

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. S2. Clustal X multiple sequence alignment of complete or near-complete IHNV/EVE genome sequences deposited in GenBank showing the IHNV 3'UTR sequence downstream of the ORF3 (capsid protein) gene targeted by the IHNV-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 IHNV strain sequence are shown.

AY355307	TAAACC	TATATAATCTATTACTATCTATACCTAC	CCTCTACACAAACCAGCTACCCAGGCAAGGTGGGACTCCGGCTACCCAGGCATGGTGGGACACTTCTCTACTATTGACGACGT	Taiwan	2003
AY102034T.....	Thailand	2002
KP742841G.....	China	2014
KF031144	Vietnam	2007
KC513422A.....	II Vietnam	2011
JN616415	Vietnam	2009
AY362547T.....G.....	Thailand	2003
GQ411199AAA.....TAT.....CCGA.....GCTG.....T.....T.....	India	2007
KM593909T.....GCT.....C.....A.....T.....GT.....	Australia	1995
KM593910T.....GCT.....C.....A.....T.....GT.....	Australia	1996
AF218266A.....GCT.....TC.....A.....T.....T.....	?	<2009
KF214742A.....GCT.....TC.....A.....T.....T.....	China	2011
JN377975A.....G.....GCT.....TC.....A.....T.....T.....C.....	South Korea	2010
JX258653A.....GCT.....TC.....A.....T.....T.....	China	2009
KJ830753A.....GCT.....TC.....A.....T.....T.....	China	<2014
KM272862T.....GCT.....TC.....A.....T.....T.....	Australia	2008
KM272863T.....GCT.....TC.....A.....T.....T.....	III Australia	2008
AY362548A.....G.....GCT.....TC.....A.....T.....T.....	Ecuador	<2003
AY355308A.....GCT.....TC.....A.....T.....T.....C.....	Taiwan	2003
KJ862253A.....GCT.....TC.....A.....T.....T.....	Brazil	2013
EF633688A.....GCT.....TC.....A.....T.....T.....	China	2007
AF273215A.....GCT.....TC.....A.....T.....T.....	Australia	2008
JX840067A.....GCT.....TC.....A.....T.....T.....	Vietnam	2012
KM272861T.....GCT.....TC.....A.....T.....T.....	Australia	2008
KM593908T.....GCT.....TC.....A.....T.....T.....	Australia	1991
AY355306	Taiwan	2003
KM593911T.....GCT.....C.....A.....T.....GT.....	Australia	2004
KM593912T.....GCT.....C.....A.....T.....GT.....	Australia	2005
GQ475529C.....C.....C.....T.....T.....A.....	I Australia	2009
KM593913AACCC.....C.....AAAGCA.....A.....A.....C.....CA.....T.....T.....G.....T.....AA.....T.....	Australia	2012
EU675312AACCC.....C.....AAAGCA.....A.....A.....C.....CA.....T.....T.....G.....T.....AA.....T.....	A Australia	1993
DQ228358AACCC.....C.....AAAGCA.....A.....A.....C.....CA.....T.....T.....G.....T.....AA.....T.....	Madagascar	<2002
AY124937	B East Africa	<2002
	CCCACAAAAAGCAAATATATCTCACTAT		GGTCCGTACCACCCTTTGGACGAT		
	IHNV-qEVEF1 ->		<- IHNV-qEVEPr1		
			TTCCACCCTGTTATTAGAGTATTACTG		
			<- IHNV-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.

