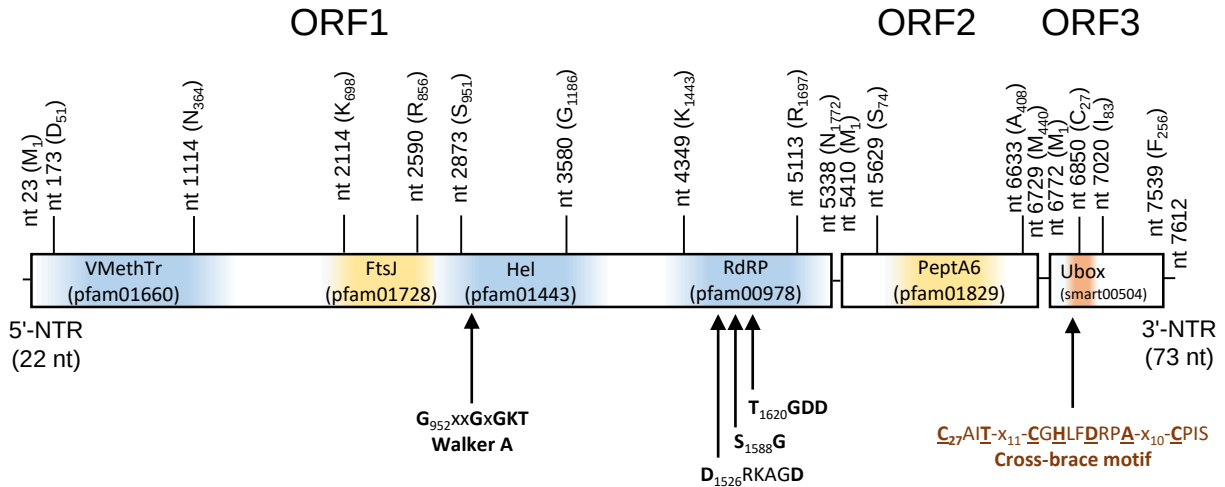
D

Havel hepe-like virus 4 (HHLV-4 strain MR644-18E/1928, OP699058, 7902nt)



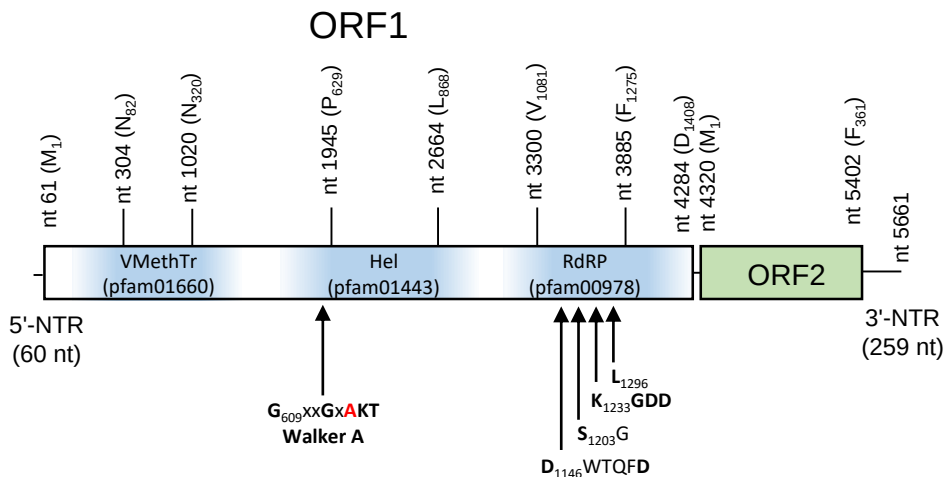
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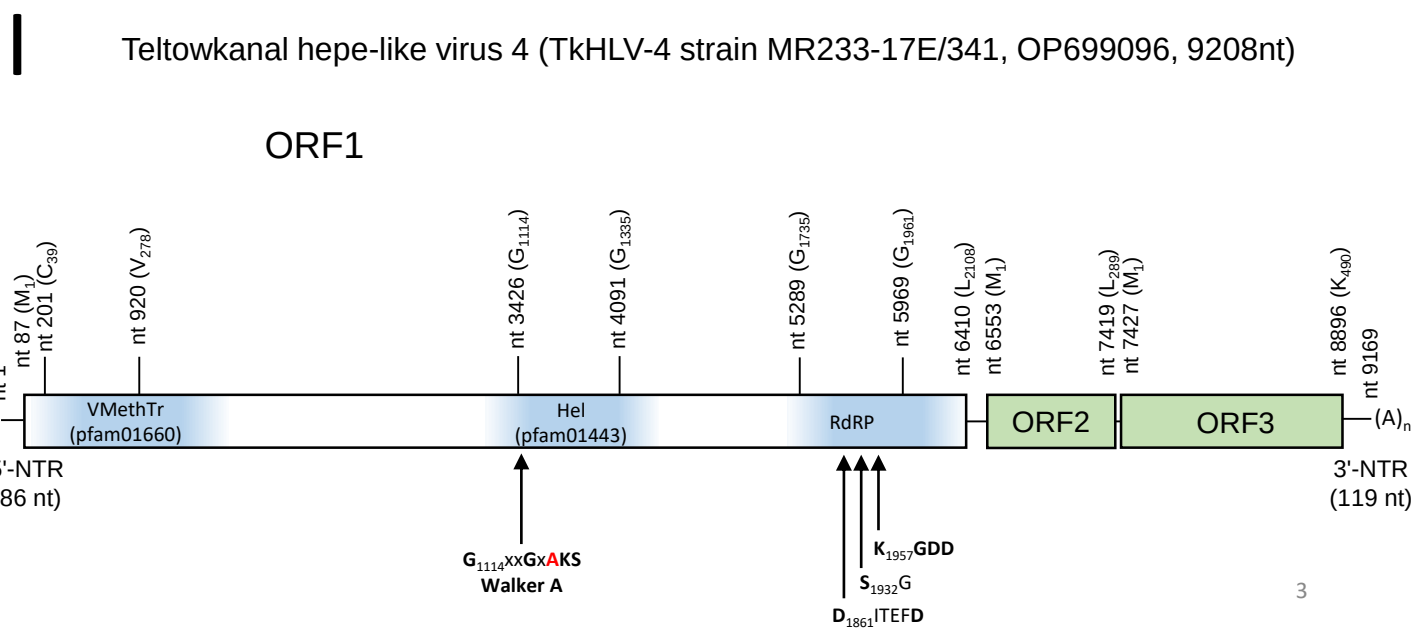
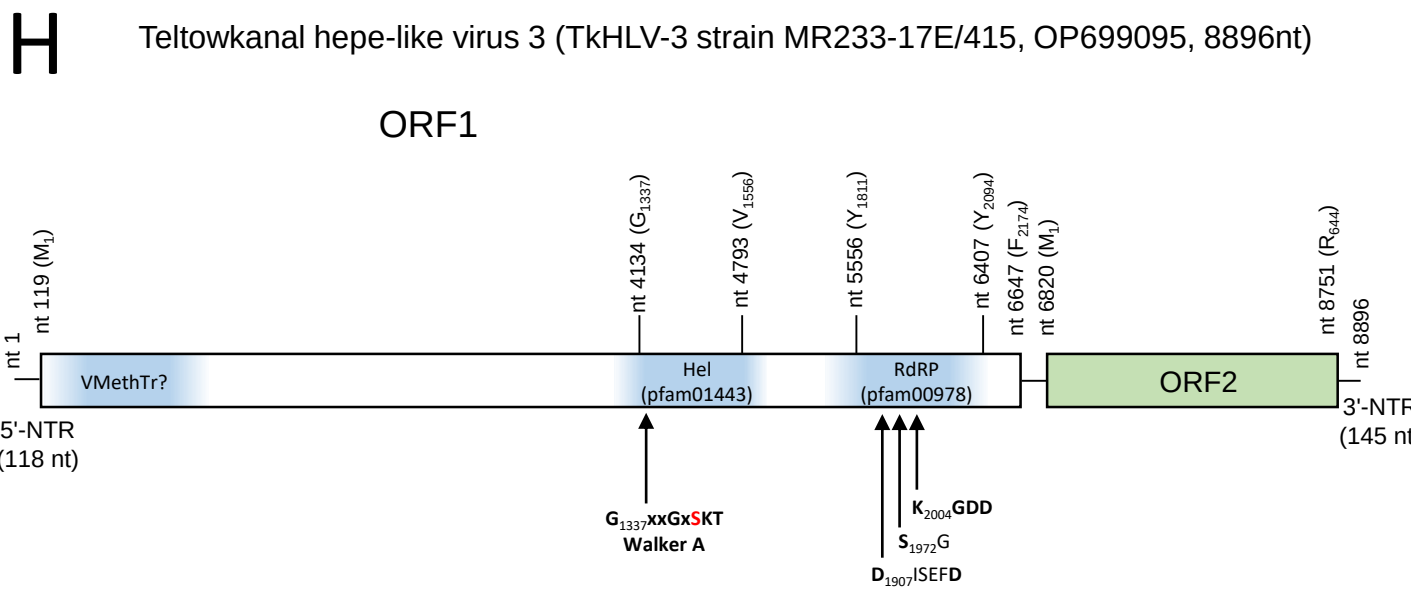
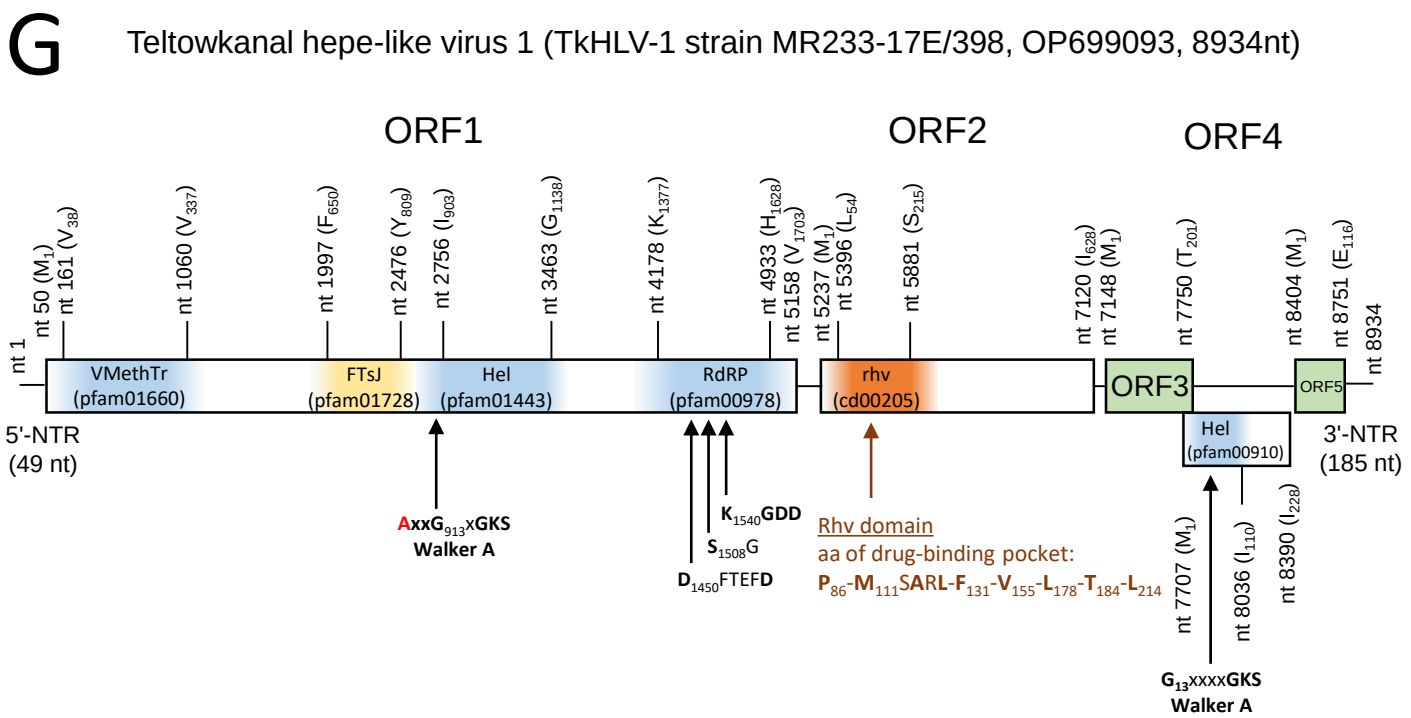
Havel hepe-like virus 5 (HHLV-5 strain MR644-18E/7839, OP699059, 7612nt)



