WHOLE-GENOME PHYLOGENETIC ANALYSIS OF HERPESVIRUSES

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Received August 20, 2007; accepted February 4, 2008

Summary. – A comprehensive phylogenetic analysis of 45 herpesviruses was performed based on whole-genome sequences. We used 4 methods, namely the alignment of conserved gene sequences (excluding 5 herpesviruses), compositional vector tree (CVTree) method, local homology analysis, and gene content analysis. The obtained results showed good consistency between the phylogenetic trees prepared by these methods and likewise, the obtained classification of the herpesviruses was consistent with their current taxonomic designation. The herpesviruses with the ambiguous classification or not assigned in the family or with the newly published genomes were also phylogenetically classified.

Key words: herpesviruses; phylogenetic analysis; whole-genome sequences

Introduction

The herpesviruses represent a group of linear double-stranded DNA viruses with genome length between 110 kbp to 230 kbp that infect vertebrates and at least one species of invertebrates. Based on details of morphology, tissue tropism, pathogenicity, and more recently on molecular phylogenetic analysis, the family *Herpesviridae* is divided into 3 subfamilies *Alphaherpesvirinae, Betaherpesvirinae,* and *Gammaherpesvirinae* (Wang *et al*., 2007; McGeoch *et al*., 2000, 2001, 2005; Gerner *et al*., 2004).

Genome sequencing projects stimulate a development of the whole-genome phylogenetic analysis that is considered superior to the single-gene phylogenetic analysis. Different genes may recount different phylogenetic relationships among the same set of organisms due to the different selective pressures. Individual genes may have different backgrounds, e.g. they may represent genetic mosaics of acquired genes from different sources, which underwent a lateral transfer, transposition, and recombination in the course of evolution (Shackelton and Holmes, 2004; Karlin *et al*., 1994). A construction of the phylogenetic tree based on the whole-genome data might reduce the interference from such inconsistencies and produce a phylogenetic tree closer to the underlying phylogeny than single-gene tree.

To date, three methods have been used in the molecular phylogenetic analysis of herpesviruses based on the whole-genome data. First method was based on the alignment of conserved gene sequences. To construct the phylogenetic trees, 26 conserved gene sequences in 19 herpesvirus genomes, 23 conserved gene sequences in 33 herpesvirus genomes, and 6 conserved gene sequences in 40 herpesvirus genomes were used (Wang *et al*., 2007; McGeoch *et al*., 2006; Alba *et al*., 2001). The results were contradictory between the number of analyzed genes and the number of the species, e.g. the more species, the less conserved genes. Further whole-genome analysis method was the gene content analysis. The herpesviruses (13 species) were analyzed with this method, and the results were compared to those obtained by using a multidimensional methodology based on a distance measures and partial orderings of dinucleotide relative abundances (Montague and Hutchison, 2000; Karlin *et al*., 1994). The last method was the CVTree analysis based on the whole-genome sequences that was initially used for the construction of phylogenetic tree for 39 herpesviruses (Gao and Qi, 2007).

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Abbreviations: CVTree = compositional vector tree; COG(s) = clusters of orthologous group(s); ICTV = International Committee on Taxonomy of Viruses

In this study, the phylogeny of 45 herpesviruses was analyzed according to the whole-genome sequences using 4 methods: the alignment of conserved genes sequences, CVTree method, local homology analysis, and gene content analysis (Deng *et al*., 2006; Xing *et al*., 2006; Qi *et al*., 2004a; Alba *et al*., 2001). In addition, several herpesviruses with recently published genomes and unclassified herpesviruses were included in our phylogenetic analysis.

Materials and Methods

Alignment of conserved gene sequences. The conserved proteins were identified using the Tatusov method (Tatusov *et al*., 2003, 1997). Clusters of orthologous groups (COGs) of proteins were recognized by an all-against-all BLASTP similarity search between 40 complete genomes (Table 1) (Altschul *et al*., 1990). Twenty proteins that were conserved in 40 herpesviruses were

