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Psittacid Herpesvirus 1 and Infectious Laryngotracheitis Virus: Comparative Genome Sequence Analysis of Two Avian Alphaherpesviruses

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Psittacid herpesvirus 1 (PsHV-1) is the causative agent of Pacheco's disease, an acute, highly contagious, and potentially lethal respiratory herpesvirus infection in psittacine birds, while infectious laryngotracheitis virus (ILTV) is a highly contagious and economically significant avian herpesvirus which is responsible for an acute respiratory disease limited to galliform birds. The complete genome sequence of PsHV-1 has been determined and compared to the ILTV sequence, assembled from published data. The PsHV-1 and ILTV genomes exhibit similar structural characteristics and are 163,025 bp and 148,665 bp in length, respectively. The PsHV-1 genome contains 73 predicted open reading frames (ORFs), while the ILTV genome contains 77 predicted ORFs. Both genomes contain an inversion in the unique long region similar to that observed in pseudorabies virus. PsHV-1 is closely related to ILTV, and it is proposed that it be assigned to the *Iltovirus* genus. These two avian herpesviruses represent a phylogenetically unique clade of alphaherpesviruses that are distinct from the Marek's disease-like viruses (*Mardivirus*). The determination of the complete genomic nucleotide sequences of PsHV-1 and ILTV provides a tool for further comparative and functional analysis of this unique class of avian alphaherpesviruses.

Avian herpesviruses comprise a wide range of pathogens and infect a wide variety of hosts. They are also remarkable for their relatively narrow host range. Herpesviruses have been isolated from a large number of avian species. Until now, only infectious laryngotracheitis virus (ILTV) (*Iltovirus*), the Marek's disease-like viruses (*Mardivirus*), and *Psittacid herpesvirus 1* (unassigned genus) have been classified as avian members of the *Alphaherpesvirinae* subfamily by the International Committee on Taxonomy of Viruses. Marek's disease virus (MDV) (*Gallid herpesvirus 2*) and herpesvirus of turkeys (HVT) (*Meleagrid herpesvirus 1*), although initially classified as gammaherpesviruses based on their lymphotrophic biological properties, were reclassified as alphaherpesviruses based on their genetic structure (9). The genomic sequences of all three serotypes of MDV have been reported (3, 33, 61).

Avian *Betaherpesvirinae* and *Gammaherpesvirinae* have not been taxonomically classified to date. However, taxonomically unassigned avian herpesviruses have been identified from a wide variety of avian species, including the bald eagle (*Acciptrid herpesvirus 1*), duck (*Anatid herpesvirus 1*), black stork (*Ciconiid herpesvirus 1*), pigeon (*Columbid herpesvirus 1*), falcon (*Falconid herpesvirus 1*), crane (*Gruid herpesvirus 1*), bobwhite quail (*Perdicid herpesvirus 1*), cormorant (*Phalacrocoracid herpesvirus 1*), owl (*Strigid herpesvirus 1*), penguin (*Sphenicid herpesvirus 1*), cardinal, finch, canary, vulture, and pheasant (1, 15, 20, 24, 26, 31, 32).

ILTV (*Gallid herpesvirus 1*) is a highly contagious and economically significant avian herpesvirus (6). Natural infections of ILTV are limited to galliform birds and cause an acute respiratory disease, which can be responsible for significant mortality and loss of productivity in the poultry industry (12, 40). In its epizootic form, the disease caused by ILTV is characterized by signs of respiratory distress accompanied by gasping and expectoration of bloody exudates (27). Infectious laryngotracheitis has been controlled through the use of live-virus vaccines, but vaccination is usually limited to areas where the disease is endemic, due to the relatively high pathogenicity of the vaccines. DNA sequencing efforts in numerous laboratories have generated the complete sequence of the ILTV genome (17, 18, 25, 28, 29, 30, 34, 49, 66, 67, 68), but to date these data have not been assembled and analyzed on a genome-wide scale.

Psittacid herpesvirus 1 (PsHV-1) is the causative agent of Pacheco's disease, an acute, highly contagious, and potentially lethal respiratory herpesvirus infection in psittacine birds (48). Amazon parrots, macaws, and cockatoos are highly susceptible to PsHV-1, which is a disease of great concern to the companion bird markets and exotic bird breeders (11, 22, 51). PsHV-1 targets hepatocytes and lymphocytes and slowly forms syncytial plaques in tissue culture. It has been previously classified both as a betaherpesvirus and as a gammaherpesvirus (31). Although there is limited PsHV-1 sequence available, phylogenetic studies based on the sequences of the PsHV-1 UL16 and UL30 genes have shown that the viruses that cause Pacheco's disease can be discriminated into four major genotypes and that PsHV-1 is most closely related to the alphaherpesvirus ILTV (or gallid herpesvirus 1) (59, 60, 62).

The genetic basis and underlying molecular mechanisms responsible for determining the pathogenicity, virulence, and host range specificity of ILTV and PsHV-1 are poorly understood. The only other avian herpesvirus genomes to be completely sequenced, the three serologically distinct members of

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TABLE 1. Previously published sequences used for the assembly of the ILTV genome

GenBank accession no.[a]	Reference	Size (bp)[b]
U80762	Johnson et al. (29)	13,700
AJ249803	Johnson et al. (30)	4,465
Y14300	Ziemann et al. (67, 68)	15,276
D00565*	Griffin and Boursnell (25)	5,395
X56093*	Poulsen et al. (49)	3,065
AY033142	Kehu et al. (unpublished data)	1,360
AF168792	Johnson (unpublished data)	31,332
AY033143	Kehu et al. (unpublished data)	378
U06635	Kingsley and Keeler (35)	1,807
Y14301	Ziemann et al. (67, 68)	1,854
AJ131832	Fuchs and Mettenleiter (18)	24,140
X97256	Fuchs and Mettenleiter (17)	10,265
L32139*	Johnson et al. (29)	8,364
U28832	Wild et al. (66)	18,900
L32139	Johnson et al. (29)	8,364
Total length		148,665

[a] *, reverse complement used in assembly.
[b] Lengths of sequence used in assembly.

the *Mardivirus* genus (MDV serotype 1 [MDV-1], MDV-2, and HVT), are biologically unique. MDV-1 can induce T-cell lymphomas and may not be representative of other broadly disseminated classes of avian herpesviruses. Comparative genome analysis can offer insight into the biological properties and phylogenetic differences of these viruses. Here we present the first complete genome sequence and analysis of both PsHV-1 and ILTV and demonstrate that they represent a phylogenetically unique class of alphaherpesviruses.

