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Original Article

First detection of white spot syndrome virus (WSSV) in wild mud crab *Scylla* spp. (de Haan, 1883) from Setiu Wetlands, Malaysia

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Abstract

In this study, tissue samples from 90 wild mud crabs (*Scylla* spp. including *S. olivacea*, *S. Tranquebarica*, and *S. paramamosain*) were collected during pre-monsoon, monsoon, and post-monsoon in the Setiu Wetlands, Terengganu, Malaysia. The tissue samples were screened for the presence of white spot syndrome virus (WSSV) by PCR. This study was conducted to detect the presence or absence of WSSV in wild *Scylla* spp. from the Setiu Wetlands at different times of sampling. WSSV DNA was detected in 36% of the mud crabs. The DNA sequence of a 941 bp genome region amplified from a crab by PCR was identified to be most similar (99% nucleotide sequence, 98% amino acid sequence) to a WSSV strain detected in Mexico (KU216744.1) and Taiwan WSSV 419 strain (AY850066.1). The data indicated that mud crabs in the Setiu Wetlands might act as a WSSV reservoir of risk to shrimp aquaculture. Our findings are the first detection of WSSV from wild mud crabs, *Scylla* spp. in the Setiu Wetlands, Terengganu, Malaysia.

Keywords: mud crab, *Scylla* spp., white spot syndrome virus, PCR, Setiu Wetlands

1. Introduction

The 23,000 ha Setiu Wetland lies between the Setiu River Basin and the larger Setiu-Chalok-Bari-Merang Basin Wetland in Terengganu on the east coast of the Malaysian Peninsular. The wetland possess high biodiversity and unique ecosystems including mangrove swamps and supports small-scale but economically important oyster farming and brackish water cage, ponds, and pen culture of mud crabs (*Scylla* spp.) (Azwad, 2013). *Scylla* spp. present in intertidal zones and estuaries in the Setiu Wetland are dominated by *Scylla olivacea* (Herbst, 1796), followed by *S. tranquebarica* (Fabricius, 1798), and *S. paramamosain* (Estampador, 1949; Keenan, Davie, & Mann, 1998; Zaidi, Hilmi, Ikhwanuddin, & Bachok, 2011).

Mud crab culture is quite extensive with extremely low input but it has been accompanied with the development of many infectious diseases, especially due to viruses, which cause mass mortalities during a disease outbreak that results in vast economic loss. Many new diseases have been reported with significant pathogenicity and a high impact on the production of mudcrabs across the world including white spot baculovirus, white spot syndrome virus (WSSV), mud crab

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reovirus, and muscle necrosis virus (Anderson & Prior, 1992; Bonami, & Zhang, 2011; Chen, Lo, Chiu, Chang, & Kou, 2000; Deng *et al.,* 2012; Jithendran, Poornima, Balasubra manian, & Kulasekarapandian, 2010; Liu, Qian, & Yan, 2011; Owen, Liessmann, La Fauce, Nyugen, & Zeng, 2010; Somboonna *et al.,* 2010; Song *et al.,* 2007; Weng, Guo, Sun, Chan, & He, 2007).

One of the most severe viral infections in *Scylla* spp. is the WSSV infection. WSSV was discovered in 1992 in Southeast Asia and has become one of the most serious viral pathogens (Flegel, 2006). To date, no decapod crustacean from marine and brackish or freshwater sources has been reported to be resistant (Flegel, 1997; Lightner, 1996; Lo & Kou, 1998; Maeda *et al.,* 2000; Stentiford, Bonami, & Alday-Sanz, 2009). WSSV has a wide host range and it has been observed not only in shrimp but also in crabs and other arthropods (Lo *et al.,* 1996a, 1996b). Among these carriers, mud crabs are considered to be a threat to shrimp farms (Forskål, 1775) because they are generally believed to be highly tolerant to WSSV and remain infected for a long period of time without signs of disease (Flegel, 2006; Somboonna *et al.,* 2010). WSSV can be reisolated from previously infected mud crabs (Flegel, 2006; Somboonna *et al.,* 2010; Villareal, 2005). *S. olivacea* and *S. tranquebarica* have been reported to be susceptible to WSSV infection (Somboonna *et al.,* 2010). *S. olivacea* and *S. paramamosain* showed wide variation in response to a challenge by WSSV. *S. olivacea* was shown to be more susceptible than *S. paramamosain* (Somboonna *et al.,* 2010).

