



Genome Sequences of a Novel Strain of Big Cypress Orbivirus Isolated from a Dead Florida White-Tailed Deer (*Odocoileus virginianus*)

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ABSTRACT Here, we report the coding sequences of Big Cypress orbivirus isolated from a dead white-tailed deer (*Odocoileus virginianus*) from Florida in 2017. To our knowledge, this is the first detection of Big Cypress orbivirus in a vertebrate host.

The genus *Orbivirus* within the family *Reoviridae* includes arboviruses with nonenveloped nucleocapsids composed of outer and inner proteinaceous layers that surround a segmented, double-stranded RNA (dsRNA) genome (1). The genes of the structural proteins of the outer capsid layer (VP2 and VP5) typically exhibit the greatest genetic variability and determine the orbivirus serotype. By comparison, the genes of the inner capsid structural proteins (VP3 coding the T2 protein) display less genetic variability and are used for species demarcation (1).

In September of 2017, a farmed white-tailed deer from Liberty County, Florida, was observed lethargic and then found dead 5 days later. A necropsy was performed on the fresh carcass, and selected specimens were submitted to the University of Florida Cervidae Health Research Initiative for evaluation. A 10% (wt/vol) spleen tissue homogenate was generated in advanced Dulbecco's modified Eagle's medium (Invitrogen Corp.) supplemented with 2 mM L-alanyl-L-glutamine (GlutaMAX, Invitrogen Corp.) and antibiotics (50 μ g/ml penicillin, 50 μ g/ml streptomycin, and 100 μ g/ml neomycin [PSN; Invitrogen Corp.]) using a manual tissue grinder (Fisher Scientific, Waltham, MA, USA). The tissue homogenate was then filtered through a sterile 0.45- μ m filter to remove particulates and contaminating bacteria and fungi and stored at -80°C . The homogenate was inoculated onto *Aedes albopictus* clone C6/36 cells where virus-induced cytopathic effects were observed 5 days postinoculation.

Viral RNA was extracted from virions in spent cell culture medium using a QIAamp viral RNA minikit (Qiagen) and served as the template for the construction of a cDNA sequencing library using a NEBNext Ultra RNA library prep kit. The resulting cDNA library was sequenced on a MiSeq (Illumina, San Diego, CA) instrument using a v3 600-cycle kit. After filtering the low-quality reads and quality trimming in CLC Genomics Workbench using default parameters, a total of 1,824,518 reads were obtained with an average read length of 237 bp. Following the removal of host sequences (*Aedes albopictus*; GenBank accession number [MNAF000000000](https://www.ncbi.nlm.nih.gov/nuccore/MNAF000000000)) using Kraken (2), a *de novo* assembly of 147,263 paired-end reads was performed in SPAdes (3). A total of 31 assembled contigs (N_{50} value, 743 bp) were screened by BLASTX in CLC Genomics Workbench using a custom virus database created from virus protein sequences

