Arch Virol (2011) 156:1835–1840
DOI 10.1007/s00705-011-1024-x

BRIEF REPORT

A novel gammaherpesvirus isolated from a black-tailed prairie dog (*Cynomys ludovicianus*)

Brandy Nagamine · Leandro Jones · Christian Tellgren-Roth · Jacqueline Cavender · Ana C. Bratanich

Received: 23 February 2011 / Accepted: 6 May 2011 / Published online: 1 June 2011

Abstract A new gammaherpesvirus, tentatively named cynomys herpesvirus 1 (CynGHV-1), was isolated from a black-tailed prairie dog (*Cynomys ludovicianus*). CynGHV-1 replicated cytopathogenically to moderate titers in various cell lines. Ten kb of the CynGHV-1 genome was sequenced using degenerate PCR and genomic cloning. Sequence similarities were found to different genes from known gammaherpesviruses. Phylogenetic analysis suggested that CynGHV-1 was in fact a novel virus closely related to representatives of different genera and unclassified members of the subfamily *Gammaherpesvirinae*. However, CynGHV-1 could not be assigned to any particular genus and therefore remains unclassified.

Keywords Gammaherpesvirus · Prairie dog · Novel virus

Introduction

Gammaherpesvirinae is a growing herpesvirus subfamily containing many members of interest for human/veterinary medicine and biomedical research. Gammaherpesviruses have been isolated and characterized in humans, various primates, ruminants, rodents, sea mammals and other animal species [14]. Some infections are not associated with clinical disease, but others have been associated with or are the direct cause of a variety of medical conditions [1]. Identification of potential new members of this subfamily [23] has sometimes been done in conjunction with amplification of conserved glycoprotein regions [3]. Classification of these unknown herpesviruses relies mainly on sequence comparison of these short segments, since these are the only data available. Isolation of many of these newly identified herpesviruses has not been accomplished. Furthermore, many of the known gammaherpesviruses are difficult to propagate *in vitro*, hindering detection as well as studies on latency and pathogenesis. In this paper, we describe a new, still unclassified gammaherpesvirus isolated from black-tailed prairie dog (*Cynomys ludovicianus*) that is easily propagated in cell culture.

In 2003, a young adult female black-tailed prairie dog (*Cynomys ludovicianus*) was trapped in Denver, CO, and was brought dead and partially eaten to the Wyoming State Veterinary Laboratory, a diagnostic facility located at the

The GenBank/EMBL/DDBJ accession numbers for the sequences reported in this paper are EU863271-863275 and EU863200.

Electronic supplementary material The online version of this article (doi:10.1007/s00705-011-1024-x) contains supplementary material, which is available to authorized users.

B. Nagamine · J. Cavender · A. C. Bratanich
Department of Veterinary Science, University of Wyoming, Laramie, WY 82070, USA

C. Tellgren-Roth
Center for Rural Health Research and Education, University of Wyoming, Laramie, WY 82070, USA

Present Address:
C. Tellgren-Roth
Rudbecklaboratoriet, Department of Genetics and Pathology, Uppsala University, 751 85 Uppsala, Sweden

L. Jones
Division of Molecular Biology, Estación de Fotobiología Playa Unión, CC 15, 9103 Playa Unión, Chubut, Argentina

Present Address:
A. C. Bratanich (✉)
Cátedra de Virología, Facultad de Ciencias Veterinarias, Universidad de Buenos Aires, Avenida Chorroarin 280, 1427 Buenos Aires, Argentina
e-mail: abratanich@fvet.uba.ar

University of Wyoming in Laramie, Wyoming. No viral lesions or histological changes indicative of viral infection were observed. Chlamydia, plague, and tularemia fluorescent antibody and culture tests were negative. The presumptive cause of death was overwhelming bacterial septicemia.

