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GENOME SEQUENCES

Draft Genome Sequence of an Adomavirus Associated with Raised Mucoid Skin Lesions on Smallmouth Bass (*Micropterus dolomieu*)

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ABSTRACT Raised mucoid skin lesions have been observed on smallmouth bass (*Micropterus dolomieu*) for years. Here, we report the draft genome of a novel adomavirus (*Micropterus dolomieu* adomavirus 2) associated with this disease. The circular genome is 17,561 bp and most similar to that of alpha-adomaviruses.

Adomaviruses are a proposed group of DNA viruses recently observed in fish hosts (1). While some adomaviruses have been identified in diseased fish, partial or complete genomes have also been recovered from metagenomic sequencing of clinically normal hosts or those of unreported health status. Phylogenetic evidence currently supports two distinct groups, alpha-adomaviruses and beta-adomaviruses (2). The genome is composed of circular double-stranded DNA (dsDNA) encoding two cassettes of transcribed genes on opposite strands, minimally 15 kb in total. The two cassettes correspond to open reading frames (ORFs) that are expressed early (EO) or late (LO) in infection. A conserved set of syntenic ORFs (EO1, LO4, LO5, LO7, LO8) has been identified; ORF content outside this core group appears to be diverse (2).

Smallmouth bass (*Micropterus dolomieu*, Centrarchidae) are prized freshwater sportfish that range throughout temperate North America. Given their recreational and cultural value, clarifying the etiology of raised mucoid skin lesions (RMSLs) is necessary for managing population health and informing the public (3, 4).

Total RNA was extracted from a pooled sample of RMSLs from smallmouth bass inhabiting areas of the upper Chesapeake Bay watershed in Pennsylvania, Virginia, and West Virginia and prepared for RNAseq. In addition, an RMSL was dissected from a different, individual smallmouth bass collected from the Susquehanna River and preserved in 96% ethanol. DNA was extracted using a DNeasy blood and tissue kit (Qiagen, Valencia, CA). Rolling-circle amplification using exonuclease-protected random hexamers was performed to amplify the circular DNA, and a sequencing library was prepared using a NexteraXT library kit (Illumina, San Diego, CA) for sequencing on an Illumina MiSeq instrument (1 × 150 bp) (5).

Transcriptome sequencing (RNA-seq) reads were trimmed of Illumina adapters and low-quality bases (Phred-scaled quality, <10) with BBDuk (6); reads less than 50 bp long were discarded. Contigs were assembled with SPAdes v. 3.13.0 (7) using a kmer value of 31 and 1× minimum coverage. Alignment to the UniRef90 database (download date 18 February 2019) with DIAMOND v. 0.9.22.123 (default parameters) (8) identified a 14,796-nucleotide (nt) contig with distant adomavirus and papillomavirus homology. This subgenomic contig lacked a complete EO1 helicase and terminated with a low-complexity sequence. The successful extension and closure of the genome reference with IVA v. 1.08 (9) (default parameters) was indicated by 285-nt terminal repeats