MATERIALS AND METHODS

Virus. The reference PsHV-1 (isolate 97-0001) was kindly supplied by David Phalen (Texas A&M University). PsHV-1 was isolated from the liver of an Amazon parrot (*Amazona oratrix*) of unknown age, with hepatic and splenic lesions characteristic of Pacheco's disease.

Virus purification and DNA isolation. PsHV-1 was propagated on confluent monolayers of primary chicken embryo fibroblast cells (CEF). Fibroblasts were isolated from 11-day-old chicken embryos by the warm trypsinization method and plated in Dulbecco's modified essential medium (DMEM/F-12) (Invitrogen Corp., Gaithersburg, MD) supplemented with penicillin (50 μg/ml), streptomycin (50 μg/ml), and 10% fetal bovine serum. Twenty hours postplating, fibroblast monolayers were infected with a 1:100 dilution of PsHV-1. Six days (144 h) postinfection, CEF monolayers exhibited 90 to 95% cytopathic effect (CPE). PsHV-1 virions were concentrated from the supernatant of infected CEF monolayers by the addition of 7% PEG 8000 and pelleted through a 30% sucrose cushion. Virions were lysed in 10% sodium dodecyl sulfate and deproteinized by incubation at 65°C in the presence of 10 mg/ml proteinase K followed by five extractions with phenol-chloroform-isoamyl alcohol (25:24:1). Virus DNA was precipitated in 100% EtOH for 48 h at −80°C and pelleted by centrifugation at 14,000 rpm for 20 min at 26°C. The virus DNA pellet was resuspended in 500 μl of TE (10 mM Tris HCl–1 mM EDTA, pH 8.0).

Virus DNA was purified by fractionation on a 0 to 40% sucrose gradient as previously described (55) and visualized by agarose gel electrophoresis. Fractions containing virus DNA were pooled, and virus DNA was precipitated and resuspended in TE. Purified virus DNA was examined for purity by restriction endonuclease digestion (data not shown) and comparison to previously published results (26).

PsHV-1 library construction, sequencing, and gap closing. Purified PsHV-1 genomic DNA (10 μg) was sheared by nebulization at 30 lb/in^2 for 50 s. Fragment ends were repaired with the End-It DNA end repair kit (Epicenter, Madison, WI) according to the manufacturer's instructions. Agarose gel-purified DNA fragments of 1.5 to 2.5 kb in length were ligated at the EcoRV site of pBluescript SK+ (Stratagene, La Jolla, CA) and transformed into SURE *Escherichia coli* electrocompetent cells (Stratagene, La Jolla, CA) by electroporation. Glycerol archive stocks of the transformed cells were prepared with a Q-Bot (Genetix Ltd., Dorset, United Kingdom) robot to pick bacterial colonies. Cultures were grown overnight in deep-well plates containing LB plus ampicillin (100 μg/ml). Plasmid DNA was purified by alkaline lysis according to the manufacturer's instructions (Perfectprep-96 Robotic Workstation; Brinkmann-Eppendorf 5 Prime, Boulder, CO). Virus DNA inserts were sequenced from both ends with M13 forward and reverse universal primers, using BigDye dideoxy chain terminator sequencing chemistry, version 3.0 (PE Biosystems, Foster City, CA), and Applied Biosystems PRISM 3700 automated DNA sequencers (PE Biosystems, Foster City, CA).

Gaps in the PsHV-1 sequence were closed by PCR amplification using viral genomic DNA as the template. Each 20-μl reaction mix contained 50 ng of genomic PsHV-1 DNA, 25 pmol of each primer (designed from flanking PsHV-1 sequence), 0.1 mM concentrations of each of the four deoxynucleoside triphosphates, 2.5 mM magnesium chloride, 0.75 U of *Taq*, and 1× buffer A (Promega, Madison, WI). Amplifications were performed on a PE Biosystems 9700 thermal cycler using an initial denaturation step of 94°C for 5 min, followed by 30 cycles of 94°C for 15 s, 55°C for 30 s, and 60°C for 4 min. PCR products were purified and sequenced as described above.

DNA sequence analysis. PsHV-1 DNA sequences were assembled with *Phrap* (16) using *Phred* quality files and default settings to produce a consensus sequence. Consensus sequences were manually edited within *Consed* (23). The ILTV DNA sequence was assembled from 14 published ILTV sequences (Table 1). The strategies used to identify PsHV-1 and ILTV genes were based on those used in the sequence analyses of other herpesviruses (3, 7, 33, 36, 52). The primary criteria for identifying a coding sequence was the presence of an open reading frame (ORF) of >100 nt. The identification of ORFs was performed with ORF Finder (21) and Vector NTI (InforMax, Inc.). Searches of predicted PsHV-1 and ILTV proteins for homology to known proteins were performed by applying BLAST (Basic Local Alignment Search Tool) (4) and PSI-BLAST (5).

Phylogenetic trees were constructed with ClustalW (58) and Treeview (47) within the Vector NTI software suite and calculated with the neighbor-joining algorithm of Saitou and Nei (54). Initially, alignments were processed by the *Seqboot* program, which generated the requested number (100) of resampled alignments for bootstrapping. The resampled alignments were then processed by the *Protdist* program, which created distance matrices for each alignment. Each of these matrices was then used by the *Neighbor* program to create a neighbor-joining tree.

Nucleotide sequence accession numbers. The complete PsHV-1 and ILTV genome sequences have been deposited in the GenBank database under accession no. AY372243 and NC_006623, respectively.