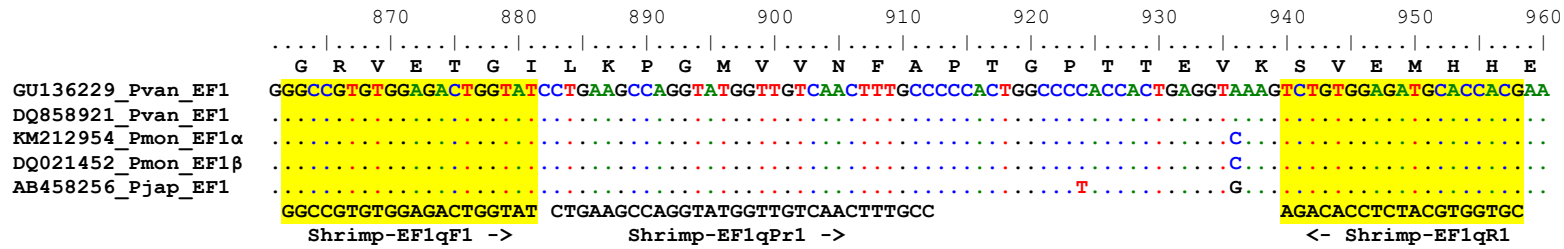


Fig. S4. Clustal X alignment of Region 1 IHHNV and IHHNV-EVE sequences amplified from *Penaeus monodon* from Malaysia (MALAY) using the PCR primer pair F1451:RP3b and cloned into pGEM-T vector as controls for the IHHNV-q309 real-time PCR test and IHHNV309F/R 1-step conventional PCR test. Included in 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.

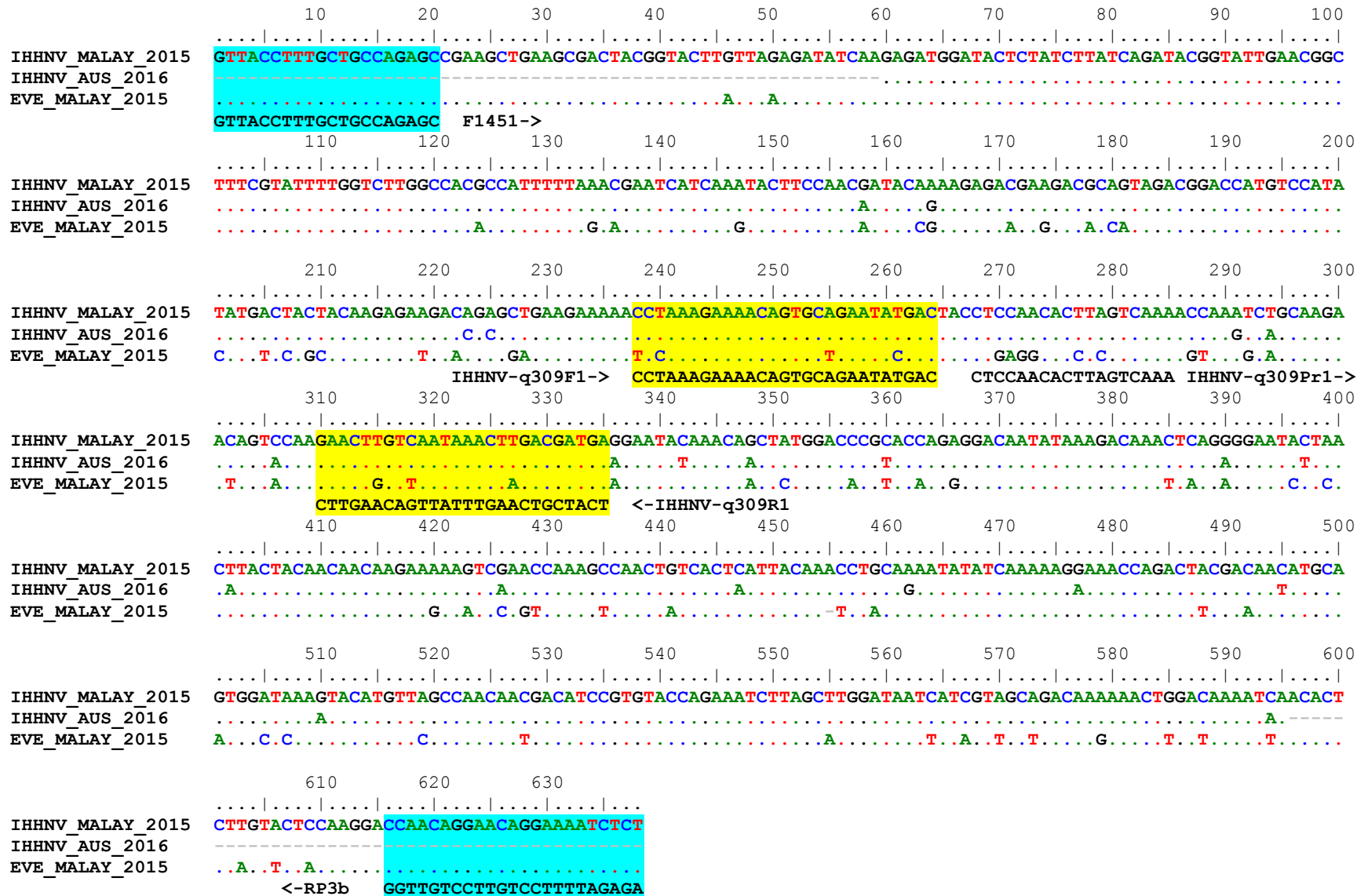


Fig. S5. Clustal X alignment of Region 2 IHNV-qEVE and IHNV sequences amplified from *Penaeus monodon* from Malaysia (MALAY) using the PCR primer pair F3031:R3782 and cloned into pGEM-T vector as controls for the IHNV-qEVE real-time PCR test. BLAST analyses of GenBank sequences showed the IHNV-EVE and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

