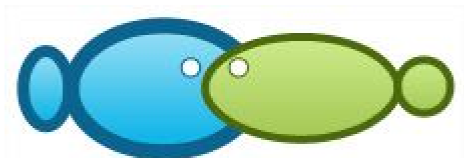

# Occurrence of infectious and non infectious Decapod Penstyldensovirus 1 (*Pst*DV-1) from tiger shrimp (*Penaeus monodon*) in South Sulawesi, Indonesia

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**Abstract**. The prevalence of Decapod Penstyldensovirus 1 (*Pst*DV-1), formerly known as infectious hypodermal and hematopoietic necrosis virus (IHHNV), was studied in post larvae and juvenile of tiger shrimp (*Penaeus monodon*) in South Sulawesi, and the occurrence of non-infectious and infectious types of *Pst*DV-1 was determined. Post larvae from shrimp hatcheries and 2 to 3 month old juvenile shrimps in grow-out ponds were examined. The DNA samples were extracted and amplified using the primers *IHHNV*-302F/R, *IHHNV*-309F/R and *IHHNV* 652-2077. Two samples (T1 and P2) which were amplified and sequenced with *IHHNV*-302F/R had 99% homology with the non infectious *Pst*DV-1 from Australia (EU675313, AY590120) and 96% homology with the non infectious *Pst*DV-1 type A from Madagascar (DQ228358); whereas the sample (P1), amplified with *IHHNV*-309F/R and (T2) with *IHHNV* 652-2270, had 98% homology with infectious types from Australia, India, Taiwan and Thailand. Phylogenetic analysis showed that the isolate P2 in the present study was in the same cluster as the non infectious type A from Australia and Madagascar, whereas the isolates P1 and T2 were in the same cluster as the infectious type from Australia. The prevalence of *Pst*DV-1 was high in all hatcheries and grow-out ponds surveyed, indicating that the virus was prevalent in aquaculture facilities at all locations with statistically significant samples. No difference in *Pst*DV-1 prevalence was found between normal and reduced growth shrimp; this could be due to a mixed infection of infectious and non infectious types of *IHHNV* in the shrimp population. This study demonstrated for the first time that both non-infectious and infectious types of *Pst*DV-1 are present together in aquaculture facilities in Indonesia.
**Key Words**: hatchery, IHHNV/*Pst*DV-1, post larvae, prevalence, tiger shrimp, virus.

**Introduction**. South Sulawesi as a centre of shrimp aquaculture development, with shrimp ponds covering 105,584 ha, contributes about 9% to the total tiger shrimp (*Penaeus monodon*) production of Indonesia (Directorate General of Aquaculture Development 2014). In Indonesia, *P. monodon* cultivated in earthen ponds are highly variable in size. Many small-sized shrimps are found at harvest, but the causes are still unknown. Possible causes of this undersize phenomenon in *P. monodon* could be related to viral infections or poor water quality. Viral diseases are a serious threat to the shrimp aquaculture industry worldwide. Viruses such as white spot syndrome virus (WSSV), monodon baculovirus (MBV), taura syndrome virus (TSV), hepatopancreatic parvovirus (HPV) and infectious hypodermal and hematopoietic necrosis virus (IHHNV) have been reported in Asian countries (Flegel et al 1999; Rai et al 2009). Decapod Penstyldensovirus-1 (*Pst*DV-1), previously known as IHHNV, is a highly pathogenic virus causing significant mortality in *Penaeus* (synonym *Litopenaeus*) *stylirostris*. This virus was first described as a causative agent of mass mortality in *L. stylirostris* in Hawaii in the early 1980s (Lightner et al 1983a) and has spread to cultured shrimps across many of the Pacific islands as well as the Americas, East and Southeast Asia and Australia (Vega-Heredia et al 2012). *Pst*DV-1 may infect other penaeid shrimps such as *Litopenaeus vannamei* and *P. monodon* with less pathogenic effect. The virus mainly causes stunted growth which results in small-sized shrimps at harvest (Kalagayan et al

1991; Rai et al 2009) however no mass mortality due to infection of this virus has been reported in *P. monodon*.

*Pst*DV-1 has a linear single stranded DNA, icosahedral, non-enveloped, comprising 4.1 kb nucleotides and three large open reading frames (ORFs). The capsid is composed of 4 proteins with molecular weights ranging from 37.5 kDa to 74 kDa (Shike et al 2000; Rai et al 2011). Four genotypes of *Pst*DV-1 have been reported, two are integrated into the host genome and non infectious, while the other two are infectious types. The origin of the first type is East Asia and Americas, the second is from South-East Asia, the third (type A) originated from Madagascar, Mauritius, India and Australia, and the fourth (type B) originated from East Africa, Mozambique and Tanzania (Walker & Winton 2010; Rai et al 2012). *Pst*DV-1 types A and B are recognized as non-infectious types, while the first and second types are infectious (Krabsetsve et al 2004; Tang & Lightner 2002; Tang et al 2003; Rai et al 2009). IHHNV infection from shrimp hatcheries was studied in this region and the prevalence of the virus infection ranged from 20% to 100% (Sriwulan & Anshary 2011). The high prevalence of infection in shrimp seed has implications for grow-out in ponds without proper seed screening protocols. Most hatcheries in Indonesia, particularly in South Sulawesi, do not routinely practice screening of seed for viral infection. This may have contributed to the stunted shrimp growth commonly observed in grow-out ponds in this region. The high prevalence of IHHNV-infected seed merits special attention in order to eliminate the viral infection through early detection of the shrimp virus in the shrimp larvae. Different primers have been used by researchers to amplify and characterize IHHNV. In this study, two previously published primers 302F/R (Khawsak et al 2008) and 309F/R (Tang et al 2007) were used. These primers and an additional primer were used to amplify and to obtain genotype sequences from the fragmented DNA of *PstDV*-1 in *P. monodon* larvae and juveniles.

