

## Detection of first gammaherpesvirus sequences in Central African bats

K. Cameron<sup>1,2</sup>, B. Hayes<sup>3</sup>, S. H. Olson<sup>1</sup>, B. R. Smith<sup>4</sup>, J. Pante<sup>4</sup>, A. Laudisoit<sup>5</sup>, T. Goldstein<sup>4</sup>, D. O. Joly<sup>1,6</sup>, R. Bagamboula MPassi<sup>7</sup> and C. E. Lange<sup>8</sup>

1) Wildlife Conservation Society, Bronx, NY, 2) United States Fish and Wildlife Service, Bailey's Crossroads, VA, USA, 3) Monadh, Inveruglas, Kingussie, UK, 4) One Health Institute, School of Veterinary Medicine, University of California, Davis, CA, 5) EcoHealth Alliance, New York, NY, USA, 6) British Columbia Ministry of Environment and Climate Change Strategy, Victoria, BC, Canada, 7) Ministry of National Defense, Congo and 8) Metabiota Inc, Nanaimo, BC, Canada

### Abstract

Herpesviruses have been identified in many species; however, relatively few bat herpesviruses are known, considering the enormous diversity of bats. We used consensus PCR to test bats from the Republic of the Congo and found DNA of two different novel bat herpesviruses. One was detected in a *Pipistrellus nanulus*, the other in a *Triaenops persicus* bat and both resemble gammaherpesviruses. On the amino acid level, the amplified sequences differ by 55% from each other, and by 27% and 25% from the next closest known viruses. The findings point towards the diversity of herpesviruses in Central African bats.

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**Keywords:** Africa, Bat, Congo, Diversity, Herpesvirus

**Original Submission:** 13 February 2020; **Revised Submission:** 23 May 2020; **Accepted:** 29 May 2020

**Article published online:** 3 June 2020

**Corresponding author:** C.E. Lange, Metabiota Inc, 425 California Street, San Francisco, CA, 94104, USA.

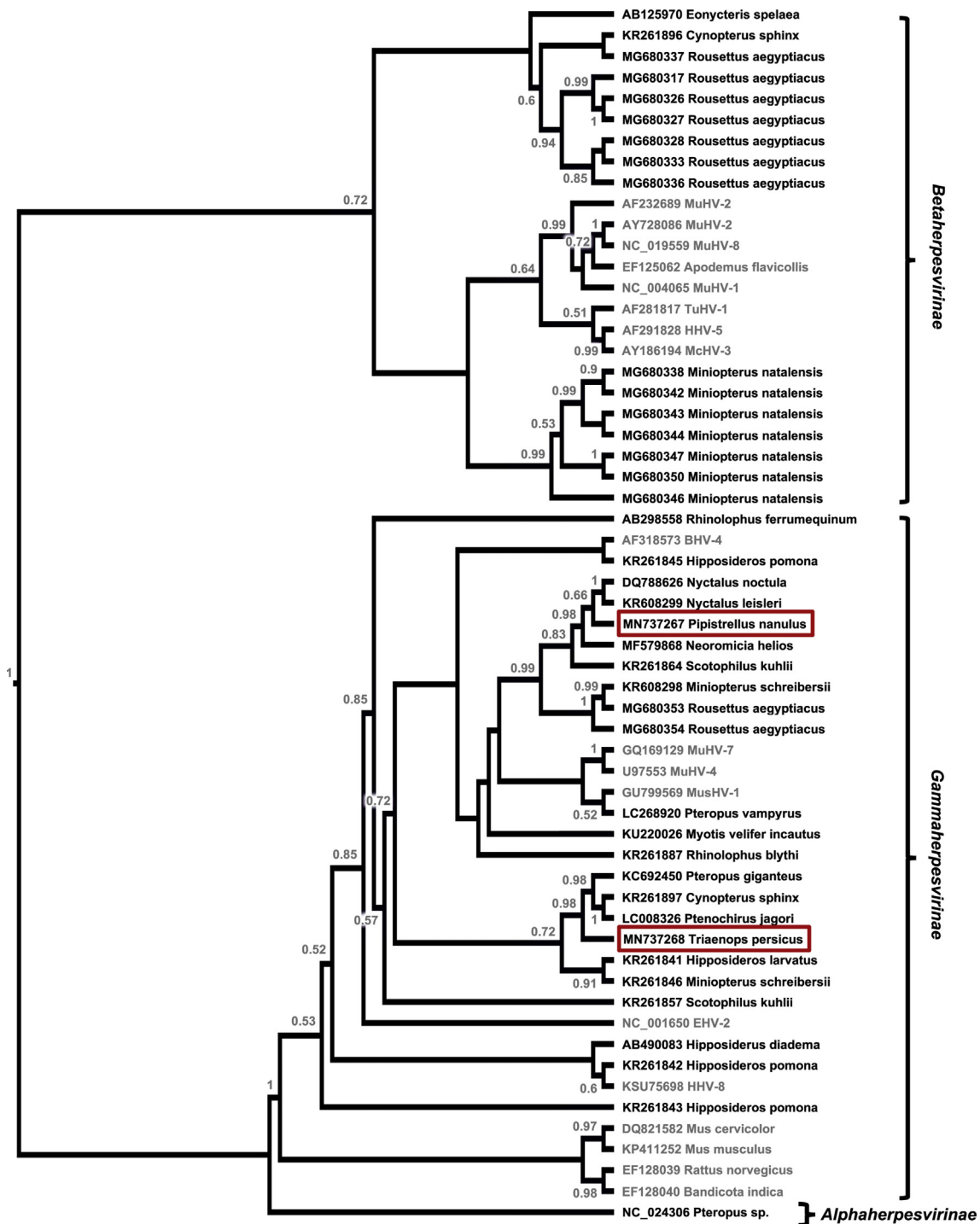
**E-mail:** [clange\\_virology@gmx.de](mailto:clange_virology@gmx.de)

