Research Article

Molecular Phylogeny of Suid Herpesvirus 1

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Pseudorabies is a disease that significantly impacts the swine industry. This disease is caused by Suid Herpesvirus 1 (SuHV-1),
which is a double-stranded DNA virus that belongs to the Herpesviridae family and the Alphaherpesvirinae subfamily and exhibits
a slow rate of genetic evolution. The aim of this study was to use both full and partial sequences of SuHV-1 genes available in
GenBank to examine the evolution and divergence of viruses isolated in different parts of the world. Partial and complete sequences
of SuHV-1 genes were obtained either from GenBank (i.e., *us6*, *us7*, *us8*, *us9*, *ul14*, *ul49.5*, and *ul44*) or from genetic sequencing
of Brazilian SuHV-1 samples. The results of this study corroborate previous phylogenetic studies of SuHV-1 that demonstrated
different evolutionary profiles of isolates from different parts of the globe, with a rapid genetic dispersion of Chinese isolates. All
of the phylogenetic trees generated in this study demonstrated a large genetic distance between SuHV-1 isolates from the Western
and Eastern regions of the world.

1. Introduction

Pseudorabies (PR) is a disease that significantly impacts the
swine industry. This disease is caused by Suid Herpesvirus 1
(SuHV-1), which is a double-stranded DNA virus that belongs
to the Herpesviridae family and the Alphaherpesvirinae
subfamily and exhibits a slow genetic evolution. The primary
hosts of SuHV-1 are domestic or wild pigs (*Sus scrofa*). This
disease also affects cows, dogs, and cats, but, in these species,
the disease is fatal in nearly 100% of cases [1]. Although PR has
been eradicated in domestic swine in many countries, such
as the United States, Germany, and England, this disease still
occurs in wild boars and other feral swine in these countries
[2].

SuHV-1 has only one serotype and can be genetically
typed using methods including whole genome enzymatic
restriction with *BamH*I, which demonstrates the presence of
four genomic types [3, 4]. However, most recent publications
also use partial sequences of the *ul44* gene to evaluate the
genetic variation of SuHV-1 isolates [5–8]. This gene encodes
glycoprotein C (gC), which is a protein that is considered

to be a potent inducer of the immune response and the
principal component of adhesion to host cell receptors [9].
Phylogenetic analysis of partial sequences of *ul44* separates
SuHV-1 into five genotypes, with specific clades that include
sequences derived from viruses isolated from domestic pigs
or wild boars [10].

The *us8* gene has also been used for phylogenetic analysis
of SuHV-1 [6, 11, 12]. This gene is more conserved than
ul44 but can also be used to detect genetic differences
between SuHV-1 isolates from different regions. *us8* encodes
glycoprotein E (gE). Although gE is not essential for viral
replication, the absence of this protein reduces the virulence
of the virus. *us8* is frequently deleted in attenuated SuHV-1
vaccine strains [13].

Sequences from other SuHV-1 genes are available in
GenBank, but these genes are not used in studies of genetic
variability. The herpesvirus genome is typically highly con-
served [3] and even genes such as *ul44* do not have sufficient
variability to generate high-resolution phylogenetic trees [5–
8]. Other genes could also be used, and some genes that
are involved in envelope formation could contain enough

TABLE 1: Genetic regions used in this work.

Gene	Protein	Function	Type	Quantity	Size (base pairs)*	Position*
ul44	gC	Cellular adhesion	Complete	24	1443	53019–54461
us6	gD	Binding to surface receptors	Complete	16	1197	118873–120075
us7	gI	Cell to cell spread	Complete	9	1086	120111–121193
			Partial	10	676	120111–120786
us8	gE	Cell to cell spread	Complete	22	1734	121297–123030
			Partial	48	404	121945–122348
us9	11 kDa	Axonal transport	Complete	19	321	123088–123384
ul14	*ul14*	Viral morphogenesis	Complete	12	480	75946–76425
ul49.5	gN	Viral morphogenesis and membrane fusion	Complete	22	297	8840–9136

*These data refer to the complete genome of the Kaplan strain (JF797218).

phylogenetic information to divide the virus into different groups and provide more information about the transmission and worldwide distribution of this virus [14].

