A REVIEW OF WHITE SPOT SYNDROME VIRUS (WSSV) FROM ECUADOR, ENDOGENOUS VIRAL SEQUENCES (EVE) OF WSSV (WSSV-EVE), AND ENDOGENOUS NIMAVIRUS *Nimav-1_LVa*: THEIR INTEGRATION IN THE GENOMES OF THE FIRST SPECIFIC PATHOGEN-FREE (SPF) *Penaeus vannamei* DOMESTICATED IN THE UNITED STATES AND *P. vannamei* FARMED IN CHINA

Acacia Alcivar-Warren*, Victoria Alday-Sanz and Kathy F. J. Tang

Environmental Genomics, Inc. P. O. Box 196, Southborough, MA 01772 USA
environmentalgenomics.warren@gmail.com

It was previously reported that mortalities of cultured shrimp, *Penaeus vannamei*, induced by white spot syndrome virus (WSSV) [Nimaviridae(1); Whispovirus(1); WSSV(1)] have occurred in Ecuador since May 1999 (Rodriguez *et al.* 2003) confirmed by histopathology and PCR, and showed 'an apparent association between lower temperature and increased mortality rates in commercial ponds'. However, it's possible that "asymptomatic" WSSV may have been present in Ecuador before 1999. Low survival in semi-intensive ponds is rarely examined by histology and therefore the start of the epidemic might have been missed. Here, we report detection of WSSV in wild and farmed *P. vannamei* of Ecuador since 1996 using histopathology and confirmed by *in situ* hybridization (Flegel, *pers. comm.*) techniques. WSSV was first detected by PCR in 5 of 38 (13%) juveniles from Bonanza, Isla Puna, Salinas River Estuary, Guayas province of Ecuador (samples provided by Empacadora Nacional in 1996, placed in liquid nitrogen immediately after collection, until DNA isolation and PCRs were performed). All 27 wild broodstock from Puerto Cayo, Manabí province, collected in 1996 and 25 broodstock from Puerto Cayo collected in 2000 were negative (0/52) by PCR, suggesting geographic differences in virus prevalence.

The sequence of WSSV from *P. vannamei* of Ecuador collected in 2015 (WSSV-EC-15098, MH090824, 288,997bp) was recently published. WSSV-like sequences (WSSV-EVE, Utari *et al.* 2017) are found in genomic libraries and expressed sequence tags isolated from the first specific pathogen-free (SPF) *P. vannamei* domesticated by the breeding program of the U.S. Marine Shrimp Farming Program (USMSFP) maintained at the Oceanic Institute in Hawaii, USA since late 1980's. DNA from juveniles of SPF Kona Line (characterized by high susceptibility to all shrimp viruses but high growth performance) was subject to a pilot whole genome sequencing using PacBio SMRT method. From ~424 Mb genomic sequence, 312 diverse repetitive families were characterized including a nimavirus, *Nimav-1_LVa*, of 279,905bp (Bao *et al.* 2020: https://pubmed.ncbi.nlm.nih.gov/31947590/) and are deposited in Repbase (www.girinst.org). *Nimav-1_LVa* is supported by 3-6 copies throughout the whole length, all flanked by the putative telomeric (GGTTA)n, with some ORFs significantly homologous to WSSV isolate of *P. japonicus* from China (CN01) collected in 1994 (NCBI reference sequence NC_003225.3, 309,286bp). Homology searches using 11 whole genome shotgun (wgs) databases in GenBank revealed that NC_003225.3 and MH090824 are not integrated in the draft genome sequence of *P. vannamei* farmed in China (assembly ASM378908v1; ~1.7Gb), but *Nimav-1_LVa* is integrated in scaffold Penaeus vannamei breed Kehai No.1 LVANscaffold_3666 (QCYY01003664, 990704bp, 428 matches). Because the genome size of SPF *P. vannamei* is expected to be ~2.87 Gb, access to a full, contiguous, whole reference genome for *P. vannamei* is urgently needed from both the founder SPF stocks and wild *P. vannamei* of Ecuador (samples available for research, collected since 1996) to study evolution and pathogenicity of WSSV and *Nimav-1_LVa*.