Although Indonesia is ranked among the top 5 shrimp producers in the world, few studies have been reported on viral infection from shrimp in this region. In this paper, the occurrence of infectious and non infectious types of *Pst*DV-1 in shrimp hatcheries and grow-out ponds in South Sulawesi, Central Indonesia, is presented and the prevalence of the *Pst*DV-1 infection is discussed.

**Material and Method**. *P. monodon* post larvae (PL7-14) were obtained from hatcheries at Takalar, Barru and Pinrang from June to November 2013 (Figure 1, Table 1). Juvenile *P. monodon* which had been cultivated for around two to three months in brackish-water grow-out ponds were sampled from sites in three centres of shrimp seed production and farming in South Sulawesi: Takalar, Pangkep and Pinrang Districts. Samples were collected during June, September, November 2012 and February 2013 (Table 2). A total of 180 post larvae and 220 juvenile shrimps consisting of 115 with normal growth and 105 with reduced growth appearance were collected (Table 2). The specimens were transported live and individually examined to the laboratory for DNA extraction.

Table 1

Sampling location, sampling time and number of post larvae examined

| *Hatchery locations* | *Sampling period* | *Stage (PL)* | *Number examined* | *PstDV-1 infected* | *Prevalence (%)* |
|---|---|---|---|---|---|
| Takalar | June, August, November | 7-14 | 80 | 62 | 77.5 |
| Barru | June, August, September | 7-14 | 50 | 34 | 68.0 |
| Pinrang | June, August, September | 7-12 | 50 | 29 | 58.0 |

Table 2

Sampling location, sampling time and number of juvenile shrimp examined (N - normal; R - reduced)

| *Sampling locations* | *Sampling period* | *Number* | | *Size X±SD* | | *PstDV-1 infected* | | *Prevalence (%)* | |
|---|---|---|---|---|---|---|---|---|---|
| | | *N* | *R* | *N* | *R* | *N* | *R* | *N* | *R* |
| Takalar | June, August, November 2012 | 50 | 50 | 22.9±3.9 | 8.7±3.9 | 21 | 25 | 42.0 | 50.0 |
| Pangkep | February 2013 | 10 | 3 | 35.4±7.2 | 10.6±3.4 | 0 | 0 | 0 | 0 |
| Pinrang | June 2012, January, February 2013 | 55 | 52 | 32.2±9.6 | 15.6±3.0 | 26 | 29 | 47.3 | 55.8 |