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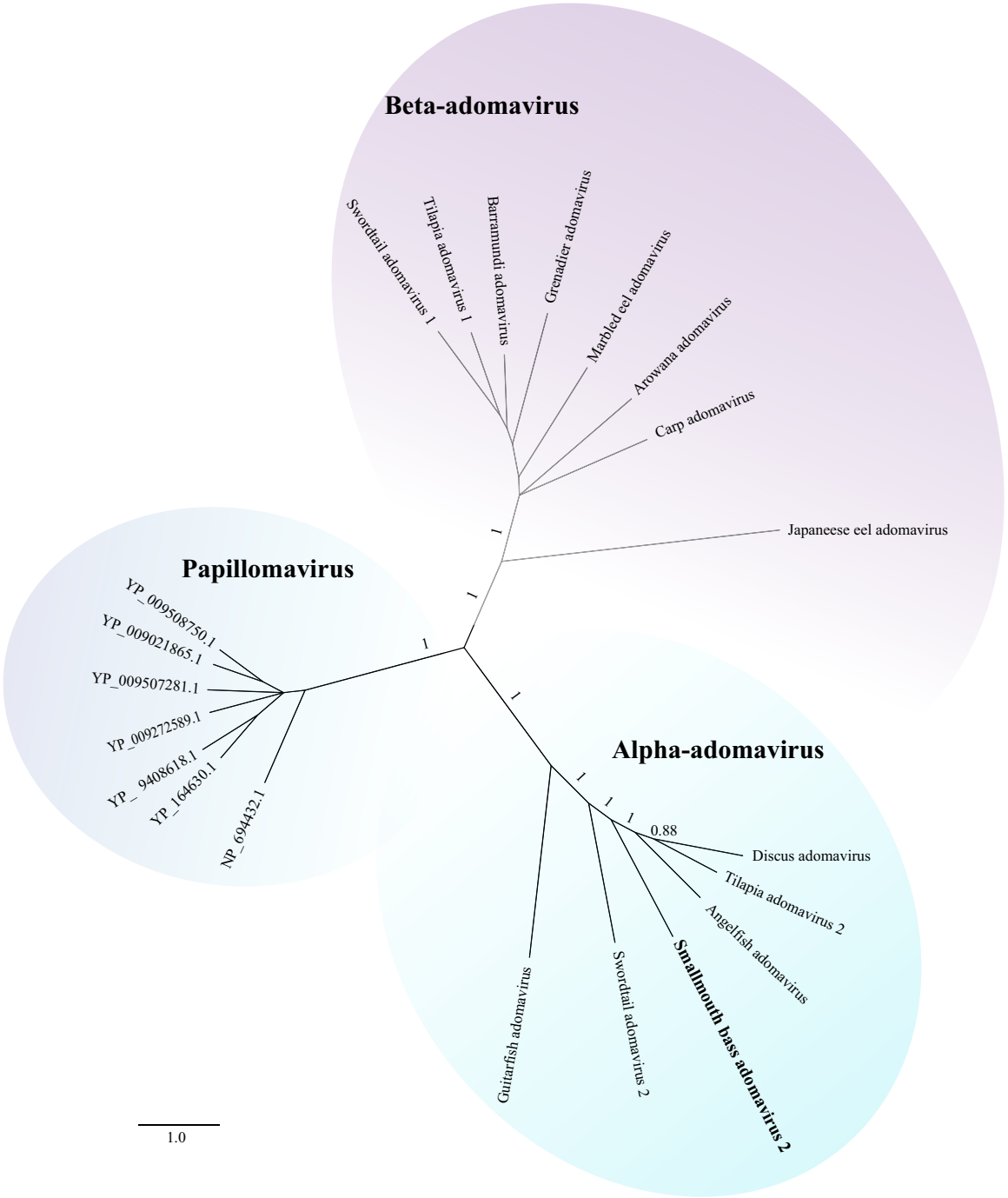

FIG 1 Bayesian phylogenetic analysis of the smallmouth bass adomavirus 2 and alpha-adomaviruses, beta-adomaviruses, and papillomaviruses based on alignments of helicase proteins (EO1 or E1). Bayesian analysis was conducted using the mtREV substitution model using MrBayes with a Markov chain Monte Carlo chain length of 250,000 and subsampling every 200 generations. Posterior probabilities are listed for select nodes. Adomavirus sequences are publicly available at https://ccrod.cancer.gov/confluence/display/LCOTF/AdintoProt. GenBank accession numbers are listed in the unrooted phylogram for representative papillomaviruses.

of normal complexity and an intact EO1 helicase. The G+C content was 44.8%. The coverage and contiguity of the genome were further evaluated by mapping rolling-circle amplicon reads with CLC Genomics Workbench v. 12.0.1. The average coverage was 117,186× (range, 3,791× to 279,719×; represented by 14 million mapped reads).

The closed alpha-adomavirus genome reference encompasses 17,561 bases and 10 discrete open reading frames annotated by homology as EO1, EO2, EO3, EO4, LO2-3,

LO4, LO5, LO6, LO7, and LO8. Putative internal ORFs (EO2+ and EO3+) were also identified. Phylogenetic analysis of the EO1 protein placed this virus in the alphaadomavirus clade (Fig. 1). This is the first complete genome of an adomavirus associated with a skin lesion of the smallmouth bass. Further analyses are required to confirm that this virus is the causative agent of the observed disease.

Data availability. The complete genome nucleotide sequence has been deposited in GenBank under accession no. MN631022. The raw sequence reads are available in the NCBI Sequence Read Archive (SRA) under BioProject accession no. PRJNA564569.

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Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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