TABLE 2. Comparison of avian alphaherpesvirus genome organizations

Virus	Length (bp)[a]							Accession no.
	TR_L	UL	IR_L	IR_S	US	TR_S	Total	
PsHV-1		119,146		13,737	16,405	13,737	163,025	AY372243
ILTV		113,039		11,202	13,232	11,202	148,665	NC_006623
MDV-1	12,584	113,563	12,584	12,120	10,847	12,120	173,818	AF147806
MDV-2	11,951	109,932	11,951	9,164	12,109	9,164	164,271	AB049735
HVT	7,072	110,694	7,072	13,610	8,615	13,610	160,673	AF282130

[a] TR_L, terminal repeat, long region; IR_L, internal repeat, long region; IR_S, internal repeat, short region; TR_S, terminal repeat, short region.

TABLE 3. ORFs identified within the PsHV-1 and ILTV genomes

PsHV1		ILTV		Length (aa)[a]		% Identity (to ILTV)	Putative function[b]
ORF	Nucleotide position	ORF	Nucleotide position	PsHV1	ILTV		
UL54	4295–2376	UL54	12082–10787	639	420	38	Posttranslational regulator of gene expression
UL53	6461–5385	UL53	13916–12855	358	337	31	Glycoprotein K; exocytosis
UL52	10335–6454	UL52	17176–13847	1,293	1,110	42	DNA helicase-primase
UL51	10319–11101	UL51	17189–17875	260	229	55	Unknown
UL50	12476–11232	UL50	19182–17935	414	416	46	Deoxyuridine triphosphatase
UL49.5	12667–13014	UL49.5	19336–19686	140	266	50	Putative viral membrane protein
UL49	13184–14038	UL49	19749–20546	283	117	50	Viral tegument protein
	Not present	UL48	20695–21882		396		Viral tegument protein (α-TIF)
UL46	14500–16323	UL46	21888–23558	606	539	44	Tegument phosphoprotein; α-TIF modulation
UL45	17426–16521	UL45	24559–23663	301	281	26	Tegument/envelope protein
ORF A	21070–22260	ORF A	25275–26405	396	376	27	Hypothetical protein
ORF B	22726–23829	ORF B	26448–27467	367	340	30	Hypothetical protein
ORF C	25174–24056	ORF C	28529–27528	372	334	33	Hypothetical protein
ORF D	25554–26654	ORF D	28639–29760	366	374	38	Hypothetical protein
ORF E	28424–27054	ORF E	31067–29838	456	410	28	Hypothetical protein
UL22	31254–28834	UL22	33539–31128	828	779	34	Glycoprotein H; fusion complexes with gL
UL23	32569–31550	UL23	34667–33576	339	363	40	Thymidine kinase
UL24	32533–33489	UL24	34556–35416	318	287	41	Unknown
UL25	33628–35511	UL25	35392–37107	627	572	47	DNA packaging protein
UL26	35709–37382	UL26	37288–39045	557	586	33	Capsid protein p40
UL26.5	37199–37891	UL26.5	38428–39045	230	206	13	Virion scaffold protein
UL27	40995–38260	UL27	41747–39099	911	873	59	Glycoprotein B
UL28	43743–41164	UL28	44013–41722	859	537	47	ICP18.5; cleavage/packaging
UL29	47426–43860	UL29	47094–44098	1,188	999	59	Major single-strand DNA binding protein
UL30	47858–51103	UL30	47271–50291	1,081	1,007	54	DNA polymerase
UL31	52171–51134	UL31	51483–50467	345	339	68	Nuclear phosphoprotein
UL32	54020–52164	UL32	53233–51479	618	582	48	Envelope glycoprotein
UL33	54019–54393	UL33	53190–53579	138	119	58	DNA packaging
UL34	54616–55440	UL34	53611–54486	300	290	62	Membrane-associated phosphoprotein
UL35	55567–55992	UL35	54515–54886	141	124	50	Capsid protein
UL36	65672–56085	UL36	62584–54917	3,209	2,556	36	Major tegument protein
UL37	69349–66437	UL37	65881–63212	970	890	34	Tegument protein
UL38	69618–71078	UL38	65973–67280	486	412	41	DNA binding; capsid protein
UL39	71339–73774	UL39	67618–69972	818	785	50	Large-subunit ribonucleotide reductase
UL40	73851–74792	UL40	69827–70915	313	310	69	Small-subunit ribonucleotide reductase
UL41	76206–74884	UL41	72176–70983	440	398	73	Virion host shutoff
UL42	76680–78182	UL42	72398–73693	519	432	34	Processivity factor for DNA polymerase
UL43	78100–79626	UL43	73756–74970	508	300	11	Unknown
UL44	80418–81806	UL44	75683–76924	462	414	29	Glycoprotein C
UL21	83761–82052	UL21	78611–77016	569	532	28	Nucleocapsid protein
UL20	84089–84835	UL20	78782–79477	248	232	30	Membrane protein
UL19	85082–89323	UL19	79664–83872	1,413	1,403	65	Major capsid protein
UL18	89684–90649	UL18	84059–85015	321	319	64	Capsid protein
UL15a	92293–90773	UL15a	86212–85103	513	764	66	Terminase; DNA packaging
UL17	92187–94634	UL17	86355–88505	815	341	38	Tegument protein
UL16	94538–95605		Not present	355			Capsid assembly
UL15b	96940–95588	UL15b	89761–88598	450	764	43	Terminase; DNA packaging
UL14	96939–97529	UL14	89595–90353	196	196	45	Unknown
UL13	97403–98785	UL13	90212–91606	486	465	45	Serine/threonine protein kinase
UL12	99023–100585	UL12	91750–93366	566	526	58	Alkaline deoxynuclease
UL11	100585–100728	UL11	93259–93502	48	80	42	Myristoylated tegument protein
UL10	102288–101047	UL10	94758–93580	413	393	46	Glycoprotein M
UL9	102386–105028	UL9	94653–97382	880	892	53	Ori binding protein
UL8	105636–107582	UL8	97378–99762	648	795	34	Helicase-primase component
UL7	108856–107690	UL7	100889–99816	388	358	41	Unknown
UL6	110909–108561	UL6	102807–100669	782	713	47	Minor capsid protein
UL5	110993–113560	UL5	102795–105314	855	840	58	Helicase-primase component
UL4	113781–114539	UL4	105403–105936	252	178	81	Unknown
UL3	114921–115523	UL3	106948–106349	200	196	66	Unknown
UL2	115671–116759	UL2	107950–107060	265	297	50	Uracil DNA glycosylase
UL1	117253–116705	UL1	107920–108279	182	131	32	Glycoprotein L
	Not present	UL0	111514–110171		447		Unknown
UL[−1]	118632–117331	UL[−1]	111670–112026	463	501	23	Unknown
ICP4a	127595–121494	ICP4	118888–114500	2,033	1,463	35	Gene regulation
US10	133103–133948	US10	122103–122936	281	278	32	Unknown
	Not present	sORF4/3	124190–123309		293		Unknown
US2	134374–134634	US2	125011–124325	85	118	34	Unknown
US3	136263–134785	US3	125100–126527	498	471	48	Protein kinase
sORF1	136535–138352	UL47	126616–128484	605	623	45	UL47
US4	138546–139388	US4	128651–129526	280	292	24	Glycoprotein G
sORF2	139667–142642	US5	129739–132693	991	985	18	Glycoprotein J
US6	142740–143891	US6	132441–133805	383	434	28	Glycoprotein D
US7	144089–145315	US7	133916–135001	492	362	34	Glycoprotein I
US8	145663–147369	US8	135198–136694	568	499	27	Glycoprotein E
	Not present	US9	136704–137483		259		Unknown
sORF4/3	148377–149249	sORF4/3	137535–138416	290	322	34	Unknown
	Not present	US10	138704–139402		232		Unknown
ICP4b	154577–160678	ICP4	142837–147225	2,033	1,463	35	Gene regulation