To attempt virus isolation, a homogenized mixture of tissue from lung, liver and spleen was overlaid on BHK-21 cells as described elsewhere [22]. On the third passage, cytopathic effect (CPE) in the form of syncytia was observed. The infected cells were examined by negative contrast electron microscopy, revealing enveloped, herpesvirus-like particles with a 100 nm capsid diameter (Fig. 1a). Since there were no published reports of known herpesviruses infecting members of this genus, a more detailed investigation was initiated. Cultures of Vero, baby hamster kidney (BHK-21) (ATCC #CRL 8544), and human rectal tumor 18 cells-clone G (HRT18-G) were permissive to CynGHV-1, as shown by the presence of syncytia (Fig. 1b). SV40-transformed Siberian ferret testis (Wyoming State Veterinary Laboratory [WSVL]), rabbit kidney 1 (ATCC #CCL 106) and bovine embryonic testicle primary lines showed no signs of CPE.

Genetic characterization was approached by two strategies. First, conserved sequences of the DNA polymerase (*pol*) gene were amplified by degenerate PCR and sequenced. Nucleic acid from virus cultures was partially purified using DNAzol® reagent (Invitrogen, CA, USA) following the manufacturer's instructions. Nested PCR amplification using degenerate primers directed to a conserved region of the herpesvirus *pol* gene was performed using methods described previously [20]. The reaction mix included 10 µl of 5X Green GoTaq® Flexi Buffer, 1 µl each of forward and reverse primers (25 pmol), 1 µl of dNTP (10 mM), 3 µl of $MgCl_2$ (25 mM), 3.75 µl of DMSO, 0.3 µl of GoTaq® DNA Polymerase (1.5 U; Promega, WI, USA), 1 µl of DNA (100 ng) and nuclease-free water adjusted to a final volume of 50 µl. Cycling conditions for the first reaction were as follows: initial incubation at 96°C for 2 min followed by 35 cycles (1 min at 94°C, 1 min at 60°C, 1 min at 72°C) and a final extension step at 72°C for 10 min. In the second round, the initial incubation was omitted. The PCR products were analyzed in 1.5% agarose gels, excised from the gel, and purified using the Wizard® SV Gel and PCR Clean-Up System (Promega, WI, USA). PCR could not be attempted on tissues, as no remaining original material was available. The 200-bp *pol* sequence that was obtained was deposited in GenBank under accession number EU863200. Preliminary BLAST analysis of this sequence indicated that CynGHV-1 belonged to the subfamily *Gammaherpesvirinae* (family *Herpesviridae*).

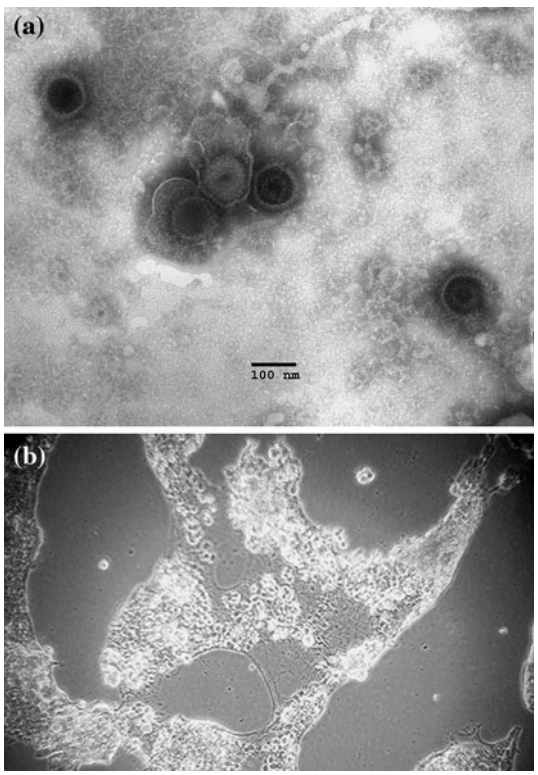

Fig. 1 **a**) Negative contrast electron microscopy of CynGHV-1 showing viral morphology indicative of herpesvirus virions. **b**) Monolayer of CynGHV-1-infected Vero cells