Table 1. Complete genomic sequences of herpesviruses

Subfamily	Genus	Virus (acronym)	Accession number	ORFs	Length (kb)
Alphaherpesvirinae	*Iltovirus*	Gallid herpesvirus 1 (GaHV-1)	NC_006623	76	149
	Mardivirus	Gallid herpesvirus 2 (GaHV-2)	NC_002229	109	174
		Gallid herpesvirus 3 (GaHV-3)	NC_002577	100	164
		Meleagrid herpesvirus 1 (MeHV-1)	NC_002641	101	159
	Simplexvirus	Cercopithecine herpesvirus 1 (CeHV-1)	NC_004812	75	157
		Cercopithecine herpesvirus 2 (CeHV-2)	NC_006560	75	151
		Human herpesvirus 1 (HHV-1)	NC_001806	77	152
		Human herpesvirus 2 (HHV-2)	NC_001798	77	155
		Cercopithecine herpesvirus 16 (CeHV-16)	NC_007653	75	156
	Varicellovirus	Bovine herpesvirus 1 (BoHV-1)	NC_001847	73	135
		Bovine herpesvirus 5 (BoHV-5)	NC_005261	73	138
		Cercopithecine herpesvirus 9 (CeHV-9)	NC_002686	72	124
		Equid herpesvirus 1 (EHV-1)	NC_001491	80	150
		Equid herpesvirus 4 (EHV-4)	NC_001844	79	146
		Human herpesvirus 3 (strain Dumas) (HHV-3)	NC_001348	73	125
		Suid herpesvirus 1 (SuHV-1)	NC_006151	77	143
	Unassigned	Psittacid herpesvirus 1 (PsHV-1)	NC_005264	73	163
Betaherpesvirinae	*Cytomegalovirus*	Cercopithecine herpesvirus 8 (CeHV-8)	NC_006150	223	221
		Human cytomegalovirus (HCMV)	NC_001347	151	230
		Human herpesvirus 5 strain Merlin (HHV-5)	NC_006273	165	236
		Pongine herpesvirus 4 (PoHV-4)	NC_003521	165	241
	Muromegalovirus	Murid herpesvirus 1 (MuHV-1)	NC_004065	161	230
		Murid herpesvirus 2 (MuHV-2)	NC_002512	167	230
	Roseolovirus	Human herpesvirus 6 (HHV-6)	NC_001664	123	159
		Human herpesvirus 6B (HHV-6B)	NC_000898	104	162
		Human herpesvirus 7 (HHV-7)	NC_001716	86	153
	Unassigned	Tupaiid herpesvirus 1 (TuHV-1)	NC_002794	158	196
Gammaherpesvirinae	*Lymphocryptovirus*	Callitrichine herpesvirus 3 (CalHV-3)	NC_004367	72	150
		Cercopithecine herpesvirus 15 (CeHV-15)	NC_006146	80	171
		Human herpesvirus 4 (HHV-4)	NC_001345	94	172
	Rhadinovirus	Alcelaphine herpesvirus 1 (AlHV-1)	NC_002531	71	131
		Ovine herpesvirus 2 (OHV-2)	NC_007646	73	135
		Bovine herpesvirus 4 (BoHV-4)	NC_002665	79	109
		Cercopithecine herpesvirus 17 (CeHV-17)	NC_003401	89	134
		Equid herpesvirus 2 (EHV-2)	NC_001650	79	184
		Human herpesvirus 8 (HHV-8)	NC_003409	82	138
		Murid herpesvirus 4 (MuHV-4)	NC_001826	81	119
		Saimiriine herpesvirus 2 (SaHV-2)	NC_001350	76	113
Unassigned	*Ictalurivirus*	Ictalurid herpesvirus 1 (IcHV-1)	NC_001493	92	143
Unassigned		Ostreid herpesvirus 1 (OsHV-1)	NC_005881	127	207
		Ateline herpesvirus 3 (AtHV-3)	NC_001987	73	108
		Ranid herpesvirus 1 (RaHV-1)	NC_008211	132	221
		Ranid herpesvirus 2 (RaHV-2)	NC_008210	147	232
Not classified*		Koi herpesvirus (KHV)	NC_009127	160	295
		Macaca fuscata rhadinovirus (MFRV)	NC_007016	171	131

*Not classified into taxonomic scheme yet (Faquet *et al*., 2005).

found, which were aligned using the ClustalW program and concatenated to form a single combined alignment covering 17285 amino acids including gaps (Table 2) (Higgins *et al*., 1994). The viruses OsHV-1, IcHV-1, KHV, RaHV-1, and RaHV-2 (viruses and their acronyms are presented in Table 1) were greatly divergent from other herpesviruses and excluded from the analysis. The phylogenetic analysis was performed using PHYLIP package version 3.6 with the neighbor-joining method (Felsenstein, 1988). The reliability of the phylogenetic relationships was evaluated statistically from 100 bootstrap replicates (Felsenstein, 1985).