The east coast fisherman in Setiu Wetlands depend on wild species such as *Portunus pelagicus*, *Scylla* spp., *Squilla mantis*, *Macrobrachium lancesteri*, and cultured species, *Litopenaeus vannamei* for their livelihoods. An outbreak of WSSV infection affected one of the shrimp farms in Setiu Wetlands in March 2015 (Unpublished). This incident raised questions whether the WSSV infections were transmitted horizontally via water to the surrounding rivers and wetlands area or the WSSV infected wild crustaceans transmitted the disease to the cultured farm.

Wild organisms in natural reservoirs might represent a potential source of viral disease. Therefore, it is necessary to understand the dynamics of viruses in the environment in terms of the mode of infection, transmission, and natural reservoir which are dependent on its prevalence (Macias-Rodriguez *et al.,* 2014). Thus, this paper describes the first detection of WSSV in wild *Scylla* spp. from Setiu Wetlands, Terengganu, Malaysia.

2. Materials and Methods

2.1 Sample collection

A total of 90 samples of wild *Scylla* spp., that included *S. olivacea* (n=69), *S. tranquebarica* (n=8), and *S. paramamosain* (n=13), were collected from Setiu Wetlands, Terengganu (N 05° 40.709, E 102° 42.774). The sampling trips were conducted during the post-monsoon (March 2015), pre-monsoon (August 2015), and monsoon seasons (December 2015). The water quality parameters (pH, temperature, salinity, and dissolved oxygen [DO]) were measured using a YSI Multiparameter Water Quality Meter and API® Freshwater Aquarium Master Test Kit (Mars Fishcare North America, Inc). The samples were kept in a polystyrene box and brought back alive to the laboratory of Aquatic Organisms Health in Universiti Malaysia Terengganu. The lengths and weights of the samples were measured and any external and internal clinical signs were observed. The gills and internal organs (hepatopancreas, stomach, and heart) were collected for sample processing. Further analysis by PCR was conducted based on Lo *et al.* (1996a, 1996b) and Nunan and Lightner (2011).

2.2 DNA extraction

The DNA extraction of the samples was conducted using NucleoSpin Tissue Extraction Kit (Macherey-Nagel) according to the protocol provided by the manufacturers. About 25 mg of the pooled organs (hepatopancreas, heart, and stomach) and gills were cut up into small pieces and placed in a 1.5 mL microcentrifuge tube. The extracted DNA was stored at –20 °C prior to PCR analysis.

2.3 PCR amplification

The conventional nested PCR was carried out according to the method by Lo *et al.* (1996a, 1996b) as described in Office International des Epizooties (2015). The primers were based on the sequence published by Lo *et al.* (1996a, 1996b): Forward primer 146F1 (5'-ACT-ACT-AAC-TTC-AGC-CTA-TCTAG-3') and reverse primer 146R1 (5'-TAA-TGC-GGG-TGT-AAT-GTT-CTT-ACG-A-3') for primary reaction with the expected size of 1447 bp, followed by the second primer set 146F2 (5'-GTA-ACT-GCC-CCT-TCC-ATC-TCC-A-3') and 146R2 (5'-TAC-GGC-AGC-TGC-TGC-ACC-TTG-T-3') in the nested reaction which were expected to yield an amplicon of 941 bp. A total of 24 µL PCR mixture containing 12.5 µL 2X MyTaqMix (Bioline), 10.5 µL Rnase-free water, 0.5 µL 146F1 (10 µM), and 0.5 µL 146R1 (10 µM) were added to 1 µL of extracted DNA. For the primary reaction, the PCR thermal profile was programmed as follows: a cycle of 94 °C for 4 min, followed by 39 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 2 min and a final 5-min extension at 72 °C.