[a] aa, amino acids.
[b] Function or property as demonstrated for the ILTV and/or HSV-1 homolog.

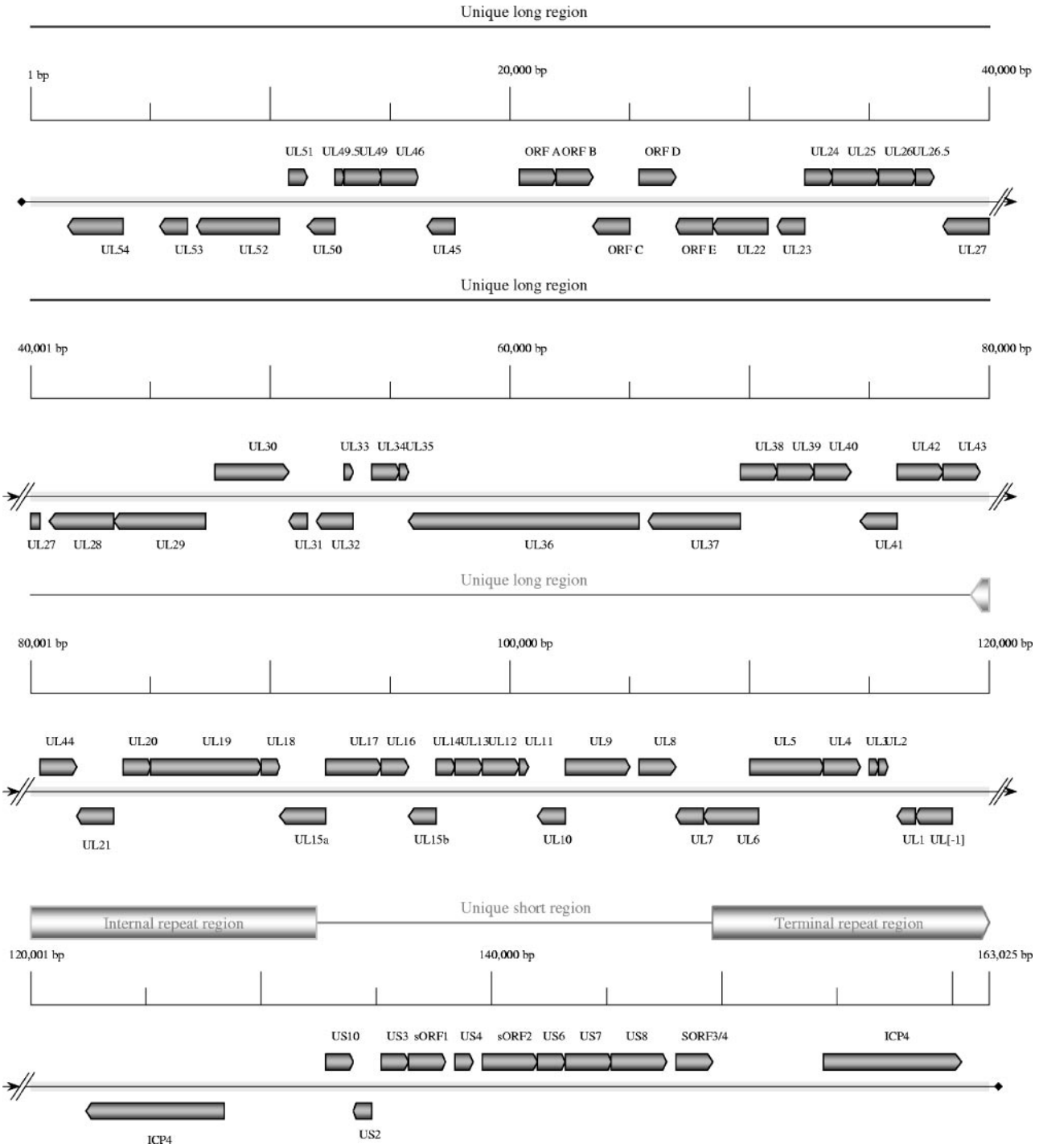

FIG. 1. Organization of the PsHV-1 genome. This map of the PsHV-1 genome shows the locations and sizes of predicted ORFs. Predicted PsHV-1 genes are labeled according to homology with characterized HSV-1 genes. Thick gray bars flanking the unique short region indicate the internal and terminal repeat regions. A "ruler" designates distances in 5,000-bp increments. Arrows on the ORF boxes indicate orientations.