This study aimed to use both full and partial sequences of SuHV-1 genes available in GenBank to examine the evolution and divergence of strains isolated in different parts of the world. We focused on alternative genes (i.e., *us6*, *us7*, *us8*, *us9*, *ul14*, and *ul49.5*) to the commonly used *ul44* to verify the genetic divergence of SuHV-1 from the Occident to the Orient.

2. Material and Methods

2.1. Genetic Sequences. Partial and complete sequences of SuHV-1 genes were obtained from GenBank (Table 1) together with all available information related to host, year, and country of isolation [6, 8, 11, 12, 15–17]. The sequences were named as the denomination of the isolate in GenBank followed by a three-letter code to identify the country of origin: Germany (GER), Argentina (ARG), Austria (AUS), Brazil (BRA), China (CHI), North Korea (SKO), Slovakia (SLK), Spain (ESP), the United States (USA), Japan (JAP), Hungary (HUN), Northern Ireland (NIR), Malaysia (MAL), and Sweden (SWE).

2.2. PCR. The *us9*, *ul14*, and *ul49.5* genes of Brazilian SuHV-1 strains and the standard Shope strain were sequenced in our laboratory to facilitate comparison. Each 20 μL reaction contained the following reagents: 8% DMSO, 200 mmol/L of DNTPs, 1 pmol/μL of each primer (Table 2), 1.5 mmol MgCl$_2$, 0.2 U of Jumpstart Taq (Sigma, USA), 4 μL of Green GoTaq 5x buffer (Promega, USA), and 2 μL of DNA. The thermocycler was programmed with the following steps and cycling times: 95°C for 5 min, followed by 35 cycles of 95°C for 50 s, 57°C for 50 s, and 72°C for 50 s and a final extension stage of 72°C for 5 min. The results were visualized on 1.5% agarose gels stained with ethidium bromide after running for one hour at 100 volts. The PCR products were sequenced using ABI 3130 equipment (Lifetech, USA).

2.3. Phylogenetic Analysis. Phylogenetic analysis was performed according to sample complexity. We first reconstructed all trees using substitution model F81 and Neighbor

TABLE 2: Primers used in this study for amplification of SuHV-1 genes *us9*, *ul14* e *ul49.5*.

Gene	Primer (5′-3′)	Amplicon (pb)
us9	F: GAGAAACCGGAAGTGACGAA	550
	R: GGGGCCCATTTATTGTGAC	
ul14	F: GCGATGGCAAAGTTGAAAAA	794
	R: GTCGAGGGTCGCGTACTG	
ul49.5	F: CCCAGGGGAACCTTATAAATC	447
	R: TTTCTCGAGCTGGACATGG	

Joining. We then tested other evolutionary models that were selected using jModelTest software [18]. The models and software programs used in this study are presented in Table 3 [19, 20]. All sequences were tested for positive selective pressure using Selecton software [21]. Because a large number of *us8* sequences are available, we used the programs of DNASP [22] and Network [23] for phylogenetic network analysis.

3. Results

Phylogenetic analysis of multiple sequences derived from different regions of the SuHV-1 genome generated trees with similar profiles; however, the *us6* gene was an exception (Figures 1, 2, 3, and 4). The primary characteristic observed was the formation of two clusters. Cluster 1 included sequences from the Occident and a small number of Asian samples, and Cluster 2 included exclusively sequences from countries in the East (i.e., China, Malaysia, Taiwan, and South Korea). The partial sequences of the *us8* gene generated a tree with a lower degree of differentiation. No differences in tree information were observed for the trees generated from the complete and partial sequences of *us7*. Some isolates, such as the Hungarian (i.e., Kaplan and Shope) and Brazilian sequences, clustered together in all trees. The Rice and Becker samples from the USA also exhibited similar profiles. The bootstrap values were low in most trees, except in cases such as Clusters 1 and 2.

