

*The following supplement accompanies the article*

**Real-time PCR tests to specifically detect IHHNV lineages and an IHHNV EVE integrated in the genome of  
*Penaeus monodon***

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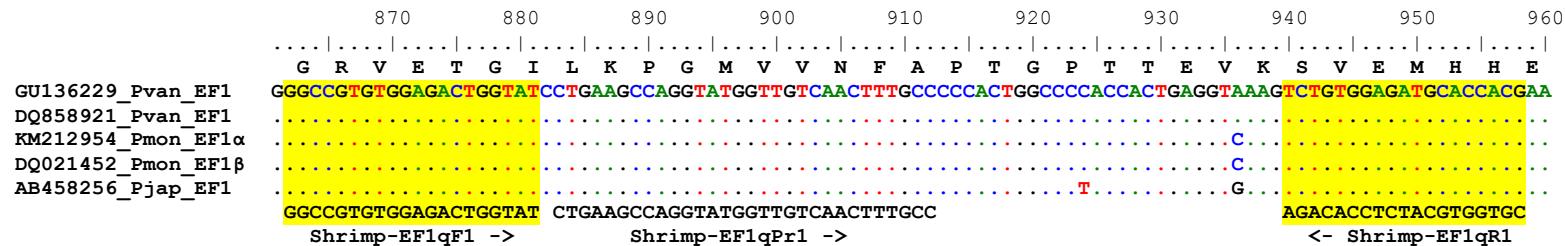
**Fig. S1.** Clustal X multiple sequence alignment of complete or near-complete IHHNV/EVE genome sequences deposited in GenBank showing the IHHNV ORF1 gene region targeted by the IHHNV-q309 TaqMan real-time PCR test. The amino acid sequence of the NS1 non-structural protein (GenBank AY355307) is shown above and PCR primer/probe sequences are shown below the alignment. The 309F PCR primer target site (Tang et al. 2007) is shown for comparison. GenBank accession numbers shown to the left and IHHNV lineages/EVE types and country/region and approximate date of identification are shown to the right. Only those nucleotides varying from the AY355307 IHHNV strain sequence are shown.

	K P K E N S A E Y D Y L Q H L V K T K S A R T V Q E L V N K L D D E E Y K Q	Taiwan	2003
AY355307	AAA <b>CCTAAAGAAAAACAGTCAGAATATGACTACCTCCAA</b> CTTAGTC <b>AAACCAAA</b> TCTGC <b>AAAGAACAGTCCAA</b> GAA <b>CTTGTC</b> A <b>ATAAACTT</b> GAC <b>GAATGAGGAAT</b> AC <b>AAACAGCT</b>		
AY102034	.....G.....	Thailand	2002
KP742841	.....	China	2014
KF031144	.....	Vietnam	2007
KC513422	.....	Vietnam	2011
JN616415	.....	Vietnam	2009
AY362547	.....	Thailand	2003
GQ411199	.....	India	2007
KM593909	.....	Australia	1995
KM593910	.....	Australia	1996
AF218266	.....	?	<2009
KF214742	.....	China	2011
JN377975	.....T.....	South Korea	2010
JX258653	.....G.....	China	2009
KJ830753	.....G.....	China	<2014
KM272862	.....G.....	Australia	2008
KM272863	.....G.....	Australia	2008
AY362548	.....G.....	III	Ecuador <2003
AY355308	.....G.....	Taiwan	2003
KJ862253	.....G.....	Brazil	2013
EF633688	.....G.....	China	2007
AF273215	.....G.....	Australia	2008
JX840067	.....G.....	Vietnam	2012
KM272861	.....G.....	Australia	2008
KM593908	.....G.....	Australia	1991
AY355306	.....G.....	Taiwan	2003
KM593911	.....	Australia	2004
KM593912	.....A.....	Australia	2005
GQ475529	.....	I	Australia 2009
KM593913	.....T.C.....T.....C.....	Australia	2012
EU675312	.....T.C.....T.....C.....	Australia	1993
DQ228358	.....T.C.....T.....C.....	Madagascar	<2002
AY124937	.....A.....T.G.....C.G.....G.A.....A.....	B	East Africa <2002
	CCTAAAGAAAAACAGTCAGAATATGAC		
	CTCCAACACTTAGTCAA		
	CTTGAACAGTTATTGAACTGCTACT		
	IHHNV-q309F1 ->		
	IHHNV-q309Pr1 ->		
	TCCAACACTTAGTCAAACCAA		
	309F ->		

**Fig. S2.** Clustal X multiple sequence alignment of complete or near-complete IHHNV/EVE genome sequences deposited in GenBank showing the IHHNV 3'UTR sequence downstream of the ORF3 (capsid protein) gene targeted by the IHHNV-qEVE real-time PCR test. PCR primer/probe sequences are shown below the alignment. GenBank accession numbers shown to the left and country/region and approximate date of strain identification are shown to the right. Only those nucleotides varying from the AY355307 IHHNV strain sequence are shown.