Next, genomic fragments were cloned for more extensive sequence studies. Ten µg of CynGHV-1 DNA was digested for 2 h at 37°C with restriction endonucleases BamHI, EcoRI, HindIII and PstI (New England Biolabs, MA, USA). Fragments were separated through a large 0.7 % agarose gel at 70 V overnight, quickly excised and eluted in 35 µl of nuclease-free water using the Wizard® SV Gel and PCR Clean-Up System (Promega, WI, USA). Fragments from 1 to 6 kb in size were chosen. Plasmid vector pUC18 was digested using the corresponding restriction enzymes, treated with calf intestinal phosphatase, separated in a 1% agarose gel, and eluted. T4 DNA ligase (Promega, WI, USA) was used to ligate the corresponding digested vector and fragments and/or vector alone following the manufacturer's instructions. Competent JM109 cells were transformed with ligated vector and fragments at $>10^8$ cfu/µg (Promega, WI, USA) according to the manufacturer's protocol and plated on LB agar containing ampicillin (50 µg/ml). Colonies were processed using the Wizard® Plus SV Minipreps DNA Purification System (Promega, WI, USA), re-digested and visualized for release of fragments.

Five genomic fragments were analyzed: a 2-kb BamHI fragment, three EcoRI fragments of approximately 1 kb, 1.8 kb and 3 kb, and one PstI fragment of approximately 1.5 kb. Potential open reading frames (ORFs),

encompassing around 8 kb of the viral genome, were found within these five genome fragments. One clone (clone B10) contained repetitive sequences that did not show significant similarities to other herpesviruses by BLAST analysis. Identified ORFs included ORF 68, ORF 69, ORF 24, ORF 25 (*major capsid protein*), ORF 9 (DNA *pol*) and ORF 22 (glycoprotein H, hereafter *gH*). These sequences were deposited into GenBank under accession numbers EU863271 (clone B10), EU863272 (ORFs 68 and 69, clone E10), EU863273 (ORF 9, clone E9-4), EU863274 (ORFs 24 and 25, clone E9), and EU863275 (ORF 22, clone H11). In further analyses (see below) *pol* sequences were combined with the 200-bp fragment sequenced by the PCR approach described above (intervening regions for which we did not compile sequence information are represented by question marks, indicating that these data are missing; Fig. S1).

Sequences were analyzed using similarity and phylogenetic methods. For both similarity and phylogenetic studies, protein sequences were aligned using Mafft [8, 9]. Similarity analysis was performed in two steps. First, we looked for sequences similar to that of CynGHV-1 among all of the sequences available in GenBank (National Center for Biotechnology Information, Bethesda, MD). This preliminary analysis, which was performed using the BLAST program [2], indicated that CynGHV-1 was most similar to members of the subfamily *Gammaherpesvirinae*. Therefore, for each of the CynGHV-1 sequences determined here, we performed a narrower similarity search using BLAST, focusing on this subfamily. After discarding duplicated and very short sequences, the sequences corresponding to hits with significant similarity to CynGHV-1 were downloaded to a local machine. Then, the hamming distances (the number of substitutions per aligned position needed to transform a given sequence into another one) from each of the CynGHV-1 sequences to each of the corresponding sequence identified by the BLAST search were calculated.