CVTree method. Amino acid sequences of all proteins in 45 herpesviruses were used to perform the CVTree program in order to analyze the phylogeny. This program used the normalized 5 aa peptide frequencies to calculate the distance of every two genomes and reconstruct the phylogenetic tree with the neighbor-joining method in the PHYLIP package.

Local homology analysis. The maximal scores of segment pairs based on an all-against-all BLASTP of genomes were used to calculate the distance matrix and reconstruct the phylogenetic tree with the neighbor-joining method in the PHYLIP package.

Gene content analysis. A pairwise hit in the definition of the COGs and a shared gene between two genomes were recorded. The total number of shared genes was used for calculation of the distance matrix and for reconstruction of the phylogenetic tree with the neighbor-joining method in the PHYLIP package.

Table 2. Conserved genes in herpesviruses

Gene of HHV-1	Accession number	Function*	Functional class**
UL2	GI:9629382	uracil-DNA glycosylase	Nuc
UL5	GI:9629385	component of DNA helicase-primase complex	Rep
UL6	GI:9629386	minor capsid protein	Str
UL7	GI:9629387	unknown	Unk
UL10	GI:9629390	virion glycoprotein M	Gly
UL12	GI:9629392	deoxyribonuclease	Nuc
UL13	GI:9629393	protein kinase	Oth
UL18	GI:9629398	capsid protein	Str
UL22	GI:9629402	virion glycoprotein H	Gly
UL24	GI:9629404	fusion protein	Str
UL25	GI:9629405	capsid associated tegument protein	Str
UL26	GI:9629406	protease	Str
UL27	GI:9629408	virion glycoprotein B	Gly
UL28	GI:9629409	DNA packaging	Str
UL31	GI:9629412	unknown	Unk
UL32	GI:9629413	virion protein	Str
UL39	GI:9629420	ribonucleotide reductase large subunit	Nuc
UL50	GI:9629432	deoxyuridine triphosphatase	Rep
UL52	GI:9629434	component of DNA helicase-primase complex	Rep
UL54	GI:9629436	immediate early protein	Trf

*Function derived from GenBank annotations.**Functional classes: Rep (replication), Nuc (nucleotide metabolism and DNA repair), Str (structural), Trf (transcription), Gly (glycoprotein), Oth (other), Unk (unknown).

Results

Phylogenetic tree deduced from the alignment of conserved gene sequences

The conserved genes identified with Tatusov method were listed in the table of the core genes defined by Davison, although some of the core genes were not identified as the conserved genes by our method that used a different and stricter homology definition, i.e. every COG should be present in all 40 species (Davison, 2002).

According to the majority rule, the consensus tree of the neighbor-joining trees inferred from the concatenation of 20 conserved genes shared by all 40 herpesviruses supported the classic taxonomy that divided the family *Herpesviridae* into three subfamilies (Fig. 1). The subfamily *Alphaherpesvirinae* was further divided into 4 genera *Simplexvirus*, *Varicellovirus, Mardivirus*, *Iltovirus,* the subfamily *Betaherpesvirinae* into 3 genera *Cytomegalovirus*, *Muromegalovirus, Roseolovirus*, and the subfamily *Gammaherpesvirinae* into 2 genera *Rhadinovirus* and *Lymphocryptovirus* with very high bootstrap support.

Several previously unclassified or ambiguously classified viruses were categorized by this method with high bootstrap support. For names and acronyms of the viruses mentioned in this paragraph, see Table 1. TuHV-1 that was previously known as unassigned virus belonging to the betaherpesviruses was found to cluster with members of the genus *Cytomegalovirus* with very high bootstrap support (bootstrap value = 100%). BoHV-4 clustered with SaHV-2 and AtHV-3 (bootstrap value = 100%). EHV-2 and AlHV-1/OHV-2 were the most divergent viruses from other rhadinoviruses, and MuHV-4 formed the most divergent branch within the gammaherpesviruses. MFRV has not been assigned in the family *Herpesviridae*, but clustered with CeHV-17 with high bootstrap support (bootstrap value = 100%) (Faquet *et al*., 2005). GaHV-1 and PsHV-1 formed the most divergent branch within the alphaherpesviriruses (bootstrap value = 100%).

Phylogenetic tree deduced by the CVTree method

The phylogenetic tree inferred by the CVTree method was properly consistent with the conserved gene tree within the three subfamilies, although inside the outgroup, the two frog herpesviruses (RaHV-1 and RaHV-2) were separated, one clustering with KHV, and the other with IcHV-1 and OsHV-1 (Fig. 2).