AY355307	TAAACCTATATAATCTATTACTATCTACCTACCCCTCTACACAAAACCAGCTACCCAGGCAAGGTGGGACTCCGGCTACCCAGGCATGGTGGGACACTTCTCTACTATTGACGACGT		Taiwan	2003	
AY102034	.....T.....		Thailand	2002	
KP742841	.....G.....		China	2014	
KF031144	.....		Vietnam	2007	
KC513422	.....A.....		Vietnam	2011	
JN616415	.....T.....G.....		Vietnam	2009	
AY362547	.....T.....T.....		Thailand	2003	
GQ411199	....AAA....TAT....CCGA....GCTG.....T.....T.....		India	2007	
KM593909	.....T.....GCT.....C.....A.....T.....GT.....		Australia	1995	
KM593910	.....T.....GCT.....C.....A.....T.....GT.....		Australia	1996	
AF218266	.....A.....GCT.....TC.....A.....T.....T.....		?	<2009	
KF214742	.....A.....GCT.....TC.....A.....T.....T.....		China	2011	
JN377975	.....A.....G.....GCT.....TC.....A.....T.....T.....		South Korea	2010	
JX258653	.....A.....GCT.....TC.....A.....T.....T.....		China	2009	
KJ830753	.....A.....GCT.....TC.....A.....T.....T.....		China	<2014	
KM272862	.....T.....GCT.....TC.....A.....T.....T.....		Australia	2008	
KM272863	.....T.....GCT.....TC.....A.....T.....T.....		III	Australia	2008
AY362548	.....A.....G.....GCT.....TC.....A.....T.....T.....		Ecuador	<2003	
AY355308	.....A.....GCT.....TC.....A.....T.....T.....		Taiwan	2003	
KJ862253	.....A.....GCT.....TC.....A.....T.....T.....		Brazil	2013	
EF633688	.....A.....GCT.....TC.....A.....T.....T.....		China	2007	
AF273215	.....A.....GCT.....TC.....A.....T.....T.....		Australia	2008	
JX840067	.....A.....GCT.....TC.....A.....T.....T.....		Vietnam	2012	
KM272861	.....T.....GCT.....TC.....A.....T.....T.....		Australia	2008	
KM593908	.....T.....GCT.....TC.....A.....T.....T.....		Australia	1991	
AY355306	.....		Taiwan	2003	
KM593911	.....T.....GCT.....C.....A.....T.....GT.....		Australia	2004	
KM593912	.....T.....GCT.....C.....A.....T.....GT.....		Australia	2005	
GQ475529	.....C.....C.....C.....T.....T.....A.....		I	Australia	2009
KM593913	.....AACCC.....C.....AAAGCA.....A.....A.....C.....CA.....T.....T.....G.....T.....AA.....T.....A.....A.....AA.....A.....AA.....		Australia	2012	
EU675312	.....AACCC.....C.....AAAGCA.....A.....A.....C.....CA.....T.....T.....G.....T.....AA.....T.....A.....A.....AA.....A.....AA.....	A	Australia	1993	
DQ228358	.....AACCC.....C.....AAAGCA.....A.....A.....C.....CA.....T.....T.....G.....T.....AA.....T.....A.....A.....AA.....A.....AA.....		Madagascar	<2002	
AY124937	.....CCCACAAAAAGCAAATATCTCACTAT		B	East Africa	<2002
	.....IHHNV-qEVEF1.....->				
	GGTCCGTACCAACCTTGGACGAT				
	<- IHHNV-qEVEPr1				
	TTCCACCCCTGTTATTAGAGTATTACTG				
	<- IHHNV-qEVER1				

