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GENOME SEQUENCES

# Genome Sequences of 26 White Sucker Hepatitis B Virus Isolates from White Sucker, *Catostomus commersonii*, Inhabiting Transboundary Waters from Alberta, Canada, to the Great Lakes, USA

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**ABSTRACT** We report 26 genome sequences of the white sucker hepatitis B virus (WSHBV) from the white sucker, *Catostomus commersonii*. The genome length ranged from 3,541 to 3,543 bp, and nucleotide identity was 96.7% or greater across genomes. This work suggests a geographical range of this virus that minimally extends from the Athabasca River, Alberta, Canada, to the Great Lakes, USA.

Hepadnaviruses are partially double-stranded DNA viruses that are known to infect mammals, birds, fish, and herptiles and contain a genome approximately 3 to 3.5 kb long (1). The white sucker hepatitis B virus (WSHBV) is 3,542 kb long and belongs to the genus *Parahepadnavirus* (2–4). WSHBV genome organization is similar to that of other hepadnaviruses, but amino acid similarity of the polymerase protein is 45% or less. In orthohepadnaviruses, which infect mammals, chronic infection is associated with the development of liver pathology, including hepatocellular carcinoma (5).

White suckers, *Catostomus commersonii*, are used as sentinel species in the Great Lakes, where the prevalence of skin and liver tumors is used as a biological metric (beneficial use impairment) to assess internationally designated areas of concern (AOCs) in the Great Lakes region (6–8). At present, the pathogenicity of WSHBV is unknown. Identification of viral genome diversity will facilitate the development of WSHBV diagnostic assays and is critical for pathobiological investigations.

Total DNA was extracted from white sucker plasma or liver samples using the DNeasy blood and tissue kit. Tissues were from archived samples of fish inhabiting six AOCs within the Great Lakes region of the United States as well as fish from the Athabasca River in Canada (see metadata in BioProject PRJNA685065 for details). Virus-positive samples were identified by quantitative PCR (qPCR) and enriched for WSHBV DNA via long-range PCR (lrPCR) using primers 1488F (5′-TGGTATCTGATGGCCTGGGA-3′) and 1265R (5′-CACCACCAGTAACACGACGA-3′) with TaKaRa PrimeSTAR GXL DNA polymerase (9, 10). The lrPCR amplicon product from individual samples was used as starting material with the Nextera XT library prep kit and individually indexed. Indexed libraries were run for 1 × 150 cycles on the Illumina MiSeq instrument. Reads were trimmed and mapped to the WSHBV reference genome (GenBank accession number NC_027922) using CLC Genomics Workbench v. 9.5.3. We sequenced and analyzed 27 genomes with this method, including a replicate of the reference genome. On average, 82% of the reads were mapped per sample (Table 1).

The 26 WSHBV genomes presented here contained the three primary hepadnaviral open reading frames (ORFs). In addition, these genomes coded a small open reading frame (smORF) that partially overlapped the C terminus of the polymerase ORF. Open reading frames were predicted using Geneious Prime v. 2020.2.3 software. This smORF differed in

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**TABLE 1** Sequencing and genome metrics associated with the WSHBV isolates