RESULTS

Genome organization. Both PsHV-1 and ILTV exhibit the structural characteristics of class D herpesvirus genomes, such as pseudorabies virus (PRV) and varicella zoster virus (VZV), which contain two domains of unique sequences (53). In this class of herpesvirus genomes, only the shorter unique sequence (US) is flanked by inverted repeats (IR and TR). The US region can then invert relative to the larger unique domain (UL), and the genome can exist in two equimolar isomeric forms. The PsHV-1 genome is 163,025 bp in length, with a base composition of 60.95% G+C. The UL sequence is 119,146 bp in length, and the US sequence is 16,405 bp (Table 2). The

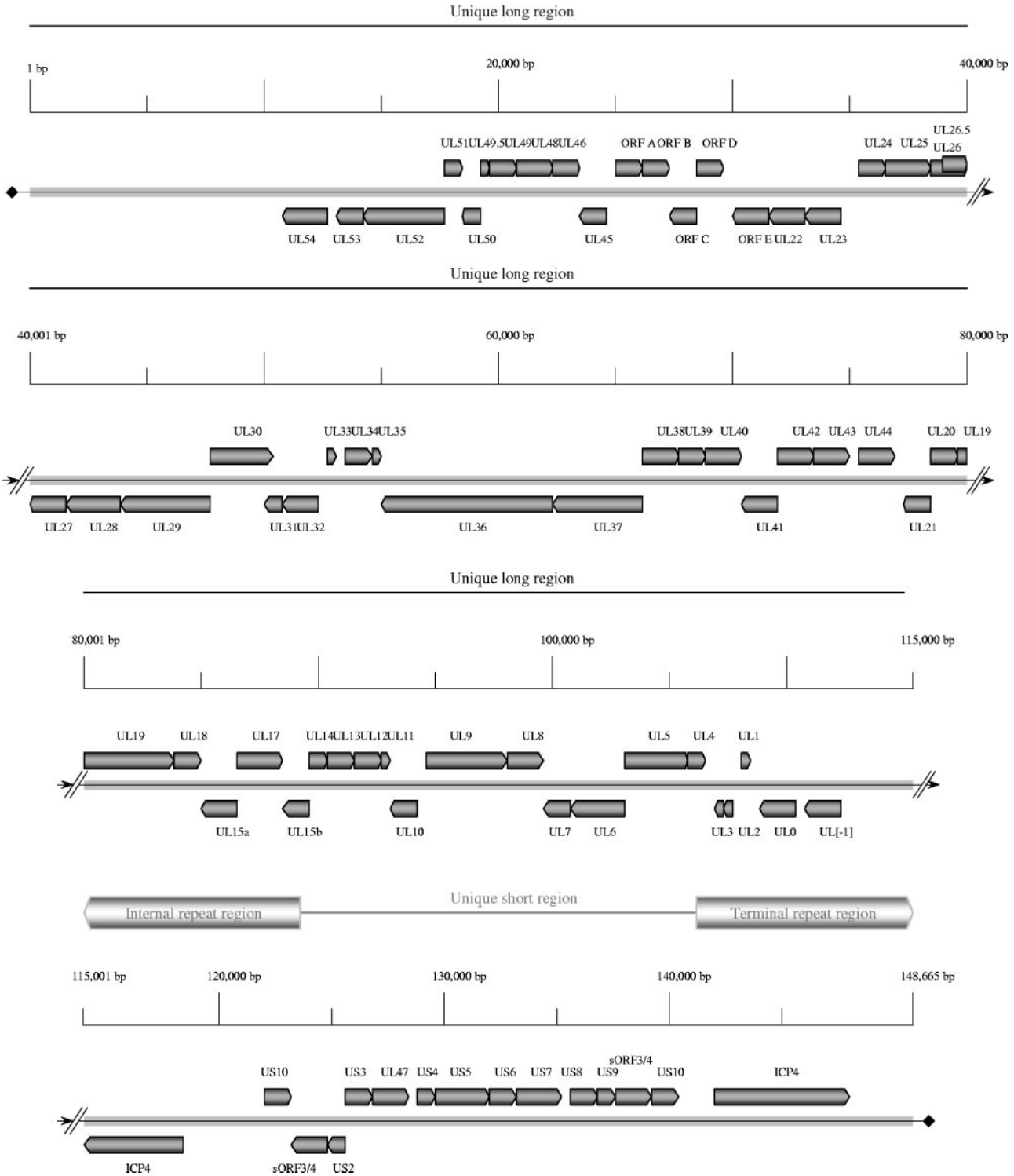

FIG. 2. Organization of the ILTV genome. This map of the ILTV genome shows the locations and sizes of predicted ORFs. Predicted ILTV genes are labeled according to homology with characterized HSV-1 genes. Thick gray bars flanking the unique short region indicate the internal and terminal repeat regions. A "ruler" designates distances in 5,000-bp increments. Arrows on the ORF boxes indicate orientations.

inverted repeat elements are each 13,737 bp in length. The ILTV genome, as assembled from 14 different published accessions (Table 1), is 148,665 bp in length, with a G+C content of 48.16%. Two sequences used in the assembly (accession no. L32139 and U28832) do not overlap. PCR amplification of ILTV DNA across the junction of these sequences indicated that there was no additional ILTV sequence present. The ILTV genome is also organized into UL (113,039 bp) and US

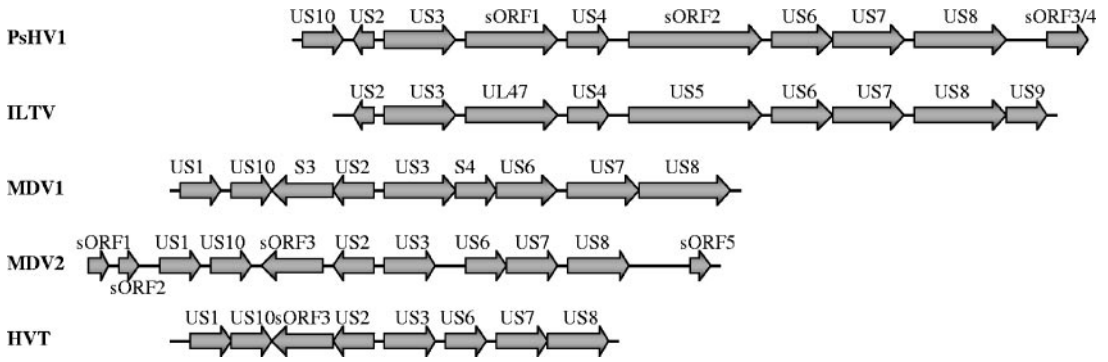

FIG. 3. Comparison of the unique short regions of five avian alphaherpesviruses. ORFs identified within the unique short regions of five avian alphaherpesviruses are indicated. Regions are aligned with respect to the conserved US2 and US3 genes. ORFs are labeled according to homology with characterized HSV-1 genes. Arrows on the ORF boxes indicate the direction of transcription.