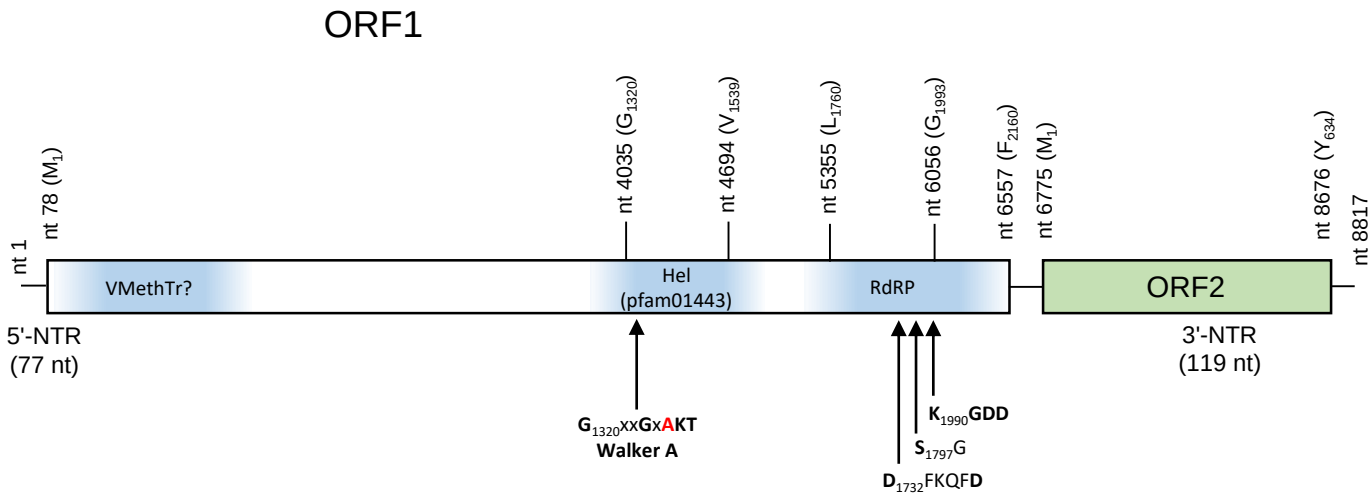
F

Havel hepe-like virus 6 (HHLV-6 strain MR644-18E/449, OP699060, 5661nt)

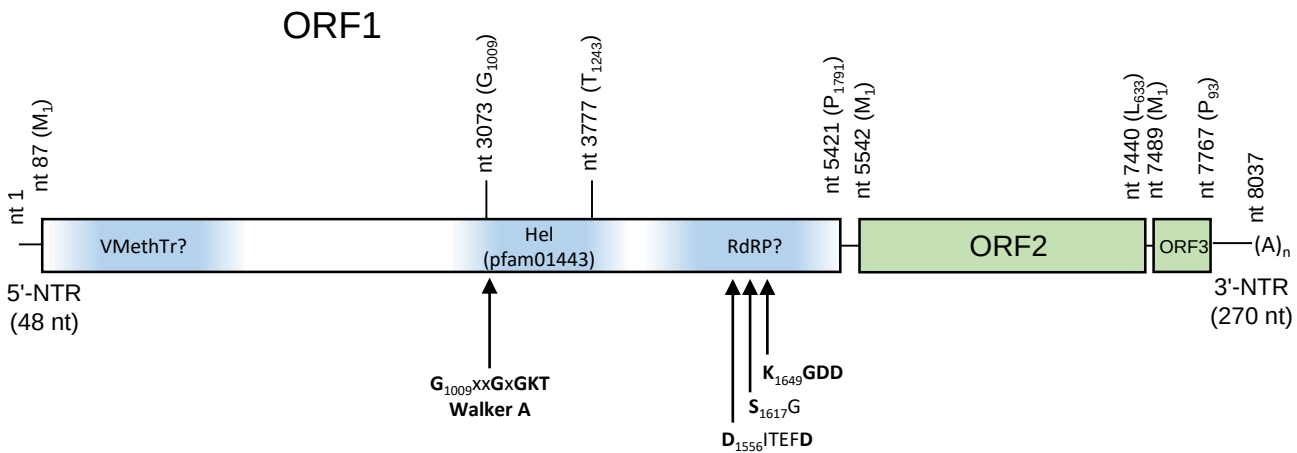




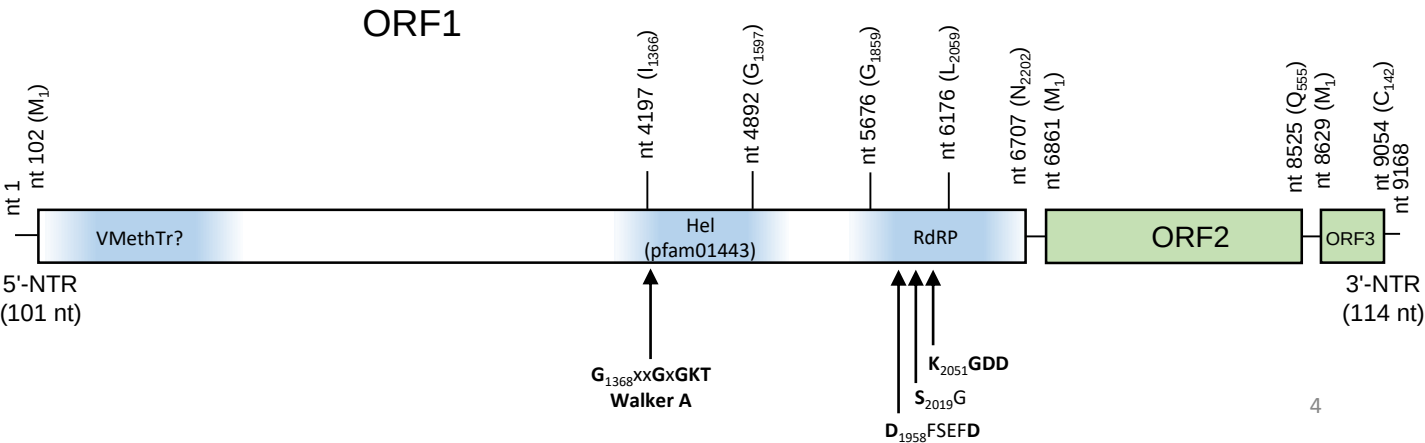
J Teltowkanal hepe-like virus 8 (TkHLV-8 strain MR233-17E/430, OP699101, 8817nt)



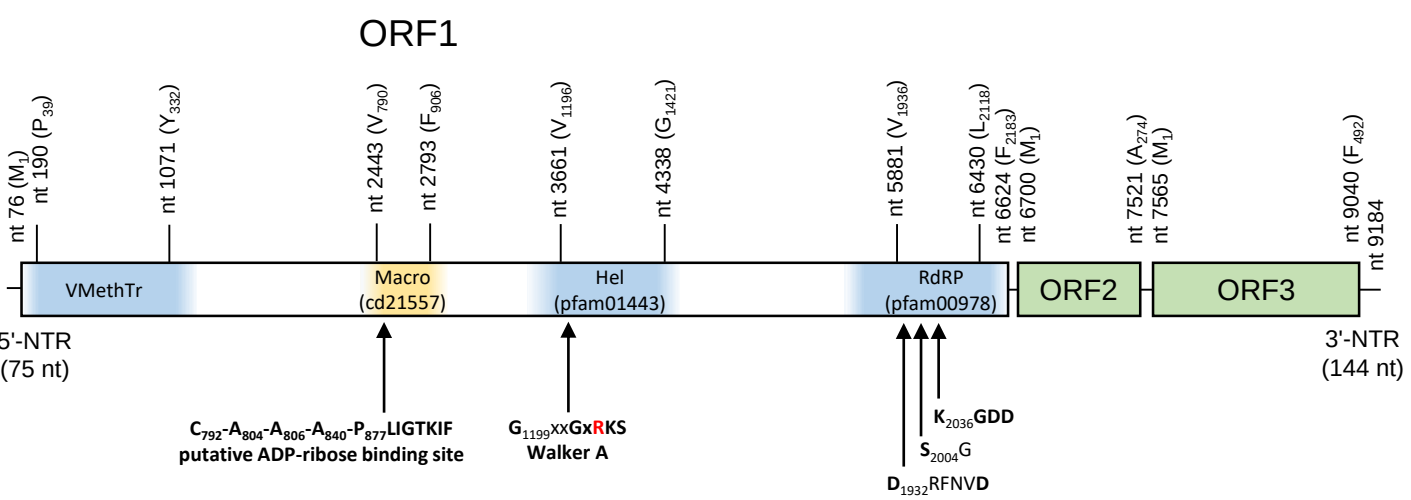
K Teltowkanal hepe-like virus 12(TkHLV-12 strain MR233-17E/582, OP699107, 8061nt)



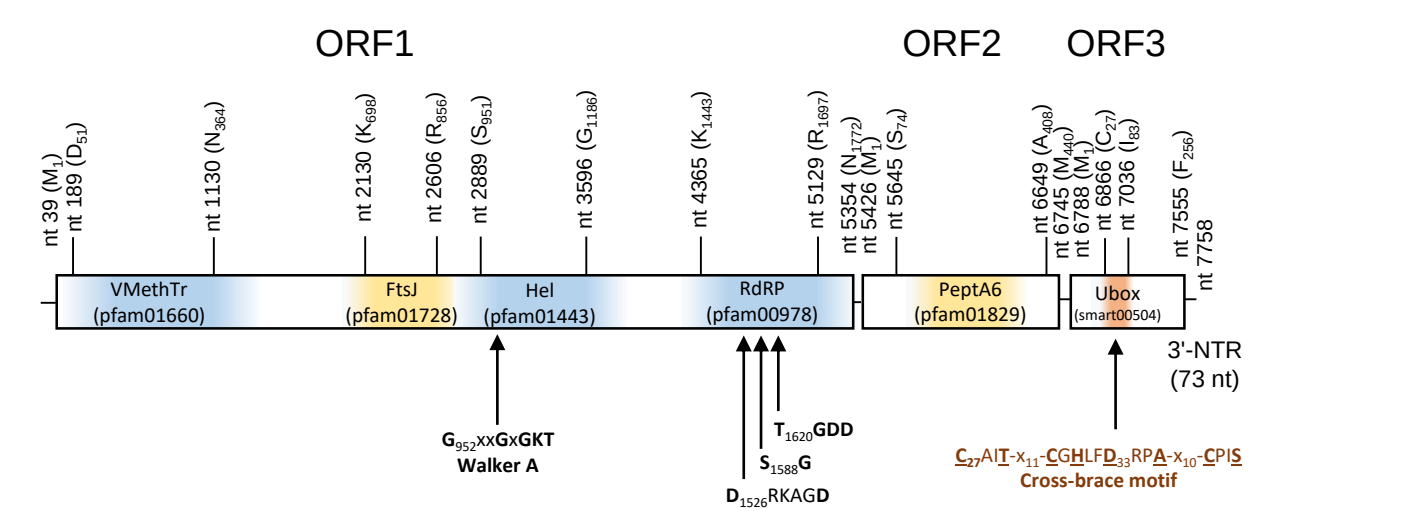
L Teltowkanal hepe-like virus 16 (TkHLV-16 strain MR233-17E/325, OP699112, 9268nt)



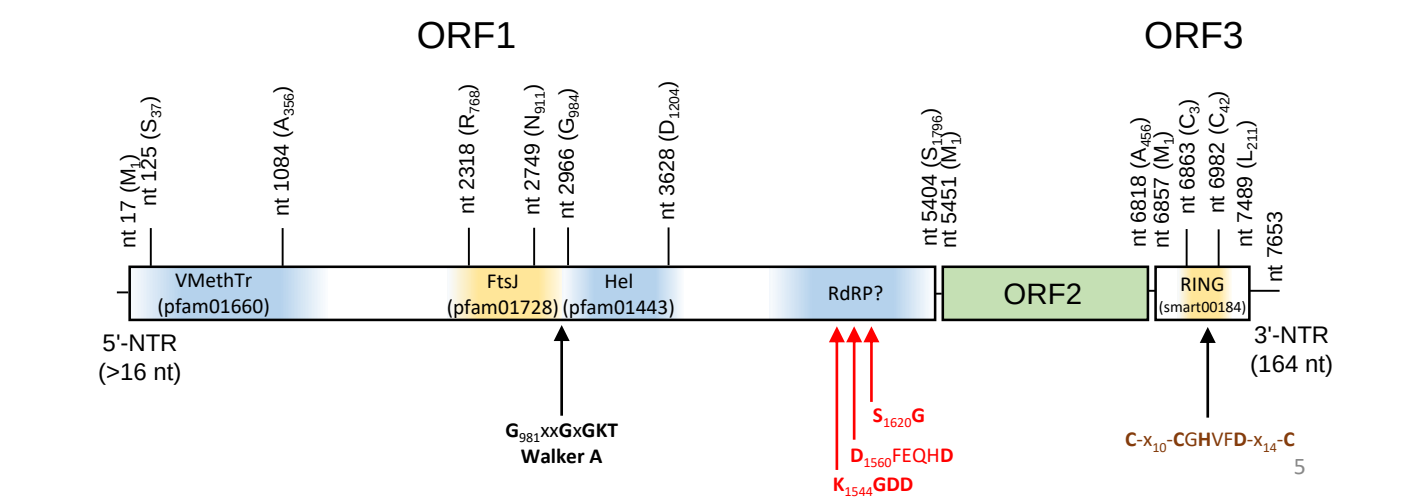
M Teltowkanal hepe-like virus 18 (TkHLV-18 strain 233-17E/349, OP699115, 9184nt)