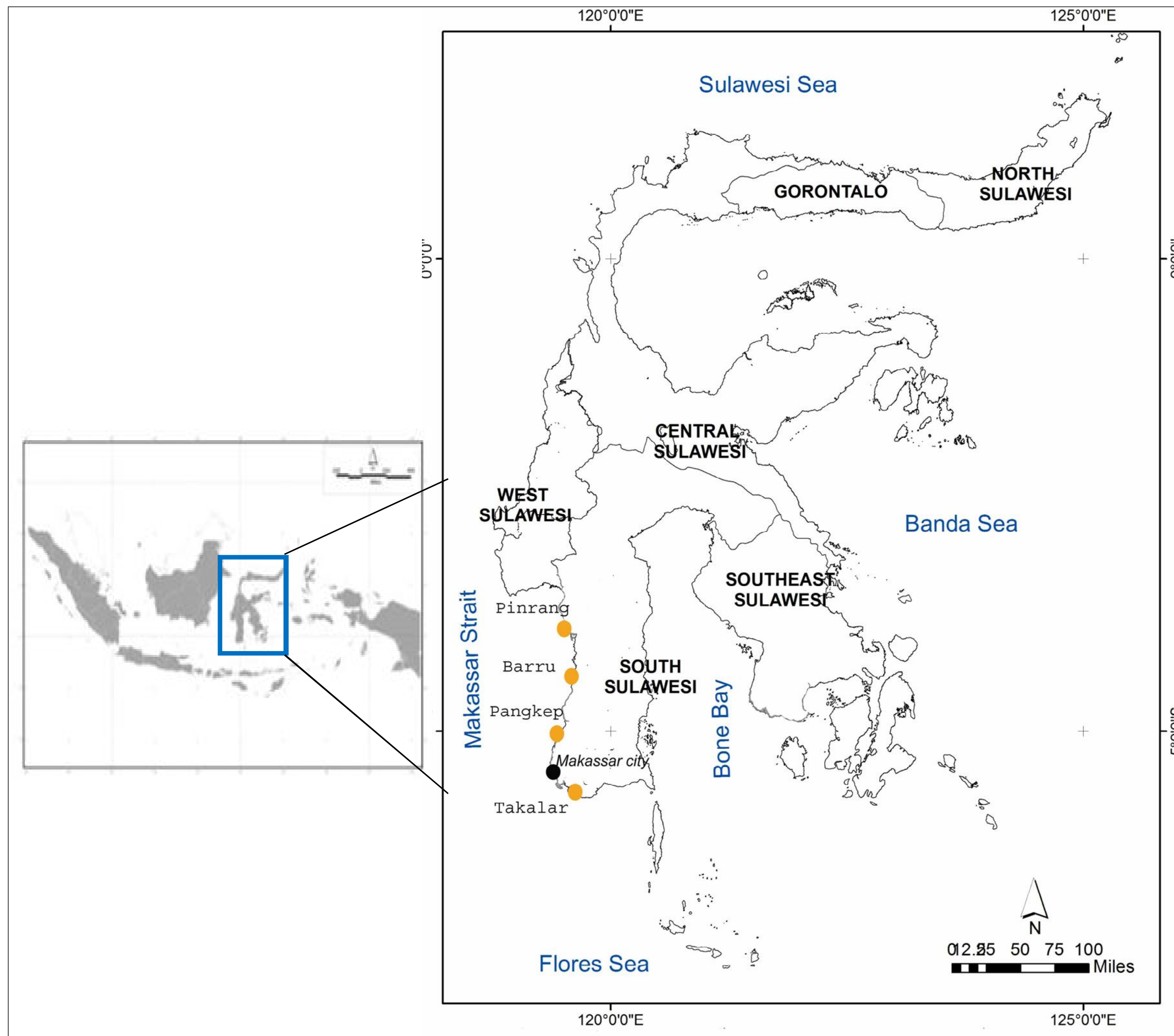


Figure 1. Map showing the sampling sites of *P. monodon* from hatcheries and grow-out ponds in South Sulawesi, Indonesia.

***DNA extraction and amplification***. DNA was collected from the gills, hepatopancreas, and pleopods of juvenile shrimp, whereas the entire body was used for post larvae. After removing the compound eyes from the eyestalks, the post larvae were individually homogenized in 1.5 mL microfuge tubes. The tissues from each juvenile shrimp were pooled and ground on ice using a mortar and pestle, then 20 mg of ground tissue was placed in a 1.5 mL microfuge tube. DNA was extracted using QiaAmp DNA mini kit (Qiagen) following manufacturer's protocols, eluted with nuclease free water and stored at –20$^{o}$C before use. DNA from each specimen was then amplified via PCR using the primer pair *IHHNV* 302F/R (F: ATTTCTCCAAGCCTTCTCACC, R: TGATGTAAGTAATTCCTCTCTGT) (Khawsak et al 2008). The PCR protocol followed was: pre-denaturation at 95$^{o}$C for 15 minutes, followed by 35 denaturation cycles at 94$^{o}$C for 30 seconds, annealing at 59$^{o}$C for 1 minute 30 seconds, extension at 72$^{o}$C for 1 minute 30 seconds, and final extension at 72$^{o}$C for 10 minutes. Total volume of each PCR mix was 25 μL, consisting of Master Mix 12.5 μL, Primer mix 2.5 μL, Q-solution 2.5 μL, and DNA Template 2.0 μL, with 5.5 μL of nuclease free water to make up the desired reaction volume. The results from electrophoresis of the PCR products, on 1.5% agarose gel with 1% TAE electrophoresis buffer stained with GelRed Nucleic Acid Gel Stain (Biotium), were examined and photographed.

***DNA sequencing and phylogenetic analysis***. Four DNA samples extracted from IHHNV-infected shrimp tissues were selected for sequencing. The samples (T1 and P2) were amplified and sequenced using the primers *IHHNV* 302F/R, the sample P1 with

*IHHNV* 309F/R (F: TCCAACACTTAGTCAAAACCAA, R: TGTCTGCTACGATGAT-TATCCA) (Tang et al 2007), and the sample T2 with a pair of primers (*IHHNV* 652F-GCCAAGGACATAC-TGCATACAC, *IHHNV* 2077R-TGTCTGCTACGATGAT-TATCCA) designed for this study from the nucleotide IHHNV-AF218266 using the PerlPrimer software program (Marshall 2004). For the primer pair *IHHNV* 302F/R, the PCR protocol outlined above was followed. For *IHHNV* 309F/R and *IHHNV* -652F/2077R the PCR protocol used was as follows: pre-denaturation at 95°C for 10 minutes, followed by 35 denaturation cycles at 94°C for 30 seconds, annealing at 50°C for 30 seconds, extension at 72°C 1 minute, and final extension at 72°C for 5 minutes. The total PCR mix volume of 50 μL comprised: 1X HotStarTaq *Plus* Master Mix, 0.5 μM of each primer and 5.0 μL DNA Template, with Nuclease free water to bring the volume up to 50 μL.