(13,232 bp) regions, with IR and TR repeats (11,202 bp) flanking the US region (Table 2). Unlike the MDV family of avian alphaherpesviruses, which exhibit genome structures similar to those of herpes simplex virus type 1 (HSV-1) and HSV-2, the UL regions of the ILTV and PsHV-1 genomes are not bracketed by inverted repeats and the US regions are considerably larger (Table 2).

Gene identification. The PsHV-1 genome contains 73 predicted ORFs (Table 3; Fig. 1), while the ILTV genome contains 77 predicted ORFs (Fig. 2 and Table 3). Sixty-one PsHV-1 genes are located within the UL region, 10 genes are within the US region, and a single copy of 1 gene (ICP4) is located within each of the repeats. The UL region of ILTV contains 62 genes, and the US region contains 9 genes. One major structural difference between the PsHV-1 and ILTV genomes occurs in the size and genetic makeup of the inverted repeat regions. The ILTV inverted repeat regions are 2,535 bp (18.5%) shorter than the PsHV-1 inverted repeats and contain two copies of three genes: ICP4, US10, and a homolog of the MDV sORF4/3 gene. Like that of HVT, neither the ILTV nor the PsHV-1 genome contains a region comparable to that in MDV-1 that has been implicated in oncogenesis.

Gene order is generally conserved within the UL and US regions of these two viral genomes, and they are colinear relative to other alphaherpesviruses. The UL regions of ILTV and PsHV-1 share two striking features. First, both genomes contain a unique block of five ORFs (designated A to E) previously found only in ILTV (64). Secondly, both ILTV and PsHV-1 contain an inversion of the genome from UL22 to UL44, which is similar to an inversion found between UL27 and UL44 in the PRV genome (36). The predicted PsHV-1 genes are 13% to 73% identical at the amino acid level to the corresponding predicted ILTV genes and have 99 to 100% identity to PsHV-1 sequences previously submitted to GenBank (59, 62).

The ILTV and PsHV-1 UL regions also exhibit differences in gene organization. PsHV-1 is predicted to contain a homolog of the UL16 gene, which is absent from the ILTV genome. Conversely, ILTV is predicted to contain the UL48 and UL0 ORFs, neither of which are found in the PsHV-1 genome.

US region. There are ten predicted ORFs within the US region of PsHV-1: US10, US2, US3, sORF1, US4, sORF2, US6, US7, US8, and sORF3/4. Gene arrangement within the PsHV-1 US region is quite similar to the corresponding region of ILTV; however, a homolog of ILTV US9 is not present in PsHV-1, nor does PsHV-1 contain the duplication of the US10 and sORF3/4 genes within the inverted repeat region, as observed in ILTV. Although the organizations of the ILTV and PsHV-1 US regions are very similar, they differ greatly from the corresponding region of the three serotypes of MDV (Fig. 3). Interestingly, neither avian herpesvirus UL region contains a homolog of UL47, a tegument phosphoprotein. However both ILTV (UL47) and PsHV-1 (sORF1) encode an ORF in the US region with weak (18%) identity to the HSV UL47 gene (38, 66).

The US regions of both ILTV and PsHV-1 are predicted to encode five structural glycoproteins. One of these genes was initially designated gp60 (37). Recent studies have adopted the HSV-1 designation of gJ (US5) for this ORF (19). The PsHV-1 US region contains a colinear ORF. The predicted translation product of this gene is a glycoprotein that has only 18% amino acid identity to the gJ glycoprotein of ILTV, although 9 of the 10 cysteine residues are conserved (Fig. 4). The PsHV-1 ORF has been designated sORF2.

Phylogenetic analysis of PsHV-1 and ILTV. ILTV and PsHV-1 share 70 conserved genes, and BLAST results indicate that PsHV-1 proteins have the strongest similarity to homologs from ILTV (average, 42.5%) compared to other herpesviruses. A phylogenetic analysis of 15 herpesvirus DNA polymerase (UL30) amino acid sequences is depicted in Fig. 5. The herpesvirus DNA polymerase exhibits the highest levels of conservation between *Herpesviridae* family members and demonstrates a distinct division of the subfamilies *Alpha-*, *Beta-*, and *Gammaherpesvirinae*. In this bootstrapped analysis, the PsHV-1 and ILTV DNA polymerase genes align into the subfamily *Alphaherpesvirinae* and are distinctly separated from the Marek's disease family of herpesviruses (*Mardivirus*) and from vulture herpesvirus (VHV). A similar analysis of five additional genes (ICP4, UL27 [gB], UL44 [gC], US8 [gE], and UL23 [thymidine kinase]) reveals the same phylogenetic pattern (data not shown). In all cases the PsHV-1 genomic sequence is most closely related to ILTV, suggesting that ILTV and PsHV-1 form a distinct clade within the *Alphaherpesvirinae*.

