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

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Title: **Novel Gammaherpesvirus Functions Encoded by Bovine herpesvirus 6 (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

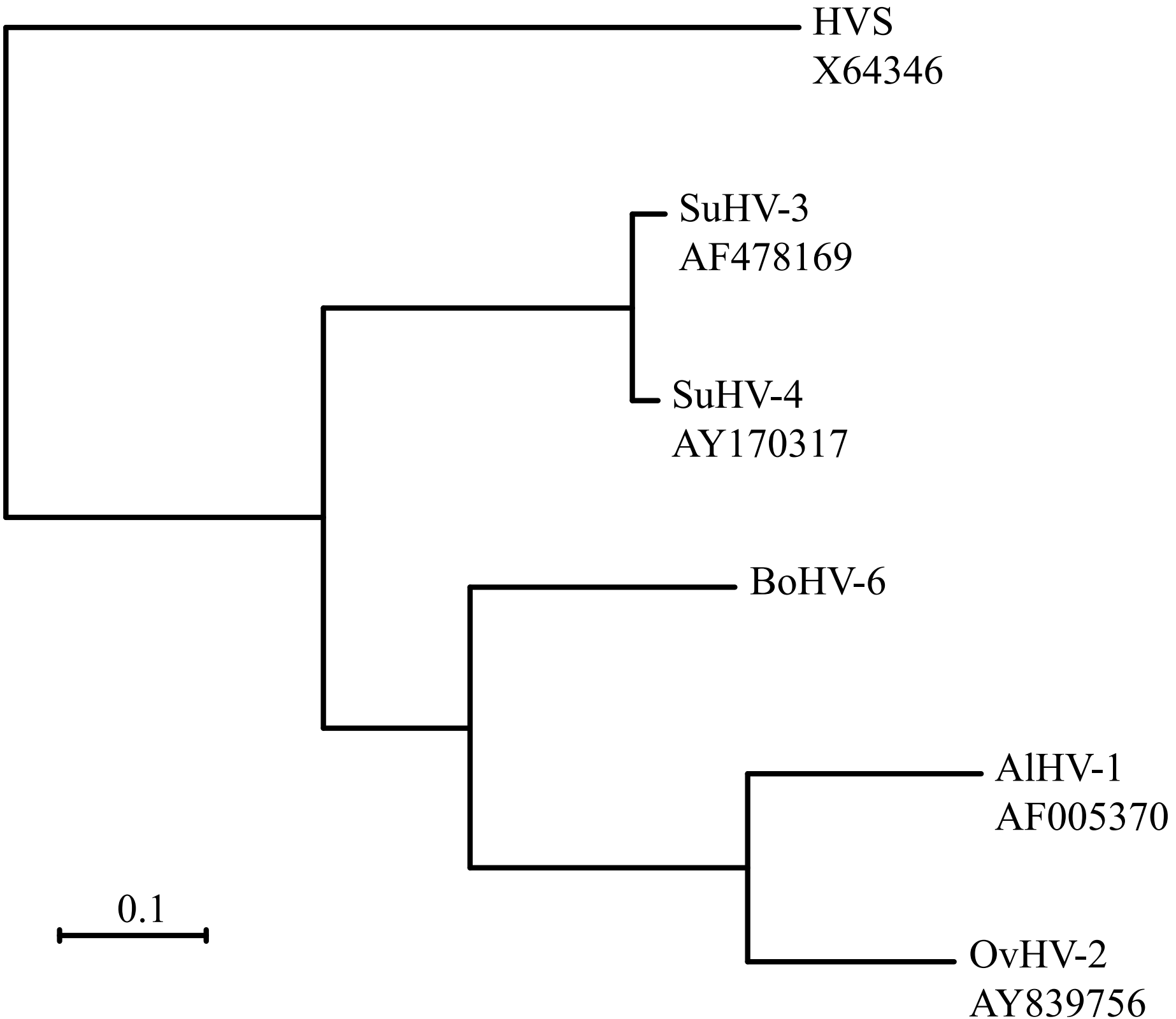
397 ¶ Str, strand