The PCR products were purified and used directly for the sequencing reaction. About 50 μL of each purified PCR sample and 25 μL of each primer were sent for sequencing to 1st BASE molecular company in Malaysia through PT Genetika Science Indonesia. The nucleotide sequence files received were edited using Bioedit Alignment Sequence Editor Ver. 7.0.5.3.software (Hall 1999) and compared with the original chromatogram when necessary. The edited/consensus nucleotides of the present samples were multiple aligned with the other IHHNV nucleotides available in GenBank and percent of similarity (homology) was calculated using DNAstar Lasergene Editseq and Megalign ver. 7.02. The aligned nucleotides were edited with Bioedit Alignment software. Phylogenetic analysis of the nucleotide sequences 702-3583 of the IHHNV-AF218266, consisting of non-structural and structural-protein, was performed using the software Molecular Evolutionary Genetics Analysis (MEGA) ver. 6.06 (Tamura et al 2013). A Maximum Likelihood Phylogenetic Tree was constructed using *Aedes aegypti* densovirus nucleotide as an out-group. DNA sequences obtained from the present study were deposited in GenBank under accession number KU215793, KU215794 and KU215795.

***Data analysis***. The prevalence of *Pst*DV-1 infection was compared between sampling locations as well as between normal and reduced growth shrimp. Non-parametric Chi-square statistical tests were applied, using the statistical software package SPSS ver. 16 for Windows.

## Results and Discussion

***PCR amplification and prevalence of PstDV-1 infection***. *Pst*DV-1 presence or absence was determined through PCR amplification using the primer pair IHHNV-302F/R on all samples from post larvae and juvenile shrimp, producing a 302 bp PCR product. The prevalence of *Pst*DV-1 infection in post larvae from three hatcheries, using the primers IHHNV-302F/R, is presented in Table 1. The prevalence of infection in post larvae ranged from 58% in Pinrang to 77.5% in Takalar. The difference in prevalence among the sampling locations was not significant ($p < 0.05$). The highest prevalence in juvenile shrimp was in Pinrang (47% in normal juvenile shrimp and 55.8% in reduced growth juveniles); however the difference in *Pst*DV-1 prevalence between juvenile shrimp with normal and reduced growth was not significant (Table 2).

***Sequencing***. The P2 and T1 samples sequenced with the primer IHHNV 302F/R showed 99% homology with *Pst*DV-1 type A from Australia (EU675313 and AY590120), 96% homology with DQ228358 from Madagascar and 91% homology with the type B *Pst*DV-1 AY124937 from Tanzania (Table 3). Nucleotide sequences of the P1 samples generated from the primer IHHNV 309F/R and T2 samples generated from the primer *Pst*DV-1 652-2077 had 98% homology with nucleotides KM593912 from wild captured *P. monodon* broodstock in Australia and 98% with AY362547 from *P. monodon* in Thailand. Both of these nucleotides are from infectious types of *Pst*DV-1. The infectious type in the present sample showed 95% homology with the type B *Pst*DV-1 AY124937 and 96% to 97% homology with *Pst*DV-1 strain from Hawaii/USA (AF218266) (Table 3).

Table 3

Pair distance of fragmented nucleotide sequences among *Pst*DV-1 including the present samples (***IHHNV*** 309, ***IHHNV*** 302, ***IHHNV*** 652). The pair distances were calculated using the sequence distance tool in MegAlign, Lasergene ver. 7.01 software

| *1* | *2* | *3* | *4* | *5* | *6* | *7* | *8* | *9* | *10* | *11* | *12* | *13* | *14* | *15* | *16* | *17* | *18* | *19* | *20* | *Nucleotides* | *No* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 96 | 96 | 96 | 96 | 96 | 96 | 92 | 86 | 98 | 88 | 86 | 91 | 45 | 94 | 95 | 92 | 99 | 98 | 97 | AY362547 | 1 |
| | | 100 | 100 | 100 | 100 | 100 | 92 | 86 | 96 | 87 | 86 | 90 | 44 | 94 | 95 | 90 | 96 | 97 | 97 | AY355308 | 2 |
| | | | 98 | 96 | 99 | 97 | 92 | 87 | 96 | 87 | 84 | 90 | 45 | 91 | 96 | 90 | 96 | 97 | 98 | AY355306 | 3 |
| | | | | 100 | 99 | 99 | 92 | 86 | 96 | 87 | 86 | 90 | 44 | 94 | 95 | 90 | 96 | 97 | 97 | EF633688 | 4 |
| | | | | | 99 | 99 | 92 | 86 | 96 | 87 | 85 | 90 | 44 | 94 | 95 | 90 | 96 | 97 | 97 | AF218266 | 5 |
| | | | | | | 99 | 92 | 86 | 96 | 87 | 85 | 90 | 44 | 93 | 95 | 90 | 96 | 97 | 97 | AY362548 | 6 |
| | | | | | | | 92 | 86 | 96 | 87 | 86 | 90 | 44 | 93 | 96 | 90 | 96 | 97 | 97 | AF273215 | 7 |
| | | | | | | | | 90 | 95 | 91 | 90 | 91 | 47 | 92 | 94 | 91 | 92 | 95 | 93 | AY124937 | 8 |
| | | | | | | | | | 85 | 97 | 99 | 96 | 45 | 86 | 87 | 96 | 86 | 88 | 87 | DQ228358 | 9 |
| | | | | | | | | | | 0 | 84 | 0 | 51 | 96 | 98 | 0 | 96 | 100 | 98 | ***IHHNV* 309** | 10 |
| | | | | | | | | | | | 100 | 100 | 41 | 92 | 86 | 99 | 88 | 90 | 88 | EU675313 | 11 |
| | | | | | | | | | | | | 99 | 44 | 86 | 87 | 99 | 86 | 88 | 87 | EU675312 | 12 |
| | | | | | | | | | | | | | 46 | 90 | 90 | 99 | 91 | 90 | 91 | AY590120 | 13 |
| | | | | | | | | | | | | | | 44 | 45 | 47 | 44 | 49 | 45 | NC012636 | 14 |
| | | | | | | | | | | | | | | | 93 | 91 | 95 | 97 | 95 | GQ411199 | 15 |
| | | | | | | | | | | | | | | | | 91 | 95 | 98 | 96 | GQ475529 | 16 |
| | | | | | | | | | | | | | | | | | 92 | 91 | 91 | ***IHHNV* 302** | 17 |
| | | | | | | | | | | | | | | | | | | 97 | 97 | AY355307 | 18 |
| | | | | | | | | | | | | | | | | | | | 98 | ***IHHNV* 652** | 19 |
| | | | | | | | | | | | | | | | | | | | | KM593912 | 20 |