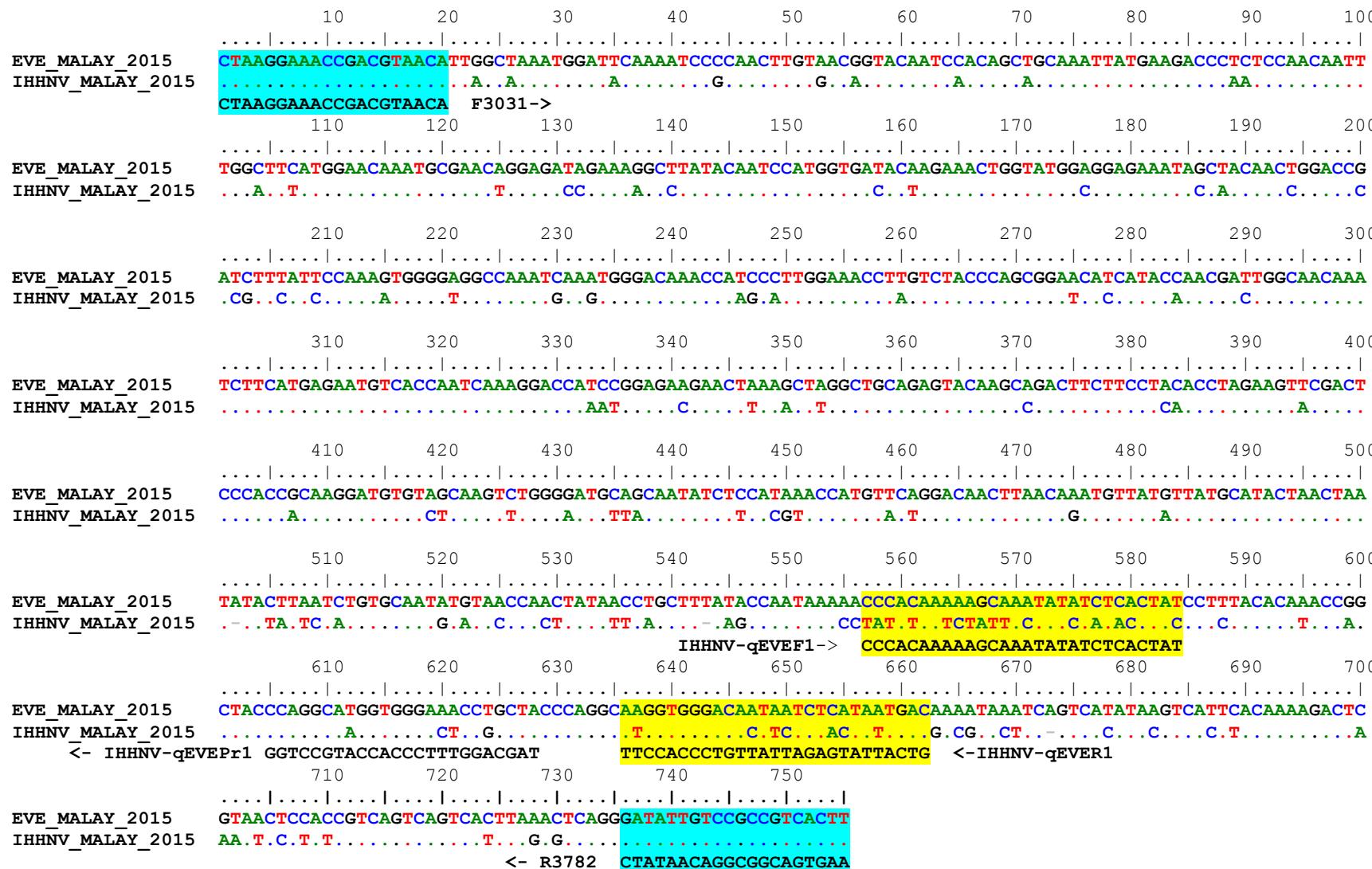
**Fig. S3.** Clustal X multiple sequence alignment of penaeid shrimp elongation factor 1 (EF1) gene sequences () showing the region targeted by the Shrimp-EF1qF1/R1 real-time PCR test. Numbering and amino acid coding sequence is shown for *Penaeus vannamei* EF1 (GU136229) and PCR primer/probe sequences are shown below the alignment. Only those nucleotides varying from the GU136229 sequence are shown.