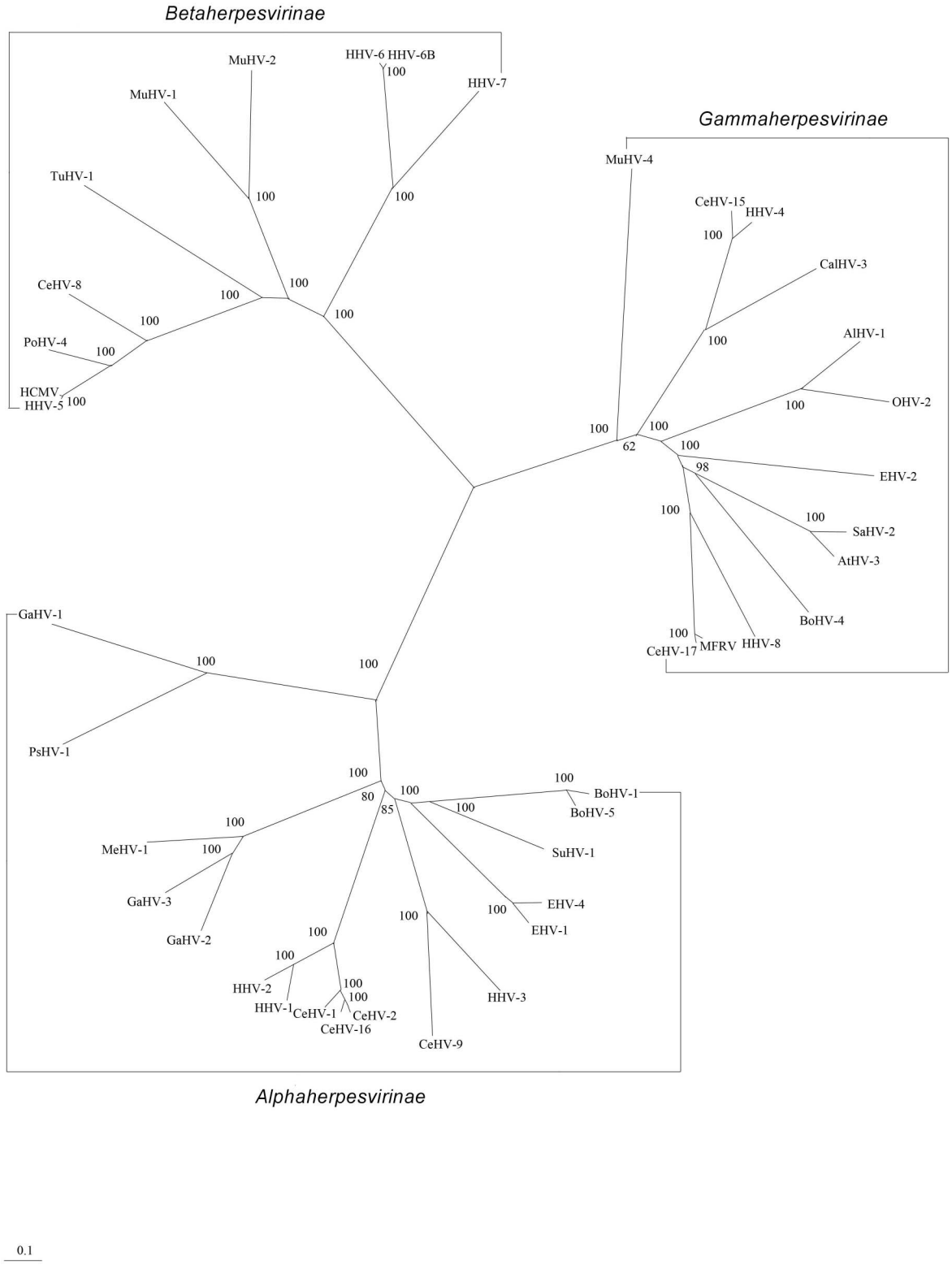

Fig. 1

Phylogenetic tree of herpesviruses inferred from the alignment of conserved gene sequences

The numbers indicate the percentage of bootstrap supporting each branch. Viruses and their acronyms are showed in Table 1.