The nested reaction was carried out with a total of 24 µL of PCR mixture containing 12.5 µL 2X MyTaqMix (Bioline), 10.5 µL Rnase-free water, 0.5 µL 146F2 (100 µM), and 0.5 µL 146R2 (100 µM) were added to 1 µL PCR product. The amplification was conducted with the following programme: one cycle at 94 °C for 4 min, followed by 39 cycles at 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 2 min, and a final 5-min extension at 72 °C. The amplified PCR products from both reactions were visualized by gel electrophoresis through 2% (w/v) agarose gel in TAE buffer and stained with SYBR® Safe DNA gel stain (Invitrogen) for 45 min at 70 volts.

2.4 Gel extraction and DNA sequencing analysis

Each sample representative from PCR positive samples from each host species was chosen for sequencing analysis to examine the sequence difference of WSSV between the different host species. The expected bands from sample 5 from site 5 (S5-5, host species: *S. paramamosain*), sample 6 from site 5 (S5-6, host species: *S. olivacea*), and

sample 1 from site 3 (S3-1, host species: *S. tranquebarica*) were excised and purified using NucleoSpin® Gel and PCR Clean-up (Macherey-Nagel) based on the standard protocols. The purified DNA was diluted in 10 ng μL^{-1}, as required by the protocol, before being sent for sequencing to the First Base Laboratory (Selangor, Malaysia).

2.5 Phylogenetic analysis

The DNA sequencing results were used for the phylogenetic analysis. The sequences were used to interrogate the National Center for Biotechnology Information BLAST database to confirm its likely identity. Then the multiple alignment were aligned using Clustal X2.0.12 with other WSSV-related sequences. Finally, the phylogenetic tree was inferred among the sequences of WSSV obtained in this study with known sequences contained in the GenBank using Molecular Evolutionary Genetics Analysis.

2.6 Statistical analysis

The effects of time of the sampling on the prevalence of virus was determined using one-way ANOVA analysis. ANOVA analyses were performed using SPSS® statistic software version 19.0. Values were identified as significantly different if the P value was less than 0.05. The Tukey HSD *post hoc* test was applied at a significance level of $\alpha \leq 0.05$, to determine differences between the explanatory variable.

3. Results

No external or internal clinical signs were observed on any of the samples. The presence of *Octolasmis* spp. was detected on the gills of 11 samples. The average weight and carapace width of the samples were tabulated according to the different seasons (Table 1).

3.1 Water parameter at sampling location

A significant difference was observed between the water parameter and the three seasons (Table 2). These results indicated the occurrence of fluctuations in water parameters during the three seasons.

3.2 Conventional nested PCR for the detection of WSSV in *Scylla* spp.

A total of 33 samples (36%) of *S. olivacea*, *S. tranquebarica*, and *S. paramamosain* sampled during post-monsoon, pre-monsoon, and monsoon season were found to be positive for the presence of WSSV. A higher number of positives were detected during the pre-monsoon and monsoon season in the Setiu Wetlands than during the post-monsoon season (26 vs. 7). Based on the results, samples of *S. olivacea* showed the highest presence (75.7%) of WSSV compared to *S. paramamosain* (12.1%) and *S. tranquebarica* (12.1%) (Table 3). The primary reaction of conventional nested PCR analysis of pooled gills and tissues (hepatopancreas, heart, and stomach) demonstrated negative results for the presence of WSSV (Figure 1). The secondary reaction of conventional nested PCR analysis of pooled samples of gills and tissues (hepatopancreas, heart, and stomach) demonstrated a positive band at the expected size of 941 bp for the presence of WSSV (Figure 1).

A significant difference was observed between the season and the prevalence of WSSV determined by one-way ANOVA (P=0.01). Tukey *post hoc* test revealed that the prevalence of WSSV infection demonstrated a significant

Table 1. Average weight and carapace length of mud crab samples during post-monsoon, pre-monsoon, and monsoon season.

	Season		
	Post-monsoon	Pre-monsoon	Monsoon
Average weight (g)	63.35	101.16	136.75
Average carapace length (cm)	7.22	7.44	8.59

Table 2. Average water parameters for each season: post-monsoon, pre-monsoon, and monsoon seasons at the sampling locations. P values were calculated using one-way ANOVA to determine the differences for the water parameter according to season. A P value was significant at less than 0.05.

