Hepatitis B viruses are partially double stranded DNA viruses that infect a wide range of hosts with host responses varying from mild infection to chronic infection and carcinogenesis. The white sucker hepatitis B virus (WSHBV) was first described in the freshwater teleost, white sucker (Catostomus commersonii) in 2015 and unofficially classified as a parahepadnavirus in 2017. At present, the significance of WSHBV infection to the health of white sucker is unknown. No other parahepadnavirus genetic diversity or effect on fish health has been studied. Here, we investigate patterns of genetic diversity and identify geographically associated variation among WSHBV genomes from white sucker inhabiting tributaries of Lake Michigan, Lake Superior, and Lake Erie, and in Alberta, Canada. We previously identified 12 of 169 White Sucker as WSHBV positive via a Nanostring codeset targeting viral core gene transcripts. These fish and an additional 16 were identified as virus positive using a qPCR method to detect viral DNA extracted from plasma and liver tissue. Subsequently, 28 WSHBV genomes were sequenced using a large amplicon sequencing (LAS) method of long-range PCR followed by amplicon
sequencing using the Illumina MiSeq. We observed inconsistencies in sequencing success of a 627 bp atypical region within WSHBV that may be explained by viral biology. The WSHBV genome and protein sequences isolated here clustered together by geography similar to that observed with geographically separated human hepatitis B virus genotypes. Although the WSHBV genomes do not meet criteria used to define subgenotypes of other hepadnaviruses, we suggest the use of the ratio of nonsynonymous/synonymous mutations (Ks/Ka) in the surface protein and total Dxy to differentiate subgenotypes. Between the four viral subgenotypes, Alberta 1522, Alberta 1538, St. Louis, and Lake Michigan the Ka/Ks was greater than 0.572 and the overall Dxy was greater than 0.00683. Each of these subgenotypes may arise from adaptive or purifying selection based on host gene expression, host immune response, or environmental exposures affecting the host and cellular processes. Similarly with other hepadnaviruses, variability between subgenotypes was clustered in the PreS and spacer domain, which is associated with immune epitopes and host specificity. This study of WSHBV genetic diversity should facilitate the development of molecular markers for future identification of genotypes and provide evidence in future investigations of possible differential disease outcomes.