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Novel Gammaherpesvirus Functions 1 Encoded by Bovine herpesvirus (Bovine Lymphotropic virus)

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Short Communication

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Abbreviations: GHV, gammaherpesvirus; ODC, ornithine decarboxylase.

Abstract

The genus *Macavirus* of the subfamily *Gammaherpesvirinae* includes viruses that infect lymphoid cells of domestic and wild ruminants and swine, causing asymptomatic latent infections in reservoir hosts. Here, we describe the genome of bovine herpesvirus 6 (BoHV-6), a macavirus ubiquitous in healthy cattle populations. The BoHV-6 genome exhibited architecture conserved in macaviruses, including a repetitive H-DNA region and unique, 141 kilobase pair L-DNA region predicted to encode 77 genes. BoHV-6 encoded in variable genomic regions a novel complement of genes relative to other characterized macaviruses, likely contributing to distinctive aspects of BoHV-6 infection biology and host range. Most notably, BoHV-6 encoded the first herpesviral protein (Bov2.b2) similar to cellular ornithine decarboxylase, an enzyme that catalyzes the first and rate-limiting step in the biosynthesis of polyamines. Bov2.b2 conceivably mediates a novel mechanism by which BoHV-6 promotes cell cycle-dependent viral replication.

The subfamily *Gammaherpesvirinae* includes human and animal herpesviruses that preferentially infect lymphoid cells, where they establish latent infections and, for select viruses, cause malignant cell transformation. The gammaherpesvirus (GHV) genus *Macavirus* currently includes nine species, the type species alcelaphine herpesvirus 1 (AlHV-1), AlHV-2, ovine herpesvirus 2 (OvHV-2), caprine herpesvirus 2 (CprHV-2), bovine herpesvirus 6 (BoHV-6), hippotragine herpesvirus 1, and suid herpesviruses (SuHV; previously known as porcine lymphotropic viruses) 3, 4 and 5. Related yet uncharacterized viruses have been detected in blood from a wide range of healthy wild ruminants (Li *et al.*, 2005).

Macaviruses infect domestic and wild ruminants and swine, causing asymptomatic infections in reservoir hosts (Ackermann, 2006). When infecting other species, however, macaviruses can cause disease. For example, AlHV-1 and OvHV-2 cause subclinical infections in reservoir wildebeest and sheep, respectively, but in cattle and other ruminants cause malignant catarrhal fever, an often-fatal lymphoproliferative disease characterized by accumulation of lymphocytes in a variety of organs (Russell *et al.*, 2009; O'Toole & Li, 2014). Other macaviruses, including BoHV-6 and SuHV-3, 4 and 5, have not been associated with natural disease in either reservoir or heterologous species despite being prevalent in cattle and swine (Van der Maaten *et al*., 1972; Rovnak *et al.*, 1998; Ehlers *et al.*, 1999; Chmielewicz *et al.*, 2003).

BoHV-6, previously known as bovine lymphotropic virus, was first isolated from leukocytes of lymphosarcomatous cattle in the United States, and subsequently reported in Europe and Canada (Van der Maaten *et al.*, 1972; Cobb *et al.*, 2006; Gagnon *et al.*, 2010; Garigliany *et al.*, 2013; Kubiś *et al.*, 2013). The US isolate, strain Pennsylvania 47, is strongly cell-associated, syncytiogenic, slow to grow in tissue culture, and serologically related to bovine GHVs (Van der Maaten *et al.*, 1972; Osorio *et al.*, 1985). Phylogenetic analysis showed that BoHV-6, together with current macaviruses, represented a group distinct from other GHVs (Rovnak *et al.*, 1998; Chmielewicz *et al.*, 2001). Notably, BoHV-6-specific DNA sequences were detected in peripheral blood mononuclear cells from 52-87% and 30% of healthy adult cattle and calves sampled, respectively (Rovnak *et al.*, 1998; Collins *et al.*, 2000; Kubiś *et al.*, 2013). Together, these results indicate that BoHV-6 is ubiquitous in healthy cattle and suggest that infection occurs at a young age. Although BoHV-6 DNA has been detected in cows with reproductive conditions, experimental data supporting a causative association between BoHV-6

and disease is lacking (Cobb *et al.*, 2006; Garigliany *et al.*, 2013; Banks *et al.*, 2008; Gagnon *et al.*, 2010). The transmission mode of BoHV-6 is unknown.