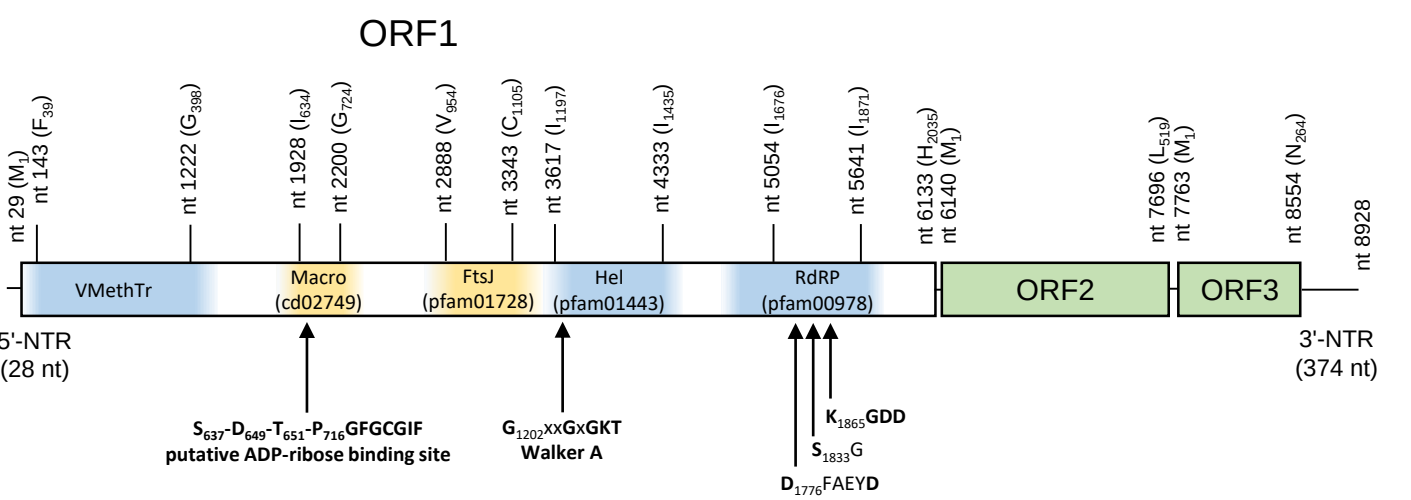
N Teltowkanal hepe-like virus 20 (TkHLV-20 strain MR233-17E/643, OP699118, 7758nt)



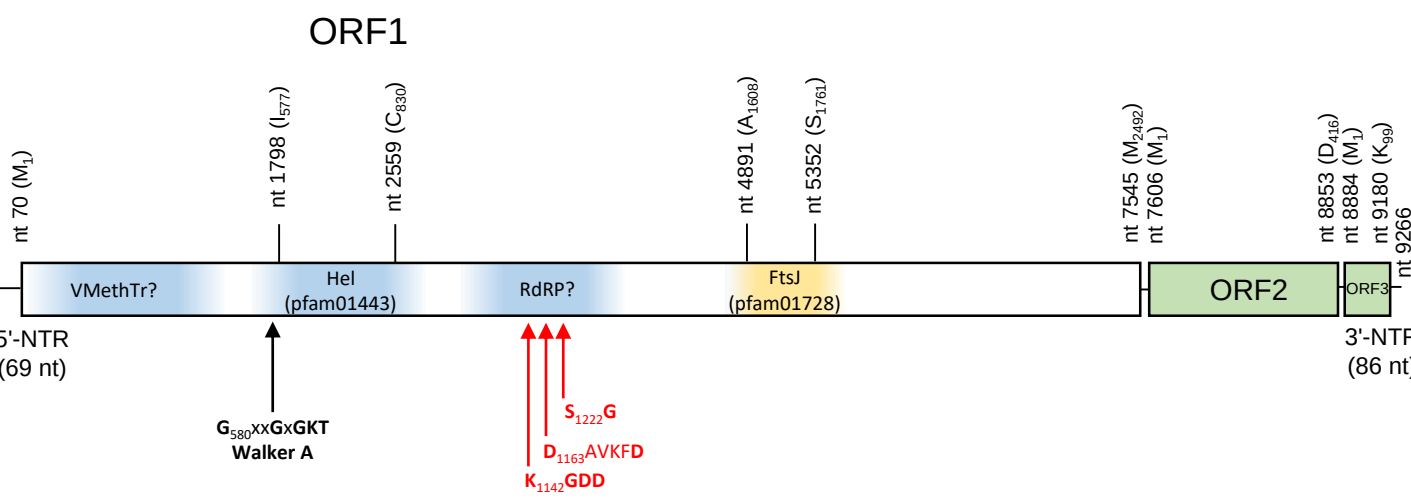
O Teltowkanal hepe-like virus 22 (TkHLV-22 strain MR233-17E/663, OP699120, 7653nt)



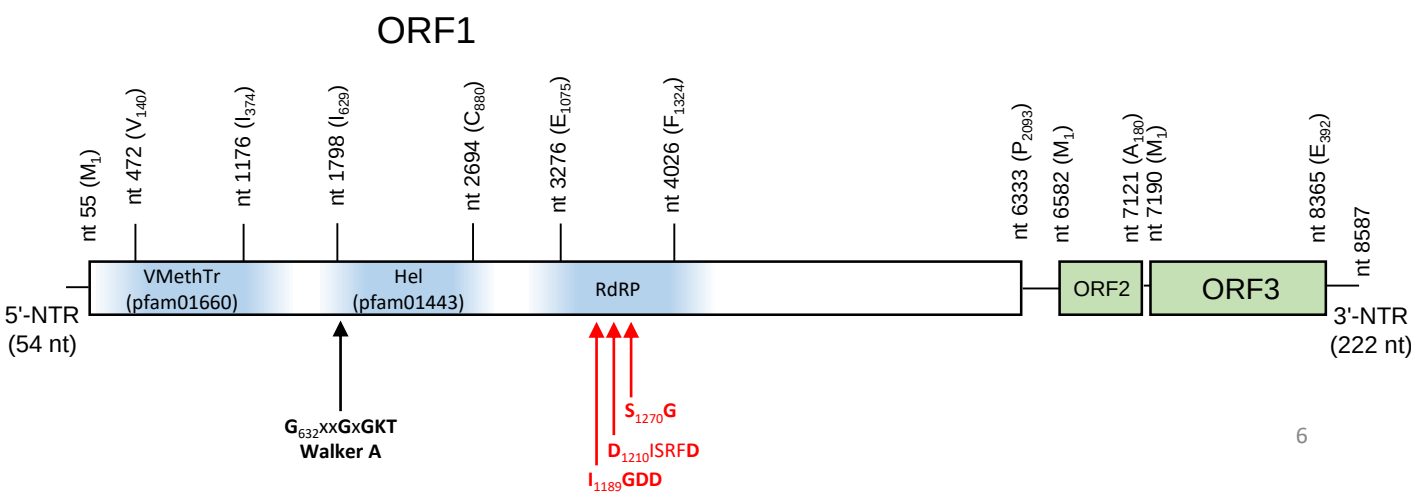
P Teltowkanal hepe-like virus 25 (TkHLV-25 strain 233-17E/401, OP699123, 8927nt)



Q Teltowkanal hepe-like virus 32 (TkHLV-32 strain MR233-17E/3264, OP699130, 9266nt)



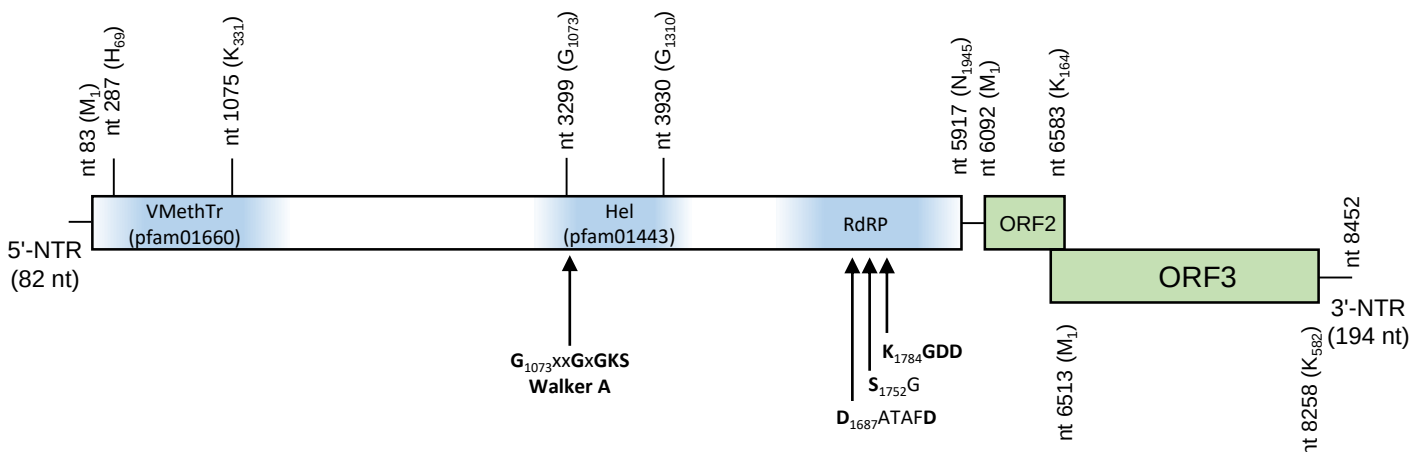
R Teltowkanal hepe-like virus 33 (TkHLV-33 strain MR233-17E/476, OP699131, 8587nt)



S

Teltowkanal hepe-like virus 34 (TkHLV-34 strain MR233-17E/510, OP699132, 8452nt)

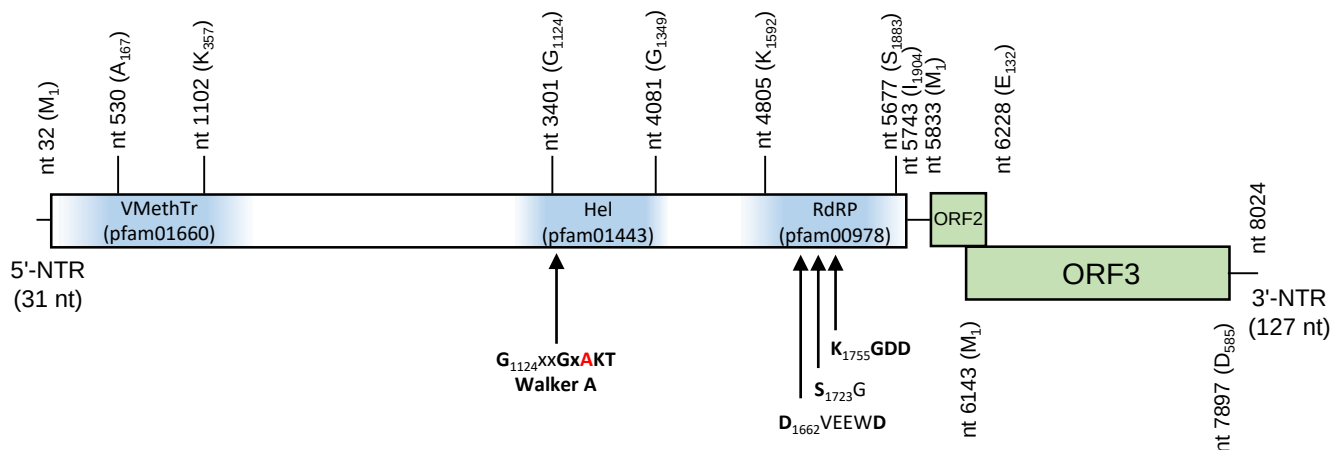
ORF1



T

Teltowkanal hepe-like virus 36 (TkHLV-36 strain 233-17E/595, OP699136, 8024nt)

ORF1

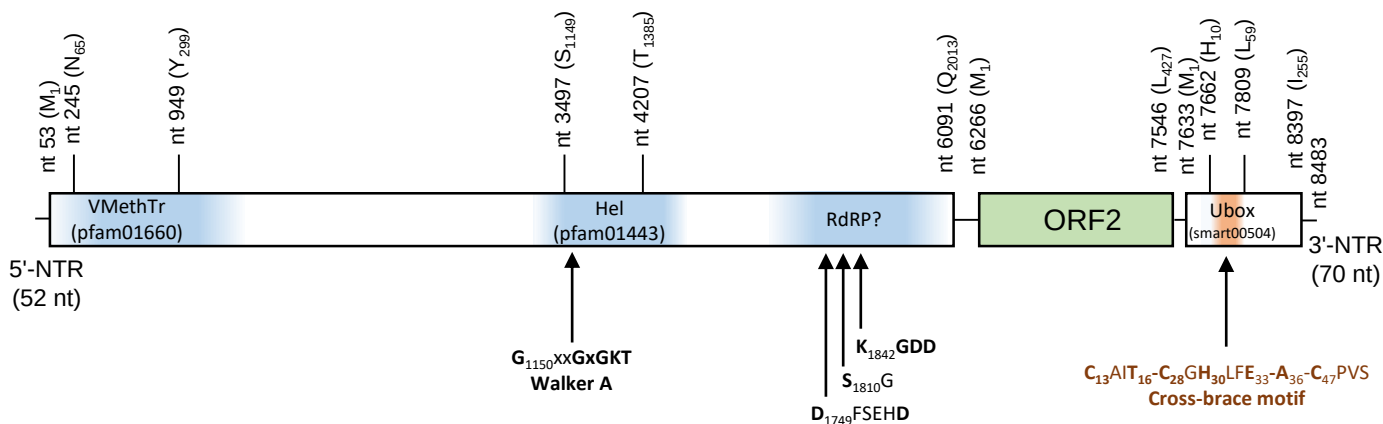


U

Teltowkanal hepe-like virus 39 (TkHLV-39 strain MR233-17E/503, OP699140, 8483 nt)

