



Short Communication

Orbivirus RNA in a Banana Serotine (*Afronycteris nanus*) Bat in the Republic of the Congo

Kenneth N. Cameron,^{1,2} Jean-Vivien Mombouli,³ Fabien R. Niama,³ Ben Hayes,⁴ Sarah H. Olson,¹ Brett R. Smith,⁵ Jasmine Pante,⁵ Sanjit Roy,^{6,7} Anne Laudisoit,⁸ Tracey Goldstein,⁵ Damien O. Joly,^{1,9} Romain Bagamboula MPassi,¹⁰ and Christian E. Lange^{6,11}

¹Wildlife Conservation Society, Bronx, NY

²United States Fish and Wildlife Service, Bailey's Crossroads, VA

³National Laboratory of Public Health, Brazzaville, Republic of the Congo

⁴Monadh, Inveruglas, Kingussie, Inverness-Shire, UK

⁵One Health Institute, School of Veterinary Medicine, University of California, Davis, CA

⁶Labyrinth Global Health, St. Petersburg, FL

⁷University of Victoria, Victoria, BC, Canada

⁸EcoHealth Alliance, New York, NY

⁹British Columbia Ministry of Environment and Climate Change Strategy, Victoria, BC, Canada

¹⁰Ministry of National Defense, Brazzaville, Republic of Congo

¹¹Metabiota Inc, Nanaimo, BC, Canada

Abstract: Orbiviruses are arthropod borne viruses of vertebrates, with some of them being important pathogens of veterinary, conservation and economic importance, while others are occasionally associated with human disease. Some apparently bat specific orbiviruses have been detected, but little is known about their distribution and diversity. We thus sampled and screened 52 bats living in the Congo Basin, and detected RNA indicative of a novel orbivirus in a single banana serotine (*Afronycteris nanus*) by PCR. The detected RNA clusters with epizootic haemorrhagic disease virus, bluetongue virus, and others. The findings highlight the need for more studies into arbovirus presence and diversity in bat species.

Keywords: Orbivirus, bat, diversity, Africa, congo

Orbiviruses have a segmented double-stranded RNA genome and belong to the *Reoviridae* family in which they constitute a genus with 27 classified species and multiple currently unclassified viruses (Maan et al. 2020; Roy 2013).

Most known orbiviruses infect vertebrate hosts and are transmitted by vectors such as ticks, mosquitos or blood sucking flies such as *Culicoides* spp. (Belaganahalli et al. 2015; Fagre et al. 2019; Jaafar et al. 2014). Orbiviruses are important animal pathogens, with bluetongue virus, epizootic haemorrhagic disease virus and African horse sickness virus being the most prominent due to their distribution and economic impact (Attoui et al. 2009a, b;

Correspondence to: Christian E. Lange, e-mail: clange_virology@gmx.de

Maclachlan and Guthrie 2010; Madani et al. 2011; Verwoerd 2012). Some orbiviruses, namely Corriparta virus, Changuinola virus, Kemerovo virus, and Orungo virus can infect humans and may be associated with febrile and neurological disease, however these viruses appear to be limited in their geographical distribution and cases appear to be sporadic (Attoui and Mohd Jaafar 2015; Doherty et al. 1970; Libikova et al. 1970; Roy 2013). While data for many of the less prominent orbiviruses are limited there is good evidence, that the host range for at least a subset may be wide and could include humans (Attoui et al. 2009a, b; Maan et al. 2020). The apparent host plasticity has important implications for the epidemiology of orbiviruses including potentially multiple reservoirs and an elevated zoonotic potential due to low host specificity and—unlike the role of the vectors—has not been studied in as much detail.

One group of animals that exhibits high species richness and global distribution are bats, and multiple bat species have been identified or proposed as the source or reservoir for emerging infectious diseases including high impact zoonoses such as Rabies, Ebola and SARS (Calisher 2015; Calisher et al. 2006; Maganga et al. 2014). While it is debated whether or not bats actually host disproportionately many (zoonotic) viruses compared to other groups of animals, or if our data is leaning towards that conclusion due to a systematic sampling error, there is little doubt about their importance in that role (Guy et al. 2019; Luis et al. 2013; Olival et al. 2017; Mollentze and Streicker 2020). A handful of orbiviruses has been detected in bats in several Asian and African countries, with sequences available for Japanaut virus (Papua New Guinea), Heramatsu orbivirus (Japan), Ife virus (Nigeria), Fomede virus (Guinea), and Bukakata orbivirus (Uganda) (Fagre and Kading 2019; Fagre et al. 2019; Kemp et al. 1988; Miura and Kitaoka 1977; Schnagl and Holmes 1975; Zhao et al. 2013). Considering the role of bats as virus reservoirs and the role of orbiviruses as pathogens we were interested in the potential presence of orbiviruses in bats living in the Congo Basin. We consequently set out to test bat samples for the presence of orbivirus RNA.