Macaviruses genomes (AlHV-1 and OvHV-2) indicate overall structure similar to genomes of viruses from the genus *Rhadinovirus* and the reference sequence from herpesvirus saimiri (HVS) (Albrecht *et al.*, 1992). This includes a single, unique coding region of low G+C content (L-DNA) and a repetitive region of high G+C (H-DNA). Comparative analyses demonstrated organizational conservation, but sequence divergence within coding regions and variable gene content located between larger conserved regions in the L-DNA fragment (Ensser *et al.*, 1997; Hart *et al.*, 2007; Goltz *et al.*, 2002). These data provide a basis for understanding differences in macavirus infection biology. Here we present genomic sequence and analysis of the genome of the macavirus BoHV-6, strain Pennsylvania 47.

High throughput sequencing of BoHV-6 was conducted to assemble complete BoHV-6 L-DNA genomic sequences. Total DNA was extracted from the supernatant of Madin Darby Bovine Kidney cells (MDBK; ATCC® CCL22™) infected with BoHV-6 strain Pennsylvania 47 using the QIAamp DNA Blood Mini Kit (Qiagen, Carlsbad, CA). DNA was used for library preparation using the Nextera XT sample preparation kit (Illumina, San Diego, CA). Sequencing was performed using the Illumina MiSeqV3 platform at the University of Illinois Biotechnology Center, and data were assembled with Ray (Boisvert *et al.*, 2010). The BoHV-6 sequence was deposited in GenBank under accession no. KJ705001.

Overall the BoHV-6 genome was similar in structure to other macavirus genomes (Essner *et al.*, 1997; Hart *et al.*, 2007), including unique L-DNA coding sequences and a repetitive H-DNA region with repeats of 1022 base pairs (bp). Data resolved the genome except across the H-DNA repeat, of which two copies assembled at each contig termini, yielding a final linear contig of 144898bp. Mapping data (1,128,744 paired-end 250bp reads mapped) to the assembled contig (Gordon & Green, 2013) allowed estimation of at least ten copies of the H-DNA repeat in the BoHV-6 genome. Additional high-scoring repeat sequences within the L-DNA segment at positions 15.5-17.7 kbp, 46.3-47.2 kbp, and 130.3-134 kbp were identified (Rice *et al.*, 2000; Betley *et al.*, 2002).

Coding potential of BoHV-6 was similar to sequenced macaviruses. ORFs were identified using EMBOSS and GeneMarkS and analyzed using BLAST and FASTA packages (Altschul *et al.*, 1990; Besemer *et al.*, 2001; Pearson & Lipman, 1988). BoHV-6 was predicted to contain 77 genes, with the majority representing homologues of conserved rhadinovirus genes (Albrecht *et al.*, 1992, Ensser *et al.*, 1997, Hart *et al.*, 2007) (Table 1). BoHV-6 contained ORFs (Bov2, ORF29, ORF40/ORF41, ORF50, Bov6, Bov8, ORF57) predicted to be spliced based on conservation with OvHV-2 and other GHV (Wang and Marín, 2006) (Table 1). BoHV-6 homologues of macavirus genes were similarly arranged in syntenic blocks of conserved GHV core genes, with rhadinovirus-specific genes and noncoding regions interspersed between and adjacent to syntentic blocks (Table 1). The sizes of these variable regions differed between macaviruses, with the block I/II and block IV/right terminal junction sequences differing in size by up to approximately 5.5kbp. Notably, the variable left-end L-DNA sequence was twice as large in BoHV-6 as in OvHV-2 and AlHV-1 (approximately 26kbp vs 12kbp). Several ORFs unique to BoHV-6 were identified in this region; however, the variable left-end sequence lacking obvious coding potential remained large (approximately 16 kbp) relative to OvHV-2 and AlHV-1.

