Draft Genome Sequence of a Novel Calicivirus from a Brown Bullhead (*Ameiurus nebulosus*) from Lake Memphremagog, Vermont/Quebec

Luke R. Iwanowicz,[a] Vicki S. Blazer,[a] Tom Jones,[b] Matthew Bodnar,[b] Korin Eckstrom,[c] Julie A. Dragon,[c] Peter Emerson[b]

[a]U.S. Geological Survey, Eastern Ecological Science Center, Kearneysville, West Virginia, USA
[b]Vermont Fish & Wildlife Department, Montpellier, Vermont, USA
[c]University of Vermont, Vermont Integrative Genomics Resource, Burlington, Vermont, USA

ABSTRACT We report a draft genome sequence of a previously undescribed calicivirus from a single brown bullhead inhabiting Lake Memphremagog, Vermont/Quebec. The genome is 7,413 nucleotides long and is most similar to the Atlantic salmon calicivirus (nucleotide identity; 64.7%).

Caliciviruses (*Caliciviridae*) are a family of small (27 to 40 nm), nonenveloped viruses that infect a broad range of terrestrial and aquatic animals and for which the International Committee on Taxonomy of Viruses (ICTV) recognizes 11 genera (1, 2). Two of these genera, *Salovirus* and *Minovirus*, infect fish hosts (3, 4). The genome of this group is a linear, positive-sense, single-stranded RNA (ssRNA) that ranges between 6.4 and 8.5 kb. They are the causative agents of diseases that range widely in clinical presentation and significance across hosts. They are sometimes observed in clinically normal hosts (5). While the more commonly recognized caliciviruses are feline calicivirus (respiratory disease), Norwalk virus (gastroenteritis in humans), and rabbit hemorrhagic disease virus (hemorrhagic disease), they are also associated with disease and covert infections in other mammals, birds, herptiles, and fishes (2–4).

Brown bullhead, *Ameiurus nebulosus*, is commonly used as sentinel species in contaminant-centric, adverse effect monitoring in the Great Lakes and elsewhere, given their association with the benthos where bioactive contaminants adsorb (6). Neoplasia is the most common biological endpoint used to evaluate exposure to mutagens in this species, particularly in the Great Lakes region, where liver tumor prevalence is associated with polyaromatic hydrocarbons in the sediment (7–10). Recently, malignant melanoma of unknown etiology has been described in brown bullhead inhabiting Lake Memphremagog, Vermont/Quebec, consequently prompting investigations into causation (11).

Total RNA was extracted from skin of normal brown bullhead and those clinically diagnosed with malignant melanoma using Omega Bio-Tek total RNA (11). The RNA was shipped to the University of Pennsylvania for paired-end library construction using the TruSeq stranded mRNA kit. Indexed libraries were run for 2 × 150 cycles on a HiSeq 2500 instrument (Illumina, San Diego, CA). Reads were assembled on a per-sample basis using MEGAHIT and screened for viral sequences in Cenote-Taker 2 (12, 13). Default parameters were used for all software analyses unless otherwise specified. The genome of a novel calicivirus consisting of 7,413 nucleotides (nt) excluding the poly(A) tail and with a GC content of 57% was recovered from a single malignant melanoma sample. Reads were mapped to the draft genome using CLC Genomics Workbench v.21.0.2 and represented 0.02% (17,705) of the total reads (99,473,704). Average coverage was 340×.