Water parameter	Season			
	Post-monsoon	Pre-monsoon	Monsoon	P value
Temperature (˚C)	30.02	34.08	29.66	0.000
Salinity (ppt)	26.74	29.11	12.66	0.000
Dissolve oxygen (mg/L)	4.50	5.73	3.73	0.018
pH	7.50	8.02	7.36	0.000

Table 3. Summary of PCR analyses for the detection of WSSV in *Scylla* spp. from Setiu Wetlands during the post-monsoon, pre-monsoon, and monsoon seasons.

Time/ Season	Species	Number tested	Positive for WSSV infection	Total positives
March 2015 (Post-monsoon)	*Scylla olivacea*	12	2	7
	Scylla tranquebarica	4	3	
	Scylla paramamosain	9	2	
August 2015 (Pre-monsoon)	*Scylla olivacea*	40	11	13
	Scylla tranquebarica	2	0	
	Scylla paramamosain	3	2	
December 2015 (Monsoon)	*Scylla olivacea*	17	12	13
	Scylla tranquebarica	2	1	
	Scylla paramamosain	1	0	
Total		90		33

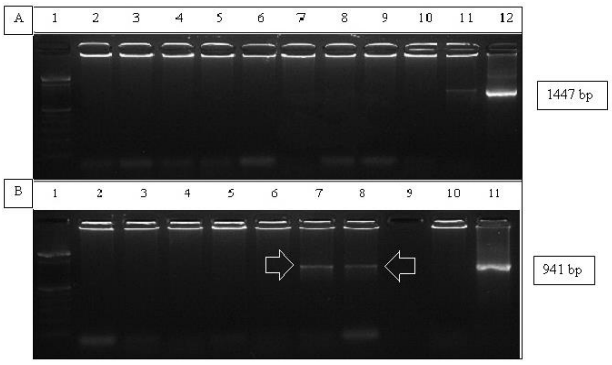

Figure 1. A) Primary PCR amplification of tissues from *Scylla* spp. from Setiu Wetlands. 1) Lane 1 (from left) 100 bp DNA ladder, 2) Lanes 2-9: samples, 3) Lane 10: Negative control, 4) Lanes 11 and 12: Synthetic positive control of WSSV, 1447 bp. B) Nested PCR amplification of tissues from *Scylla* spp. from Setiu Wetland 1) Lane 1 (from left): 100 bp DNA ladder, 2) Lanes 2-8: Tissue samples, 3) Lane 10: Negative control, 4) Lane 11: Synthetic positive control of WSSV, 941 bp. 5) Lanes 7 and 8: Positive results for the presence of WSSV 941bp, 6) Lanes 2-6: Negative results for the presence of WSSV.

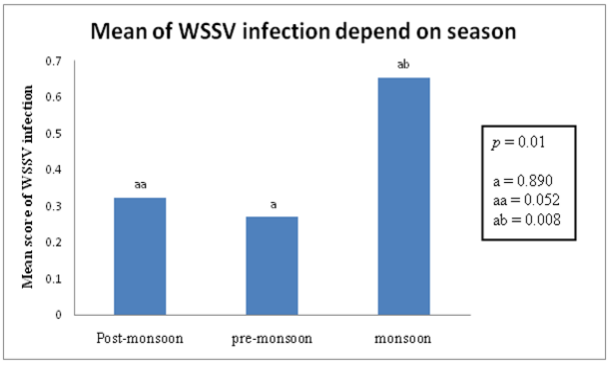

Figure 2. Mean score of the prevalence of WSSV infection in wild mud crab depend on the season (P=0.01). A different lower case letters denote a significant differences for a Tukey *post hoc* test, α<0.05. Letters a and aa: no significant differences, α>0.05; a=0.890, aa = 0.052 and Letter ab: significant difference, α<0.05; ab=0.008.

difference between the pre-monsoon and monsoon season (α=0.008). The total prevalence of WSSV infection was not influenced by the different number of mud crabs sampled during the post-monsoon season (n=25), pre-monsoon season (n=45), or monsoon season (n=20). A comparison of total positives for WSSV infection based on the season showed the highest prevalence during the monsoon season (Figure 2).