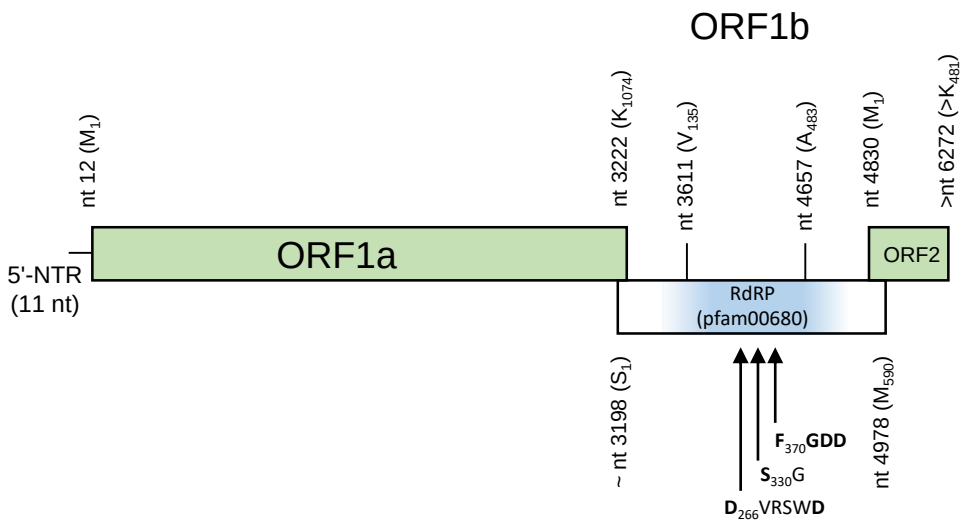
ORF1

ORF3



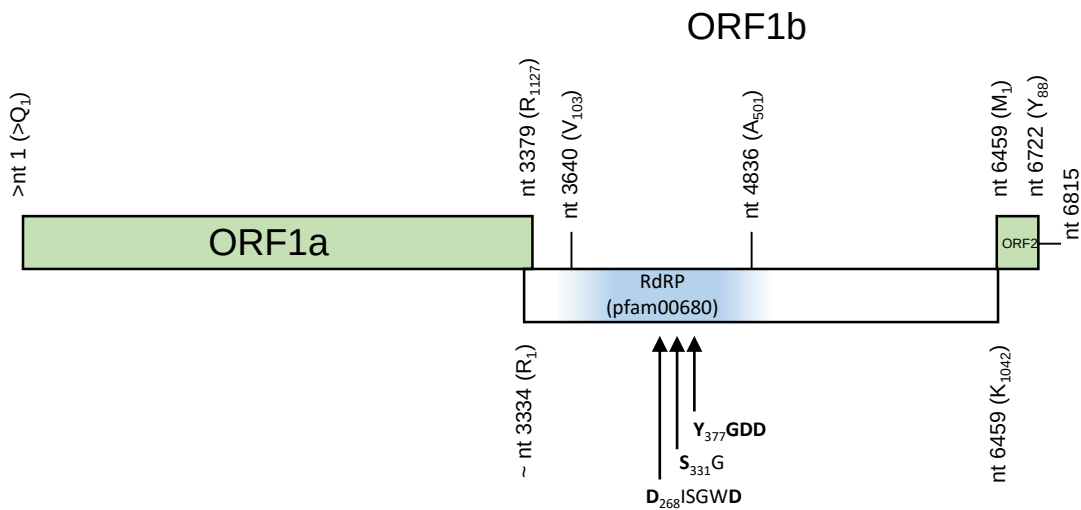
V

Teltowkanal astro-like virus 1 (TkALV-1 strain MR233-17E/955, OP699151, 6353nt)