Figure 1. Map of the Republic of the Congo highlighting the area where sampling took place.

Samples from apparently healthy bats were collected during sampling for a faunal inventory study, commissioned in association with a proposed iron ore mine feasibility study for the Zanaga mine development project (Fig. 1, Table 1) (Bates et al. 2013). The samples were also part of a set of bat samples that were screened for the presence of Coronavirus RNA, Rhabdovirus RNA, and Herpesvirus DNA (Cameron et al. 2020, 2021; Kumakamba et al. 2021). As part of the original faunal inventory study, a limited number of animals were sacrificed as voucher specimens, while all other bats were released after sampling. Sample collection was approved by the government of Republic of the Congo (permit number 018/MRSIT/DGRST/DMAST issued by the Republic of the Congo Ministry of Scientific Research and Technical Innovation). Bats were captured by mist-netting during the rainy season. Nets were placed at heights of 1–3 m along forest trails and streams in lowland forest in July and August 2012 in the Lékoumou department of Republic of the Congo. Bat species were identified based on field guides and verified by Paul Bates of the Harrison Zoological Museum, Sevenoaks,

Table 1. Species Sampled by Count, Sex, Sample Type and Orbivirus RNA Test Result.

Species	Bats sampled	Sex	Sample types	Orbivirus RNA
<i>Afronycteris nanus</i>	1	Male	Lung (1), oral swab (1), rectal swab (1)	Positive
<i>Glauconycteris alboguttata</i>	1	Unknown	Lung (1)	Negative
<i>Glauconycteris beatrix</i>	1	Female	Lung (1)	Negative
<i>Hipposideros caffer</i>	1	Male	Lung (1), oral swab (1), rectal swab (1)	Negative
<i>Hipposideros ruber</i>	6	Male (6)	Rain (1), lung (3), oral swab (4), rectal swab (4)	Negative
<i>Hipposideros sp.</i>	3	Female (2), unknown (1)	Oral swab (3), rectal swab (3)	Negative
<i>Hypsugo crassulus</i>	1	Male	Lung (1)	Negative
<i>Megaloglossus woermanni</i>	1	Female	Oral swab (1), rectal swab (1)	Negative
<i>Mimetillus moloneyi</i>	1	Male	Lung (1)	Negative
<i>Miniopterus inflatus</i>	6	Male (3), female (3)	Lung (2), oral swab (5), rectal swab (6)	Negative
<i>Neoromicia tenuipinnis</i>	2	Unknown (2)	Lung (2)	Negative
<i>Nycteris hispida</i>	1	Female	Lung (1)	Negative
<i>Pipistrellus nanulus</i>	1	Male	Lung (1)	Negative
<i>Triaenops afer</i>	26	Male (19), female (9)	Brain (3), lung (4), oral swab (25), rectal swab (24)	Negative
Total	Bats 52	Male (31), female (17), unknown (4)	Samples 103	Positive (1), negative (51)

Sample positive for orbivirus RNA is highlighted in bold

Kent, UK (Kingdon 2005; Monadjem et al. 2010). Oral and rectal swabs were collected from live and voucher specimens using sterile micro-tipped polyester swabs (Puritan™ Medical Products Company, LLC), placed in 1.5 mL NucliSens® lysis buffer (bio- Mérieux, Inc.) in 2.0 mL cryotubes. Tissue samples from voucher animals were collected in 2.0-mL cryotubes without medium. All were stored at ambient temperature for up to 3 h before being frozen in liquid nitrogen and later transferred to -80°C until further processing.

RNA was either manually extracted using Trizol®, or using a Qiagen Viral RNA Mini Kit and stored at -80°C . Afterward RNA was reverse transcribed using random hexamers with a Maxima H Minus First Strand cDNA Synthesis Kit (Thermo Scientific), and cDNA stored at -20°C until analysis. Samples were tested with four conventional consensus PCR assays, targeting the same approximately 188 nucleotide (nt) fragment of the conserved viral protein 1 (VP1) gene that encodes for the viral polymerase. The four PCRs had different forward primers (VP1/F2494/1: TCTGAGATGTAYGTYGGAGATGATA;

VP1/F2494/2: TCTGAGATGTAYGTYGGTGATGACA;
VP1/F2494/3: TCGGAACARTAYGTVGGNGAYGATA;
VP1/F2494/4: TCNGARCARTAYGTKGGNGAYGACA)
but the same reverse primer (VP1/R2682: CCYT-GYTTNGCRTGNGTYTGYGTYTTYTC) (Palacios et al. 2011). PCR products were examined by gel electrophoresis and products excised, cloned and the sequence determined by Sanger sequencing at the University of California Davis DNA sequencing facility. Geneious (version 7.1) was used to assess sequence quality, trim off primer binding sites and align sequencing results. Consensus sequences were compared to the GenBank database (BLAST N).