***Phylogenetic tree analysis***. A total of 4 samples (P2, T1, P1, T2) were sequenced using the three primer sets used for DNA amplification: IHHNV-302F/R (302 bp), IHHNV-309F/R (308 bp) and IHHNV 652-2077 (1308 bp). The phylogenetic analysis constructed a maximum likelihood tree from the genome nucleotide fragments amplified, which showed a clear pattern between infectious and non infectious type A and type B *Pst*DV-1 (Figures 2 and 3). The P2/T1 sample using IHHNV -302F/R was in the same cluster as the non infectious *Pst*DV-1 type A from Madagascar (DQ228385) and from Australia (EU675312, EU675313, and AY590120) (Figure 2). The P1 sample (primer pair IHHNV -309F/R) and the T2 sample (primer pair IHHNV 652-2077) were in the same cluster as infectious *Pst*DV-1 from Australia (GQ475529) (Figure 3).

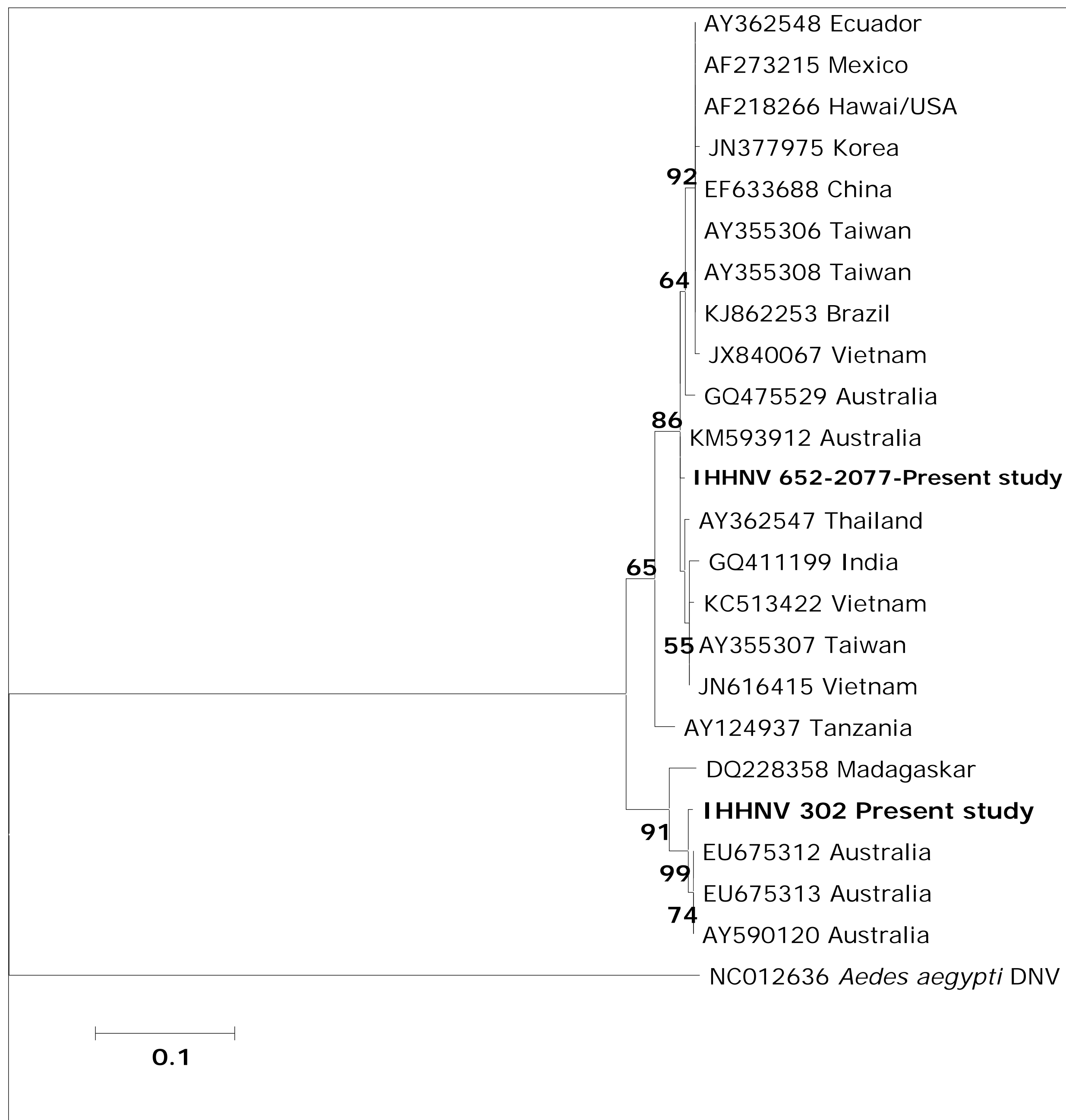


Figure 2. Phylogenetic tree of *Pst*DV-1 including the non infectious type isolate (IHHNV-302) from *P. monodon* based of fragments sequenced of genomes. Maximum Likelihood tree was constructed using MEGA version 6.06 (Tamura et al 2013), using Kimura-2 parameter model and Nearest-Neighbor-Interchange (NNI) with 1,000 bootstrap number with complete deletion. Percentages ≥ 50% are shown at the internal nodes.