The Asian SuHV-1 genome sequences presented two patterns. The first pattern was close to that of the Western samples and was distributed among different subgroups, close to a group of atypical strains that are most frequently

TABLE 3: Substitution models, reconstruction of phylogenetic tree and programs used for each group of sequences studied in this work.

Gene	Type	Evolutionary models	Phylogeny	Software
ul44	Complete	GTR Γ4+I	Bayesian Inference	Mr.Bayes[a]
	Partial			
us6	Complete	GTR	Maximum Likelihood	Seaview[b]
us7	Complete	HKY+Γ	Maximum Likelihood	Seaview
	Partial			
us8	Complete	GTR+Γ	Maximum Likelihood	Seaview
	Partial	HKY	Maximum Likelihood	Seaview
us9	Complete	F81	Neighbor Joining	Seaview
ul14	Complete	F81	Neighbor Joining	Seaview
ul49.5	Complete	TrN	Neighbor Joining	Seaview

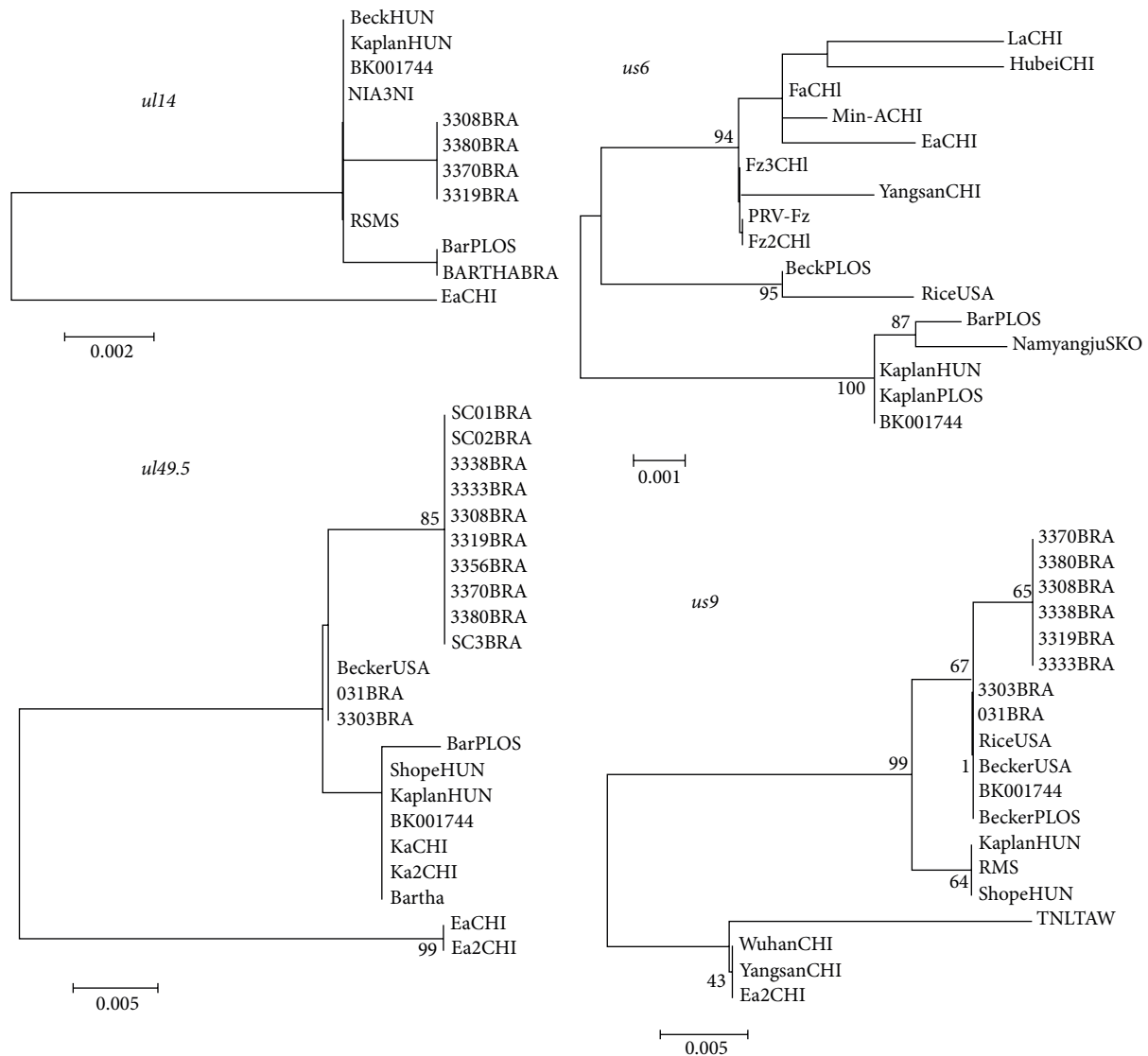[a]Huelsenbeck e Ronquist, 2001 [b]Gouy et al., 2010 [20].