For probabilistic phylogenetic analysis, amino acid substitution models were estimated using the program ModelGenerator [10]. Maximum-likelihood trees were inferred with PhyML version 3.0 [4] using a BIONJ starting tree that was reordered by SPR for searching the tree space. Bayesian analysis was performed with the MrBayes program [7, 19]. Analyses were run independently twice, using Metropolis-coupled Markov Chain Monte Carlo (MCMC) to enhance the tree-climbing capabilities of the Markov chains [7]. Parsimony analysis was performed with the TNT program, which is a relatively new program implementing novel technologies for phylogenetic analysis aimed to provide a thorough exploration of the tree space, ensuring that all of the possible phylogenetic hypotheses that could be supported by the data are considered. For tree searches, we built 100 Wagner trees by random addition sequence (RAS) of terminals, and the resulting topologies were subjected to tree bisection reconnection (TBR) branch swapping. Ten trees were held while swapping, and ambiguously supported branches were automatically collapsed during tree searches. This analysis resulted, in all cases, in fully stable consensus trees (i.e., trees that no longer changed upon the addition of further RAS+TBR cycles), indicating that every possible consensus topology that could be supported by the data was represented among the found trees [5].

Given the wide range of sequence variability among the groups under study, all sequence analyses were performed on deduced amino acid sequences. Similarity searches across GenBank sequences confirmed that CynGHV-1 was similar to members of the subfamily *Gammaherpesvirinae* (family *Herpesviridae*). In the case of the complete *pol* gene (Fig. S1, Table 1), combined CynGHV-1 *pol* sequences (EU863200 plus EU863273, 266 aa plus 68 aa) were most similar (81%) to a partial *pol* (55 aa) from sanguinine herpesvirus 1 (SHV-1), an unclassified virus that was isolated from New World primates (callitrichid herpesvirus 1) [16, 18]. Pairwise nucleic acid and amino acid identity values between CynHV1 and SHV1 *pol* sequences were 0.7452 and 0.8125, respectively. In this gene, CynGHV-1 was also somewhat similar to members of the genus *Rhadinovirus*, though the genetic distances between these sequences and CynGHV-1 sequences did not seem to be significantly smaller than those observed for the unclassified gammaherpesviruses (Table 1). For the *major capsid protein*, *gH*, ORF24 and ORF69 sequences, CynGHV-1 was also more similar to sequences from rhadinoviruses, but the genetic distances to the rhadinoviruses were also slightly smaller than the distances to members of the other genera. The CynGHV-1 ORF68 sequence was most similar to a sequence from the genus *Macavirus*, though it was also close to rhadinovirus and percavirus sequences (data not shown).

In order to compare the similarity patters of CynGHV-1 to those of other members of the subfamily *Gammaherpesvirinae*, we created box plots that summarize the genetic distances from each sequence analyzed here against all of the other sequences [13, 15]. This analysis showed that CynGHV-1 was quite divergent within the subfamily, even if only unclassified gammaherpesviruses were considered. These ones usually displayed genetic distances to other gammaherpesviruses that were greater than the large majority of the distances observed for CynGHV-1 (data not shown).

In herpesviruses, it is usual for different genes to have contrasting phylogenetic histories [6, 14, 21]. Furthermore, the number of sequences with significant similarity to CynGHV-1 was disparate among the genes that were studied, a

Table 1 Genetic distances from CynGHV-1 *pol* sequence to other *pol* sequences available in GenBank