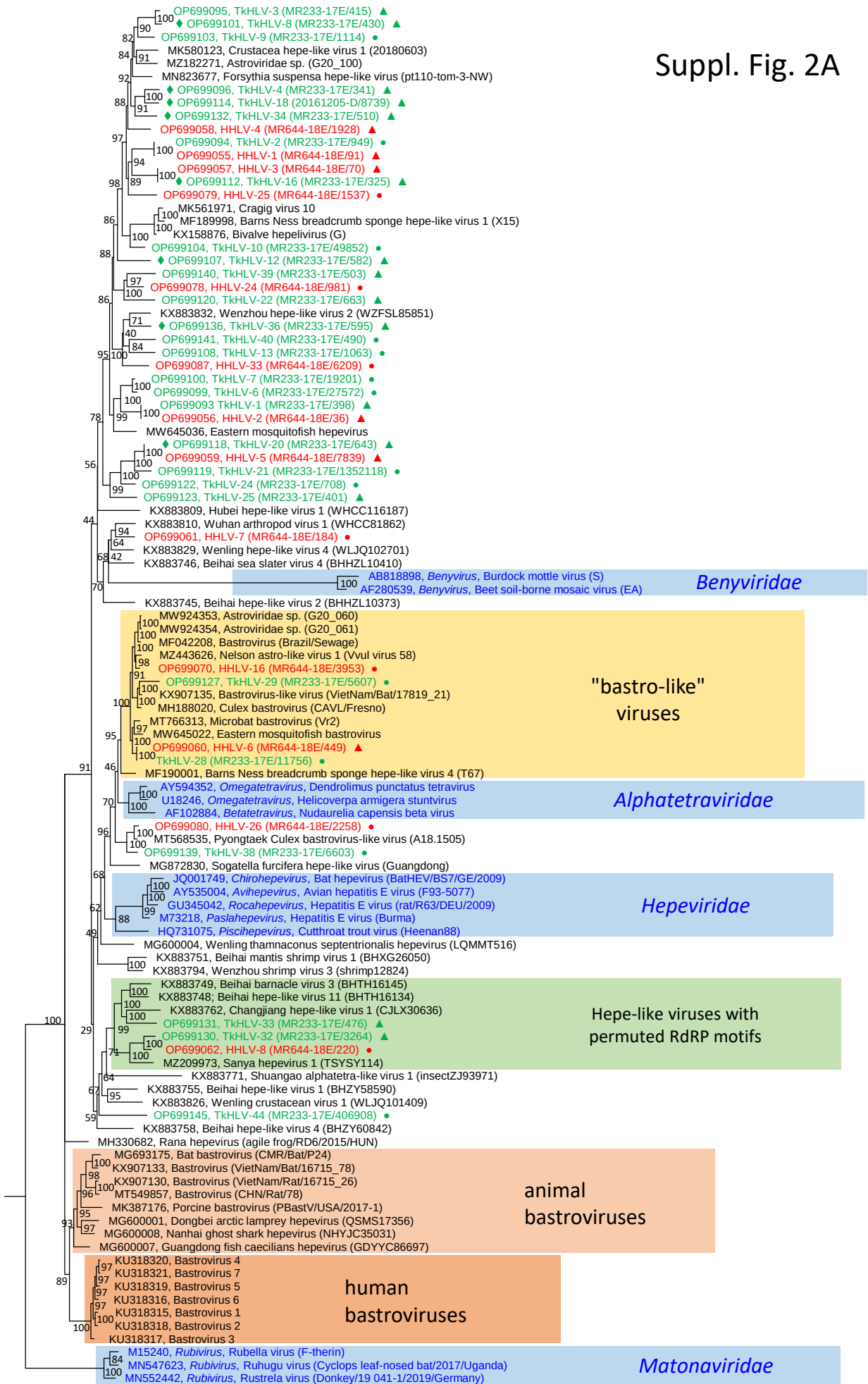


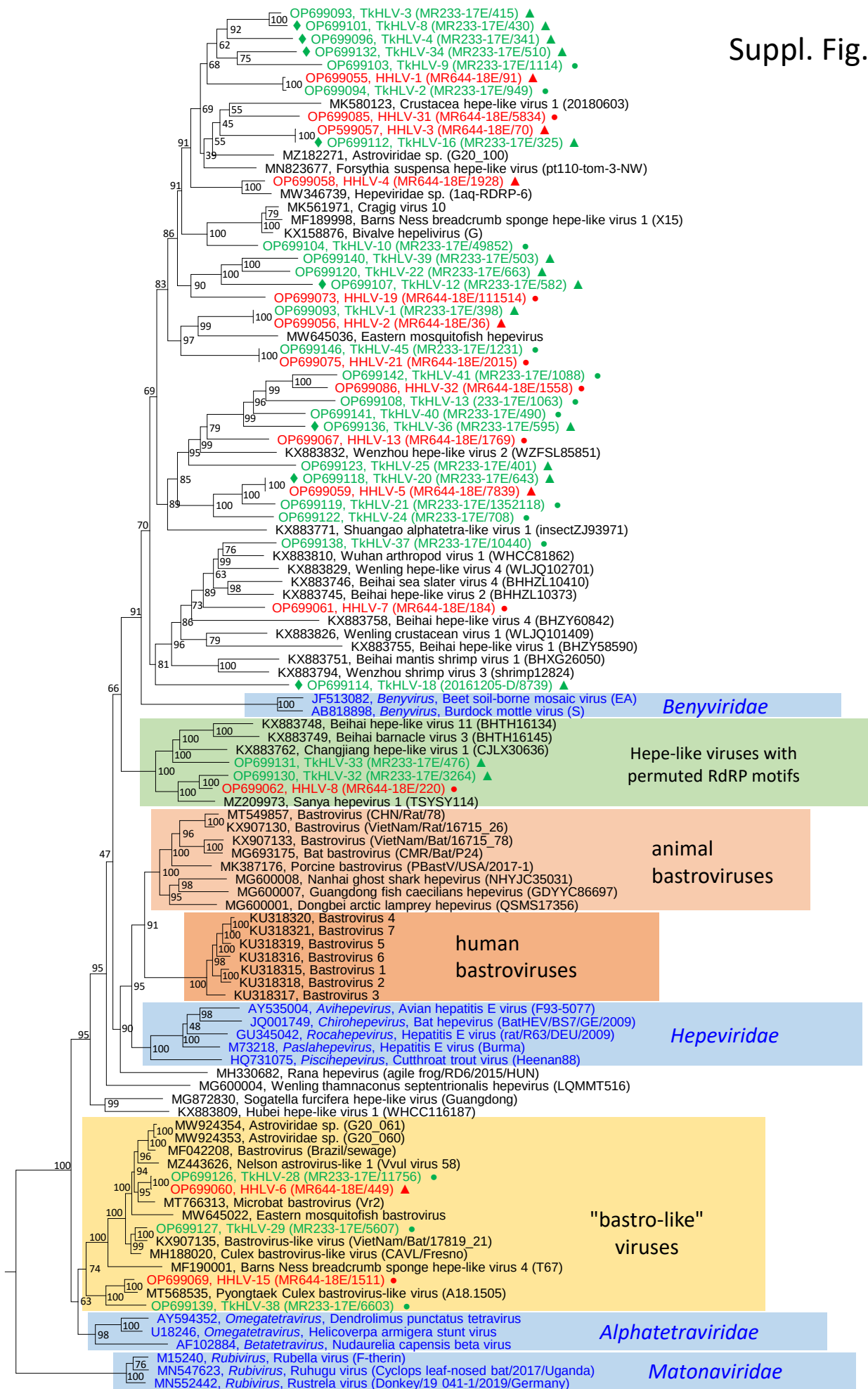
W

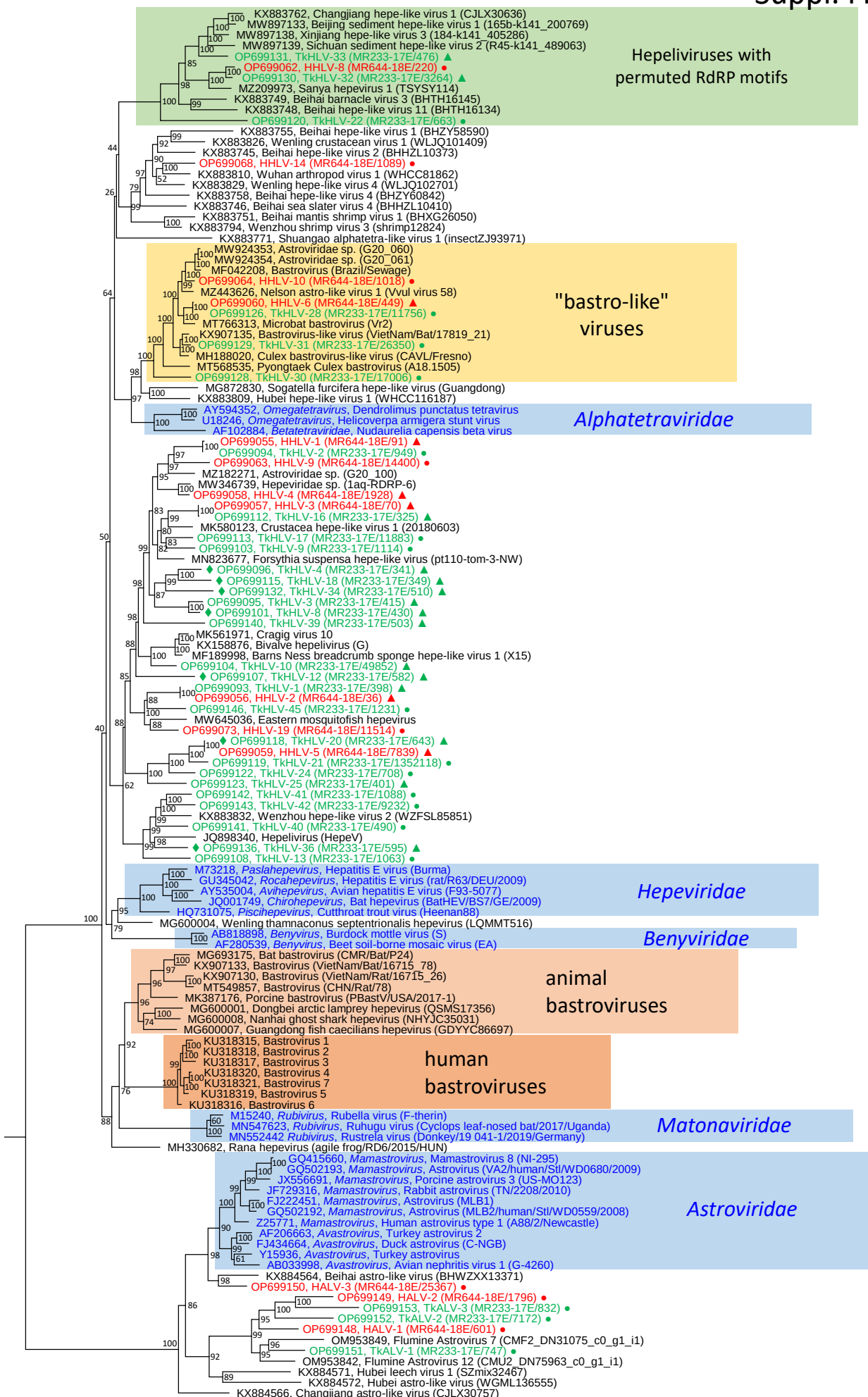
Teltowkanal astro-like virus 3 (TkALV-3 strain MR233-17E/832, OP699153, 6815nt)

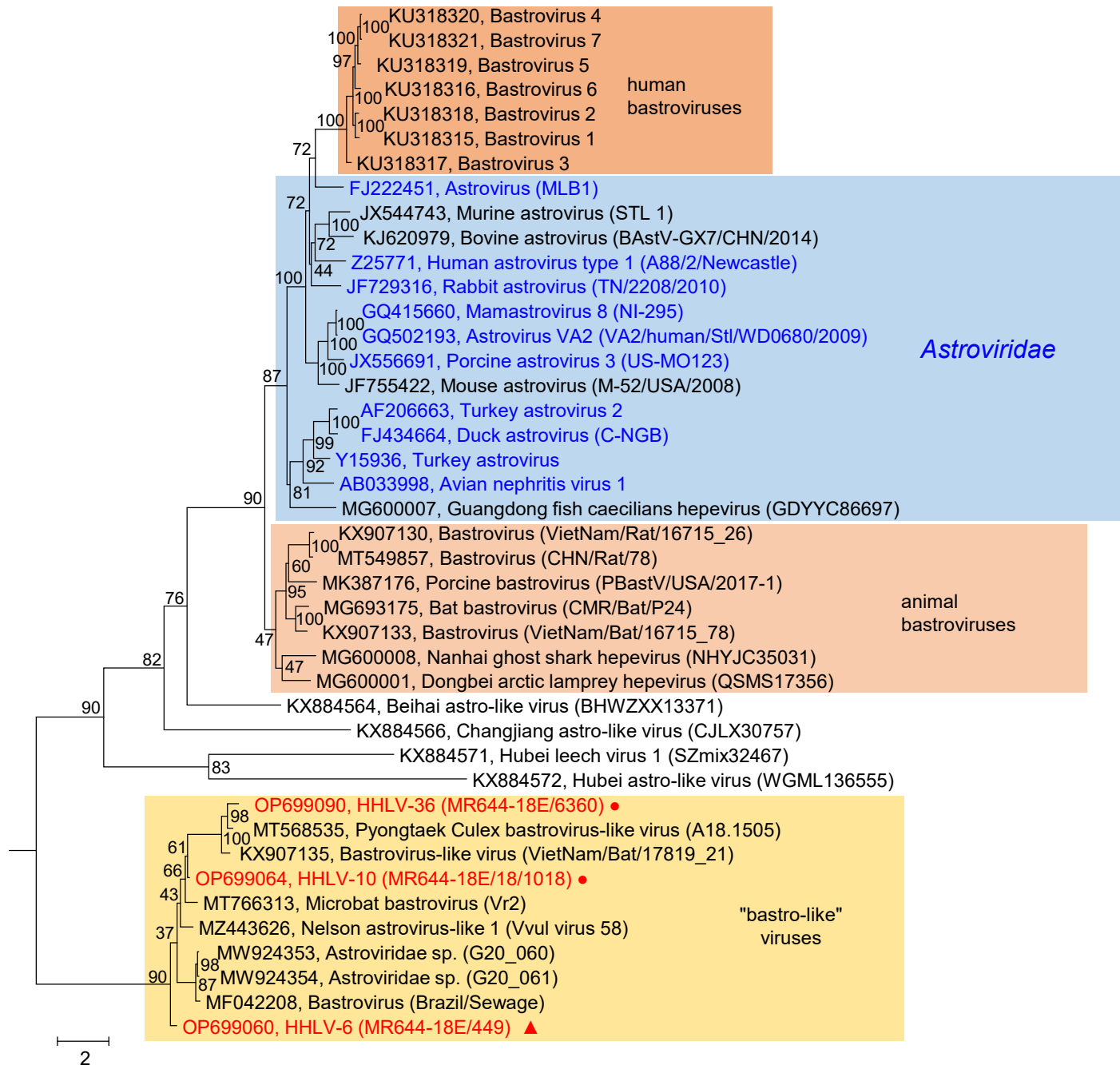


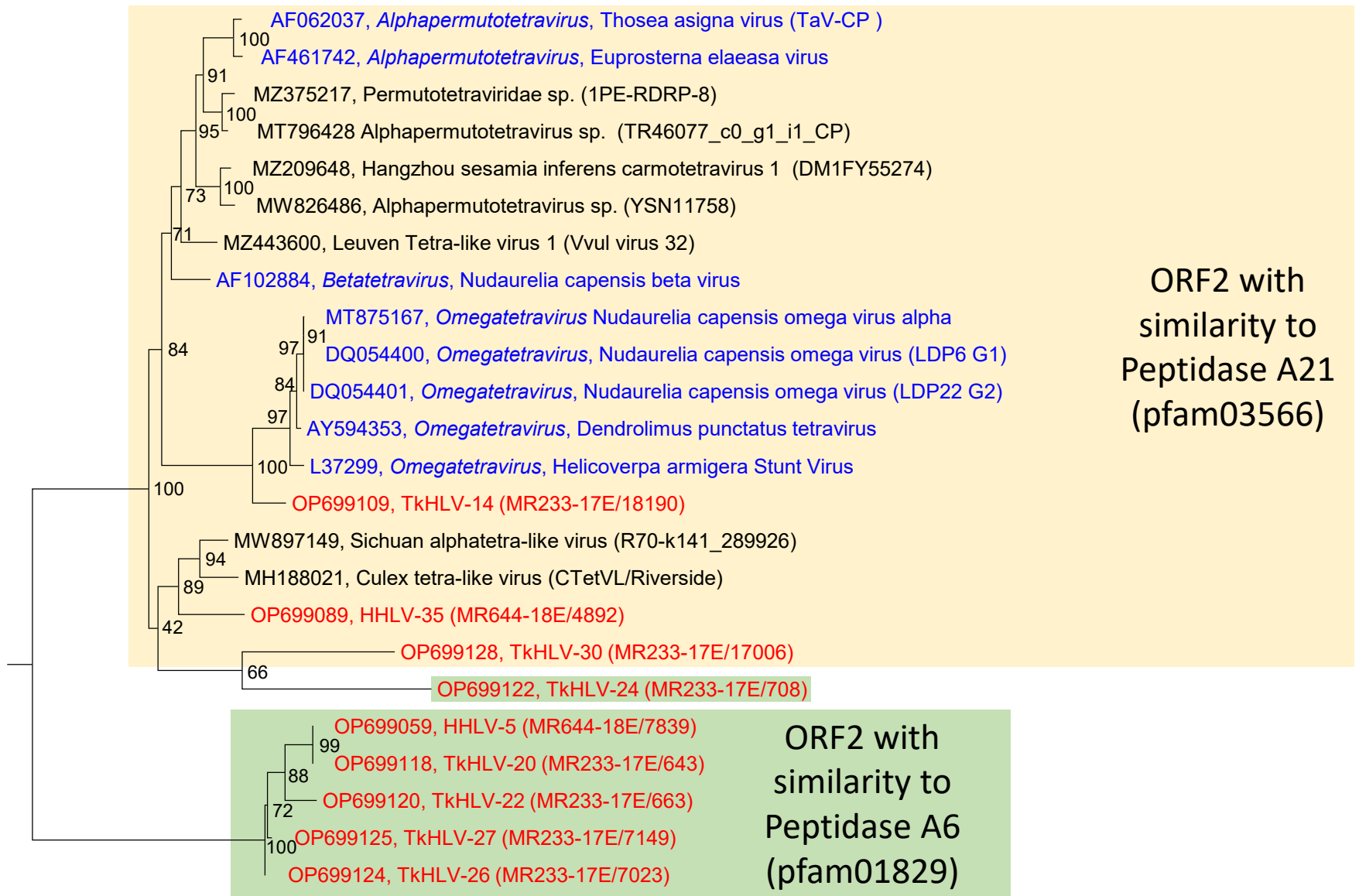
Suppl. Fig. 2A











[illegible]

TkHLV-4 MR233-17E/341
TkHLV-34 MR233-17E/510
MW346739 Hepeviridae sp. 1aq-RDRP-6
HHLV-3 MR644-18//1928
HHLV-9 MR644-18E/14400
HHLV-1 MR644-18E/91
TkHLV-2 MR233-17E/949
TkHLV-17 MR233-17E/11883
HHLV-4 MR644-18E/70
MN823677 Forsythia suspensa hepe-like virus pt110-tom-3-NW
MK580123 Crustacea hepe-like virus 1 20180603
MF189998 Barns Ness breadcrumb sponge hepe-like virus 1 X15
MK561971 Cragig virus 10
TkHLV-1 MR233-17E/398
HHLV-2 MR2018E/36
HHLV-10 MR644-18E/1018
HHLV-6 MR644-18E/449
MH188020 Culex bastrovirus-like virus CAVL/Fresno
KX907135 Bastrovirus-like virus VietNam/Bat/17819_21
TkHLV-31 MR233-17E/5834
MT568535 Pyongtaek Culex bastrovirus A18.1505
TkHLV-30 MR233-17E/17006
KU318315 Bastrovirus 1
KU318318 Bastrovirus 2
KU318317 Bastrovirus 3
MK387176 Porcine bastrovirus PBastV/USA/2017-1
MG693175 Bat bastrovirus CMR/Bat/P24
MT549857 Bastrovirus CHN/Rat/78
AY594352, *Omegatetravirus*, *Dendrolimus punctatus tetravirus*
U18246, *Omegatetravirus*, *Helicoverpa armigera stunt virus*
AF102884, *Betatetravirus*, *Nudaurelia capensis beta virus*
M73218, *Paslahepevirus*, *Hepatitis E virus Burma*
GU345042, *Rocahepevirus*, *Hepatitis E virus rat/R63/DEU/2009*
AY535004, *Avihepevirus*, *Avian hepatitis E virus F93-5077*
JQ001749, *Chirohepevirus*, *Bat hepevirus BatHEV/BS7/GE/2009*
HQ731075, *Piscihepevirus*, *Cutthroat trout virus Heenan88*
MN15240, *Rubivirus*, *Rubella virus F-therin*
MN547623, *Rubivirus*, *Ruhugu virus Cyclops leaf-nosed bat/2017/Uganda*
MN552442, *Rubivirus*, *Rustrela virus Donkey/19_041-1/2019/Germany*
TkHLV-22 MR233-17E/663
HHLV-8 MR644-18E/220
TkHLV-32 MR233-17E/3264
MZ209973 *Sanya hepevirus 1 TSYSY114*
TkHLV-33 MR233-17E/476
KX883762 *Changjiang hepe-like virus 1 CJLX30636*
KX883749 *Beihai barnacle virus 3 BHTH16145*
KX883748 *Beihai hepe-like virus 11 BHTH16134*
MW897133 *Beijing sediment hepe-like virus 1165b-k141_200769*
MW897139 *Sichuan sediment hepe-like virus 2 R45-k141_489063*
MW897138 *Xinjiang hepe-like virus 3 184-k141_405286*