BoHV-6 herpesvirus gene orthologues were generally most similar to those from AlHV-1 and OvHV-2, sharing an average of 50% amino acid identity. This was consistent with previous analysis of nearly identical sequence from the virus initially characterized as BoHV-6 (99% identity to GenBank accession no. AF031808 within the DNA polymerase gene) (Rovnak et al., 1998), which demonstrated BoHV-6 to cluster within the macavirus tree, closer to but distinct from a AlHV1/OvHV2/CprHV2 subgroup relative to SuHV-3, 4 and 5 (Chmielewicz *et al.*, 2003; Ehlers & Lowden, 2004). This relationship was confirmed by analysis of multigene data available using genomic sequence presented here, suggesting that BoHV-6 is a macavirus distinct from porcine and AlHV-1/OvHV-2 sublineages (Fig. 1). ORF019 through ORF046 were concatenated, aligned (Katoh & Kuma, 2002), screened for conserved sequence (Castresana, 2000), and 7710 aligned amino acids used for maximum likelihood analysis (Guindon & Gascuel, 2003). Novel viruses that group closely with BoHV-6 relative to OvHV-2 and AlHV1, defining sublineages of ruminant herpesviruses, have been described (Ehlers & Lowden, 2004). Thus, the BoHV-6 sequence presented here likely represents a prototype for one of these sublineages.

BoHV-6 contains a novel complement of genes relative to characterized macaviruses and GHVs. These included Bov2, Bov4.5, Bov5, Bov6, Bov7, Bov8, and Bov9 (Table 1). Like AlHV-1 and OvHV-2, BoHV-6 contained two genes, Bov4.5 and Bov9, encoding Bcl-2 homologues, with Bov4.5 a homologue of EBV BALF1, known to affect apoptosis *in vitro* and *in vivo* (Bellows *et al.*, 2002). Bov5 encoded a G protein-coupled receptor (GPCR) homolog of GHV proteins, including those affecting viral oncogenesis and pathobiology as constitutively active GPCRs and/or mediators of immune evasion (Paulsen *et al.*, 2005; Zuo *et al.*, 2009). Bov8 was a homologue of putative macavirus glycoproteins and positionally similar to rhadinovirus cell-binding glycoproteins, including BoHV-4 Bo10 which is alternatively spliced to affect cell tropism (Machiels *et al.*, 2011; Machiels *et al.*, 2013). Bov2 and Bov6 shared limited similarity to spliced GHV genes which encode known or predicted transcription factors. Notably, BoHV-6 contained sequences (position 10594-10822) similar to the C-terminal two (of five) exons of cellular and OvHV-2 (Ov2.5) Interleukin-10 (IL-10). While C-terminal peptides of cellular IL-10 may exhibit a range of immunological properties (Gesser, 1997), presence of these sequences in BoHV-6 in the absence of obvious N-terminal exons suggests that novel spliced viral IL-10 variants may occur in BoHV-6. BoHV-6 lacked obvious homologues of genes present in AlHV-1 and OvHV-2, including the semaphorin present in other GHVs and speculated to involve modulation of host immune responses. Absent in BoHV-6 were macavirus ORFs of unknown function, including A1 from AlHV-1, ORF3.5 putative secreted protein from OvHV-2, ORF8.5 repeat protein from OvHV-2, and A10/Ov10 putative nuclear protein from AlHV-1 and OvHV2. Contributing to the novel BoHV-6 gene complement were BoHV-6 ORFs absent in other macaviruses (Table 1). Four of these were small, novel ORFs dispersed across the large left terminal genomic region. Other BoHV-6-specific genes were located between conserved blocks II/III (Bov11.b2) and in the right terminal region. These novel ORFs conceivably confer novel function to BoHV-6 relative to macavirus relatives.

Most notably, BoHV-6 encoded a protein (Bov2.b2) similar to cellular ornithine decarboxylase (ODC). ODC-like proteins include ODC, which catalyzes the first and rate-limiting step in the biosynthesis of polyamines, and antizyme inhibitor (AZI) of ODC, an ODC-like protein involved in ODC regulation but lacking decarboxylase activity. Polyamines are small cationic organic molecules affecting many cellular processes, including cell proliferation (Cohen, 1998).

Active ODC is a homodimer bound to essential cofactor pyridoxal phosphate (PLP). ODC is regulated at the transcriptional, translational, and posttranslational levels, the latter operating through protein degradation (Pegg, 2006). ODC is highly labile and has a very short half-life, with its abundance regulated by a family of polyamine-induced proteins called antizymes, which bind to and inactivate ODC by preventing dimerization and targeting enzyme monomers for ubiquitin-independent, 26S proteasome-dependent proteolysis (For reviews, see Coffino, 2001; Pegg, 2006). In ODC, an N-terminal domain is required for high affinity antizyme binding, while C-terminal PEST element and adjacent sequences control antizyme-mediated proteolysis (Ghoda *et al*., 1989, Li & Coffino, 1992). Indirect control of ODC activity is mediated by AZI, which binds antizyme to release, and effectively prevent degradation of, ODC. AZI thus is a positive regulator of the polyamine pathway.