Name	GI[a]	Distance[b]	Genus[c]
Sanguinine herpesvirus 1	gi1495884	0.187	Unclassified
Asinine herpesvirus 5	gi225356610	0.405	Unclassified
Sperm whale gammaherpesvirus	gi282154890	0.406	Unclassified
Equid herpesvirus 7	gi157931528	0.417	Unclassified
Truchechid herpesvirus 1	gi116090371	0.421	Unclassified
Saimiri sciureus rhadinovirus 1	gi37729020	0.421	*Rhadinovirus*
Pan troglodytes rhadinovirus 1b	gi10798916	0.424	*Rhadinovirus*
Mandrillus herpesvirus 1	gi11611796	0.424	*Rhadinovirus*
Pan rhadinoherpesvirus 2	gi15723248	0.424	*Rhadinovirus*
Elephant Gammaherpesvirus 3B	gi297499058	0.424	Unclassified
Elephant Gammaherpesvirus 3A	gi297499062	0.424	Unclassified
Hylobates leucogenys rhadinovirus 2	gi42495018	0.424	*Rhadinovirus*
Hawaiian monk seal herpesvirus	gi68342479	0.424	Unclassified
Elephantid herpesvirus 5	gi144602794	0.430	*Rhadinovirus*
Elephantid herpesvirus 6	gi145751444	0.430	Unclassified
Phocid herpesvirus 2	gi258649651	0.430	Unclassified
Colobus polykomos Lymphocryptovirus 1	gi265509540	0.436	*Lymphocryptovirus*
Asinine herpesvirus 4.1	gi17221152	0.437	Unclassified
Otariid herpesvirus 2	gi258649643	0.437	Unclassified
Egyptian fruit bat Gammaherpesvirus	gi267922539	0.437	Unclassified
Phacochoerus africanus rhadinovirus 1	gi37781552	0.437	*Rhadinovirus*
Zebra herpesvirus	gi4809212	0.437	Unclassified
Pan troglodytes rhadinovirus 1a	gi10798912	0.439	*Rhadinovirus*
Pan troglodytes rhadinovirus 3	gi261343331	0.441	*Rhadinovirus*
Procavid herpesvirus 1	gi117582192	0.446	*Rhadinovirus*
Elephantid herpesvirus 4	gi80978873	0.446	Unclassified
Saimiriine herpesvirus 2	gi9625965	0.448	*Rhadinovirus*
Human herpesvirus 8 type M	gi1718257	0.450	*Rhadinovirus*
Human herpesvirus 8	gi2246468	0.450	*Rhadinovirus*
Plecotus auritus rhadinovirus 1	gi111118346	0.453	*Rhadinovirus*
Ateline herpesvirus 2	gi1495882	0.453	*Rhadinovirus*
Stejnegers beaked whale gammaherpesvirus	gi282154892	0.453	Unclassified
Hippopotamus amphibius rhadinovirus 1	gi57648931	0.453	*Rhadinovirus*
Macaca nemestrina rhadinovirus 2	gi8925896	0.453	*Rhadinovirus*
Gorilla rhadinovirus 1	gi171988558	0.454	*Rhadinovirus*
Chlorocebus rhadinovirus 2	gi6469148	0.454	*Rhadinovirus*
Pan troglodytes rhadinovirus 2	gi156144452	0.456	*Rhadinovirus*
Pan troglodytes rhadinovirus 1	gi156254730	0.459	*Rhadinovirus*
Chlorocebus rhadinovirus 1	gi6469146	0.460	*Rhadinovirus*
Retroperitoneal fibromatosis-associated herpesvirus	gi8925893	0.460	*Rhadinovirus*

[a] GenBank accession number

[b] Genetic (hamming) distance to CynGHV-1. The 30 most similar sequences identified by BLAST are shown. For the complete distance list, see Table S1

[c] NCBI taxonomy

fact that would introduce a further element of uncertainty in phylogenetic analysis [17, 24]. Thus, phylogenetic analysis was performed separately for each gene. These analyses confirmed that CynGHV-1 was a gammaherpesvirus (Fig. 2), although they were unable to clearly indicate the relationships of CynGHV-1 to a particular gammaherpesvirus genus