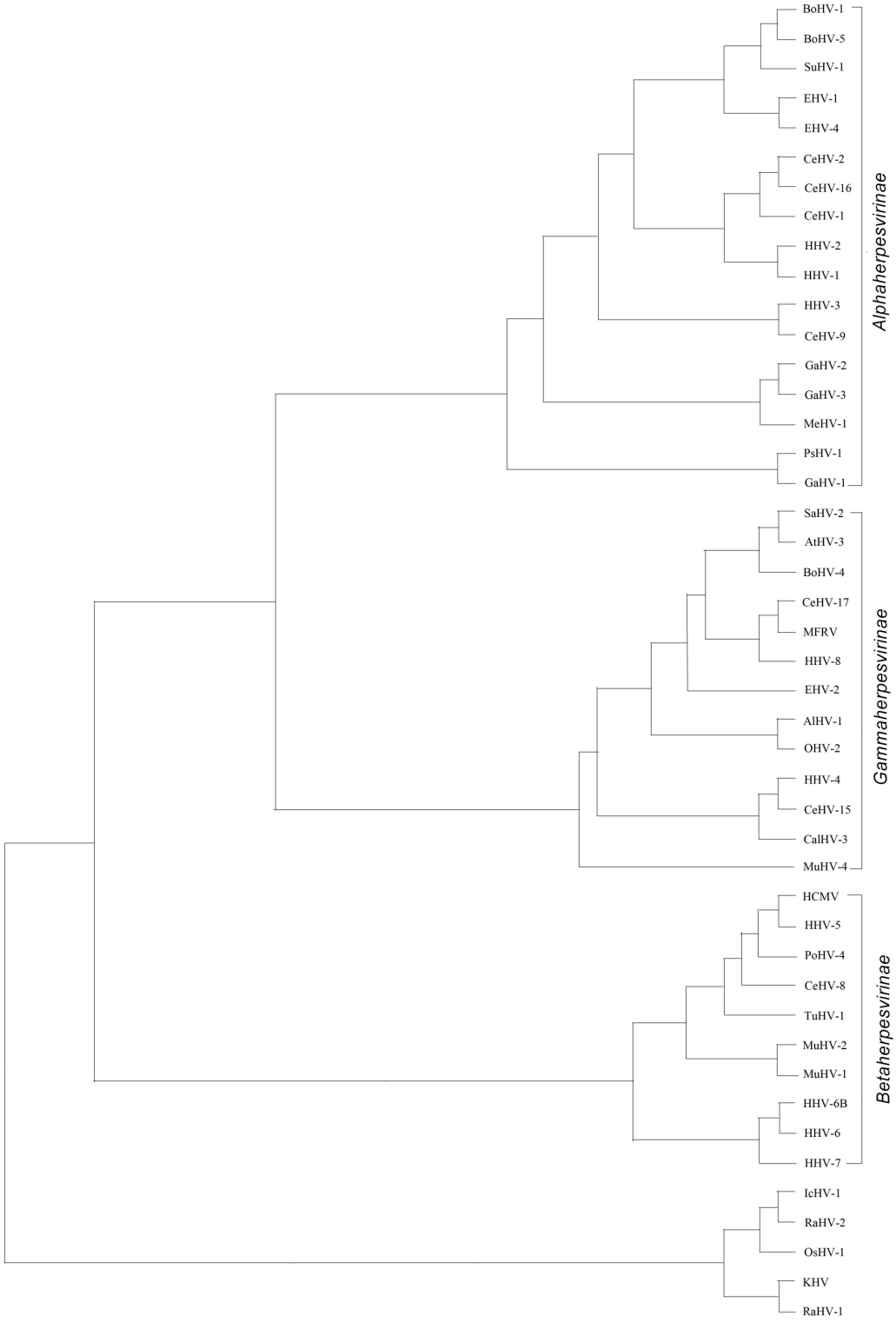

Fig. 2

Phylogenetic tree of herpesviruses inferred by the CVTree method

Viruses and their acronyms are showed in Table 1.