| Isolate | GenBank accession no. | Total no. of reads after QC[a] | No. of mapped reads | Avg coverage (×) | Genome size (bp) | GC content (%) | smORF size (bp) |
|---|---|---|---|---|---|---|---|
| ABR1522 | MW161131 | 2,141,370 | 1,937,078 | 75,689.34 | 3,541 | 42.4 | 195 |
| ABR1538 | MW161132 | 2,863,981 | 2,822,813 | 112,253.79 | 3,542 | 42.2 | 195 |
| SBR13 | MW161133 | 2,191,318 | 2,125,155 | 64,755.67 | 3,542 | 42.3 | 231 |
| SBR16 | MW161134 | 1,497,387 | 1,457,566 | 47,939.84 | 3,542 | 42.3 | 231 |
| SBR17 | MW161135 | 2,181,071 | 1,392,955 | 45,092.31 | 3,542 | 42.3 | 231 |
| SBR2 | MW161136 | 1,827,035 | 1,155,429 | 36,247.59 | 3,542 | 42.3 | 231 |
| SBR20 | MW161137 | 2,168,462 | 1,672,284 | 56,452.84 | 3,542 | 42.3 | 231 |
| SBR3 | MW161138 | 1,289,092 | 1,232,824 | 40,272.46 | 3,542 | 42.3 | 231 |
| SBR4 | MW161139 | 1,560,236 | 1,171,939 | 36,130.67 | 3,542 | 42.3 | 231 |
| SBR6 | MW161140 | 1,888,684 | 1,697,465 | 55,603.12 | 3,542 | 42.3 | 231 |
| SBR7 | MW161141 | 2,371,126 | 2,223,101 | 71,787.57 | 3,542 | 42.3 | 231 |
| SBR8 | MW161142 | 2,295,355 | 179,627 | 5,264.56 | 3,542 | 42.3 | 231 |
| SBR9 | MW161143 | 2,131,580 | 1,388,757 | 44,117.56 | 3,542 | 42.3 | 231 |
| SLR27 | MW161144 | 2,655,865 | 2,555,274 | 96,156.14 | 3,541 | 42.1 | 231 |
| SLR41 | MW161145 | 1,553,252 | 1,545,424 | 46,650.82 | 3,541 | 42.2 | 231 |
| SLR13 | MW161146 | 1,489,690 | 1,475,648 | 53,887.92 | 3,541 | 42.2 | 231 |
| SLR8 | MW161147 | 1,683,950 | 1,668,967 | 57,808.29 | 3,541 | 42.2 | 231 |
| SWC57 | MW161148 | 2,396,868 | 520,345 | 16,764.23 | 3,542 | 42.1 | 231 |
| FXR8 | MW161149 | 4,585,006 | 3,541,498 | 133,512.02 | 3,542 | 42.4 | 231 |
| MWR1 | MW161150 | 1,462,527 | 1,455,494 | 51,113.01 | 3,542 | 42.4 | 231 |
| MWR12 | MW161151 | 1,416,716 | 1,375,538 | 53,072.39 | 3,542 | 42.3 | 231 |
| MWR15 | MW161152 | 1,520,880 | 1,465,009 | 54,029.38 | 3,543 | 42.3 | 279 |
| MWR18 | MW161153 | 1,037,823 | 1,036,230 | 38,759.37 | 3,542 | 42.4 | 231 |
| MWR5 | MW161154 | 2,926,955 | 2,912,649 | 110,665.44 | 3,542 | 42.3 | 231 |
| MWR8 | MW161155 | 1,174,913 | 1,092,535 | 40,702.67 | 3,542 | 42.3 | 231 |
| RR154 | MW161156 | 1,398,141 | 1,290,718 | 53,167.09 | 3,542 | 42.3 | 231 |
| RR173 | NC_027922 | 3,703,912 | 3,217,633 | 129,053.27 | 3,542 | 42.3 | 231 |

[a] QC, quality control.

size between the genomes from Canada (195 bp) and those from the United States (231 bp or 279 bp). Genome size (3,542 bp) was conserved in all but 7 genomes in which insertions or deletions at single sites were observed within the putative noncoding region of the genome (range, 3,541 to 3,543 bp). The genomes had a maximum nucleotide difference of 3.5% observed in the polymerase ORF and 3.3% in the surface ORF. In comparison, human hepadnaviruses are classified into genotypes by >8% genome nucleotide differences and >4% differences in nucleotides encoding the surface protein (11). Although these new genomes would be considered a single genotype by the standard convention, WSHBV represents a new genus for which diversity may need to be addressed differently.

**Data availability.** The complete genome sequences have been deposited in GenBank under accession numbers MW161131, MW161132, MW161133, MW161134, MW161135, MW161136, MW161137, MW161138, MW161139, MW161140, MW161141, MW161142, MW161143, MW161144, MW161145, MW161146, MW161147, MW161148, MW161149, MW161150, MW161151, MW161152, MW161153, MW161154, MW161155, and MW161156. The raw reads were deposited under BioProject number PRJNA685065.

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