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GENOME ANNOUNCEMENT

Complete Genome Sequence of the European Sheatfish Virus

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Viral diseases are an increasing threat to the thriving aquaculture industry worldwide. An emerging group of fish pathogens is formed by several ranaviruses, which have been isolated at different locations from freshwater and seawater fish species since 1985. We report the complete genome sequence of European sheatfish ranavirus (ESV), the first ranavirus isolated in Europe, which causes high mortality rates in infected sheatfish (*Silurus glanis*) and in other species. Analysis of the genome sequence shows that ESV belongs to the amphibian-like ranaviruses and is closely related to the epizootic hematopoietic necrosis virus (EHNV), a disease agent geographically confined to the Australian continent and notifiable to the World Organization for Animal Health.

Ranaviruses are emerging pathogens causing serious disease in wild and farmed cold-blooded animals (6). They are icosahedral viruses of the *Iridoviridae* family with circular double-stranded DNA (dsDNA) genomes ranging from 105 to 140 kbp and a complex nucleocytoplasmic replication cycle. One of their most intriguing biological properties is their wide host tropism, which includes amphibians, fish, and reptiles.

Complete ranavirus genomes from different hosts and locations show the existence of two groups, one corresponding to the fish viruses grouper iridovirus (GIV) and Singapore grouper iridovirus (SGIV), isolated in Asia, and the second to the amphibian-like ranaviruses (ALRVs), which include viruses isolated from amphibians or reptiles in North America, Asia, and Europe as well as one species, the epizootic hematopoietic necrosis virus (EHNV), isolated from diseased fish in Australia.

The first fish ranavirus to be isolated in Europe was European sheatfish ranavirus (ESV), which was obtained from moribund sheatfish (*Silurus glanis*) fry in Germany in 1989 (2, 8). A second ranavirus isolated from catfish (*Ameiurus melas*) in France (12) and Italy (4) was found to be very closely related to the former (3, 9, 10) and may represent an isolate of the same virus. Experimental ESV infection in sheatfish results in 100% mortality by 8 days after challenge (1) and causes high mortality in pike (*Esox lucius*) (11), too. Other species, such as black bullhead (*Ameiurus melas*) (7) and rainbow trout (3), are apparently not affected, although viral replication has been detected.

To obtain the complete genome sequence of ESV, the reference virus stock from the Spanish Central Veterinary Laboratory was plaque purified on ZF4 cells to ensure clonality and DNA from viral particles was extracted. Sequencing was performed using Genome Sequencer FLX Titanium equipment (Roche 454 Life Sciences) at the Parque Científico de Madrid. A total of 71,527 viral reads with an average length of 355 bp was assembled with Newbler 2.5.3 in 7 contigs flanked by repeated regions that were joined into a single contig by PCR and Sanger sequencing.

The final genome sequence of ESV is 127,732 bp long, which is slightly longer than that of the EHNV genome, and it has a GC content of 54.23%. Annotation of the ESV genome was performed with genome annotation transfer utility (GATU) software (13) using the genome of EHNV as a template. A total of 136 putative open reading frames, including all of the iridovirus- and ranavirus-specific genes as well as 12 out of 13 ALRV-specific genes, were identified. Global multiple alignments with other ranaviruses using LAGAN software (5) show that ESV has the highest degree of conservation with EHNV (88%) and *Ambystoma tigrinum* virus (ATV) (79%). Dot plot analyses show large colinearity stretches with both the EHNV and ATV genomes. Compared to ATV, ESV shows 9 major sequence insertions, while in comparison with EHNV, ESV shows three insertions, one deletion, and one inversion. Altogether, these results place ESV as a novel ALRV belonging to the ATV/EHNV group.

Nucleotide sequence accession number. The complete genome sequence of European sheatfish virus was deposited in GenBank under accession number JQ724856.

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ERRATUM

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Volume 86, no. 11, p 6365–6366. Page 6356, column 1, lines 3 and 4: "circular doubled-stranded DNA" should read "circularly permuted and terminally redundant double-stranded DNA."

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