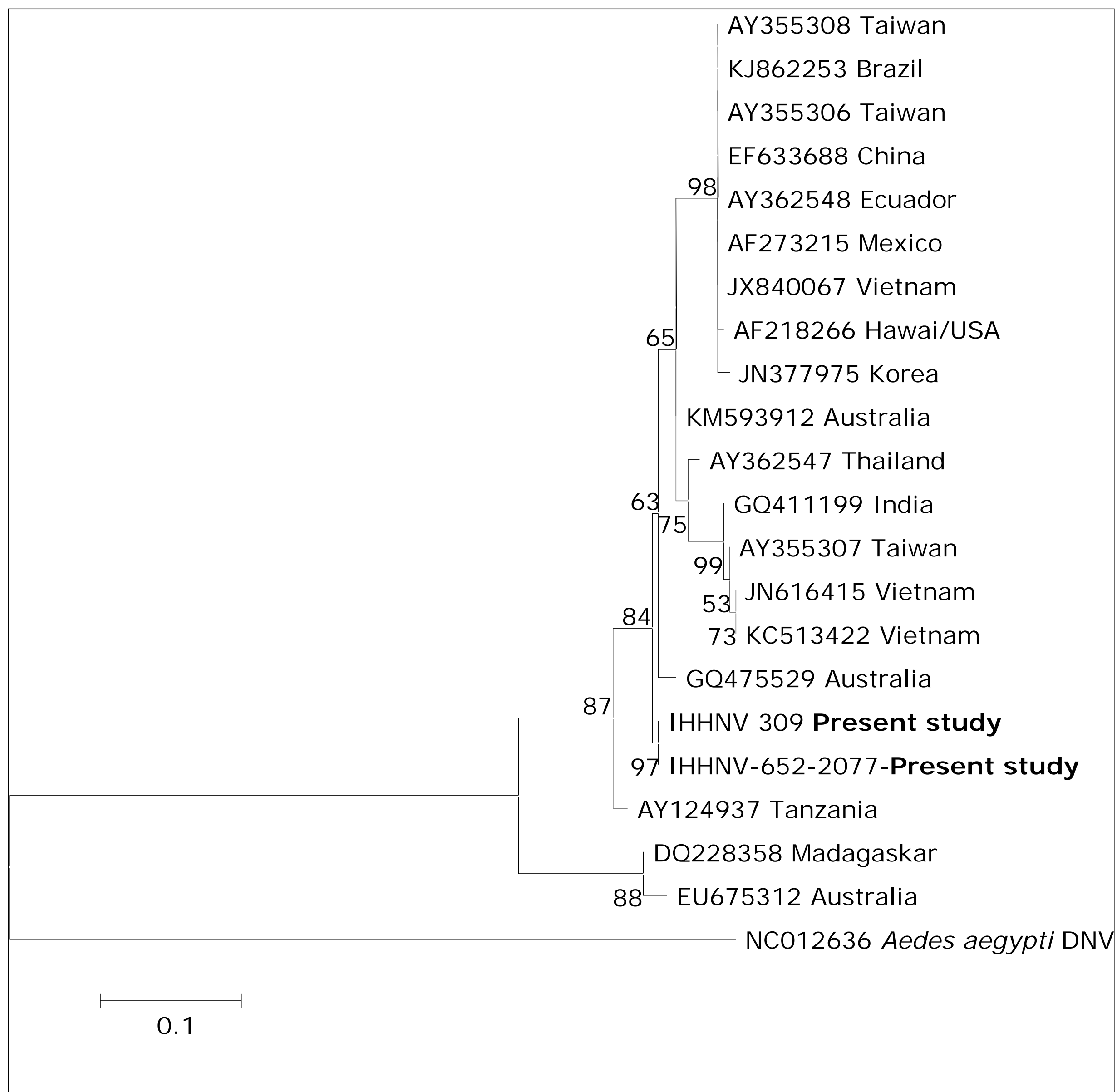


Figure 3. Phylogenetic tree of *Pst*DV-1 including the present infectious type isolate (IHHNV-309 and IHHNV 652-2077) from *P. monodon* based on sequenced genome fragments. The Maximum Likelihood tree was constructed using MEGA version 6.06 (Tamura et al 2013), using the Kimura-2 parameter model and Nearest-Neighbor-Interchange (NNI) with 1,000 bootstrap replications and complete deletion. Percentages ≥ 50% are shown at the internal nodes.

**Discussion**. Prevalence of IHHNV infection was high in all hatcheries surveyed, suggesting a widespread distribution of the virus in hatchery facilities in South Sulawesi region. The virus can be transmitted horizontally through cannibalism, contaminated water, carriers, or vertically from broodstock that pass the virus to their progeny (Lightner et al 1983b; Withyachumnarnkul et al 2006; Lightner et al 2012). In Indonesia, wild-caught *P. monodon* broodstock are the only source of spawners in hatcheries. Screening of broodstock for viral infections is not a routine practice in many Indonesian hatcheries, potentially allowing viral transmission and the contamination of aquaculture facilities. Thus, the high prevalence of IHHNV/*Pst*DV-1 infection in all three hatcheries and grow-out ponds could be due to using wild *P. monodon* broodstock naturally infected with the virus or to other wild crustaceans entering the shrimp ponds during culture. Therefore, the high prevalence of *Pst*DV-1 in juvenile shrimp in grow-out ponds could be a consequence of low awareness regarding viral screening in hatcheries, meaning that shrimp seed released for stocking would carry a high potential risk of being infected by multiple viral infections contracted in the hatchery. This study also confirmed a previous report that IHHNV/*Pst*DV-1 is prevalent in Indonesia (Lightner et al 1990). High prevalence of this viral infection was also reported in Indian hatcheries, with around 76% prevalence of *P. monodon* post larvae infection by IHHNV (Joseph et al 2015). In addition, as in Indonesia, shrimp larvae in Indian hatcheries were also found to be infected by different kinds of viruses concurrently (Joseph et al 2015; Sriwulan & Anshary 2011).