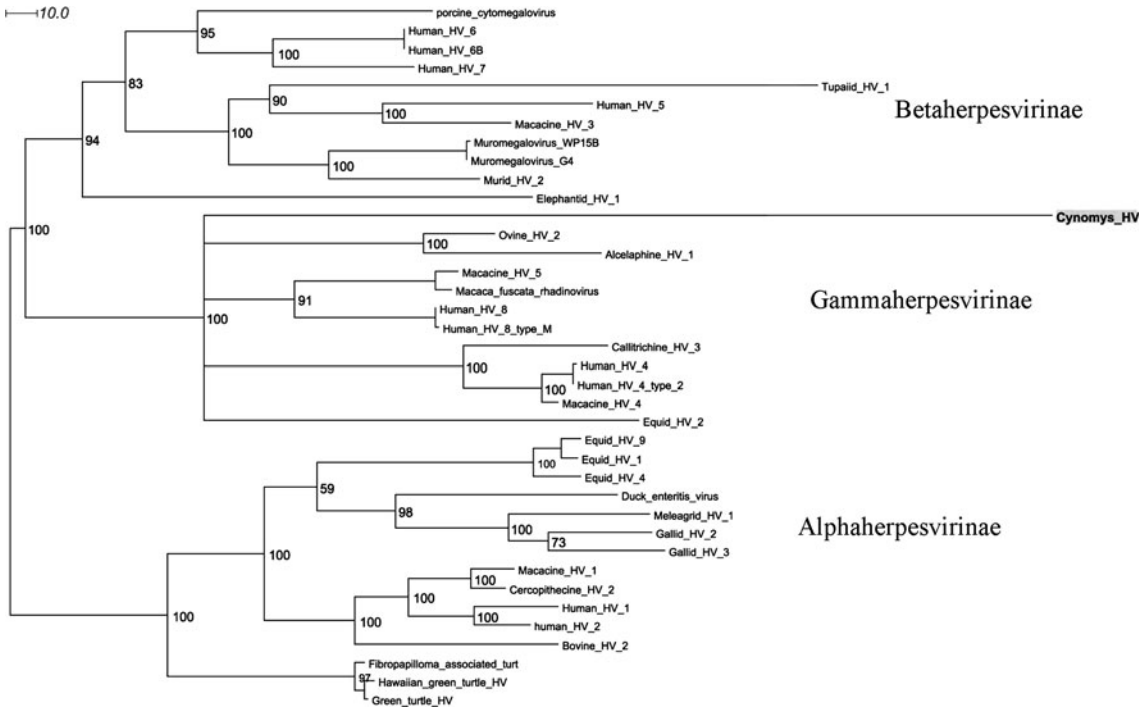

Fig. 2 Phylogenetic tree of representative members of the family *Herpesviridae* based on sequences from the complete DNA *pol* gene. Numbers close to tree nodes indicate bootstrap (n=100) support. Branch lengths are proportional to the number of amino acids substitutions (bar = 10 substitutions). The tree was constructed using the TNT program. Equivalent results were obtained by maximum-likelihood and Bayesian analyses

(data not shown). Phylogenetic trees based on complete *pol* sequences were highly unresolved due to the high proportion of missing nucleotides observed for many sequences (Fig. S1) and, as expected, these trees grouped CynGHV-1 with SHV-1 (S2). Trees derived from the *major capsid protein*, *gH*, ORF24, ORF-68 and ORF-69 sequences placed CynGHV-1 close to some members of the genus *Rhadinovirus* as seen with *pol* sequences (data not shown). Nevertheless, the genera *Rhadinovirus* and *Lymphocryptovirus* were poly- or paraphyletic in these trees (data not shown). In the case of the *pol gene*, we also faced the problem of having high proportions of missing data for many of the taxa studied, a fact that can result in obtaining unresolved phylogenetic trees [17, 25].

In conclusion, sequencing and comparison of different genomic clones indicated that CynGHV-1 belonged to the subfamily *Gammaherpesvirinae*. Regardless of the phylogenic method used (parsimony, maximum-likelihood, Bayesian) the overall branching pattern of the trees constructed did not allow to establish with certainty a particular genus as being the closest to CynGHV-1. Box-plot analyses to explore genetic distances were also inconclusive. Thus, a consistently observed tendency to branch closer to rhadinoviruses could not be confirmed by any of the approaches used here. Furthermore, one genomic clone did not show any relevant similarity to herpesviruses (clone B10, data not shown). Thus, the present data would indicate that sequencing of the entire CynHV-1 genome will be necessary.