### Introduction

Herpesviruses are large DNA viruses with a long evolutionary history that infect many diverse vertebrate hosts. They are largely host species specific, and it is expected that the diversity of herpesviruses is very high as a result of co-speciation. Consequently, rodents and bats are especially likely to harbour numerous undetected herpesviruses, making them a valuable source of information about virus biology and evolution. This is supported by numerous recent studies [1–17]. The diversity of bats is very high in Central Africa, yet few bat herpesviruses have been reported from this area [2]. The activities of the PREDICT project in the Republic of the Congo (ROC) provided a unique opportunity to surveil bats for herpesviruses. The goal was to evaluate whether the selected sampling techniques and consensus PCRs would yield viral

DNA and compare genetic relatedness to known bat herpesviruses.

Samples from apparently healthy bats were collected in August 2012 in ROC during a sampling expedition for a faunal inventory study, commissioned in association with a proposed iron ore mine feasibility study (see [Supplementary material, Appendix S1](#)) [18]. As part of that study, a limited number of animals were killed as voucher specimens and all bats were sampled for the PREDICT project. Non-lethal sample collection under PREDICT was approved by the Institutional Animal Care and Use Committee (IACUC, UC Davis) and the government of ROC (permit number 018/MRSIT/DGRST/DMAST issued by the ROC Ministry of Scientific Research and Technical Innovation). Bat captures were conducted by mist-netting during the rainy season. Nets were placed at heights of 1–3 m along forest trails and streams in lowland forest. Oral and rectal swabs were collected from live and voucher specimens using sterile micro-tipped polyester swabs (Puritan™ Medical Products Company, LLC, Guilford, ME, USA), placed in 1.5 mL NucliSens® lysis buffer (bioMerieux Inc., Marcy l'Étoile, France) in 2.0-mL cryotubes and stored at ambient temperature for up to 3 hours before being frozen in liquid nitrogen and later transferred to –80°C until further processing.



**FIG. 1.** Maximum likelihood phylogenetic tree of herpesviruses comparing a conserved 169-nucleotide sequence fragment of DNA polymerase gene [19]. The tree includes the two novel sequences (red boxes) and previously known sequences of other bat (black) and non-bat (grey) herpesviruses. GenBank accession numbers and either virus abbreviation or host species (in the absence of ICTV approved name) are shown. Bat herpesvirus sequences differing by less than 2% from others were excluded. Bootstrap support above 0.5 indicated at nodes.

DNA was manually extracted using Trizol® and stored at  $-80^{\circ}\text{C}$  until analysis. Samples were tested with two nested consensus PCR assays, one targeting an approximately 374-

nucleotide fragment of the DNA packaging terminase subunit I gene (Term) and the other targeting an approximately 175-nucleotide fragment of the conserved catalytic subunit of the

DNA polymerase gene (DPOL) [19,20]. PCR products were examined by gel electrophoresis and products were excised and cloned; the sequence was determined by Sanger sequencing at the UC Davis DNA sequencing facility. Obtained sequences were analysed in Geneious, and manually edited. Consensus sequences were compared with the GenBank database (BLAST).