Comparison of prevalence of the virus infection between the normal and undersized shrimp showed no statistically significant difference. *Pst*DV-1 is known to be highly pathogenic causing mass mortality in *L. stylirostris*; it has been implicated in stunted growth and runt deformity syndrome in *L. vannamei* (Bell & Lightner 1984; Gunalan et al 2014) as well as slow growth syndrome in *P. monodon* (Rai et al 2009). In the present study, although the prevalence of *Pst*DV-1 in reduced growth shrimp was more than 50% while the prevalence in the normal growth shrimp was about 40%, this difference was not statistically significant ($p > 0.05$). Based on this finding, six samples were sequenced using the published primer pairs IHHNV 302F/R and IHHNV 309F/R as well as the specially designed primer IHHNV 652-2077F/R to identify the *Pst*DV-1 genotype present in isolates from the fragmented DNA. Phylogenetic analysis showed that the P2 (IHHNV -302) samples were in the same cluster as the non-infectious *Pst*DV-1 type A from Madagascar and Australia, and had a high similarity with *Pst*DV-1 type A. Using the primers 309F/R and IHHNV 652-2270, the other juvenile shrimp samples showed a 98% similarity with the infectious type of *Pst*DV-1 reported from Australia (Saksmerprome et al 2010; Jaroenram et al 2015) and Thailand (Hsia et al 2003). Phylogenetic analysis placed the infectious type of *Pst*DV-1 from South Sulawesi in the same cluster as *Pst*DV-1 from Australia (GQ475529). Our results show that both infectious and non-infectious types of *Pst*DV-1 co-exist in aquaculture facilities in South Sulawesi. To date, the known geographic ranges of non infectious *Pst*DV-1 are Africa, Australia and western Indo-Pacific (Tang & Lightner 2006; Tang et al 2007), whereas infectious *Pst*DV-1 has been reported from the Americas, Asia and Southeast Asia, Australia, Middle East and many Pacific islands (OIE 2009; Teixeira-Lopes et al 2011; Martorelli et al 2010). A report showed that the epizootic spread of *Pst*DV-1 in America originated from *Pst*DV-1- infected *P. monodon* imported from the Philippines (Tang et al 2003).