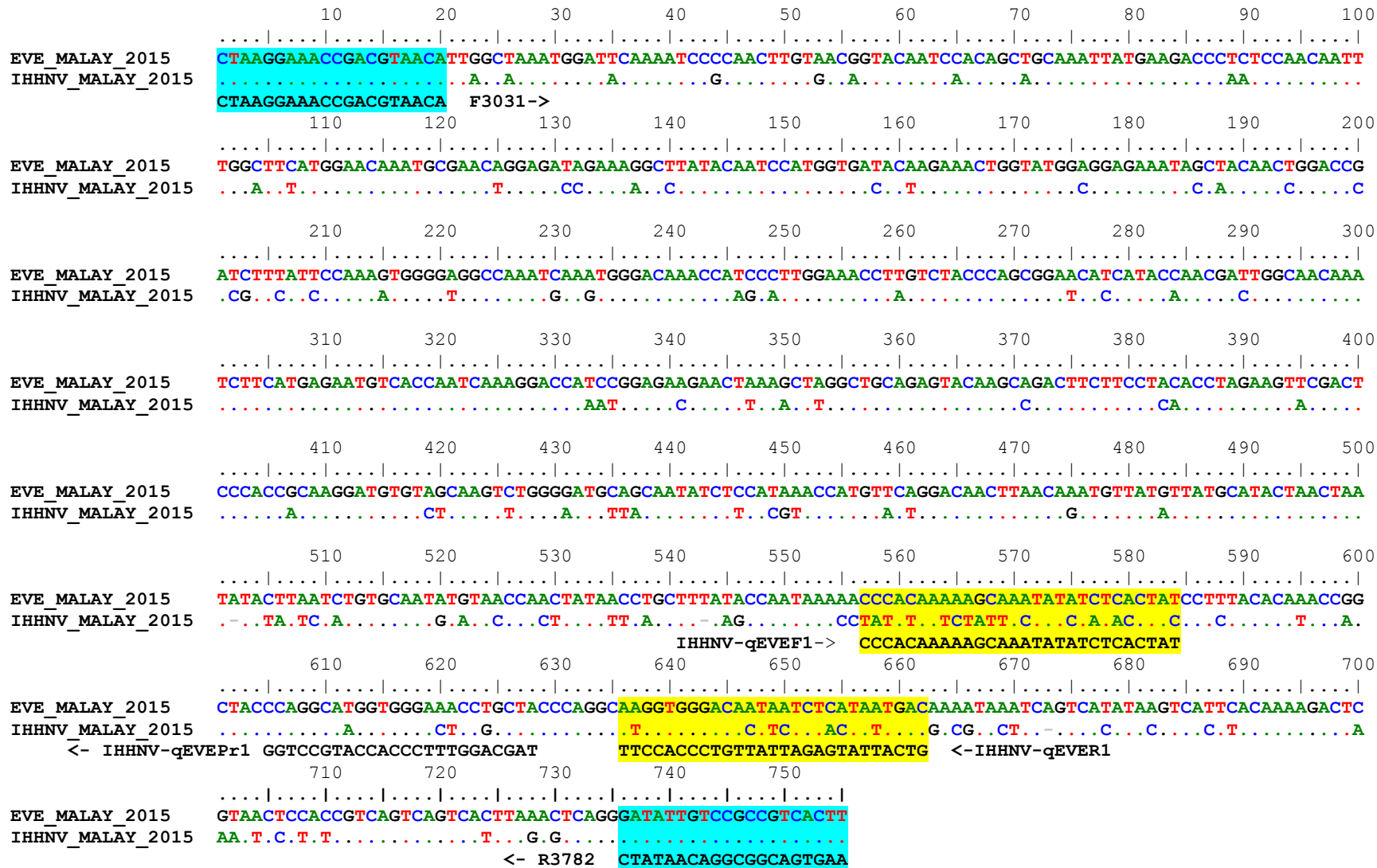
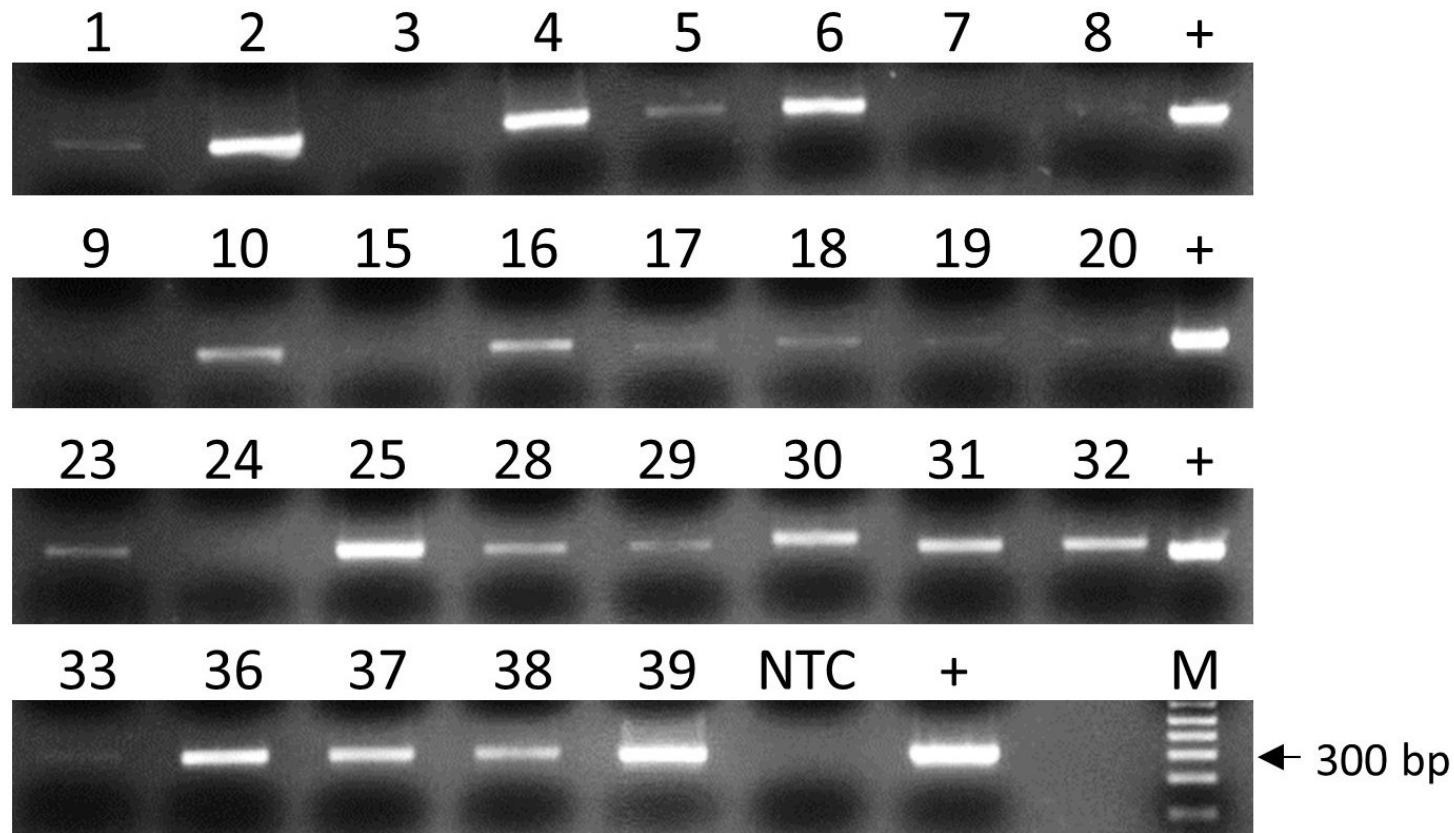


Fig. S6. Agarose gel electrophoresis of the ~0.3 kb DNA amplified using the IHHNV309F/R 1-step PCR and samples of *P. monodon* from Vietnam and Malaysia as described in Table 4. An 8 μ L aliquot of each 25 μ L reaction was analysed. NTC = no template control reaction, M = 1 kb PLUS DNA ladder (Life Technologies).



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