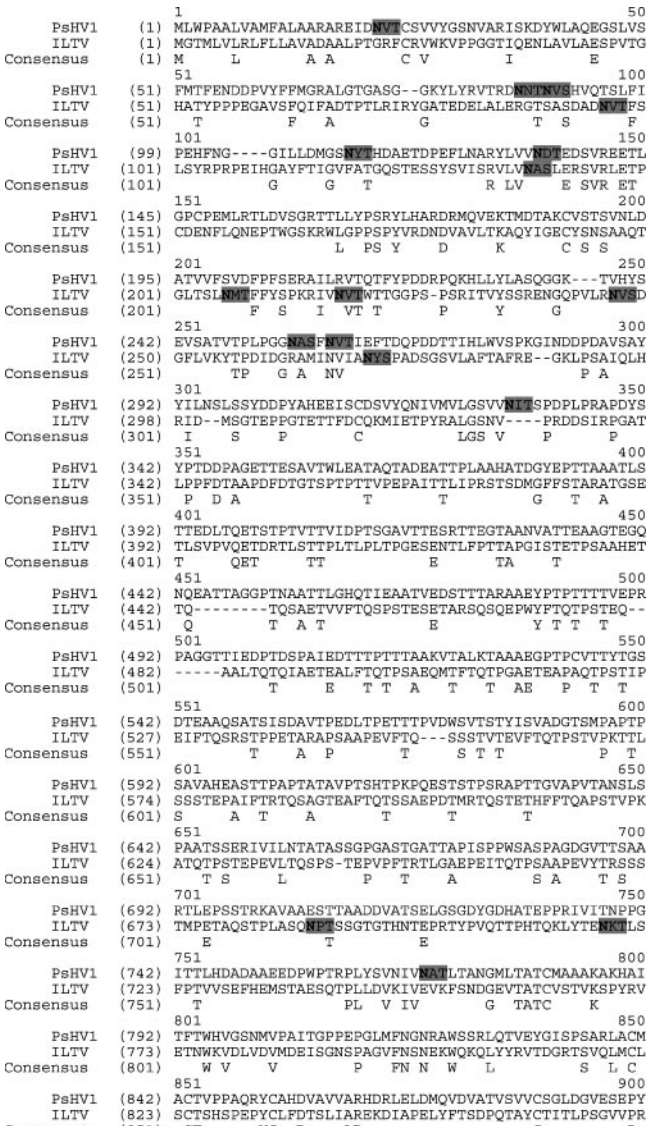

FIG. 4. Amino acid alignment of the predicted PsHV-1 sORF2 and ILTV gJ proteins. Amino acid sequences were aligned with the AlignX program (Vector NTI). Gaps in the alignments are represented as dashes. The consensus sequence is shown below the alignment. Potential glycosylation sites are indicated by shaded boxes, with asparagines (N) indicated in boldface type.

DISCUSSION

Herpesviruses of mammals and birds clearly descend from a common ancestor, but their genomes exhibit significant variation with respect to nucleotide sequence, gene content, and genomic organization (2, 10, 13, 14, 41, 42, 43, 65). Among avian hosts, the herpesviruses also tend to be species specific with respect to susceptibility, pathogenicity, and virulence. Although they differ significantly in G+C content—61% for PsHV-1 and 48% for ILTV—phylogenetically, PsHV-1 and ILTV are closely related. While their genomic structure, content, and organization are similar to other alphaherpesviruses, they represent a unique class of avian alphaherpesviruses. Similarities between PsHV-1 and ILTV can be seen in the UL inversion, the absence of repeats flanking the UL region, the conserved structure of the US region, and a conserved cluster of five unique ORFs in the UL region. The similarity of the PsHV-1 and ILTV genomes suggests that PsHV-1 (psittacid) and ILTV (gallid) may represent a class of avian alphaherpesviruses that diverged early from a common ancestor and are distinct from the Marek's disease family of alphaherpesviruses, as previously suggested by other researchers (33). Based on this evidence, we propose that PsHV-1 be formally assigned to the *Iltovirus* genus of the *Alphaherpesvirinae*.

To date, avian herpesviruses have been isolated from the owl (strigid); chicken and pheasant (gallid); turkey (meleagrid); pigeon (columbid); falcon (falconid); vulture and crane (gruiformid); duck (anatid); quail (perdicid); cardinal, finch, and canary (passeriform), and parrot (psittacid) (1, 26). With the advent of rapid and cost-effective sequencing strategies, it is now feasible to sequence representative herpesvirus isolates from different avian genera in order to determine whether the ILTV/PsHV-1 genus of the alphaherpesviruses is broadly represented. As evidenced by the VHV DNA polymerase analysis (Fig. 5), as more avian herpesvirus sequence becomes available additional avian herpesvirus genera may be identified.

Herpesviruses have several common properties, including a large number of conserved enzymes involved in nucleic acid metabolism, DNA synthesis, and protein processing. As expected, PsHV-1 and ILTV resemble other alphaherpesviruses in genome organization and gene content. The ILTV and PsHV-1 genomes also share several unique characteristics with respect to genome arrangement and content, which may provide clues to differences and similarities in their tissue tropism, pathogenicity, and host range. One characteristic that sets PsHV-1 and ILTV apart from the other alphaherpesviruses is the presence of five unique, conserved ORFs in the UL region (ORF A to ORF E), which Veits et al. (64) have found to be dispensable for ILTV replication in tissue culture. They have suggested that these genes may play a role in immune evasion or species specificity.

Herpesvirus-encoded structural glycoproteins play major roles in host range and pathogenicity. Both ILTV and PsHV-1 potentially encode 10 structural glycoproteins. ILTV and PsHV-1 contain structurally similar glycoprotein C (gC) proteins, which appear to lack consensus alphaherpesvirus glycoprotein-encoded heparan binding domains (34). Earlier experiments have shown that the initial attachment step of ILTV to susceptible cells does not involve interactions with heparan- or chondroitin sulfate-containing proteoglycans (35). This observation has also been confirmed for PsHV-1 (data not shown).