As the phylogenetic analysis indicates that both infectious and non-infectious types of *Pst*DV-1 of Indonesian isolates share a common ancestor with Australian isolates, it is likely that the Indonesian strain could have originated from Australia or *vice versa*. According to Krabsetsve et al (2004), *Pst*DV-1 is an endemic virus and has been present in the Australian aquaculture environment for long period of time. In addition, Australia has implemented a strict quarantine system which does not allow the importation of live animals. It is thus more likely that the Indonesian isolate might have evolved from the Australian *Pst*DV-1 strain. This study showed that the infectious *Pst*DV-1 strain found had a high divergence from the infectious type from Hawaii, and closer similarity with the Australian strain. The consequences of infection by a mixture of

infectious and non infectious *Pst*DV-1 types are unknown. Tang & Lightner (2006) stated that the non infectious type of *Pst*DV-1 integrates into the host genome and becomes part of the *P. monodon* genome; furthermore, shrimps exposed to non-infectious *Pst*DV-1 types did not show Cowdry A body inclusion in their tissues, a typical sign of pathogenic and infectious *Pst*DV-1. However, recent studies show that the *P. monodon* and the *L. vannamei* infected with infectious *Pst*DV-1 survive longer when exposed to WSSV infection than uninfected shrimp or shrimp infected with non infectious *Pst*DV-1 (Bonnichon et al 2006; Molthathong et al 2013; Melena et al 2015). The mechanisms of such interference phenomena are not always clear. One possible explanation is that the initial infection by *Pst*DV-1 may block the entry of WSSV by down regulating production of cellular receptors or through competition for a common receptor (Molthathong et al 2013; Melena et al 2015). In grow-out ponds, multiple viral infections of IHHNV and WSSV are commonly found, and may have a similar effect to those observed under laboratory conditions. Withyachumnarnkul et al (2006) reported that low-level infections by IHHNV have low impact on *P. monodon* growth and reproductive performance, while Jaroenram et al (2015) recently reported a mutated *Pst*DV-1 strain in Australia in which a 1 bp deletion occurred in ORF1/NS1, a mutation which is believed to reduce the virulence of this virus.

The primer 302F/R used to determine the prevalence of *Pst*DV-1 could theoretically amplify both infectious and non infectious *Pst*DV-1. BLAST analyses of the primer pair (using NCBI BLAST tool and alignment with infectious and non infectious types of *Pst*DV-1 using Multalin Multialignment online software) indicated that the 302F/R primer could attach to the nucleotides of both infectious and non infectious *Pst*DV-1 and hence could possibly amplify DNA nucleotides for either of the virus types. Therefore, as both infectious and non infectious types of *Pst*DV-1 were found in aquaculture facilities in this region, the primer intended to amplify the infectious types of *Pst*DV-1 might have amplified the non-infectious type as well, making it difficult to directly relate the effect of *Pst*DV-1 infection between normal and reduced growth of juvenile shrimp in the present study. The existence of both infectious and non-infectious types of *Pst*DV-1 in Indonesian aquaculture facilities has implications for the selection of suitable primers for detection of infectious *Pst*DV-1. The 302F/R primer pair, proven capable of amplifying the non-infectious type, is not appropriate for amplifying the infectious type of *Pst*DV-1 in Indonesia. On the other hand, the 309F/R primer pair has proven successful in amplifying the infectious type, and therefore it is suitable to use for routine *Pst*DV-1 diagnosis in Indonesia, in particular for screening shrimp seed as well as in selective breeding and broodstock domestication programs. Although Saksmerprome et al (2011) considered that the 309F/R primer pair might cause false positives, OIE (2016) recommends the 309F/R primer pair for screening of *Pst*DV-1 in shrimp as only the infectious type of IHHNV is amplified.

In some regions, especially in Java (the main centre of *P. monodon* production), many Indonesian farmers have switched from *P. monodon* to *L. vannamei* due to shortage of high quality *P. monodon* broodstock and seed. In South Sulawesi, on the other hand, shrimp farmers have remained faithful to black *P. monodon* although in some districts cultivation of *L. vannamei* has begun. Since the ban on the use of trawl nets in Indonesia, it has been difficult to obtain high quality shrimp broodstock from deep waters; many fishermen can only catch broodstock from shallow waters where they are more vulnerable to various virus infections. As shrimp hatcheries have experienced difficulties in obtaining high quality broodstock, the quality of seed produced has tended to decline; efforts to raise quality broodstock from hatchery-produced seed have not been successful to date. One possible solution to overcome this problem might be to catch broodstock from areas far from shrimp aquaculture activities, then properly screen them for viruses before use in breeding programs.

**Conclusions**. *Pst*DV-1 commonly occurs in cultured *P. monodon* in South Sulawesi, Indonesia. *P. monodon* sampled were infected by non-infectious type A *Pst*DV-1, similar to type A *Pst*DV-1 reported from Australia and Madagascar, as well as by infectious *Pst*DV-1 similar to IHHNV from Australia. These findings indicated that infectious and non

infectious *Pst*DV-1 are prevalent in cultured shrimp in Indonesian aquaculture. The implementation of biosecurity in hatcheries and grow-out ponds, including the routine testing of broodstock and seed for presence of viral agents, will help reduce the spread of viral infections within and between Indonesian aquaculture facilities and regions.

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