A phylogenetic tree based on the VP1 coding region was constructed including the sequence obtained from the sample, 28 other orbiviruses and 2 rotaviruses as the out-group. Multiple sequence alignments were made in Geneious (ClustalW Alignment). Bayesian phylogeny of the VP1 gene was inferred using MrBayes (version 3.2) with the following parameters: Datatype = DNA, Nucmodel = 4by4, Nst = 1, Coavion = No, # States = 4, Rates = Equal, 2 runs, and 4 chains of 10,000,000 genera-

tions (Ronquist et al. 2012). The final average standard deviation of split frequencies was below 0.0032. The first 10% of the trees was discarded and the remaining ones combined using TreeAnnotator (version 2.5.1; <http://bea.st.bio.ed.ac.uk>) and displayed with FIGTREE (1.4.4; <http://tree.bio.ed.ac.uk/>) (Bouckaert et al. 2019).

We collected a total of 103 samples from 52 bats (51 insectivorous and 1 fruit bat) belonging to at least 13 different species, (Fig. 1, Table 1). The collected samples were primarily rectal (40) and oral swabs (40), but also included lung (19), and brain (4) samples. Male bats numbered 31, female 17 and the sex of 4 remained unidentified. All samples were tested and orbivirus RNA was detected in one lung sample of an adult male banana serotine (*Afronycteris nanus*).

The 188 nt VP1 sequence obtained showed highest similarity with Epizootic haemorrhagic disease virus (73% nucleotide identities at a 97% coverage), Wallal virus (72% nucleotide identities at an 86% coverage) and Bluetongue virus (80% nucleotide identities at a 44% coverage) based on BLASTN. Phylogenetic analysis placed the sequence in the same clade as these viruses along with Eubenangee virus, Warrego virus, Changuinola virus, Japanaut virus and Ibaraki virus (Fig. 2). The sequence was stored in GenBank with accession number MW167122.

We detected RNA indicative of a previously unknown orbivirus in a banana serotine captured in the Republic of the Congo using consensus PCR (Fig. 1). The genetic sequence obtained from this lung sample shares highest identities and clusters with orbiviruses of major veterinary importance (Bluetongue virus and Epizootic haemorrhagic disease), however, given the short length of the PCR product this placement may be seen as tentative despite reasonable bootstrap support. The same clade also contains Changuinola virus which is associated with human disease and Japanaut virus, the first orbivirus detected in bats (Fig. 2) (Attoui and Mohd Jaafar 2015; Jafaar et al. 2014; Schnagl and Holmes 1975). We assume that the virus of which we detected the RNA is likely transmitted by biting midges or sandflies such as *Culicoides* spp., given the knowledge of the other viruses that it clusters with (Fagre

and Kading 2019; Jafaar et al. 2014). What role this virus plays in its supposed natural host and if its host range extends beyond (*Afronycteris*) bats remains unknown, but the finding highlights that bats can harbour orbiviruses such as orbiviruses (Fagre and Kading 2019). Bats may play a unique role in the dissemination of such viruses, since they are widely distributed and numerous, especially in tropical climates, allowing the vectors to pick up and pass on the viruses readily. Vector mobility may also be less important for the spread of bat-orbiviruses, as some bat species cover large geographic distances, and share habitats with humans, domestic and other wild animals (Monadjem et al. 2010). Banana serotines often live very close to humans, roosting frequently in and around plantations, gardens, and parks in rural but also urban environments. The banana serotine (*Afronycteris nanus*, Peters 1852), is a small (3 g) insectivorous bat belonging to the *Vespertilionidae* family. It ranges over much of sub-Saharan Africa (Hayman and Hill 1971; Happold and Happold 1996). The typical habitat of this bat in southern Africa is considered to be savanna woodland (Taylor 2000), however, it is also commonly documented from lowland and montane forests (Monadjem et al. 2016). It is most commonly found roosting in the new unfurled leaves of banana plants, close to human habitation (Kingdom 1974), where groups of up to six or seven individuals can sometimes be found in one leaf (Taylor 2000). The banana serotine is known to host their own species of hantavirus (Sumibcay et al. 2012).

