Complete Genome Sequence of a Novel Aquareovirus That Infects the Endangered Fountain Darter, *Etheostoma fonticola*

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Here, we report the complete genome of a novel aquareovirus isolated from clinically normal fountain darters, *Etheostoma fonticola*, inhabiting the San Marcos River, Texas, USA. The complete genome consists of 23,958 bp consisting of 11 segments that range from 783 bp (S11) to 3,866 bp (S1).

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The fountain darter, *Etheostoma fonticola*, is a small benthic fish endemic to the headwaters of the San Marcos and Comal Rivers of central Texas, USA (1). This fish is currently listed as federally endangered, and the current recovery plan requires biological research necessary for monitoring, management, and restoration (2–4). During 2003, an unknown viral agent was isolated from a pool of clinically normal fountain darters collected from the San Marcos River. It is unknown if this virus is a pathogen of concern. Identification of this virus was a critical component of disease management for this species. Here, we present the complete genome sequence of this novel aquareovirus.

Aquareoviruses are nonenveloped icosahedral virions that contain a genome composed of 11 segments of double-stranded RNA that code for 12 proteins. At present, seven species of *Aquareovirus* are recognized by the International Committee on Taxonomy of Viruses based on nucleotide or amino acid identity of segment 2 or segment 10, and from the results of RNA-RNA hybridization experiments (5, 6). For decades, aquareoviruses have been isolated from moribund and clinically normal fish and shellfish (7, 8). The pathogenicity of aquareoviruses ranges from apathogenic to highly virulent. Pathogenic aquareoviruses are most commonly observed in fishes maintained for aquaculture (9).

Ribosomal RNA was depleted from total RNA isolated from infected CHSE-214 cells using a Ribo-Zero kit. High-throughput sequencing was performed using a combination of Ion Torrent PGM and Illumina MiSeq platforms. Genome assembly was conducted using CLC Genomics Workbench (version 7.5) and CAP3 (10). Dideoxy sequencing was used to close sequence gaps. The composite average mapping coverage of each segment ranged from 241 to 9,133×. Blastx queries of all segments in the NCBI database best matched aquareoviruses that infect fish but clearly identified this virus as unique. We confirmed full-length sequences via the identification of the conserved 5′- and 3′-terminal sequences (GUUU and CAUC, respectively). The complete genome of this virus is 23,958 nucleotide bp, and the size of the segments are as follows S1, 3,947 bp; S2, 3,866 bp; S3, 3,687 bp; S4, 2,554 bp; S5, 2,242 bp; S6, 2,057 bp; S7, 1,317 bp; S8, 1,317 bp; S9, 1,118 bp; S10, 987 bp; and S11, 783 bp. The G+C content was 56.6%, which is consistent with that of other aquareoviruses (11). A single open reading frame (ORF) was identified in each segment, with the exception that S7 contained two partially overlapping ORFs. A total of 12 ORFs were identified that encoded putative structural and nonstructural proteins typical of aquareoviruses. Here, we name this novel virus *Etheostoma fonticola aquareovirus* (EFAReV). The public availability of this complete genome sequence will facilitate the development of diagnostic molecular markers.

Accession number(s). The genome sequence of the *Etheostoma fonticola* aquareovirus was submitted to GenBank with the accession numbers KU194213 to KU194223 that correspond to genomic segments 1 to 11, respectively.

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