



Near-Complete Genome Sequences of 12 Peruvian Strains of Infectious Hypodermal and Hematopoietic Necrosis Virus Infecting the Shrimp *Penaeus vannamei*

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ABSTRACT Infectious hypodermal and hematopoietic necrosis virus (IHHNV) is a shrimp virus listed by the World Organisation for Animal Health (OIE). In this study, we report the genomic sequences of 12 IHHNV strains obtained from shrimp samples from aquaculture cultures from the Tumbes region of Peru.

Penaeus vannamei is the shrimp with the highest commercial value, and therefore it is the most widely cultivated species around the world, including Peru (1, 2).

Infectious hypodermal and hematopoietic necrosis virus (IHHNV; genus, *Penstylidensovirus*; subfamily, *Densovirinae*; family, *Parvoviridae*) is one of the smallest penaeid shrimp viruses (3). This virus was first reported in the 1980s in Hawaii (4, 5). Phylogenetic analysis revealed the introduction of this virus to the American continent in the 1970s (6). Interestingly, IHHNV causes high mortality in *Penstylidensovirus stylirostris*; however, in *P. vannamei* and *P. monodon*, mortality has not been reported. In these species, IHHNV causes slow growth and deformities in the exoskeleton (called Runt deformity syndrome) (7).

IHHNV is a 22-nm parvovirus with a nonenveloped icosahedral capsid; it has a genome of ≈ 3.9 kb of single-stranded DNA encoding 3 open reading frames (ORFs). IHHNV infection is considered benign and of low risk, but recent studies in *P. monodon* show that high viral loads can produce a significant economic impact (8, 9).

Sequences from Peruvian IHHNV strains have not been described. In this work, we report 12 partial genomic sequences of IHHNV strains detected in the Aquaculture Health Laboratory of the National Fisheries Health Agency (SANIPES), from shrimp from different productive zones and natural environments of the Tumbes region in Peru.

The sampling area included culture systems (9 samples) and natural areas (3 samples) from the north, center, and south of the coast of the Tumbes Department. DNA was isolated from 25 mg of homogenized pleopod tissue from *P. vannamei* using the DNeasy blood and tissue kit (Qiagen, Germany). The DNA was quantified with the Qubit 4 fluorometer (Invitrogen, USA). The samples were screened by endpoint PCR using the primers 309F/R, following the recommendations described in the Manual of Diagnostic Tests for Aquatic Animals from the World Organisation for Animal Health (OIE) (Fig. 1) (10).

Subsequently, to cover the IHHNV genome, 8 endpoint PCRs were performed per sample with specific primers to cover 3.9 kb using 50 to 100 ng of DNA (Fig. 1), following the protocols of Silva et al. (2014), with modifications to the amplification time (11). The PCRs were performed with high-fidelity *Taq* platinum (Thermo Scientific, USA). Sequencing was carried out using dideoxy-chain termination sequencing with the fluorescent nucleotides method. The sequences were assembled using Geneious Prime 2020.0.5 software (Biomatters, Inc.), generating a consensus sequence of ≈ 3.7 kb. The base calling was done with a quality score of 99.9% accuracy ($Q > 30$). The sequencing covered 96% of the genome (compared to the reference Hawaii IHHNV strain; GenBank

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We dedicate this article to the memory of biologist Juan José Bonifacio (1985 to 2020), who actively participated in the execution of this project.

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