Bov2.b2 was 53-56% and 42-44% amino acid identical to vertebrate ODC and AZI, respectively, and encoded 238-residue N-terminal PLP-binding (PFAM PF02784.8) and 114-residue C-terminal (PFAM PF00278.14) domains. Bov2.b2 contained the 18 amino acids required for decarboxylase activity (Fig. 2), including residues homologous to human Lys69, critical for PLP binding, and Cys360, believed to perform the nucleophilic attack of ornithine, and several residues involved in PLP stabilization, substrate interaction, protein dimerization, and domain structure (Peg, 2006; Ivanov *et al.*, 2010). Among the latter are four residues (Asp88, Arg154, Arg277, and Tyr389) that are not conserved in AZI. Likewise, the putative antizyme binding site in Bov2.b2 was more similar to the homologous site in ODC than in AZI (68% vs 54% amino acid identity, respectively). The only residue directly contacting substrate and differing between Bov2.b2 and mammalian ODC was at position 332 (Figure 2), one of several residues affecting substrate preference (Shah *et al.*, 2004). Similar to AZI, Bov2.b2 lacked 23 C-terminal residues that comprise most of the mammalian ODC PEST element. Also lacking in Bov2.b2 and AZI were the last five amino acids of mammalian ODC (ASINV), which have been shown to affect ODC stability (Ghoda *et al.*, 1989; Ghoda *et al.*, 1992; Macrae & Coffino, 1987). Together, data suggest that Bov2.b2 encodes a *bona fide* decarboxylase that might exhibit enhanced stability in virus-infected cells relative to host ODC. However, a possible role for Bov2.b2 as a novel AZI can not be excluded.

Bov2.b2 is the first reported ODC-like protein encoded in a fully nuclear-replicating

virus. Viral ODC-like genes have been previously reported only in three nucleocytoplasmic large DNA viruses (NCLDVs). These include chlorella viruses, which infect chlorella-like green algae, Yoka poxvirus, a virus isolated from mosquitos, and mimivirus *Cafeteria roenbergensis* virus, which infects zooplankton (Lu *et al.*, 1996; Zhao *et al.*, 2011; Fisher *et al.*, 2010). Catalytic activity has only been demonstrated for decarboxylase of Paramecium bursaria chlorella virus 1 (PBCV-1) (Morehead *et al*, 2002). Bov2.b2 was less similar to NCLDV ODCs (29% and 40% amino acid identity) than to mammalian ODC, however, likely reflecting independent acquisitions of host genes and potentially a novel role during infection.

The role(s) of viral ODC/AZI (or even polyamines) during infection remains unknown. In terms of polyamine dynamics, viral expression of either an ODC or an AZI should in principle lead to increased polyamine synthesis. Polyamines are essential molecules implicated in various cellular functions, including cell cycle and proliferation. The pro-proliferative role of polyamines has received particular attention, as ODC and polyamines are required for G_1 progression and cell transformation, and ODC is a critical target for the oncogene Myc, known for its ability to drive quiescent cells into the cell cycle (Auvinen *et al.*, 1992; Gerner & Meyskens, 2004; Nilsson *et al.*, 2004). Responsiveness of mammalian ODC to Myc relies on two conserved Myc-binding sites (E boxes, CAYGTG) mapping to ODC regulatory sequences (Bello-Fernandez *et al.*, 1993). Notably, three E-boxes are found in sequences upstream *Bov2.b2* at positions -1770 and -2352 (CACGTG), and at position -471 (CATGTC) relative to the translational start, suggesting that Bov2.b2 expression is controlled by Myc. Herpesviruses are known for inducing cellular changes associated with cell cycle entry, thus creating an environment suitable for viral DNA replication. Such reprogramming seems particularly important for viruses infecting quiescent cells. Conceivably, either as an ODC or as an AZI, Bov2.b2 might mediate a novel, perhaps complementary strategy by which BoHV-6 promotes cell cycle-dependent viral replication.