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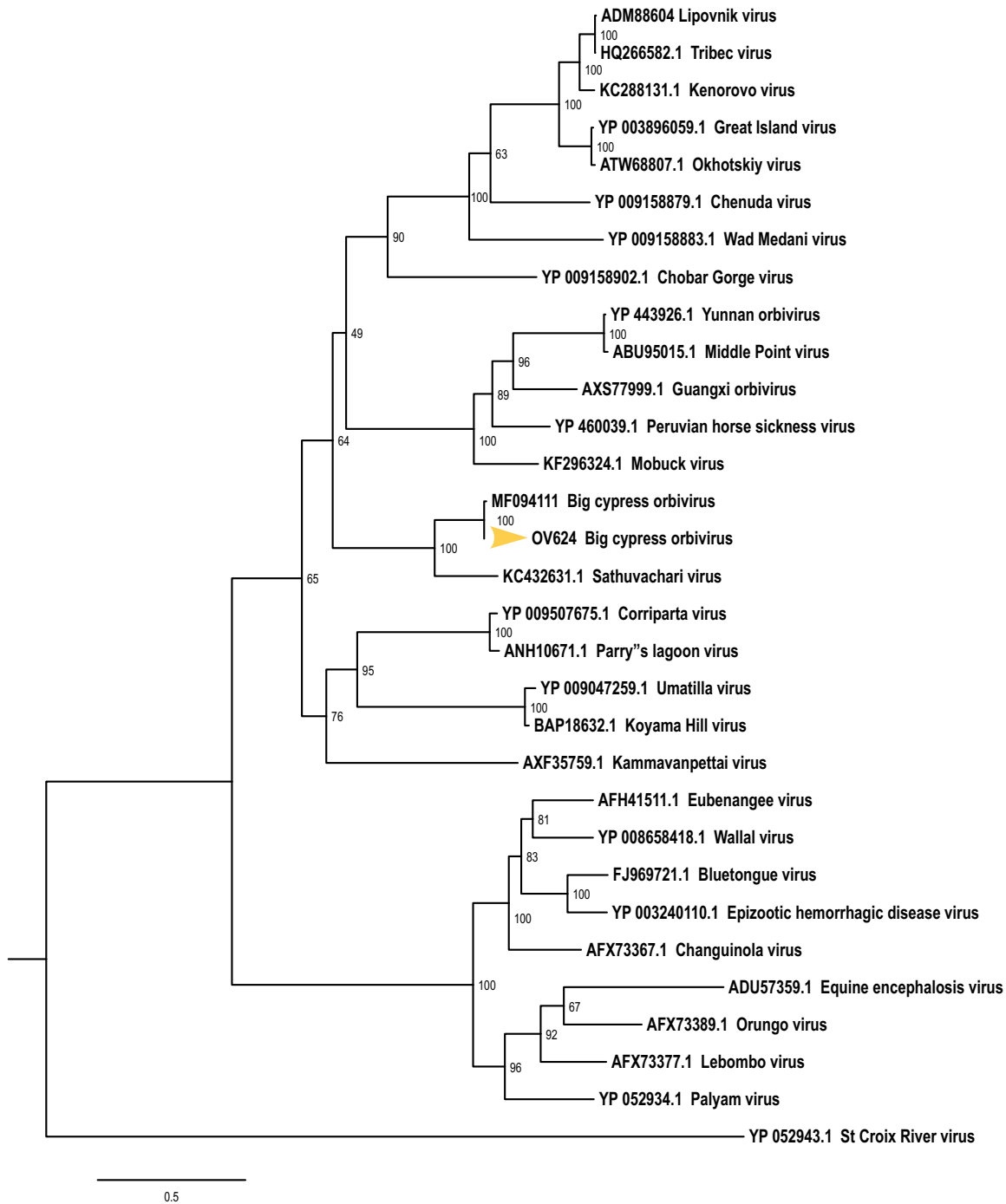


FIG 1 Maximum likelihood phylogram, based on the amino acid sequence alignment of the T2 proteins, depicting the relationship of Big Cypress orbivirus strain OV624 to 30 orbiviruses. Bootstrap values are given at each node. Branch lengths represent the number of inferred substitutions, as indicated by the scale.

retrieved from the UniProt Knowledgebase (<https://www.uniprot.org/uniprot/>). The BLASTX analysis identified all 10 segments of an orbivirus closely related to Big Cypress orbivirus (BCPOV) previously isolated in C6/36 cells from *Psorophora columbiae* mosquitoes collected in Big Cypress National Preserve (BCNP-2-151) in southern Florida (4). The total length of the nearly complete coding sequences of the 10 BCPOV strain OV624 (BCPOV-OV624) segments was 17,751 bp, with a G+C content of 41.73%. The integrity of the BCPOV-OV624 coding sequences was verified by mapping the reads

using Bowtie 2 (5) and inspecting the alignments in Tablet 1.17.08.17 (6). The average coverage of the BCPOV-OV624 genome was 1,699 reads/nucleotide. The BCPOV-BCNP-2-151 displayed a similar nearly complete coding sequence length of 17,854 bp and a G+C content of 41.53% (4). The untranslated regions of the 10 segments of BCPOV-OV624 were not determined, and only the partial coding sequence for the VP2 protein was determined.

The inner (T2; used to determine orbivirus species) and outer capsid (VP2; used to determine orbivirus serotype) proteins of BCPOV-OV624 showed 99% and 35% amino acid identity, respectively, compared with BCPOV-BCNP-2-151. Thus, BCPOV-OV624 represents a new BCPOV strain and likely a new serotype. Maximum likelihood phylogenetic analysis performed in IQ-TREE (7), based on the amino acid sequence alignment of the T2 proteins from 30 orbiviruses, supported BCPOV-OV624 as the closest relative to BCPOV-BCNP-2-151 (Fig. 1).

BCPOV has been proposed as the prototype of a new orbivirus species (4). The isolation of BCPOV-OV624 from a dead white-tailed deer suggests it may represent a previously unknown mosquito-borne cervid pathogen. Further study is needed to determine the vertebrate host range of BCPOV, including its potential role in disease among farmed and wild white-tailed deer populations.

Data availability. The genome sequences for BCPOV-OV624 and raw sequence data have been deposited in GenBank (accession numbers [MK105769](#) to [MK105778](#)) and the Sequence Read Archive (accession number [PRJNA508942](#)).

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