FIGURE 1: Phylogenetic trees for genes *ul14*, *ul49.5*, *us6*, and *us9*. The models used in the reconstruction of each tree are listed in Table 3. Only bootstrap values above 70 are shown. Topologies are similar with the exception from the one constructed from *us6*, indicating conflicting signals in the analysis.
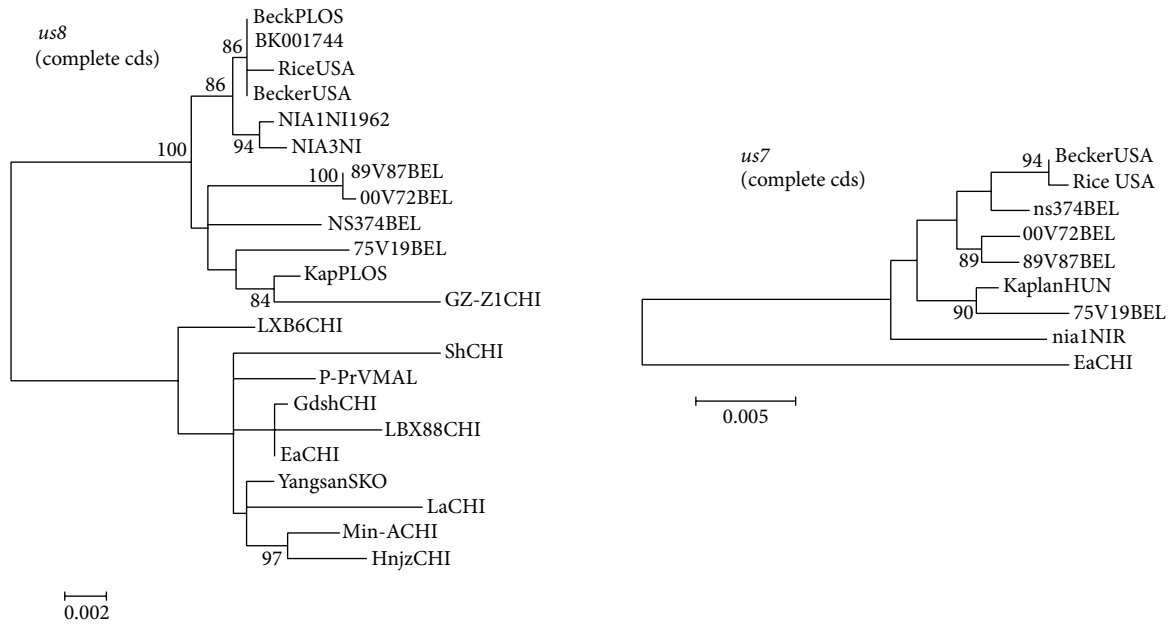
FIGURE 2: Phylogenetic trees for genes *us7* and *us8*. The models used in the reconstruction of each tree are listed in Table 3. Only bootstrap values above 70 are shown.

found in Brazil but also occur in the USA and Europe. The second pattern is more distant and acts as a root for all trees reconstructed in this work, with the exception of *us6*. In both cases, the sequences of Asian origin exhibited a greater degree of genetic variation than sequences within other clades.