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Figure Legends

Figure 1. Phylogenetic analysis of BoHV-6. Maximum likelihood tree from concatenated protein datasets (ORF19 through ORF46). GenBank accession nos. are noted with appropriate taxa. Bootstrap analysis (100 replicates) indicated 100% support at all nodes.

Figure 2. Clustalw alignment of BoHV-6 and select mammalian ODC amino acid sequences. Numbers on the right indicate amino acid positions for human ODC. Full- and dash-lined boxes indicate PLB-binding and C-terminal domains, respectively. Columns highlighted in grey indicate key residues associated with decarboxylase activity as determined by crystalographic and mutagenesis analysis of ODC (Ivanov et al., 2010). The underlined sequence corresponds to the antizyme binding site as determined for mouse ODC; indicated with an arrow (↓) is position 332 associated with substrate preference. Asterisks [*], colons [:], and periods [.] below the alignment indicate fully, strongly, and weakly conserved, residues, respectively.

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TABLE 1: BoHV6 ORFs

Gene	BoHV-6					Description/Putative function	OvHV-2 ϕ			AlHV-1			SuHV-4		
Block	ORF *	Exon	Str ¶	Nucleotide Position	len		len	% Id #	Hit len	len	% Id	Hit len	len	% Id	Hit len
Left	Bov1.b1		+	2840-3331	164										
	Bov1.b2		+	4522-5184	221										
	Bov2	2	-	9763-8687											
	Bov2	1	-	10092-9874	432	Basic leucine-zipper motif protein	186	26	97	199	30	86			
	Bov2.5		+	10763-10822	20	Interleukin-10 fragment	182	50	14						
	Bov2.b1		-	**17950-17441**	170		390	35	97						
	Bov2.b2		+	20087-21379	431	ornithine decarboxylase									
	Bov2.b3		-	22017-21499	173										
	ORF3		+	24552-28688	1379	tegument protein/v-FGAM-synthetase	1361	37	1324	1369	35	1391	1378	36	1406
	Bov4.5		+	28868-29413	182	vBcl-2; EBV BALF1 homolog	212	34	165	231	35	165	178	38	174
I	ORF6		+	29569-32967	1133	single-stranded DNA binding protein	1129	66	1133	1127	63	1133	1126	59	1133
	ORF7		+	33024-35072	683	terminase subunit	682	54	677	680	55	684	675	55	688
	ORF8		+	35068-37638	857	glycoprotein B	863	65	751	854	62	803	876	59	790
	ORF9		+	37914-40904	997	DNA polymerase	998	67	994	1026	66	996	1004	65	1001

	Bov5		+	41188-42117	310	G protein-coupled receptor	417	42	291	302	41	279	325	32	282
	ORF10		+	42184-43392	403		406	31	391	404	33	402	401	34	405
	ORF11		+	43436-44677	414		410	46	389	406	46	404	409	49	400
	Bov11.b1		+	**47040-47585**	182										
II	ORF17		-	49784-48159	542	protease; capsid protein	552	43	570	524	44	553	500	42	552
	ORF17.5		-	49010-48159	284	capsid scaffold protein	275	33	292	524	34	283	500	34	281
	ORF18		+	49795-50580	262		276	57	259	275	58	259	261	49	260
	ORF19		-	52209-50569	547	tegument protein	559	58	559	556	59	553	549	55	547
	ORF20		-	52750-52043	236		250	53	226	250	52	224	275	54	213
	ORF21		+	52752-54437	562	thymidine kinase	569	41	562	561	37	561	580	47	563
	ORF22		+	54476-56683	736	glycoprotein H	750	45	687	733	44	736	778	46	691
	ORF23		-	57888-56686	401		400	43	400	401	42	401	398	58	401
	ORF24		-	60115-57869	749		729	59	722	745	57	728	736	53	734
	ORF25		+	60117-64205	1363	major capsid protein	1367	69	1365	1370	69	1371	1372	71	1371
	ORF26		+	64264-65175	304	capsid triplex subunit 2	304	63	304	306	63	303	304	59	304
	ORF27		+	65188-66099	304		293	47	290	292	47	291	294	44	292
III	ORF29	2	-	67645-66500											
	ORF30		+	67661-67906	82		83	56	66	85	45	75	79	51	69
	ORF31		+	67834-68502	223		224	52	223	225	56	205	206	53	201
	ORF32		+	68454-69881	476		476	38	475	474	36	492	453	37	395