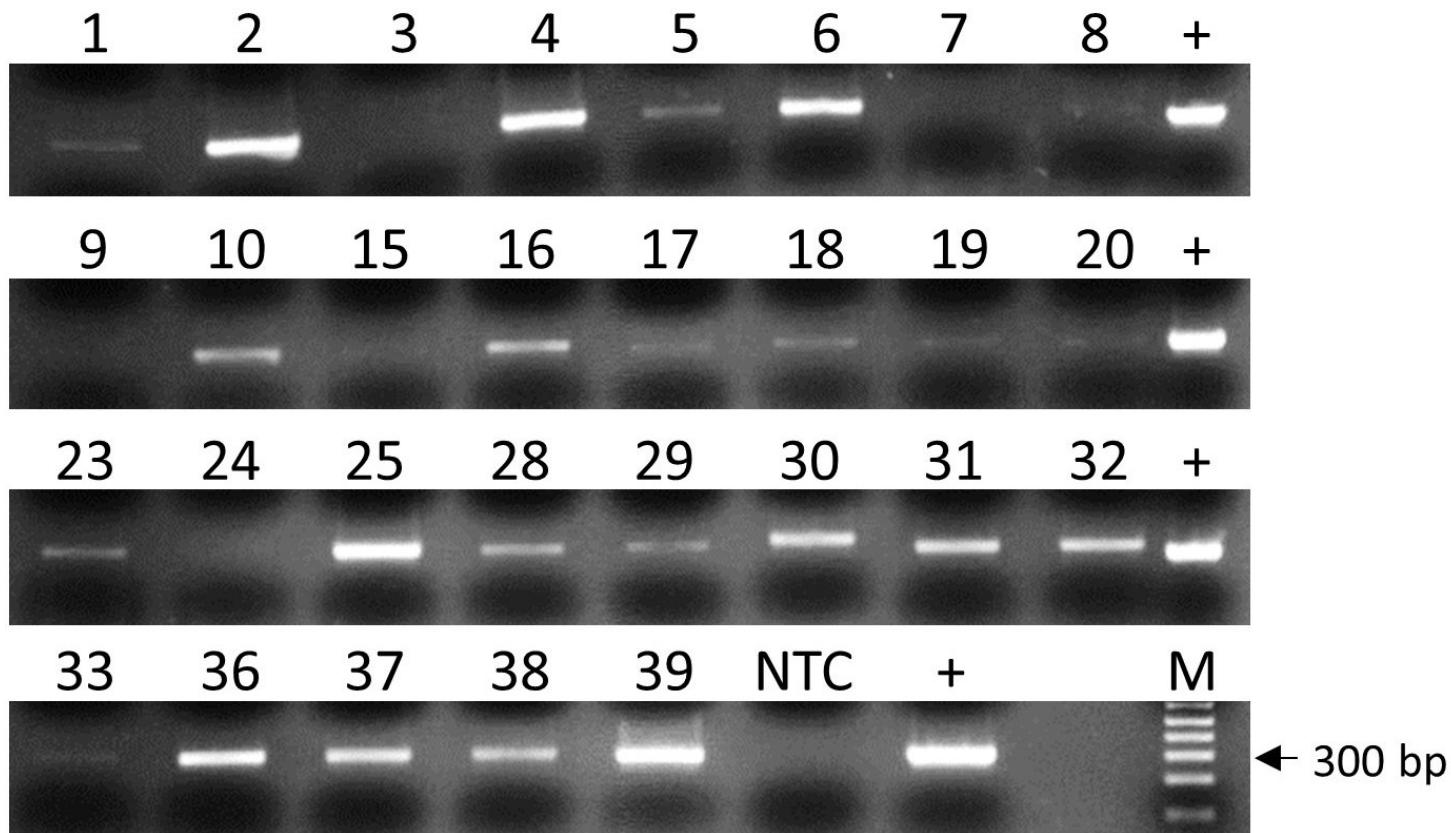
**Fig. S4.** Clustal X alignment of Region 1 IHHNV and IHHNV-EVE sequences amplified from *Penaeus monodon* from Malaysia (MALAY) using the PCR primer pair F1451:RP3b and cloned into pGEM-T vector as controls for the IHHNV-q309 real-time PCR test and IHHNV309F/R 1-step conventional PCR test. Included in the alignment is a F1451:RP3b PCR product sequence amplified from *P. monodon* for Australia (AUS) trimmed of illegible sequence. BLAST analyses of GenBank sequences showed the IHHNV sequences from Australia and Malaysia and to be most closely related to viruses classified in IHHNV lineages II and III, respectively, and the IHHNV-EVE sequence from Malaysia to cluster with IHHNV-EVE Type A sequences. PCR primer and probe sequences are shaded.



**Fig. S5.** Clustal X alignment of Region 2 IHHNV-qEVE and IHHNV sequences amplified from *Penaeus monodon* from Malaysia (MALAY) using the PCR primer pair F3031:R3782 and cloned into pGEM-T vector as controls for the IHHNV-qEVE real-time PCR test. BLAST analyses of GenBank sequences showed the IHHNV-EVE and IHHNV sequences from Malaysia to be most closely related to Type A EVEs and viruses classified in IHHNV Lineage III, respectively. PCR primer and probe sequences are shaded.



**Fig. S6.** Agarose gel electrophoresis of the ~0.3 kb DNA amplified using the IHHNV309F/R 1-step PCR and samples of *P. monodon* from Vietnam and Malaysia as described in Table 4. An 8 µL aliquot of each 25 µL reaction was analysed. NTC = no template control reaction, M = 1 kb PLUS DNA ladder (Life Technologies).



#### LITERATURE CITED

- ✉ Tang KFJ, Navarro SA, Lightner DV (2007) PCR assay for discriminating between infectious hypodermal and hematopoietic necrosis virus (IHHNV) and virus-related sequences in the genome of *Penaeus monodon*. Dis Aquat Org 74: 165–170