Phylogenetic analysis using only partial sequences of the *us8* gene in DNAsp identified a total of 31 haplotypes; 23 of these haplotypes were formed exclusively by sequences from China. Only 11 polymorphic sites were observed in Western haplotypes (i.e., 5 singleton variable sites and 6 parsimony informative sites). In contrast, 53 polymorphic sites were observed in Eastern haplotypes (i.e., 38 singleton variable sites and 15 parsimony informative sites). The phylogenetic network demonstrated the divergence of these two main groups of SuHV-1 (Figure 5). Three main haplotypes form the base of the network. The first haplotype has a low frequency and is represented by a GenBank isolate named Kaplan, which is a strain that typically clusters with sequences derived from SuHV-1 isolates from wild boars. The other two groups occur more frequently. The Western cluster was formed by 15 sequences from Europe (i.e., Italy and Belgium), a single sequence from the USA, and a single sequence from Brazil. The Eastern cluster in the base of the network is formed by nine sequences from China and one sequence from South Korea.

4. Discussion

Molecular analyses to characterize SuHV-1 isolates are commonly performed using partial sequences of the *ul44* gene [10]; other genes are rarely studied. The purpose of this study was to analyze partial and complete sequences of SuHV-1 genes available in GenBank. All of the phylogenetic trees generated in this study demonstrated a large genetic distance between strains isolated in the Western and Eastern regions of the globe.

SuHV-1 has only one serotype, but this virus can be classified into four genomic types by restriction enzyme digestion analysis of the complete genome [4]. This analysis requires large amounts of viral DNA and can be laborious and time consuming. For this reason, partial gene sequencing has been increasingly used to replace full genome sequencing analysis [5–8, 10–12]. Phylogenetic analysis of partial *ul44* sequences demonstrated that SuHV-1 has a slow rate of genetic evolution and can be divided into five groups that are designated as A, B, C, D, and E [10]. Genetic groups A and C are formed by strains isolated from outbreaks of PR related to feral pigs. Groups B and D are formed by strains isolated from outbreaks related to domestic pigs. Group E is the most genetically diverse group and includes strains from Eastern regions, primarily China. Phylogenetic analysis using complete sequences of *ul44* generated genetic groups similar to those described in previous studies that used only partial sequences [10, 12].

Phylogenetic analysis of sequences available in the GenBank revealed a deep genetic distance between Clusters 1 and 2. Cluster 1 was divided into at least two clades and was always separated from the Eastern SuHV-1 genomic sequences. The low genetic diversity of these isolates is consistent with the slow evolution of herpesviruses. In some cases, SuHV-1 strains exhibit the same genetic profile for up to twenty years in the same region [6, 8]. The low genetic diversity of SuHV-1 is also responsible for the low bootstrap values observed in the trees constructed in this study and in previous studies [5, 7, 8]. The smaller size of the partial sequences of the *us8* gene generated a tree with a lower degree of differentiation,