Given that orbiviruses are important pathogens in animals (and to some extent in humans) a better understanding of their presence and prevalence in different hosts and vectors is needed. While the scope of this particular study was limited it certainly underlines the importance and need for larger scale investigations to unveil the distribution and diversity of orbiviruses in bats globally. Screening bats that roost and forage in peridomestic environments such as the banana serotine on a larger scale and on a regular basis for potential zoonotic pathogens including orbiviruses could be a cornerstone in proactive research and surveillance in a One Health context.

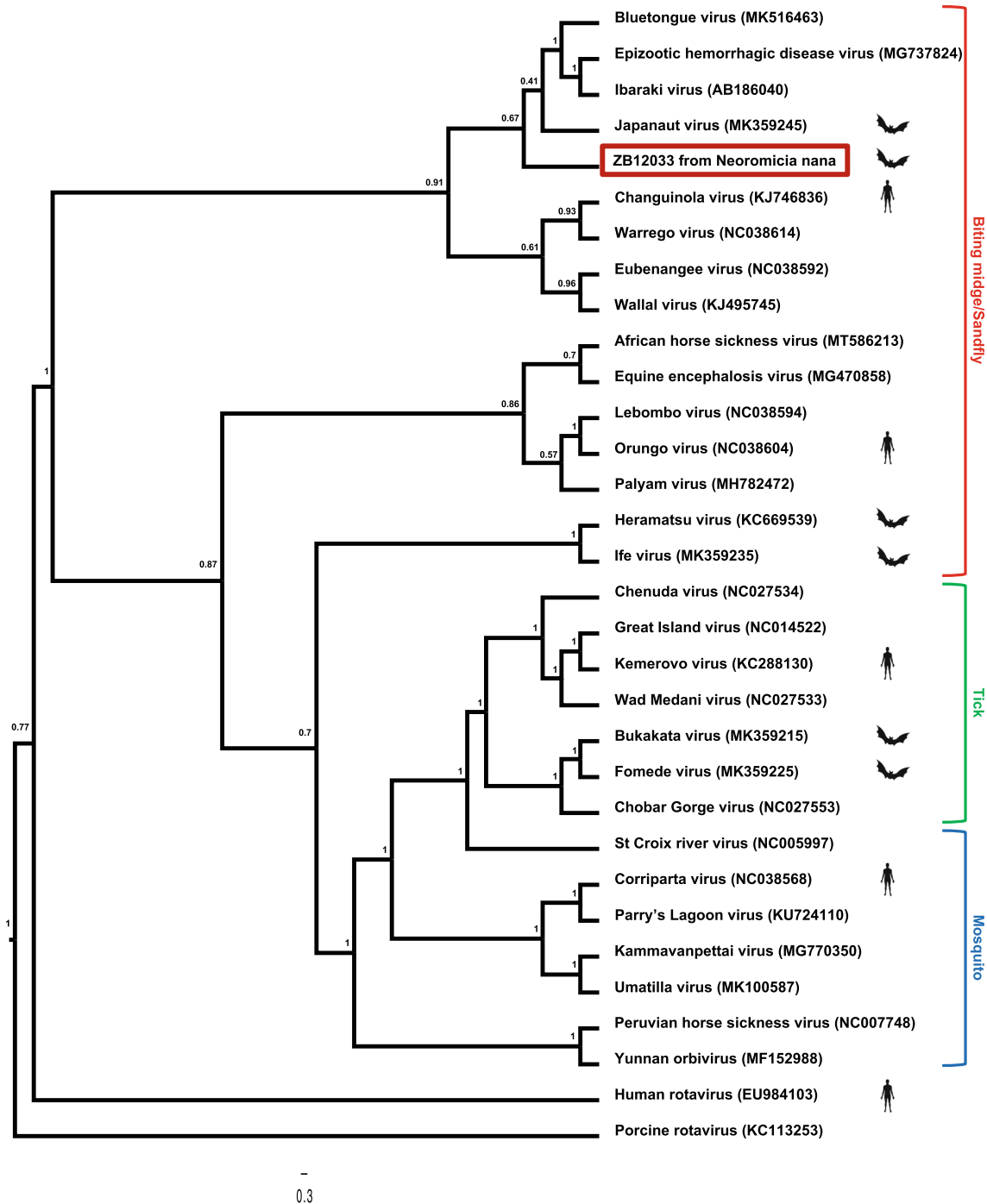


Figure 2. Maximum likelihood phylogenetic tree of orbivirus sequences presented as a proportional cladogram, based on the nucleotide sequence of the VP1 gene. The tree includes the sequence detected during the project (box), and indicates association with bats and humans as well as known types of vectors. GenBank accession numbers are listed for previously published sequences. Numbers at nodes indicate bootstrap support.

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DECLARATIONS

CONFLICTS OF INTEREST 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.

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