										motif B		canonical motif C	
LSQQGFNSKA	IGFMANMRSD	WYCATNALINS	KLVRQIKFDV	SYVMTSGALF	TLSGNTFVNM	AIIGASFDFI	NLQFGAFKGD	DSHV--VAGG	IKTIKHGNML				
AKSAGAPART	VDVHHVQREK	WTATAMFDVD	QRVQHALEVS	RYMNTSGHLV	TLTFNTMYSM	TIIGGGFKYT	NLRFAMFKGD	DSVV--CCDK	CEERYDQGT				
LSMLGFNECK	IKLVVNLRLK	WVMALRGSSL	QHDITITLLK	VFKKHSGEPA	TLCGNFWFNR	AVTCLCVTLF	NPYICAFKGD	DSLII--VADS	VTVVRSQVDY				
LRMLKMPECK	VKLYITKLRL	WVMALRGSSL	QDDTITLLRG	VFKKHSGEPA	TLDGNTWFNR	AVTGMCTITK	NPYIAGFKGD	DSII--IADE	ITVERHGYDS				
LYNPMSEFT	IQFMEKMRQS	WTLRMRIISGK	TMMASALQKG	HWKQSGQIHL	TLGGNTLYSM	GAICMCFNFG	DYIAAGFKGD	DSFI--LCHK	YSEVKEYNTS				
YEMGWFSFLPA	YKLMFTQQRQS	WKMSLIVDNQ	TITSHSFLTG	NFMQASGQPD	TLGSNTFYDM	AAVGMCID-I	DFVAAFFKGD	DSYI--IARR	WHYAVFKGRK				
YEMGWFSFLPA	YKLMFTQQRQS	WKMSLIVDNQ	TITSHSFLTG	NFMQASGQPD	TLGSNTFYDM	AAVGMCID-I	DFVAAFFKGD	DSYI--IARR	WHYAVFKGRK				
LKGGCISRKRI	IDFYVDKRSK	WMSVNIITGTP	NETLSTVLNG	QWKQSGQOPF	TLDGNTMYNM	WLIGICYKFT	DLFSASFKGD	DSYI--CATK	ID-----				
LRQLGFNEKV	LGVYLEMREK	WKLVSATDS-	--SFVMVMCN	WMQSSGQAF	TLAGNTQHNM	IVMGAAIYIK	NIVFAMFKGD	DSLII--VCEG	IREAKIEGVL				
LKMMFGDGDGL	VNFYMAKRKD	WTLINQDNDF	NMPLRTMLDG	EYMQHSGQPF	TLDGNTFMNM	SCMGCMCFEW	NMIFASFKGD	DSYI--LAES	IKPIMVGHDT				
LRMMGCNQKLI	LGYYLKLRSQ	WTLSSVSDNG	PDNISMFILDG	VWKQSGQOPF	TLDGNTFMNM	WAIMGCMDFV	YLDAATFKGD	DSAL--IGBE	FERIMHDITR				
FGLLGVNAKI	INFYSSMRTK	WCNLYQCREG	----ITMLHG	EYMQHSGQPL	TICGNTLLNM	AVLGYYARIE	NMLYASFKGD	DSTI--RAKK	ISTVPGKTKA				
FGLLGVNHKI	IDFYAKMRTE	WCNLYQCNQG	----ISMLNG	LYMQHSGQPL	TITGNTFILNM	AVLGYYARIFQ	EMLYAAFKGD	DSHI--RAKK	VSVVQGRKTA				
LTLDIGVTDEC	RLDYLYEQRMD	WTVVYPG---	----FMVLKG	KMKKQSGEIF	TIDFNTAVCM	AANGYMLDIV	DLVLAQFKGD	DSNI--AAED	IR--LSKEGRE				
LTLDIGVTDEC	RQLYLYEQRMD	WTVVYPG---	----FMVLKG	KMKKQSGEIF	TIDFNTAVCM	AANGYMLDIV	DLVLAQFKGD	DSNI--AAED	IR--LSKEGRE				
LKIKGVPHFL	LEAFMDQLRN	RRICCE----	----VSLIV	NDKKDSGAPH	TLIDNCLFNL	AICLDLMDVF	LYLY--IKGD	DSFA--RGPK	VRFDHERMSR				
LRVIGCPBIL	LEAFIEQITS	RRICCE----	----QSSLNV	NDKKDSGAPH	TLIDNCLFNL	AICLDLMDVF	EHLV--IKGD	DSLA--RGRN	VRFDSHRMND				
LIRIGCPAKL	VSFYCEQLKT	RPLSA?-----	----QSSLVV	NDKKDSGAPH	TLVDNCLFNL	AICLDVMTDF	DKLY--IKGD	DSLA--RGNP	VAFNMERLNR				
LLRIGAPPIL	VSFYIEQLKT	RRICAA-----	----QSSLVV	NDKKDSGAPH	TLVDNCLFNL	AICLDVMDQF	DKLY--IKGD	DSLA--RGPD	VSNFLPRNLH				
LLRIGCPQLL	VSFYIEQLKT	RRICAA-----	----QSSLVV	NDKKDSGAPH	TLVDNCLFNL	AICLDVMDQF	DKLY--IKGD	DSLA--RGRN	VSNFLSRIND				
LLRIGCPPEL	VELFCMLQSE	RSVVDV-----	----FISVCA	YDKKDSGAPH	TLVDNCLFNL	AVILDVLSDF	RVLL--IKGD	DSIA--FGEN	IRFNKRASW				
LAALGCPQL	RNNFLAMMLR	RNVQSR----	----VGTVQV	QNKKDSGRVD	TLIGNTLFNA	AILLSCIDRT	QIQYALMKQG	DGLI--IGKA	VRPNYKRIEE				
LKRIGLDPAV	LDLYRDYRLS	AKYSGPG---	----ADIPAA	--GMRMSEAN	TLGNTMTVTG	ILHCAVYNQE	PAWMM--FKGD	DAIM--AGPD	SMNVDDVS--				
LKRIGLDPAI	LDLYRDYRLA	AKYSGPG---	----ADIPAA	--GMRMSEAN	TLLGNTFLVTG	ILHCAVYNQE	PAWMM--FKGD	DAIM--AGPD	SMNVDDVET--				
LKRVGIDTQI	AELYTEFRKN	ATYSGPG---	----ADLAAD	--GMRMSEAN	TLLGNTFLVTG	ILHCAVYNQE	PAWMM--FKGD	DAII--AGPP	EMELDCET--				
LRCTGVPSQI	CNAYRALRKN	AWMTAVD---	----HAFSAD	--HARMSEAN	TLFGNTAISL	AVSSLLLPGE	WACAA--FKGD	DSFV--QSDC	ABLATDK---				
LRDLGVPTDI	VRTYRALRES	ARVSAGQ---	----VEAEAS	--YARMSEAN	TLFGNTFVVTM	AVNALLFPGR	FDWAA--FKGD	DSII--CNPQ	NFSDVDI---				
LRWLGVPSDV	CQLYLALRAA	ARIESTM---	----LCVDAP	--NARMSEAN	TLFGNTFVTM	AVNALLPGRS	FSWAA--FKGD	DSIV--AGPE	HEAPVGG---				
LARVGTAAAA	VTLFRQRCGK	RTLRAKG---	----IGSVEV	DLGLDSGAVN	TILGNTTFSA	VVMLTLFRGV	KFAA--FKGD	DBLL--VGSQ	LFTTPARLHM				
LERIGTPAAA	VNLFRERCCK	RTLRAKG---	----LGSVEV	DLGLDSGAVN	TFGNTTFISA	VVMLTLFRGV	KFAA--FKGD	DBLL--CGSH	LAFDASRLHM				
CDR-GCPEHL	IYEFKRSKS	RTLSSR----	----IGSVDV	SFMLSBSGAVN	TIARNTLFAS	GLMLFLFVGV	DFIA--ARGD	DVFL--AGNN	LILDAERLRM				
MEECGMPQWL	IRLYHLIRSA	WILQAP----	----KESLRG	FKKKHSGEFG	TLGNNTVWNM	VITHCYDFDR	DFQVAAFKGD	DSIV--LCSE	YR--QSPGAIV				
MKEAGAPEWM	WRLYHLRLSG	WVLQAP----	----QESLRG	RKKKHSGEFG	TLGNNTVWNM	AVIMHCYRFD	GLAVAAFKGD	DSIV--CCRF	YQ--QEPRAAA				
MRRFGMPDWM	VALYHLVRSY	WLLVAP----	----KEALRG	CKKKHSGEFG	TLGNNTVWNM	VVLHHVYEFD	RPSVLCFKGD	DSVV--VCSB	VR--ARPEGVS				
LTETGMPDWM	VRLYHLQRAY	WNLVAP----	----DACLRG	CKKKHSGEFG	TLGNNTVWNM	VVNACVYFTF	DAVHLVYKGD	DSVV--LCRS	FE--QEPDAHH				
LVQCGMPPAL	AEVYFNRLRK	WTLRAR----	----VASLRG	TSKKHSGEFG	TLGNNTVWNM	AVFHTTSTFT	BMTLALFKGD	DSIV--CCDS	SI--LTPTAKE				
SASL-LGLPC	AEDYRALRAG	SYCTLRE----	----LGSTET	GLERTSGEPA	TLGNNTFVAM	MMAMRMVPGK	VRWAGIFQGD	DMVIFLPEGA	RSAALKWTPA				
SASL-LGLPS	IDYKALRAG	SYCLLRD----	----IASTET	GLERTSGEPA	TLGNNTLVGM	MMAMRMVPGK	VRWAGIFQGD</						

Supplementary Figure 5 (continued)