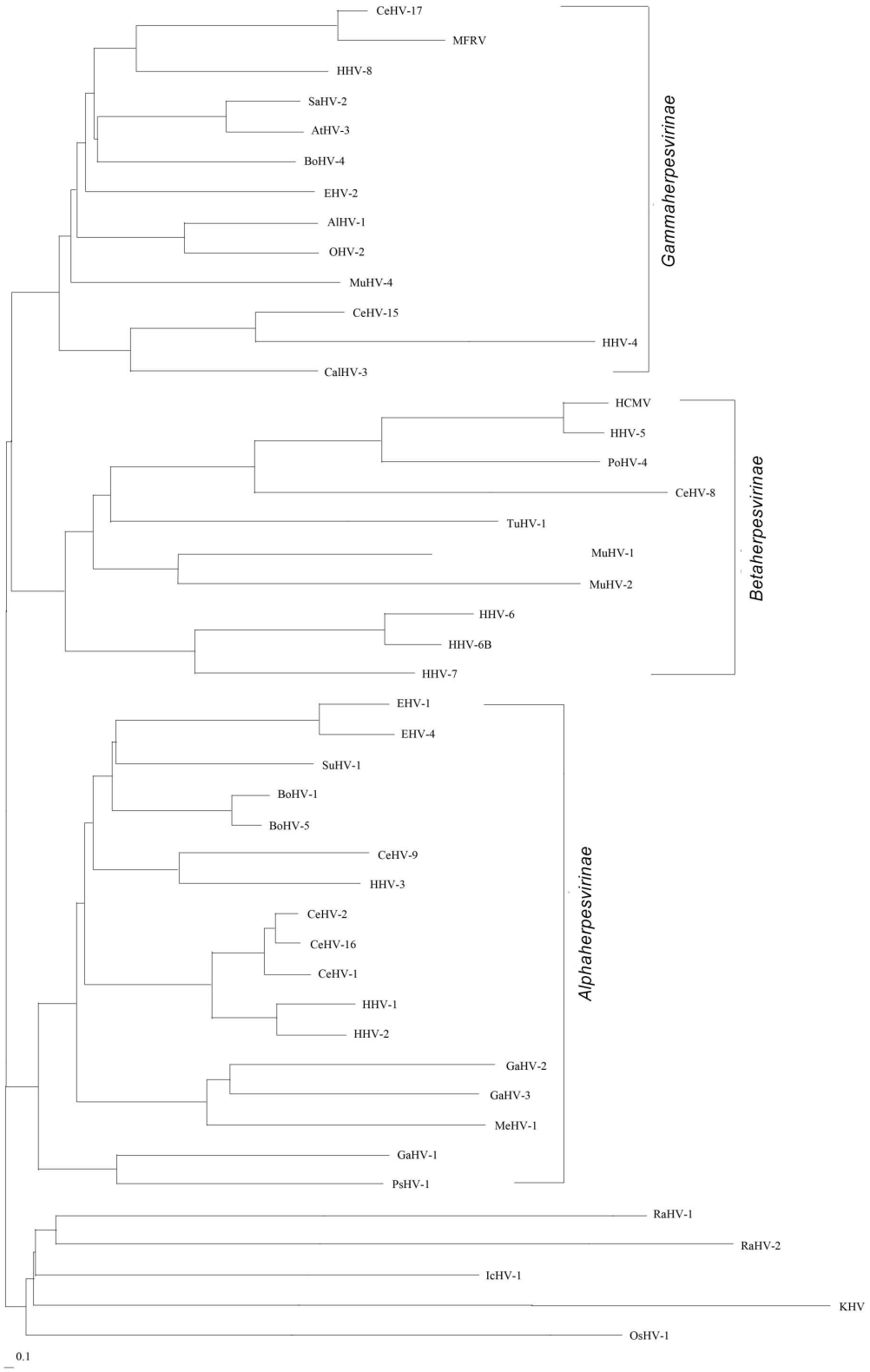

Fig. 3

Phylogenetic tree of herpesviruses inferred from local homology analysis

Viruses and their acronyms are showed in Table 1.

Phylogenetic tree inferred by local homology analysis

The tree generated from local homology analysis, which was rooted with RaHV-1, RaHV-2, KHV, IcHV-1, and OsHV-1 (viruses and their acronyms are presented in Table 1) was adequately consistent with those from alignment of conserved gene sequences or by the CVTree method with the exception for the MuHV-4 and the root. MuHV-4 was closer to the rhadinoviruses in the tree by local homology analysis than in other two trees (Fig. 3). Within the root, the two frog herpesviruses formed a sister-group with the fish herpesvirus IcHV-1 and clustered with the other fish herpesvirus KHV, which has not been assigned to the family *Herpesviridae* yet (Faquet *et al*., 2005). Finally, all these viruses were clustered with the single invertebrate herpesvirus OsHV-1.

Phylogenetic tree inferred from the gene content analysis

The phylogenetic tree inferred from the gene content analysis that was rooted with RaHV1, RaHV-2, KHV, IcHV-1, and OsHV-1 was consistent with the previous trees in general except for some differences inside the subfamilies (Fig. 4). The cluster inside the root was the same as at the tree made by the local homology analysis.