The gJ homolog in ILTV encodes an N- and O-linked modified glycoprotein with significant homology to equine herpesvirus type 1 gp2 (19). Veits et al. (63) have indicated that gJ (US5) may be a dominant antigen for the humoral immune response against ILTV in chickens. More-recent studies conducted with gJ-negative ILTV mutants have demonstrated that the gJ gene plays a role in determining the severity of infection

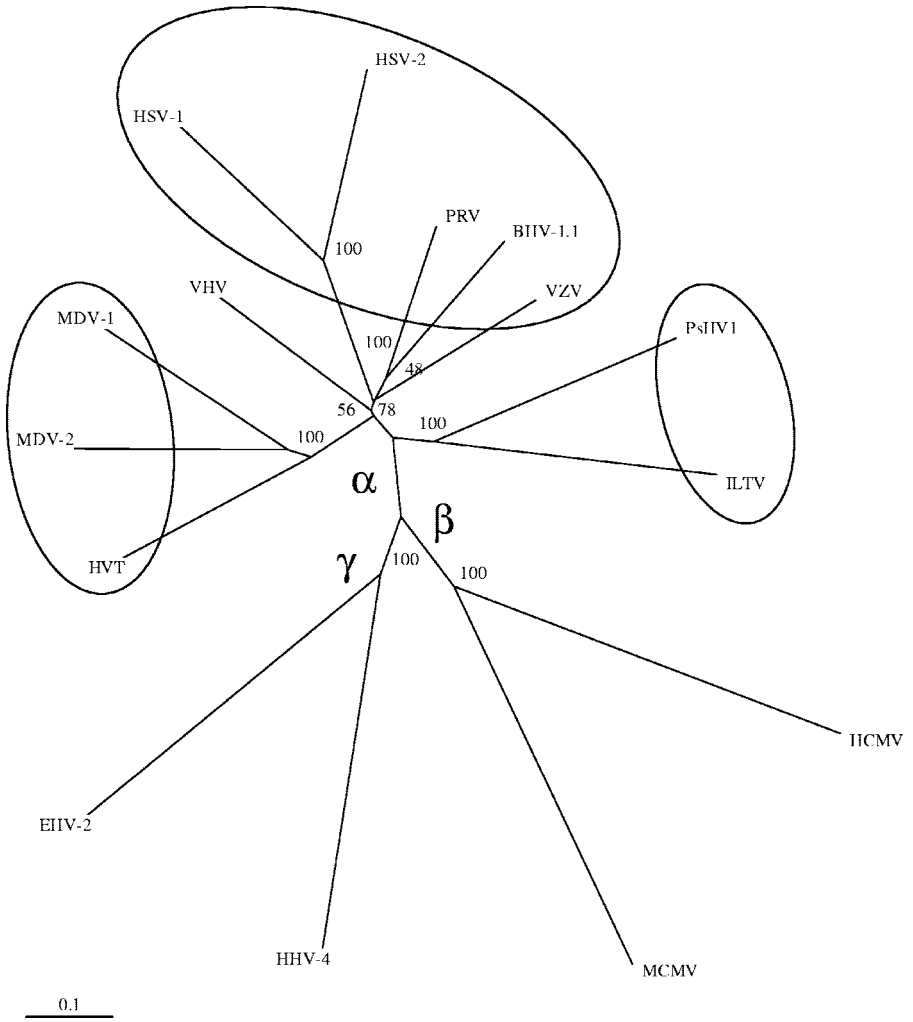

FIG. 5. Phylogenetic analysis of herpesvirus DNA polymerase (UL30) proteins. A phylogenetic tree of UL30 (DNA polymerase homolog) for representative alpha-, gamma-, and betaherpesviruses is shown. The tree was constructed and bootstrap analysis was performed as described in Materials and Methods. The measure of divergence is presented as a scale at the lower left. Accession numbers of the protein sequences used in tree assembly are as follows: AY372243 (PsHV-1), NC006623 (ILTV), AB024414.1 (MDV-2, strain HPRS24), AF282130.1 (HVT), P04293 (HSV-1), P07918 (HSV-2), P09252 (VZV), NC006151 (PRV), AJ004801 (bovine herpesvirus type 1.1), P08546 (human cytomegalovirus), P27172 (murine cytomegalovirus), P03198 (human herpesvirus 4; Epstein-Barr virus), P52367 (equine herpesvirus type 2); AAT79466 (VHV), AAF66765 (MDV-1, strain GA).

and suggest that gJ may be of interest as a target for live-virus vaccine development in ILTV (19). These two gene products (gC and gJ) may determine host and/or tissue preferences for these viruses. However, the potential PsHV-1 homolog of US5 (sORF2) appears to be mainly conserved only by position, exhibiting only 18% amino acid identity to ILTV gJ. A comparison of mutant viruses containing deletions of the gC or gJ genes may help determine their roles in virus growth and replication in vitro and in vivo.

ILTV and PsHV-1 also differ with respect to specific genes known to be involved in virus growth and pathogenicity. ILTV lacks a homolog to UL16, a tegument protein that interacts with UL11 and is involved in nucleocapsid processing (39). Conversely, a homolog to α-TIF (UL48) is not found in the PsHV-1 genome but is present in ILTV. UL48 is best known for its role in enabling virus-specific immediate early (IE) gene transcription (8). In certain isolates of HSV-1 lacking viral α-TIF, amounts of IE proteins sufficient to promote virus growth and replication are made, although CPE in tissue culture progresses more slowly (50). While ILTV infection of primary cell cultures (chicken hepatocyte or chicken kidney) results in >90% CPE after 16 h, PsHV-1 infections to >90% CPE in CEF are considerably slower (~144 h). It is possible that the lack of an α-TIF gene product in PsHV-1 may result in lower replication efficiency and a subsequently reduced rate of CPE. This slow growth in tissue culture might also account for some early confusion regarding the classification of PsHV-1. In addition, the UL48 protein appears to be involved in the final maturation process of virion formation in the cytoplasm (45, 46). This activity may be modulated by the association of UL48 with UL47, another tegument protein (44). In both PsHV-1 and ILTV, an ORF exhibiting limited homology to UL47 has been identified in the US region (sORF1, UL47).

The PsHV-1 and ILTV genome sequences provide tools for

subsequent studies designed to examine the expression and analyze the functions of these virus genomes. In addition, these sequences will aid in the study of these two herpesviruses. Based on a phylogenetic analysis, Styles et al. (57) have identified four "genotypes" of PsHV. Similarly, Sellers et al. (56) have identified the presence of novel ILTV subgroups that may play a role in the propagation and subsequent outbreaks of ILTV. Comparative sequence analysis will aid in understanding the apparent diversity within these related groups of avian alphaherpesviruses. The complete nucleotide sequences of the psittacid herpesvirus 1 (PsHV-1) and gallid herpesvirus 1 (ILTV) genomes offer researchers the opportunity to begin a comparative analysis of a unique class of avian herpesviruses that are biologically and phylogenetically distinct from the *Mardivirus* genus.

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