TkHLV-4 MR233-17E/341	TSTYLGHKKL AE-MNF-FS- ---EFIANFI	TPIG----FF	PDVIRRASRV	ISKVYT-HPD	DWNKIRISVA	DSLMTVITNDN	Q-
TkHLV-34 MR233-17E/510	LMQWCQYILK DS-VDV-VP- ---EFIANFL	NPWG----FF	PDVLRVRVSRV	VGRIVT-HPE	QWEEMRRSVA	DCLDVINNNS	E-
MW346739 Hepeviridae sp. 1aQ-RDRP-6	LWKHLGYKFK VS-FLN-YP- ---EYIANII	TPEG----FF	PDLVRRASRV	ISKIYN-DKV	DWAE LRSTA	DSLAVINNGY	S-
HHLV-3 MR644-18//1928	LWKHLGYQFK VN-LVK-YP- ---EYIANII	TPEG----FF	PDLIRRTSRV	VSKIYG-DRM	DWEE TRMSTA	DSL SVINNAY	S-
HHLV-9 MR644-18E/14400	IYKRAGDFDK IE-TPV-IP- ---EYIANIV	TPFG----FV	PDLLRRVSRV	VSKVYQ-TKA	DWDQIKLSTA	DALAVTPQAN	M-
HHLV-1 MR644-18E/91	VVDIMGYQFK ID-TPE-IG- ---EFIANIV	TPIG----FV	PDYLRRVSRV	VSKIYE-TNS	DWNEIRLSTA	DALSVVQQNN	L-
TkHLV-2 MR233-17E/949	VVDIMGYQFK ID-TPE-IG- ---EFIANIV	-----	-----	-----	-----	-----	--
TkHLV-17 MR233-17E/11883	-ERLYGEK-K VR-----	---DFCGYKI	KDFR-----	PI-----	-----	-----	--
HHLV-4 MR644-18E/70	FTEHVGKIK IH-EVD-IA- ---EYIANIV	TPHG----WF	PDVIRRVTRV	LTKIYT-TKD	DWLEIRKSMS	DALDVID-PE	NR
MN823677 Forsythia suspensa hepe-like virus pt110-tom-3-NW	LYAILSYKLL YD-FQN-IS- ---EYIANII	TPTG----FF	PDVLRRTSRI	LTKIYT-VAD	DWEEIKKSTA	DALDVIADDQ	E-
MK580123 Crustacea hepe-like virus 1 20180603	YIEITGYKIK AF-YVP-IL- ---EYISNIV	TPAGK---FF	PDVIRRVSRV	VSKIYT-TQT	DWEEQKLSIT	DSL DVINTPE	D-
MF189998 Barns Ness breadcumb sponge hepe-like virus 1 X15	IYADHGYKLL IS-FEK-VS- ---EFIANFI	TPFG----FF	PDVVRRAVKA	VSKVYE-DEE	SWEESRINKL	EVL SMVNSAE	K-
MK561971 Cragig virus 10	IYVEHGYKLL IS-FEK-VS- ---EFIANFV	TPYG----FF	PDVVRRSVKA	VSKVYE-DEE	SWEESRVNLK	EVL SMVNTAD	K-
TkHLV-1 MR233-17E/398	FLINGGMKPK LH-IHEGSG- ---EFTGYIQ	TPYG----PY	PD TIRRVAKA	FSKVFK-DEK	DLDEFKLAMH	DYVSGINNHA	Q-
HHLV-2_MR2018E/36	FLINGGMKPK LH-IHEGSG- ---EFTGYIQ	TPYG----PY	PD TIRRVAKA	FSKVFK-DEK	DLDEFKLAMH	DYVSGINNHA	Q-
HHLV-10 MR644-18E/1018	YLKNCGYKFK PN-QHT-SG- ---SFVSFLV	NQQG----VA	LDLPRMCAKV	TSRAYT-NVE	DFLNYQE AIS	AQLKNIDMQA	G-
HHLV-6 MR644-18E/449	YI?KCG?VEK PE-KHT-SG- ---SFVSFLI	NQNG----VA	LDLPRICAKV	TSRAYT-NIG	DYHNYCDA?A	GTLKTVDMQA	G-
MH188020 Culex bastrovirus-like virus CAVI/Fresno	YNKQCGFQFK PN-SSA-VG- ---QFVSFLV	SPRG----VA	LDLARIAAKI	TSRAYN-NKE	DYDNYASALA	GTLKPIDIDA	G-
KX907135 Bastrovirus-like virus VietNam/Bat/17819_21	YNKTCGFQFK PN-TSS-VG- ---QFVSFLV	SPRG----VA	LDLARIAAKV	TSRAYT-NKE	DYDNYALALA	GTLKPIDVDA	G-
TkHLV-31 MR233-17E/5834	YNKKCGFQFK PN-SSS-VG- ---QFVSFLV	SPRG----VA	LDLARIAAKV	TSRAYT-NKE	DYDNYALALA	GTLKPIDVDA	G-
MT568535 Pyongtaek Culex bastrovirus A18.1505	YTKNCNYQFK PD-VSN-SG- ---EFVSFII	NSEG----AS	YDLRLSSKV	FSRVYT-SPQ	DFRDYRDAVG	VTLAHNPLTA	G-
TkHLV-30 MR233-17E/17006	LEKNCSYKLL LT-VGK-SA- ---EFTSFII	NGNG----AA	LNIPRIA AKV	LTRNYT--AD	KYADYVAVG	DLIKSSN-NV	G-
KU318315 Bastrovirus 1	MHKDYGMKAK LE-RME-VP- ---EFVSFFV	SPYG----LL	PDFFRIANKM	VSN DHQDTPE	WREASAAANAR	AVLELADNPA	--
KU318318 Bastrovirus 2	MHKDYGMKAK LE-RME-VP- ---EFVSFFI	SPYG----LL	PDFFRIANKM	CSNDH QDTPE	WREASAAANAR	AILELTDNPA	--
KU318317 Bastrovirus 3	MQEDYGMKAK PE-RME-VP- ---EFVSFFV	SPYG----LL	PDFFRIANKM	VSN DHQPSPE	WREASAAANAR	AILRMVTNPA	--
MK387176 Porcine bastrovirus PBastV/USA/2017-1	IEDNIGMICK LE-RQE-VP- ---EFVSFLV	A-PH----FA	PDLKRCVGKL	LGKFPFKE--	-VEELQQA VE	NKLLIHDP E	--
MG693175 Bat bastrovirus CMR/Bat/P24	ISMQTMGMCK IE-ENH-VA- ---EFVGLLV	SSDH----VF	PDLRRRVGKL	TGRGYPGDPL	ARDQFAQSVK	DTLALIPPAE	--
MT549857 Bastrovirus CHN/Rat/78	VEQLTGMI CK VE-KPE-VP- ---EFVGLVI	S-DR----AY	PDLRRAVGKL	LGKGYPGDPI	ARADLMRSVN	ERLWLIPKDR	--
AY594352, Omegatetravirus, Dendrolimus punctatus tetravirus	GEHYAKAHLK VE-TEV-VV- ---PYIGLLV	SAEQ----VV	BDPVRVALKV	FGRCYS-THT	LYGKYVDAVA	DLVSSWADAA	N-
U18246, Omegatetravirus, Helicoverpa armigera stunt virus	GERYKTKHLK VE-VQK-IV- ---PYIGLLV	SAEQ----VV	LDPVRSALKI	FGRCYT-SEL	LYSKYVEAVR	DITKGWSDAR	Y-
AF102884, Betatetravirus, Nudaurelia capensis beta virus	GSYLAANNLK IE-KTA-VV- ---SFIGFIV	SQAA----VT	ADVRLATRT	YGRSYK-NAD	DLAKYKIAIA	DHCKLFRSPR	T-
M73218, Paslahepevirus, Hepatitis E virus Burma	LIAGCGLKLL VD-FRP-IG- ---LYAGVVV	APGLG---AL	PDVVRFAGRL	TEKNWGPGE	RAEQLRLAVS	DFLRKLTNVA	Q-
GU345042, Rocahepevirus, Hepatitis E virus rat/R63/DEU/2009	LITGCGLKLL VN-FSD-VG- ---SYAGLLV	ACGLG---VT	PDVVRFLGRL	SEKNWGPVKE	RKEDLEQSVR	DFVARLRNVT	A-
AY535004, Avihepevirus, Avian hepatitis E virus F93-5077	LVADCGLKMK DK-TGP-CG- ---AFSNLLI	FPGAG---VV	CDLLRQWGRL	TDKNWGPDIQ	RMQDLEQACK	DFVARVVTQG	K-
JQ001749, Chirohepevirus, Bat hepevirus BatHEV/BS7/GE/2009	LITSCGLKLL VQ-FAG-IG- ---VFSHYIV	APGEG---VV	KDLLRTWGRM	TEKNFS-DSE	RSHDL CVA AQ	DFVNSVTSQG	K-
HQ731075, Piscihepevirus, Cutthroat trout virus Heenan88	FISQNLVLMK QK-TSH-LG- ---SFTCSLV	TR-TS---YV	PDVIRFACRL	NNKDFT-DTK	RIEDLQRATD	DFIKRATVTT	N-
M15240, Rubivirus, Rubella virus F-therin	EVGLFGFHIP VKHVTPTP- ---SFCGHVG	TAAG----LF	HDVMHQAIKV	LCRRFD--PD	VLEEQQVALL	DRLRGVYAAL	PD
MN547623, Rubivirus, Ruhugu virus Cyclops leaf-nosed bat/2017/Uganda	ELGLYGFKIP VKHVATATP- ---SFCGHVG	SADG----LF	ADVLHLAIKL	LCRKWD--PE	VLGEQQTAML	DRLMTVYAAL	PA
MN552442, Rubivirus, Rustrela virus Donkey/19_041-1/2019/Germany	ELDLPGFVIP IKRVATATP- ---SFCGQLG	SEAG----LF	VDVLHLAVKL	LCRRVD--PA	LETERQEAML	DKMKRVYGAL	PD
TkHLV-22 MR233-17E/663	LQNAFGFRLK ED-FGN-PA- ---EFICNFI	TDYG----FF	PDVVRRSARY	ISKVYQ-SVE	DFQLTRDNIR	DAVSTVTSDA	--
HHLV-8 MR644-18E/220	MKAALGASLK MH-QGP-IG- ---DFVGYII	QDFS----LR	PDIFRLCAKV	TSRK FALGTS	VPNGLKAVYE	QEYPALRN--	--
TkHLV-32 MR233-17E/3264	MKAALGAALK VH-EGP-IG- ---DFVGYII	QDFS----LR	PDIFRLSAKV	ISRK FSLGTS	VSAGLRAAYE	ADYPELKS--	--
MZ209973 Sanya hepevirus 1 TSYSY114	LKKVFGAQLK LH-EGE-IA- ---DFVGFLI	KDWR----AY	PDIFRLSAKL	LNRRFVDRCD	VPKQTAGSYV	SACKPIGR--	--
TkHLV-33 MR233-17E/476	VEKLFGEFEVK FG-AGE-IG- ---DFAGNVF	ANGK----VY	TDVMRMTAKT	MCRRFL-LNR	ALSKEQLAEM	VADGYTPAE--	--
KX883762 Changjiang hepe-like virus 1 CJLX30636	AEDMFGVQVK TG-AGV-VG- ---SFVGILY	HEGA----VV	PDLLRMTGKF	LDRQIY-SEA	NIPKLDKMTL	EEEGYKEHE--	--
KX883749 Beihai barnacle virus 3 BHTH16145	AQDLFDMVIK HEFTHN-IG- ---EFQGYLF	VAEANI PKFV	PNIVKRVTK E	LGRAYK--PQ	DVKSQNKLVL	QELREMGVHG	--
KX883748 Beihai hepe-like virus 11 BHTH16134	AEKLLCMEYK YHRVNHVAGG	AAADFTGFTA	HDLAGTVREF	PDLPKRAAKE	LTRKYV----	PFEDN--PQP	EDLK-----
MW897133 Beijing sediment hepe-like virus 1165b-k141_200769	AEEMFGVQVK TG-AGA-IG- ---SFVGILV	HEGA----VV	PDLLRMTGKF	LERQYF-SEA	NVPKIDRL EL	ENQGF RDNE--	--
MW897139 Sichuan sediment hepe-like virus 2 R45-k141_489063	TEELFGVKIK VE-HGE-VA- ---SFIGYLI	KD-----	-----	-----	-----	-----	--
MW897138 Xinjiang hepe-like virus 3 184-k141_405286	VEELFGVQIK HH-SGK-IG- ---SFVGYLL	HAGR----MV	PDLFRITGKF	MARRLF-ANR	AIDAAAELEM	KALGYRGAD--	--