ORF33		+	69874-70890	339	tegument protein	339	47	339	335	43	339	339	70	339
ORF34		+	71796-72773	326		337	50	337	343	48	331	326	49	317
ORF29	1	-	71797-70883	687	DNA packaging terminase subunit	687	70	688	686	69	688	683	67	681
ORF35		+	72754-73212	153		153	38	152	152	34	149	151	41	148
ORF36		+	73106-74494	463	putative tyrosine kinase	440	48	429	454	49	452	453	48	452
ORF37		+	74430-75884	485	alkaline exonuclease	485	67	485	485	67	485	485	69	485
ORF38		+	75842-76030	63		61	42	57	59	48	58	63	59	59
ORF39		-	77233-76103	377	glycoprotein M	408	68	377	374	71	378	378	61	379
ORF40	1	+	77373-78671	616	helicase-primase complex	634	37	615	478	37	452	455	32	460
ORF40/41	2	+	78752-79300			634	34	44	175	37	61	162	23	99
ORF42		-	80119-79289	277		257	56	241	257	56	231	266	54	245
ORF43		-	81764-80091	558	minor capsid protein	562	71	548	557	73	547	567	65	555
ORF44		+	81754-84114	787	helicase-primase subunit BBLF4	830	71	787	783	70	787	782	66	787
ORF45		-	85006-84188	273		261	30	272	235	26	208	223	36	230
ORF46		-	85798-85043	252	uracil DNA glycosylase	251	66	251	252	68	252	251	66	250
ORF47		-	86210-85782	143		151	41	128	168	36	147	142	35	132
ORF48		-	87606-86221	462		446	30	442	419	30	420	498	36	309
ORF50	1	+	87771-87824	545	R-transactivator	573	40	353	619	42	330	510	42	466
ORF49		-	88865-87879	329	transcriptional control protein Na	324	42	311				309	27	292
ORF50	2	+	88898-90478											

	Bov6	1	+	90829-91395	272		256	48	118	210	16	102	172	33	110
	Bov6	2	+	91493-91597											
	Bov6	3	+	91703-91846											
	Bov7		+	93323-94099	259	putative glycoprotein	121	36	69	243	35	140	234	45	242
	Bov8	1	+	94108-96067	750	putative major envelope glycoprotein	473	27	412	683	25	771	725	24	662
	Bov8	2	+	96163-96452											
IV	ORF52		-	96896-96483	138		136	30	136	125	33	124	136	55	134
	ORF53		-	97296-96976	107		102	71	53	103	47	107			
	ORF54		+	97382-98251	290	dUTPase	293	53	288	298	48	295			
	ORF55		-	98957-98301	219		218	67	210	220	68	210			
	ORF56		+	98956-101448	831	helicase-primase primase subunit BSLF1	837	55	834	837	55	836			
	ORF57	1	+	101595-101646	459	transcriptional control protein Mta	433	44	457	436	43	458			
	ORF57	2	+	101753-103077											
	ORF58		-	104914-103862	351		351	54	351	351	50	350			
	ORF59		-	106043-104919	375	processivity factor	389	58	379	411	55	347			
	ORF60		-	107058-106144	305	ribonucleotide-reductase, small subunit	305	76	305	305	77	305			
	ORF61		-	109491-107140	784	ribonucleotide-reductase, large subunit	785	61	776	780	60	783			
	ORF62		-	110530-109514	339	capsid triplex subunit 1	335	52	329	334	53	328			
	ORF63		+	110532-113318	929	tegument protein	947	49	936	952	46	944			
	ORF64		+	113380-121221	2614	large tegument protein	2624	36	2284	2606	37	741			

	ORF65	-	121874-121266	203	capsid protein	211	35	150	252	29	128
	ORF66	-	123233-121923	437		435	46	433	437	45	434
	ORF67	-	123949-123143	269	tegument protein	258	63	268	263	59	262
	ORF67a	-	124279-123962	106		84	55	84	84	48	84
	ORF68	+	124395-125789	465	putative major envelope glycoprotein	472	51	463	468	52	468
	ORF69	+	125792-126652	287		284	67	283	280	68	261
Right	ORF73	-	**134364-133036**	443	putative immediate early protein	495	38	393	1300	34	252
	ORF75	-	139068-135124	1315	FGAM-synthase	1316	52	1316	1315	50	1326
	Bov9	+	139907-140425	173	Bcl-2	206	29	135	168	27	107
	Bov9.b1	-	141411-140839	191							
	Bov9.b2	-	141765-141421	115							