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

401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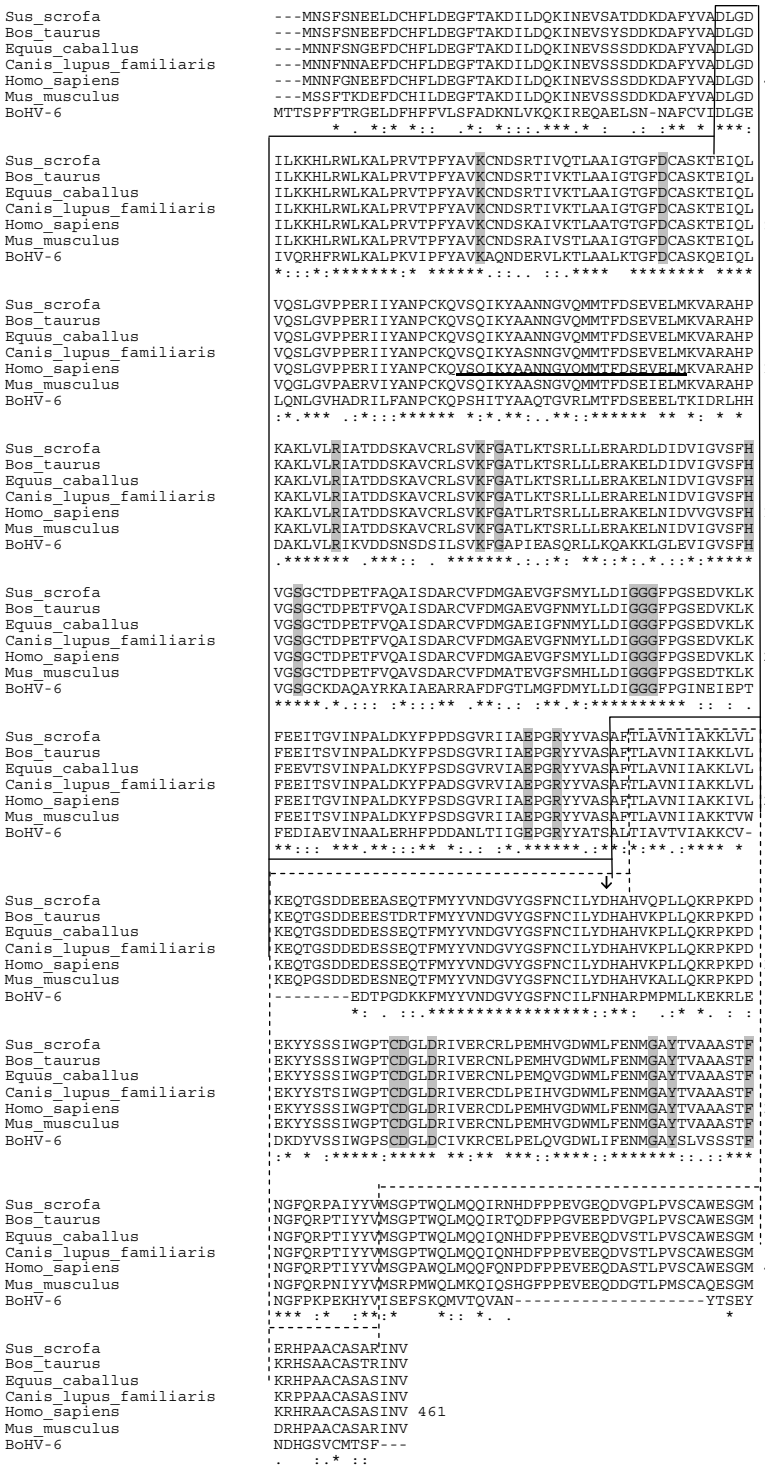
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Bos_taurus
Equus_caballus
Canis_lupus_familiaris
Homo_sapiens
Mus_musculus
BoHV-6