Supplementary Table 1: Virus strains										
Virus	GenBank accession number	Strain designation	Genome size	Coverage	Included in phylogenetic analyses of			Presence of contigs with >95% nt identity		Biological Sample (NCBI SRA)
					VMethylTr	Helicase	RdRp	Havel river sample	Teltowkanal samples	
A. Havel hepe-like viruses										
HHLV-1	OP699055	MR644-18E/91	8936 nt (cg)	43.9	+	+	+		TkHLV-2	MR644-18 (SRR21869516)
HHLV-2	OP699056	MR644-18E/36	8932 nt (cg)	18.0	+	+	+		TkHLV-1	MR644-18 (SRR21869516)
HHLV-3	OP699057	MR644-18E/70	9102 nt (cg)	13.1	+	+	+		TkHLV-16	MR644-18 (SRR21869516)
HHLV-4	OP699058	MR644-18E/1928	7902 nt (cg)	73.6	+	+	+		+	MR644-18 (SRR21869516)
HHLV-5	OP699059	MR644-18E/7839	7612 nt (cg)	20.9	+	+	+		TkHLV-20	MR644-18 (SRR21869516)
HHLV-6	OP699060	MR644-18E/449	5661 nt (cg)	13.0	+	+	+		TkHLV-28	MR644-18 (SRR21869516)
HHLV-7	OP699061	MR644-18E/184	4894 nt (par)	14.6	+	+	n.i.		-	MR644-18 (SRR21869516)
HHLV-8	OP699062	MR644-18E/220	4478 nt (par)	13.3	+	+	+		-	MR644-18 (SRR21869516)
HHLV-9	OP699063	MR644-18D/14400	1780 nt (par)	9.2	n.i.	n.i.	+		-	MR644-18 (SRR21869516)
HHLV-10	OP699064	MR644-18E/1018	2088 nt (par)	7.7	n.i.	n.i.	+		+	MR644-18 (SRR21869516)
HHLV-11	OP699065	MR644-18E/3095	1287 nt (par)	7.8	n.i.	n.i.	n.i.		-	MR644-18 (SRR21869516)
HHLV-12	OP699066	MR644-18E/3665	1204 nt (par)	8.0	n.i.	n.i.	n.i.		-	MR644-18 (SRR21869516)
HHLV-13	OP699067	MR644-18E/1769	1608 nt (par)	5.6	n.i.	+	n.i.		-	MR644-18 (SRR21869516)
HHLV-14	OP699068	MR644-18E/1089	5064 nt (par)	15.1	n.i.	n.i.	+		-	MR644-18 (SRR21869516)
HHLV-15	OP699069	MR644-18E/1511	1724 nt (par)	8.5	n.i.	+	+		-	MR644-18 (SRR21869516)
HHLV-16	OP699070	MR644-18E/3953	1375 nt (par)	4.4	+	n.i.	n.i.		+	MR644-18 (SRR21869516)
HHLV-17	OP699071	MR644-18E/1353	1819 nt (par)	47.2	n.i.	n.i.	n.i.		-	MR644-18 (SRR21869516)
HHLV-18	OP699072	MR644-18E/835	2309 nt (par)	14.7	n.i.	n.i.	n.i.		-	MR644-18 (SRR21869516)
HHLV-19	OP699073	MR644-18E/111514	2141 nt (par)	15.3	n.i.	+	+		-	MR644-18 (SRR21869516)
HHLV-20	OP699074	MR644-18E/1277	1874 nt (par)	7.4	n.i.	n.i.	n.i.		-	MR644-18 (SRR21869516)
HHLV-21	OP699075	MR644-18E/2015	1526 nt (par)	10.5	n.i.	+	n.i.		TkHLV-45	MR644-18 (SRR21869516)
HHLV-22	OP699076	MR644-18E/2223	1468 nt (par)	14.7	n.i.	n.i.	n.i.		-	MR644-18 (SRR21869516)
HHLV-23	OP699077	MR644-18E/31806	1541 nt (par)	3.1	n.i.	n.i.	n.i.		-	MR644-18 (SRR21869516)
HHLV-24	OP699078	MR644-18E/981	2125 nt (par)	5.6	+	n.i.	n.i.		+	MR644-18 (SRR21869516)
HHLV-25	OP699079	MR644-18E/1537	1709 nt (par)	9.7	+	n.i.	n.i.		+	MR644-18 (SRR21869516)
HHLV-26	OP699080	MR644-18E/2258	1459 nt (par)	6.6	+	n.i.	n.i.		-	MR644-18 (SRR21869516)
HHLV-27	OP699081	MR644-18E/3029	1300 nt (par)	4.9	n.i.	n.i.	n.i.		TkHLV-48	MR644-18 (SRR21869516)
HHLV-28	OP699082	MR644-18E/3455	1231 nt (par)	6.7	n.i.	n.i.	n.i.		-	MR644-18 (SRR21869516)
HHLV-29	OP699083	MR644-18E/5050	1067 nt (par)	7.4	n.i.	n.i.	n.i.		-	MR644-18 (SRR21869516)
HHLV-30	OP699084	MR644-18E/5051	1067 nt (par)	8.0	n.i.	n.i.	n.i.		-	MR644-18 (SRR21869516)
HHLV-31	OP699085	MR644-18E/5834	1006 nt (par)	5.1	n.i.	+	+		-	MR644-18 (SRR21869516)
HHLV-32	OP699086	MR644-18E/1558	1702 nt (par)	12.1	n.i.	+	+		-	MR644-18 (SRR21869516)
HHLV-33	OP699087	MR644-18E/6209	980 nt (par)	6.8	+	n.i.	n.i.		-	MR644-18 (SRR21869516)
HHLV-34	OP699088	MR644-18E/3095	1287 nt (par)	6.1	n.i.	n.i.	n.i.		-	MR644-18 (SRR21869516)
HHLV-35	OP699089	MR644-18E/4892	1080 nt (par)	7.3	n.i.	n.i.	n.i.		-	MR644-18 (SRR21869516)
HHLV-36	OP699090	MR644-18E/6360	970 nt (par)	7.5	n.i.	n.i.	n.i.		-	MR644-18 (SRR21869516)
HHLV-37	OP699091	MR644-18D/77135	1011 nt (par)	4.4	n.i.	n.i.	n.i.		TkHLV-33	MR644-18 (SRR21869516)
HHLV-38	OP699092	MR644-18E/10756	1964 nt (par)	7.4	n.i.	n.i.	n.i.		TkHLV-6	MR644-18 (SRR21869516)
B. Teltowkanal hepe-like viruses										
TkHLV-1	OP699093	MR233-17E/398	8934 nt (cg)	62.1	+	+	+	HHLV-2		MR233-17 (SRR21869517)
TkHLV-2	OP699094	MR233-17E/949	6377 nt (par)	13.6	+	+	+	HHLV-1		MR233-17 (SRR21869517)
TkHLV-3	OP699095	MR233-17E/415	8896 nt (cg)	76.9	+	+	+	+		MR233-17 (SRR21869517)
TkHLV-4	OP699096	MR233-17E/341	9208 nt (cg)	761.9	+	+	+	+		MR233-17 (SRR21869517)
	OP699097	20161205-D/3212	8995 nt (cg)	120.6	n.i.	n.i.	n.i.	-		20161205 (SRR21869518)
TkHLV-5	OP699098	MR233-17E/15319	1572 nt (par)	7.4	n.i.	n.i.	n.i.	-		MR233-17 (SRR21869517)
TkHLV-6	OP699099	MR233-17E/27572	2715 nt (par)	13.3	+	n.i.	n.i.	HHLV-38		MR233-17 (SRR21869517)
TkHLV-7	OP699100	MR233-17E/19201	1403 nt (par)	5.8	+	n.i.	n.i.	+		MR233-17 (SRR21869517)
	OP699101	MR233-17E/430	8817 nt (cg)	566.0	+	+	+	+		MR233-17 (SRR21869517)
	OP699102	20161205-D/10029	8717 nt (par)	147.1	n.i.	n.i.	n.i.	-		20161205 (SRR21869518)
TkHLV-9	OP699103	MR233-17E/1114	5982 nt (par)	37.5	+	+	+	-		MR233-17 (SRR21869517)
TkHLV-10	OP699104	MR233-17E/49852	7452 nt (par)	15.4	+	+	+	-		MR233-17 (SRR21869517)
TkHLV-11	OP699105	MR233-17E/15966	1539 nt (par)	7.7	n.i.	n.i.	n.i.	-		MR233-17 (SRR21869517)
	OP699106	20161205-D/2309	4574 nt (par)	414.9	n.i.	n.i.	n.i.	-		20161205 (SRR21869518)
	OP699107	MR233-17E/582	8061 nt (cg)	1987.1	+	+	+	+		MR233-17 (SRR21869517)
TkHLV-13	OP699108	MR233-17E/1063	6083 nt (par)	15.0	+	+	+	+		MR233-17 (SRR21869517)
TkHLV-14	OP699109	MR233-17E/18190	1442 nt (par)	8.8	n.i.	n.i.	n.i.	+		MR233-17 (SRR21869517)
TkHLV-15	OP699110	MR233-17E/10927	1864 nt (par)	25.3	n.i.	n.i.	n.i.	-		MR233-17 (SRR21869517)
	OP699111	20161205-D/18546	6893 nt (par)	23.3	n.i.	n.i.	n.i.	HHLV-4		20161205 (SRR21869518)
TkHLV-16	OP699112	MR233-17E/325	9268 nt (cg)	189.7	+	+	+	HHLV-4		MR233-17 (SRR21869517)
TkHLV-17	OP699113	MR233-17E/11883	1788 nt (par)	6.9	n.i.	n.i.	+	-		MR233-17 (SRR21869517)
	OP699114	20161205-D/8739	9158 nt (cg)	129.1	n.i.	n.i.	n.i.	HHLV-27		20161205 (SRR21869518)
TkHLV-18	OP699115	MR233-17E/349	9184 nt (cg)	439.9	+	+	+	HHLV-27		MR233-17 (SRR21869517)
TkHLV-19	OP699116	MR233-17E/20784	1348 nt (par)	5.34	n.i.	n.i.	n.i.	+		MR233-17 (SRR21869517)
	OP699117	MR137-16D/77	7678 nt (cg)	681.8	n.i.	n.i.	n.i.	HHLV-5		MR137-16 (SRR21869519)
TkHLV-20	OP699118	MR233-17E/643	7758 nt (cg)	4444.4	+	+	+	HHLV-5		MR233-17 (SRR21869517)
TkHLV-21	OP699119	MR233-17E/1352118	5461 nt (par)	63.0	+	+	+	-		MR233-17 (SRR21869517)
TkHLV-22	OP699120	MR233-17E/663	7653 nt (cg)	448.9	+	+	+	+		MR233-17 (SRR21869517)
TkHLV-23	OP699121	MR233-17E/14711	1602 nt (par)	8.2	n.i.	n.i.	n.i.	-		MR233-17 (SRR21869517)
TkHLV-24	OP699122	MR233-17E/708	7785 nt (par)	71.5	+	+	+	+		MR233-17 (SRR21869517)
TkHLV-25	OP699123	MR233-17E/401	8927 nt (cg)	37.9	+	+	+	-		MR233-17 (SRR21869517)
TkHLV-26	OP699124	MR233-17E/7023	2338 nt (par)	30.8	n.i.	n.i.	n.i.	-		MR233-17 (SRR21869517)
TkHLV-27	OP699125	MR233-17E/7149	2315 nt (par)	41.4	n.i.	n.i.	n.i.	-		MR233-17 (SRR21869517)
TkHLV-28	OP699126	MR233-17E/11756	5672 nt (par)	16.6	+	+	+	HHLV-6		MR233-17 (SRR21869517)
TkHLV-29	OP699127	MR233-17E/5607	2640 nt (par)	13.9	+	+	n.i.	+		MR233-17 (SRR21869517)
TkHLV-30	OP699128	MR233-17E/17006	2613 nt (par)	6.0	n.i.	n.i.	+	+		MR233-17 (SRR21869517)
TkHLV-31	OP699129	MR233-17E/26350	1200 nt (par)	14.5	n.i.	n.i.	+	-		MR233-17 (SRR21869517)
TkHLV-32	OP699130	MR233-17E/3264	9266 nt (cg)	18.5	+	+	+	+		MR233-17 (SRR21869517)
TkHLV-33	OP699131	MR233-17E/476	8587 nt (cg)	52.7	+	+	+	HHLV-37		MR233-17 (SRR21869517)
	OP699132	MR233-17E/510	8452 nt (cg)	237.1	+	+	+	+		MR233-17 (SRR21869517)
TkHLV-34	OP699133	MR137-16D/328	8220 nt (par)	243.6	n.i.	n.i.	n.i.	+		MR137-16 (SRR21869519)
	OP699134	20161205-D/3071	1203 nt (par)	21.8	n.i.	n.i.	n.i.	+		20161205 (SRR21869518)

TkHLV-35	OP699135	MR233-17E/33892	1062 nt (par)	5.3	n.i.	n.i.	n.i.	-		MR233-17 (SRR21869517)
TkHLV-36	OP699136	MR233-17E/595	8024 nt (cg)	1217.9	+	+	+	+		MR233-17 (SRR21869517)
	OP699137	20161205-D/4059	7940 nt (cg)	224.7	n.i.	n.i.	n.i.	+		20161205 (SRR21869518)
TkHLV-37	OP699138	MR233-17E/10440	1908 nt (par)	9.8	n.i.	+	n.i.	-		MR233-17 (SRR21869517)
TkHLV-38	OP699139	MR233-17E/6603	2417 nt (par)	7.2	+	+	n.i.	+		MR233-17 (SRR21869517)
TkHLV-39	OP699140	MR233-17E/503	8483 nt (cg)	371.4	+	+	+	+		MR233-17 (SRR21869517)
TkHLV-40	OP699141	MR233-17E/490	8541 nt (par)	81.5	+	+	+	+		MR233-17 (SRR21869517)
TkHLV-41	OP699142	MR233-17E/1088	6035 nt (par)	21.2	n.i.	+	+	-		MR233-17 (SRR21869517)
TkHLV-42	OP699143	MR233-17E/9232	2031 nt (par)	9.8	n.i.	n.i.	+	-		MR233-17 (SRR21869517)
TkHLV-43	OP699144	MR233-17E/10363	1915 nt (par)	9.4	n.i.	n.i.	n.i.	+		MR233-17 (SRR21869517)
TkHLV-44	OP699145	MR233-17E/406908	1458 nt (par)	14.1	+	n.i.	n.i.	-		MR233-17 (SRR21869517)
TkHLV-45	OP699146	MR233-17E/1231	5700 nt (par)	15.8	n.i.	+	+	HHLV-21		MR233-17 (SRR21869517)
TkHLV-46	OP699147	MR233-17E/14194	1631 nt (par)	9.5	n.i.	n.i.	n.i.	-		MR233-17 (SRR21869517)
C. Astro-like viruses										
HALV-1	OP699148	MR644-18E/601	3273 nt (par)	7.2	n.i.	n.i.	+		+	MR644-18 (SRR21869516)
HALV-2	OP699149	MR644-18E/1796	1589 nt (par)	7.1	n.i.	n.i.	+		+	MR644-18 (SRR21869516)
HALV-3	OP699150	MR644-18E/25367	539 nt (par)	2.6	n.i.	n.i.	+		-	MR644-18 (SRR21869516)
TkALV-1	OP699151	MR233-17E/955	6353 nt (par)	153.6	n.i.	n.i.	+	+		MR233-17 (SRR21869517)
TkALV-2	OP699152	MR233-17E/7172	2312 nt (par)	2.8	n.i.	n.i.	n.i.	-		MR233-17 (SRR21869517)
TkALV-3	OP699153	MR233-17E/832	6815 nt (par)	440.6	n.i.	n.i.	+	+		MR233-17 (SRR21869517)

Abbreviations: cg, almost complete genome; par, partial genome; n.i., not included; RdRp, RNA-dependent RNA polymerase; VMethylTr, viral methyltransferase