Discussion

This study represented a comprehensive phylogenetic analysis of herpesviruses based on the whole-genome information that supported the classical division of herpesviruses into subfamilies and the majority of subdivisions into genera. Additionally, presented analyses successfully defined the classification of several previously unclassified or ambiguous viruses or viruses with whole-genomes published recently.

TuHV-1 is an unclassified herpesvirus that was classified in our 3 phylogenetic trees as a sister taxon of the genus *Cytomegalovirus* in the subfamily *Betaherpesvirinae,* what was consistent with the result of the previous study (Faquet *et al*., 2005; Bahr and Darai, 2004). Our results showed that the unassigned MFRV was most related to the CeHV-17, and the unassigned alphaherpesvirus PsHV-1 was closest to GaHV-1 in accordance with the previous study (Thureen and Keeler, 2006). These results confirmed the newest ICTV proposals that have not been passed into accepted taxonomy yet, but they were employed (McGeoch *et al*., 2006). Two avian herpesviruses, PsHV-1 and GaHV-1, are far divergent from other avian herpesviruses such as GaHV-2, 3 and MeHV-1 and were included into genus *Iltovirus*.

Another herpesvirus OHV-2 with the genome recently published, clustered with AlHV-1 in all our methods, in accordance with the phylogenetic analysis based on glycoprotein B sequence (Dunowska *et al*., 2001). BoHV-4 was ambiguous in previous studies (Wang *et al*., 2007; McGeoch *et al*., 2005; McGeoch, 2001). In our three phylogenetic analyses, BoHV-4 clustered with AtHV-3/SaHV-2. The placement of two most divergent branches EHV-2 and AlHV-1/OHV-2 within the genus *Rhadinovirus* was in accordance with other analyses (McGeoch *et al*., 2005; McGeoch, 2001).

Five lower-vertebrate herpesviruses (RaHV-1, RaHV-2, IcHV-1, KHV, and OsHV-1) were so divergent from other mammalian and avian herpesviruses that they were not analyzed by previous phylogenetic methods based on the specific genes. They clustered together and were selected as the outgroups in our study. Inside the cluster, local homology analysis and gene content analysis revealed unanimous relationships. Two frog herpesviruses RaHV-1 and RaHV-2 clustered together and formed the center of the fish clade close to the IcHV-1, the placement consistent with the outcome of other genomic study for these two viruses (Davison *et al*., 2006, 1999). The other fish herpesvirus KHV with the largest genome among the herpesviruses was the most divergent from the fish clade. Owing to the unusual large genome, early studies suggested that it did not belong to herpesviruses (Ronen *et al*., 2003). However, comparisons conducted on a more appropriate basis found that KHV was related to the fish herpesvirus (Waltzek *et al*., 2005). OsHV-1, the only member of the herpesviruses that has an invertebrate host, was the most divergent branch from other viruses inside the root cluster. Due to the specific host and high divergence, previous paper demonstrated that it represented a third major class of the herpesviruses other than the mammalian, avian, and fish herpesviruses (McGeoch *et al*., 2006). This assignment corresponded with the genomic analysis (Davison *et al*., 2005).

Although the analysis based on the conserved genes included sequences of 20 conserved genes, some data such as the special genes of some organisms was still neglected. For example, MuHV-4 is highly divergent. The 20 conserved genes could not reflect its detailed, special characteristics, but only its common characteristics within the gamma-herpesviruses. Therefore, MuHV-4 was placed at the bottom of the genus *Lymphocryptovirus.* By the analysis based on local homology, the position of MuHV-4 was pushed into the genus *Rhadinovirus*, though it was still the most divergent. In general, this result was consistent with the conclusion of McGeoch *et al*., 2005, who put the BoHV-4, MuHV-4, AtHV-3/HVS-2, and HHV-8/CeHV-17 into a multifurcated branch.

The CVTree method made the distance less apparent and is suitable for the more divergent species, for example the bacteria (Qi *et al*., 2004b). However, for the related species, it could be inappropriate. In our study, most of the