FIGURE 3: Phylogenetic trees for partial sequences of gene *us8*. The models used in the reconstruction of each tree are listed in Table 3. Only bootstrap values above 70 are shown.
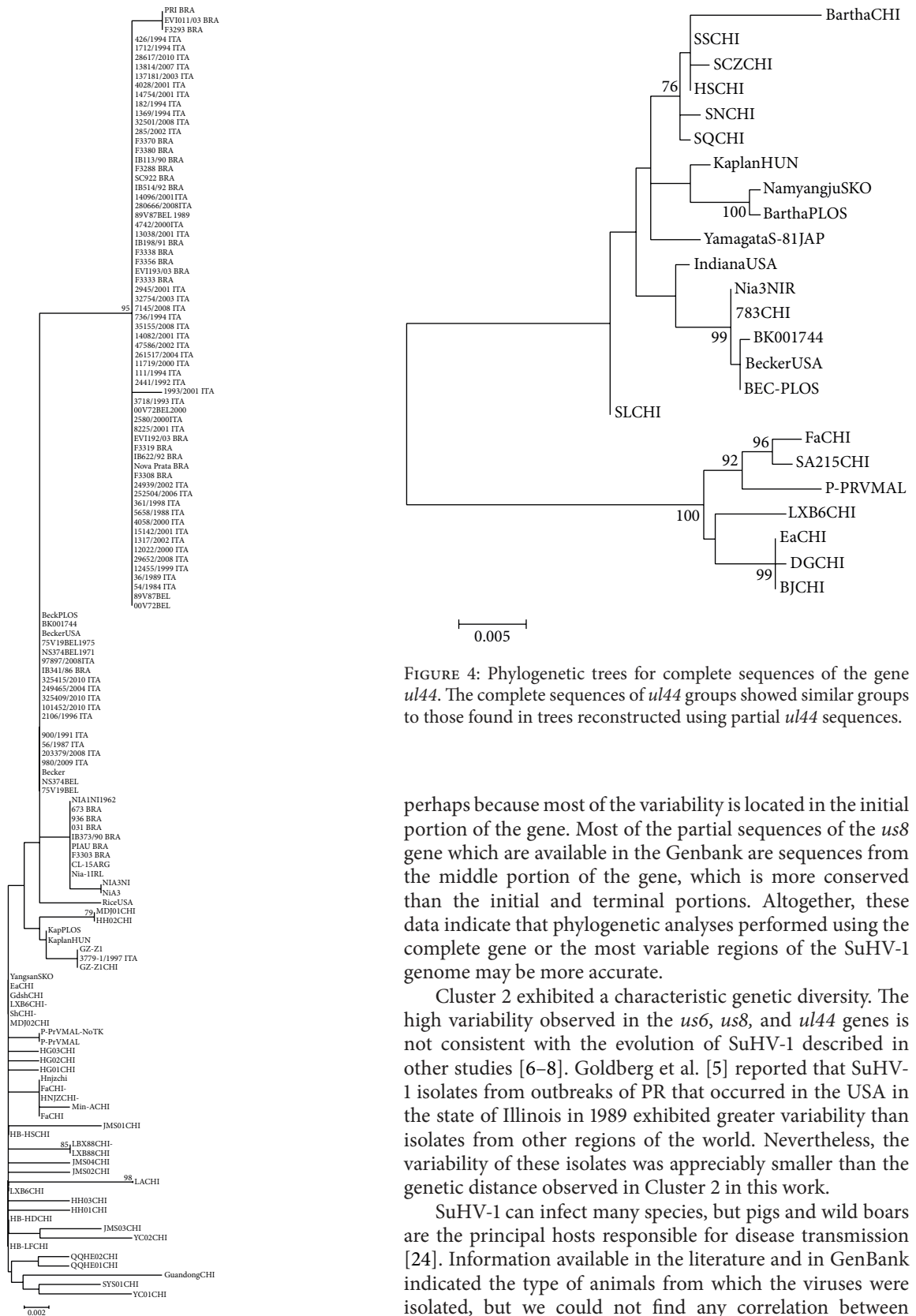

FIGURE 4: Phylogenetic trees for complete sequences of the gene *ul44*. The complete sequences of *ul44* groups showed similar groups to those found in trees reconstructed using partial *ul44* sequences.

perhaps because most of the variability is located in the initial portion of the gene. Most of the partial sequences of the *us8* gene which are available in the Genbank are sequences from the middle portion of the gene, which is more conserved than the initial and terminal portions. Altogether, these data indicate that phylogenetic analyses performed using the complete gene or the most variable regions of the SuHV-1 genome may be more accurate.

Cluster 2 exhibited a characteristic genetic diversity. The high variability observed in the *us6*, *us8*, and *ul44* genes is not consistent with the evolution of SuHV-1 described in other studies [6–8]. Goldberg et al. [5] reported that SuHV-1 isolates from outbreaks of PR that occurred in the USA in the state of Illinois in 1989 exhibited greater variability than isolates from other regions of the world. Nevertheless, the variability of these isolates was appreciably smaller than the genetic distance observed in Cluster 2 in this work.