The genomic RNA was organized into two partially overlapping open reading frames (ORFs), ORF1 (polyprotein, nt 35 to 7117) and ORF2 (putative minor structural
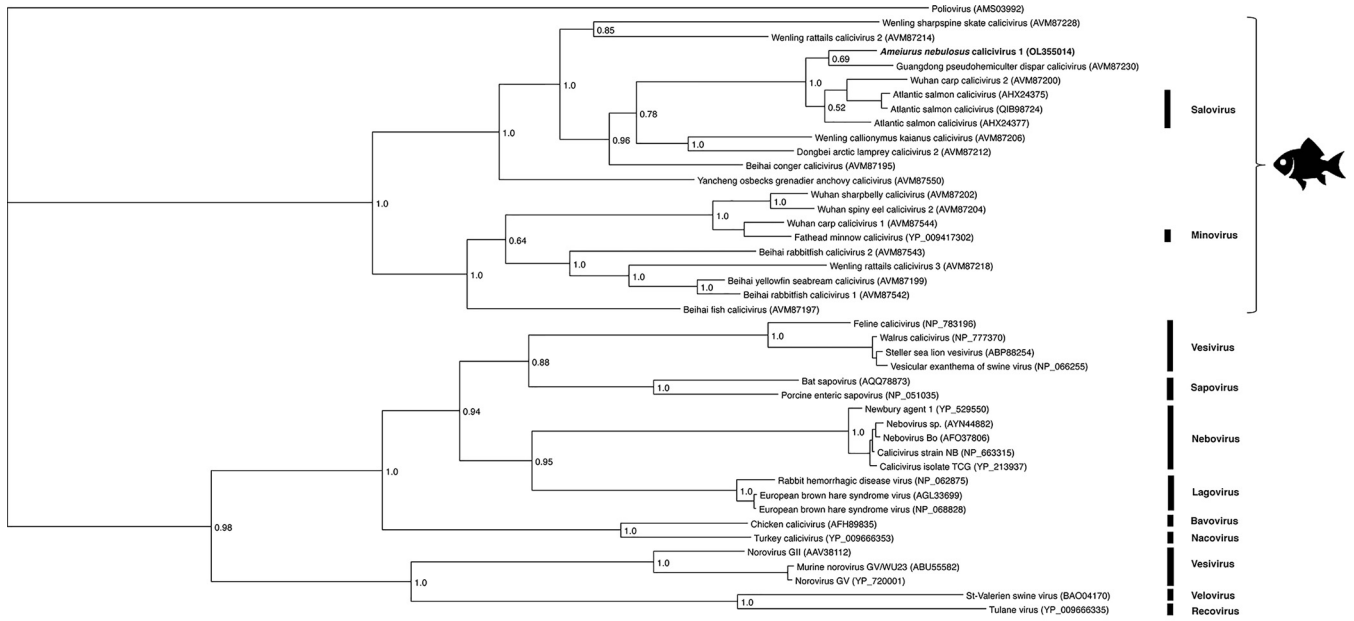
FIG 1 Bayesian phylogenetic analysis of the conserved domain within the *Ameiurus nebulosus* calicivirus 1 RNA-dependent RNA-polymerase (RdRp) protein. Phylogenies were constructed using the LG+G+I amino acid substitution model using MrBayes with a Markov chain Monte Carlo chain length of 200,000 and subsampling every 200 generations. Posterior probabilities are listed for select nodes. Sequences used for phylogenetic analysis are publicly available in GenBank. GenBank accession numbers are listed in the unrooted phylogram. The conserved domain of the poliovirus RdRp was set as an outgroup. Genera for which an official classification is available are denoted along the right margin. Viruses associated with piscine hosts are also indicated.

protein, nt 6952 to 7317). These ORFs were predicted using Geneious Prime v.2020.2.3. The 5′ and 3′ untranslated regions were 34 and 96 nt, respectively. We anticipate that 8 bases are missing from the 5′ end. The highest nucleotide identity of the genome was to the *Salovirus*, Atlantic salmon calicivirus isolate Nordland/2011 (GenBank accession number NC_024031; 64.7%). Nucleotide identity was determined using pairwise alignments in MUSCLE v.3.8.425 bundled within Geneious Prime v.2020.2.3 (14). Phylogenetic analysis of the RNA-dependent RNA-polymerase protein conserved domains identified the placement of this novel virus (*Ameiurus nebulosus* calicivirus 1) in a strongly supported clade that included Atlantic salmon calicivirus and two unclassified caliciviruses with fish hosts (Fig. 1).

Viral sequence was identified in only 1 of 8 melanoma samples, and it was not present in normal skin. It is unlikely that this novel calicivirus is associated with malignant melanoma of brown bullhead, and an association with significant disease is unknown. Given that health evaluations of this species are used to assess environmental health, it is critical to catalogue potential microbial pathogens to more comprehensively document disease.

Data availability. The genome sequence has been deposited in GenBank under accession number OL355014. The raw reads were deposited under BioProject number PRJNA777583, BioSample number SAMN22865800, and SRA number SRR16816962. Alignments and ancillary metadata are available at https://doi.org/10.5066/P9MPFVMX.

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