Although we included in our comparisons a significant fragment from the *pol* gene, which is highly conserved and usually utilized for herpesvirus classification [3], we could not assign CynHV-1 to any particular genus. This could be due in part to the fact that many of the *pol* sequences deposited in GenBank are partial ones. Sequencing of glycoprotein B in conjunction with a region of the *pol* gene has allowed identification of new lineages within the subfamily *Gammaherpesvirinae* [3], but our work preceded this published approach, and thus, it was not applied. Our sequencing data, using individual genes or concatenated fragments, put CynHV-1 closer to different herpesvirus genera or even unclassified ones, suggesting that CynHV-1 could be a representative of a new lineage within this subfamily. Accurate phylogenetic location of a potential new gammaherpesvirus is usually complicated due to the transfers of genetic material that have occurred between widely distinct hosts [3, 11, 12, 14]. The significance of the strong similarity between CynHV-1 and SHV-1, based on a 68-aa fragment of the *pol* gene, can only be speculative at this time. The lack of additional sequence data from SHV-1 makes conclusions regarding the phylogenetic relationship between these two viruses very difficult.

The prevalence of this virus in the prairie dog population in unknown, as is the primary host species. Gammaherpesviruses are sometimes a predisposing factor for bacterial infections, which could have been the case in this animal. We attempted detection of neutralizing antibodies in serum samples from 100 black-tailed and white-tailed prairie dogs from locations in Wyoming and Colorado, but the results were negative (data not shown). However, since no serum sample ever neutralized CynGHV-1, we could not obtain a positive control for our test. Thus, there is no certainty that CynGHV-1 can be neutralized. It is possible, as well, that long periods of viral latency may preclude the possibility of detecting any serologic evidence of infection using this kind of approach. Since fresh tissues from the original animal were not available, the detection and location of viral antigens in tissues was not possible.

In summary, this research provides the first evidence of a novel gammaherpesvirus isolated from prairie dog (*Cynomys ludovicianus*), which we have tentatively named cynomys herpesvirus 1. CynGHV-1 was cultured on cell lines from three species (African green monkey, Syrian golden hamster and human) and is capable of infecting both epithelial and fibroblastic cells. Distance and phylogenetic analyses were not conclusive enough to determine with certainty the genus to which CynGHV-1 belongs, emphasizing the need for more extensive sequencing. Further studies are necessary to determine if the prairie dog is the host and to assess the extent of this infection within this species.

Acknowledgments We would like to thank Mrs. Carol E. Hearne for the electron microscopy data. We are also very grateful to Dr. Geoff J. Letchworth for his advice and enthusiasm throughout the duration of the project as well as critical review of this manuscript. The authors also wish to thank Dr. Charles H. Calisher and Dr. J. Jeffrey Root, (CSU, Fort Collins) for providing the prairie dog serum samples. Bioinformatic analysis by CT-R was supported by NIH Grant P20 RR016474 from the INBRE Program of the National Center for Research Resources.