A phylogenetic tree based on the DPOL PCR target region was constructed. First multiple sequence alignments were made in Geneious (ClustalW Alignment), and alignment regions with <50% support were removed. Bayesian phylogeny of DPOL was inferred using MRBAYES (version 3.2); Markov Chain Monte Carlo with SYM substitution matrix, variable gamma rates, invariant sites, two runs, four chains of 10 000 000 generations, and trees were sampled after every 1000 steps during the process to monitor phylogenetic convergence [21]. The final average standard deviation of split frequencies was <0.0073. The first 10% of the trees were discarded and the remaining ones were combined using TreeAnnotator (version 2.5.1; <http://beast.bio.ed.ac.uk>) and displayed with FIGTREE (1.4.4; <http://tree.bio.ed.ac.uk>) [22].

Twenty-one samples (nine oral swabs, nine rectal swabs, one lung sample, one liver sample and one faecal sample) were collected from ten bats at three different locations in ROC's Bambama district (see [Supplementary material, Appendix S1](#)). Three of the animals were identified as *Tri-aenops persicus*, two as *Hipposideros ruber* and one each as *Hypsugo crassulus*, *Neoromicia brunnea*, *Nycteris hispida*, *Pipistrellus nanulus* and *Triaenops afer*. Herpesvirus DNA was amplified from the oral swabs of two bats with the DPOL PCR, whereas none was detected with the Term PCR in any of the samples. The closest related sequences were herpesvirus sequences from other bats. On the nucleotide level the sequence that was obtained from *P. nanulus* had 70% similarity to *Nyctalus noctula rhadinovirus I* (DQ788626) found in a bat in Germany, while the sequence obtained from a *T. persicus* had 64% similarity to bat herpesvirus isolate HL/11HNI (KR261841) found in a *Hipposideros larvatus* in China. On the amino acid level (BlastP) the *P. nanulus* viral sequence was closest to *Nyctalus leisleri gammaherpesvirus I* DNA polymerase (AMY98787, Spain) with 75% identity, and the *T. persicus* sequence closest to bat herpesvirus DNA polymerase ALH21055 (*Miniopterus schreibersi*, China) with 73%. Upon phylogenetic analysis both sequences clustered with gammaherpesviruses, in two separate branches with other bat herpesvirus sequences (Fig. 1).

Despite the small sample size (ten bats), we were able to detect the DNA of two novel bat herpesviruses. This outcome is not unexpected, as other studies have found prevalence for herpesvirus DNA up to 45% in oral samples of bats [11].

Studies are often difficult to compare because of different sampling techniques, different extraction methods and different PCR assays used for viral detection. The DPOL PCR has been successful in detecting bat herpesviruses before, but reports using the Term PCR are missing, although it is known to work well in other species [1,3,5,6,17]. It has been reported, that both PCR targets do not necessarily work equally well, or at all, for the same herpesviruses [17]. The combination of oral swab sampling and consensus DPOL PCR certainly appears to be the most promising first step in further investigating bat herpesvirus prevalence and diversity based on our and other's data [11].

Considering the results from the sequence comparison and the phylogenetic analysis, both novel sequences probably belong to different gammaherpesviruses, that are related, though not closely, to other bat gammaherpesviruses. This marks the detection of the first two gammaherpesviruses from the Congo Basin, where so far only two bat alphaherpesviruses have been described; in an *Eidolon helvum* from Cameroon and in an unidentified bat from the Central African Republic [2]. Even across continental Africa short sequences of only six bat gamma-herpesvirus isolates have been published, all deriving from South Africa along with 36 bat betaherpesvirus isolates [14,15]. Considering the diversity of bats species in Central Africa and the number of bat herpesviruses detected elsewhere, especially in tropical regions of Asia, we conclude, that our findings support the hypothesis that a high herpesvirus diversity in Central African bat species can be expected [5,8,10,12,13,18,23,24]. Including larger numbers of diverse bat hosts and following up on detected herpesviruses with next-generation sequencing techniques to learn more about virus and host biology and evolution would be desirable for future studies.

## Conflicts of interest

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None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

## Acknowledgements

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The authors would like to thank: the government of the Republic of the Congo for the permission to conduct this study; the staff of the Wildlife Conservation Society; especially Ashley Vosper, and the staff of the University of California in Davis who assisted in sample collection and testing; any other involved members of the PREDICT-I consortium (<https://ohi.vetmed.ucdavis.edu/programs-projects/predict-project/authorship>). The study was undertaken as part of the global

USAID-funded Emerging Pandemic Threats (EPT) PREDICT project that focuses on enhancing the global capacity for the detection and discovery of potentially zoonotic viruses at the human–animal interface. It was made possible primarily by the generous support of the American people through the United States Agency for International Development (USAID) Emerging Pandemic Threats PREDICT programme (cooperative agreement number GHN-A-OO-09-00010-00). The contents are the responsibility of the authors and do not necessarily reflect the views of USAID or the United States Government.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.nmni.2020.100705>.

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