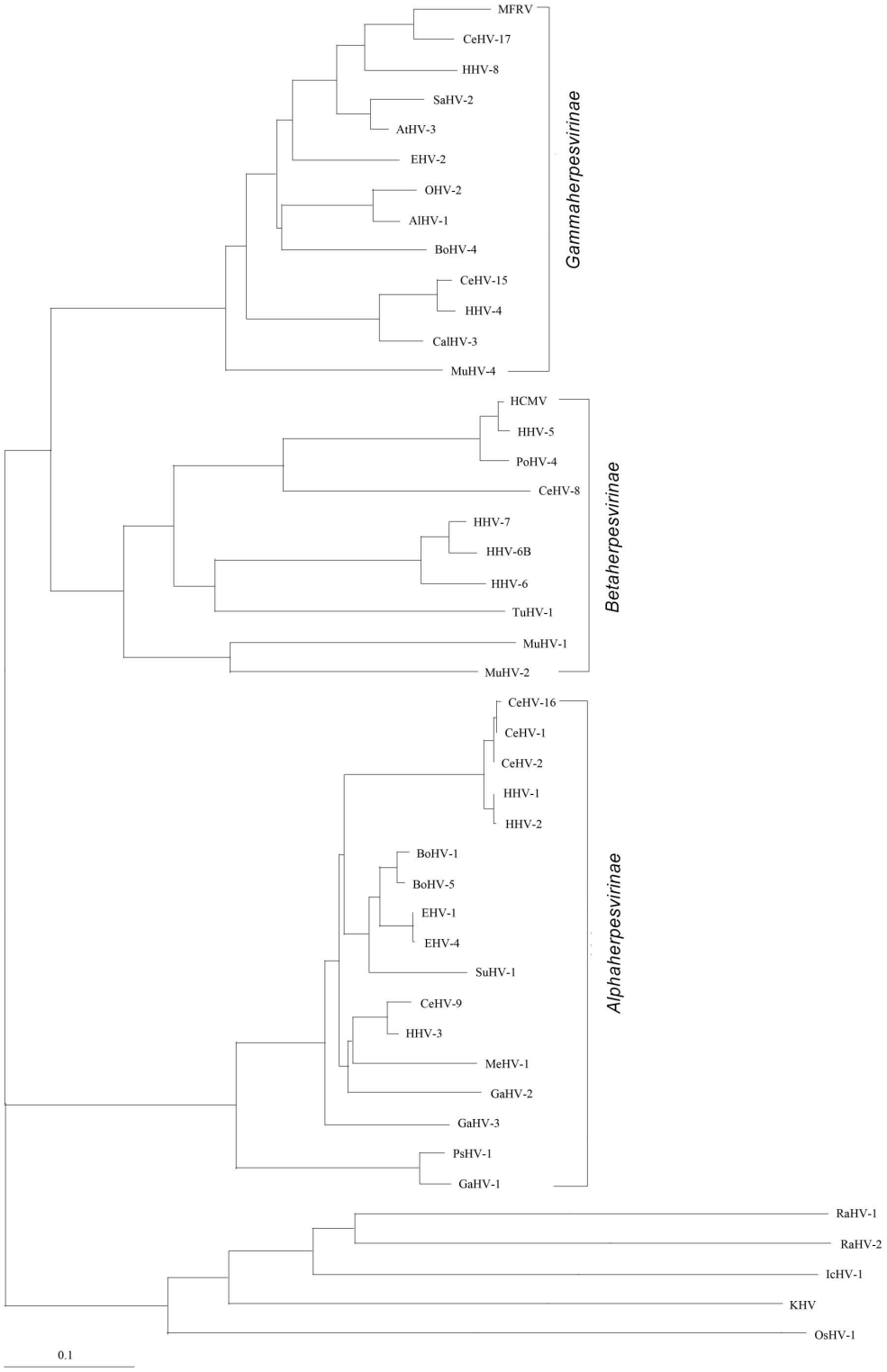

Fig. 4

Phylogenetic tree of herpesviruses inferred from gene content analysis

Viruses and their acronyms are showed in Table 1.

phylogenetic relationships resolved by this method were sufficiently consistent with the previous studies except for the fish, frog, and the invertebrate herpesviruses (inside the root).

Another method based on genome information that we used in this study was the local homology analysis that was fast in the tree formation. The phylogenetic trees inferred by this method were consistent well with the classic taxonomy except for the detailed branch of SuHV-1. This method combines the detailed information of conserved and non-conserved genes. Nevertheless, it ignores the order and the orientation of the genes. The orientation of some SuHV-1 genes was different from that of the other herpesviruses in the same genus, so this fact resulted in the unusual placement of SuHV-1. Anyway, for the phylogenetic analysis of herpesviruses this method based on the whole-genome information was suitable, especially for the genes having little difference in the arrangement inside the genera.

In general, the result by gene content method was consistent with the classic taxonomy with a slight alteration that was probably caused by the methodology that identified only the presence or absence of the conserved gene neglecting the detailed gene sequences and gene arrangement.

Acknowledgement. This study was supported by the grant 04009785 from the National Science Foundation of Guangdong Province.

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