394 * "ORF" names correspond to numbering of Herpesvirus saimiri homologues present in other macaviruses. "Bov" names correspond to numbering of
395 homologues present in OvHV2 and AlHV1, "BovX.5" names correspond to numbering of homologues in OvHV2, and "BovX.bX numbering corresponds to
396 ORFs unique to BovVH6. ORFs associated with genomic repeats have positions in Bold text

397 ¶ Str, strand

398 § len, length in amino acids

399 # %Id, percent amino acid identity

400 ϕ OvHV-2, AlHV-1, SuHV-3, GenBank Accession nos. AY839756, AF005370, AF478169, respectively

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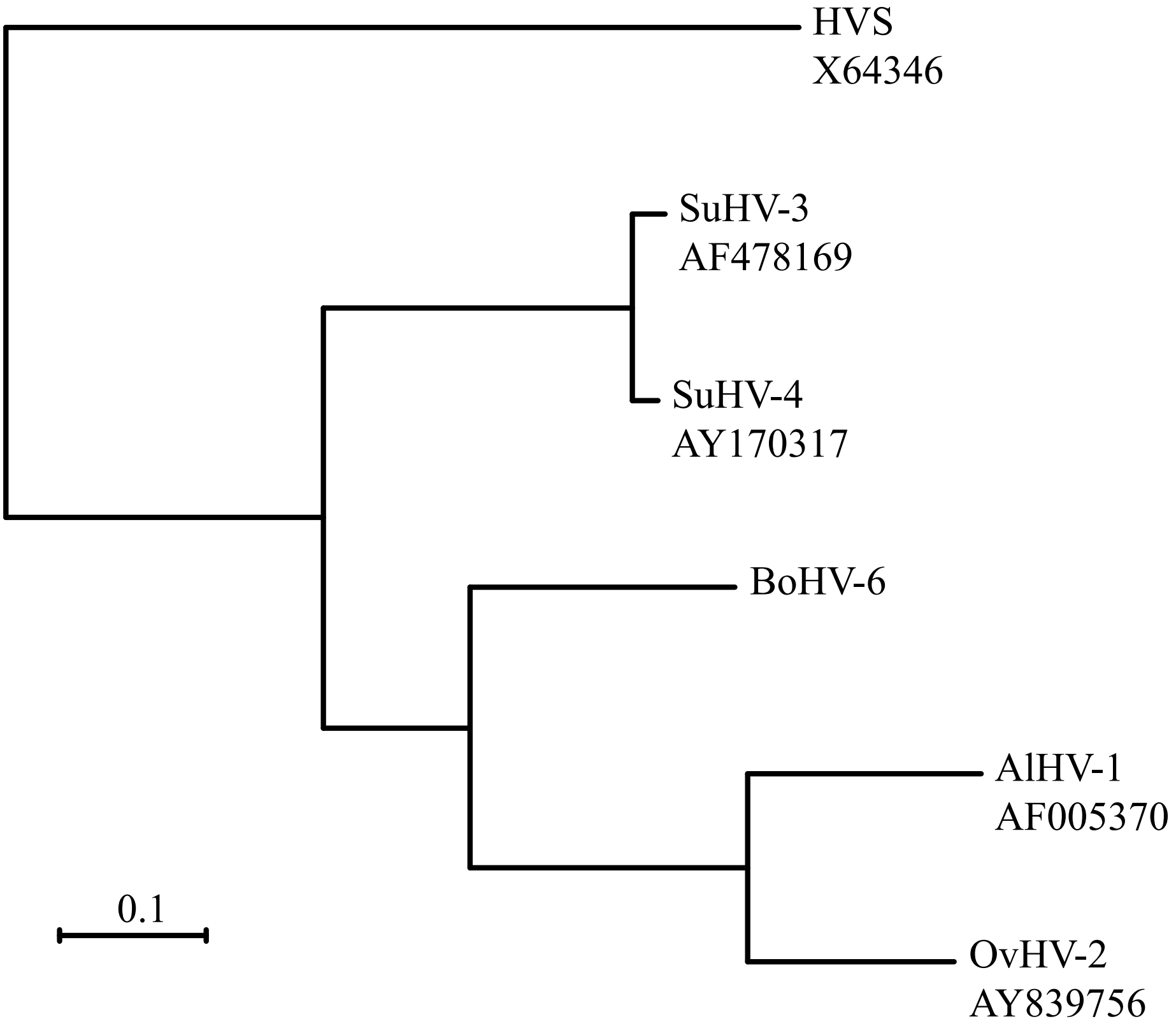
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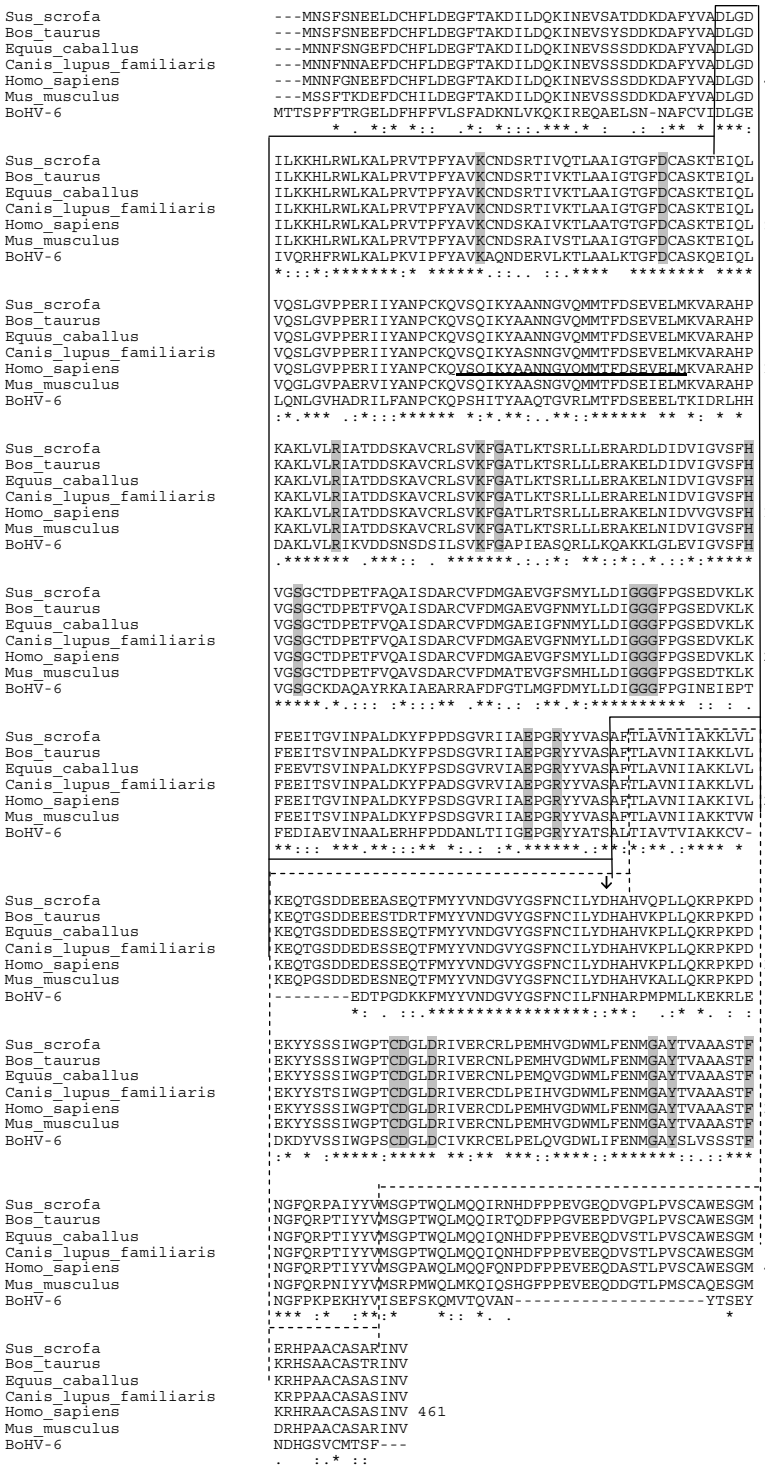
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Bos_taurus
Equus_caballus
Canis_lupus_familiaris
Homo_sapiens
Mus_musculus
BoHV-6