SuHV-1 can infect many species, but pigs and wild boars are the principal hosts responsible for disease transmission [24]. Information available in the literature and in GenBank indicated the type of animals from which the viruses were isolated, but we could not find any correlation between clusters, genetic variability, and species (data not shown). Other studies reported differences between strains isolated from pigs and from wild boars [10, 12], but no specific viruses have been isolated from cows, dogs, or cats to date [5, 10].
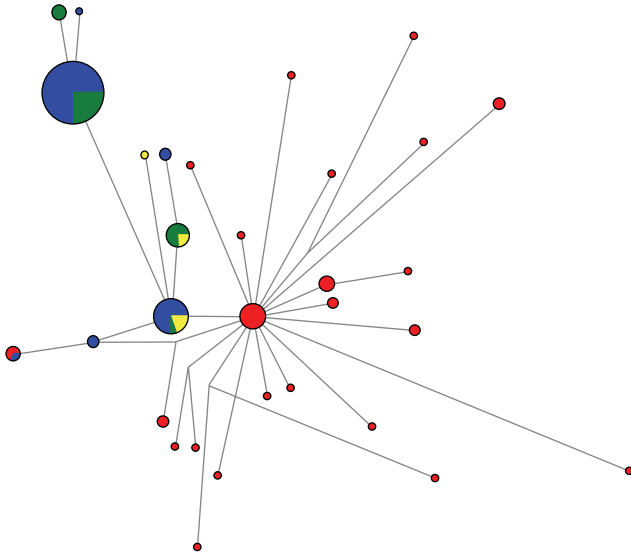
FIGURE 5: Phylogenetic network reconstructed using network from *us8* sequences of SuHV-1 isolates. Each circle is colored according to the number of sequences from different regions grouped in that haplotype. Red represents sequences from strains isolated in Asia, green from strains isolated in South America, yellow from strains isolated in USA, and blue from strains isolated in Europe. The base of the network is formed by three haplotypes. Western and Eastern sequences are connected by the two most frequent haplotypes present.

Most studies of SuHV-1 phylogeny are based only on reconstructed phylogenetic trees. We used phylogenetic networks to analyze genetic variability in partial *us8* sequences. Three haplotypes were found in the base of the network; one haplotype formed exclusively by sequences from Europe, another haplotype formed by sequences from Europe, the United States, and South America, and a third haplotype formed by sequences from China. The phylogenetic network generated in this study demonstrates a low dispersion of SuHV-1 in the Western part of the world and a rapid dispersion of SuHV-1 from a homogeneous group of samples to a higher frequency of haplotypes in China. Only the *us8* and *ul44* genes exhibited evidence of positive selective pressure; thus, natural selection alone does not explain the genetic diversity of SuHV-1 isolates from China. The high genetic diversity of Cluster 2 may be associated with the high prevalence of SuHV-1 in China [25].

5. Conclusion

The results of this work corroborate other phylogenetic studies that demonstrated different evolutionary profiles of SuHV-1 isolates from the Eastern and the Western regions of the globe, with a rapid genetic dispersion of Chinese isolates.

Conflict of Interests

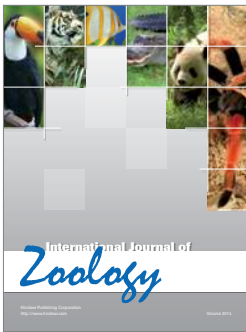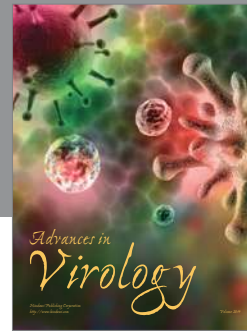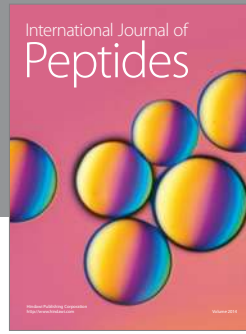The authors declare that there is no conflict of interests regarding the publication of this paper.

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