References

1. Ackerman M (2004) Herpesviruses, a brief overview. Methods Mol Biol 256:199–219
2. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410
3. Ehlers B, Dural G, Yasmum N, Lembo T, de Thoisy B, Ryser-Degiorgis MP, Ulrich RG, McGeoch DJ (2008) Novel mammalian herpesviruses and lineages within the gammaherpesvirinae: cospeciation and interspecies transfer. J Virol 82:3509–3516
4. Guindon S, Gascuel O (2003) A simple, fast and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol 52:696–704
5. Goloboff PA, Farris JS (2001) Methods for quick consensus estimations. Cladistics 17:S26–S34
6. Hayward GS, Zong JC (2007) Modern evolutionary history of the human KSHV genome. Curr Top Microbiol Immunol 3:1–42
7. Huelsenbeck JP, Ronquist F (2001) MrBayes: Bayesian inference of phylogeny. Bioinformatics 17:754–755
8. Katoh K, Misawa K, Kuma K, Miyata T (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res 30:3059–3066
9. Katoh K, Asimenos G, Toh H (2009) Multiple alignment of DNA sequences with MAFFT. Methods Mol Biol 537:39–64
10. Keane TM, Creevey CJ, Pentony MM, Naughton TJ, Mclnerney JO (2006) Assessment of methods for amino acid matrix selection and their use on empirical data shows that ad hoc assumptions for choice of matrix are not justified. BMC Evol Biol 6:29
11. Kruger JM, Venta PJ, Swenson CL, Syring R, Gibbons-Burgener SN, Richter M, Maes RK (2000) Prevalence of bovine herpesvirus-4 infection in cats in Central Michigan. J Vet Intern Med 14:593–597
12. Martinez-Guzman D, Rickabaugh T, Wu TT, Brown H, Cole S, Song MJ, Tong L, Sun R (2003) Transcription program of murine gammaherpesvirus 68. J Virol 77:10488–10503
13. Martins AN, Medeiros SO, Simonetti JP, Schatzmayr HG, Tanuri A, Brindeiro RM (2008) Phylogenetic and genetic analysis of feline immunodeficiency virus gag, pol, and env genes from domestic cats undergoing nucleoside reverse transcriptase inhibitor treatment or treatment-naive cats in Rio de Janeiro, Brazil. J Virol 82:7863–7874
14. McGeoch DJ, Gatherer D, Dolan A (2005) On phylogenetic relationships among major lineages of the gammaherpesvirinae. J Gen Virol 86:307–316
15. McGill R, Tukey JW, Larsen WA (1978) Variations of box plots. Am Stat 32:12–16
16. Melendez LV, Daniel MD, Barahona HH, Fraser CE, Hunt RD, Garcia FG (1971) New herpesviruses from South American monkeys. Preliminary report. Lab Anim Sci 21:1050–1054
17. Philippe H, Snell E, Bapteste E, Lopez P, Holland P, Casane D (2004) Phylogenomics of eukaryotes: impact of missing data on large alignments. Mol Biol Evol 21:1740–1752
18. Ramer JC, Garber RL, Steele KE, Boyson JF, O'Rourke C, Thomson JA (2000) Fatal lymphoproliferative disease associated with a novel gammaherpesvirus in a captive population of common marmosets. Lab Anim Sci 50:59–68
19. Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574
20. Rose TM, Strand KB, Schultz ER, Schaefer G, Rankin GW Jr, Thouless ME, Tsai CC, Bosch ML (1997) Identification of two homologs of the Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) in retroperitoneal fibromatosis of different macaque species. J Virol 71:4138–4144
21. Shin YC, Jones LR, Manrique J, Lauer W, Carville A, Mansfield KG, Desrosiers RC (2010) Glycoprotein gene sequence variation in rhesus monkey rhadinovirus. Virology 400:175–186
22. Taylor SK, Vieira VG, Williams ES, Pilkington R, Fedorchak SL, Mills KW, Cavender JL, Boerger-Fields AM, Moore RE (1996) Infectious keratoconjunctivitis in free-ranging mule deer (*Odocoileus hemionus*) from Zion national Park, Utah. J Wildl Dis 32:326–330
23. VanDevanter DR, Warrener P, Bennett L, Schultz ER, Coulter S, Garber RL, Rose TM (1996) Detection and analysis of diverse herpesviral species by consensus primer PCR. J Clin Microbiol 34:1666–1671
24. Wiens JJ (2006) Missing data and the design of phylogenetic analyses. J Biomed Inform 39:34–42
25. Wu GA, Jun S, Sims GE, Kim SH (2009) Whole-proteome phylogeny of large dsDNA virus families by an alignment-free method. Proc Natl Acad Sci USA 106:12826–12831