3.3 Sequencing and phylogenetic analysis

A multiple alignment of nucleotide sequences of the amplified PCR products confirmed that samples S5-5 (*S. paramamosain*), S5-6 (*S. olivacea*), and S3-1 (*S. tranquebarica*) were from the same member of WSSV. These results confirmed that all positive samples were from the same member of WSSV. S5-6 (*S. olivacea*) and S3-1(*S. tranquebarica*) showed 99%, while S5-5 (*S. paramamosain*) showed 98% sequence similarity to the whole genome of WSSV Mexican strain WSSV-MX08 (GenBank accession no. KU216744.1).

Multiple alignment of nucleotide sequences of the amplified PCR product confirmed that sample S5-5 (*S. paramamosain*) was from the same lineage as the WSSV Mexican strain (WSSV-MX08) (Rodriguez-Anaya *et al.*, 2016), sample S5-6 (*S. olivacea*) was from the same lineage as the Taiwan WSSV419 strain (Reville *et al.*, 2005), and sample S3-1 (*S. tranquebarica*) was the WSSV complete genome strain (van Hulten *et al.*, 2001) with minor variations (Figure 3).

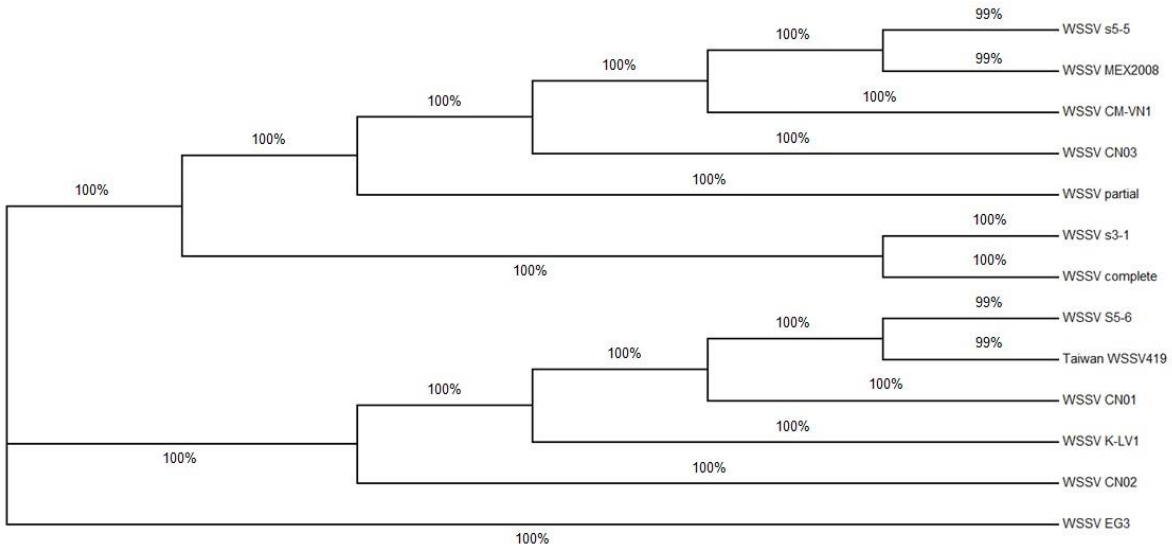

Figure 3. Phylogenetic neighbour-joining tree deduced from analysis nucleotide sequences of WSSV strain. A BLAST search of the WSSV_S5-5, WSSV_S5-6 and WSSV_S3-1 segments showed similarity sequence identities with MX08 (KU216744.1), CN01 (KT995472.1), CN02 (KT995470.1), CN03 (KT995471.1), EG3 (KR083866.1), K-LV1 (JX515788.1), Taiwan WSSV 419 (AY850066.1), CM-VN1 (JX564899.1), white spot syndrome virus with partial sequence (AF361753.1), and WSSV complete genome (AF369029.1).

4. Discussion

The water quality parameters taken at each of the sampling locations included temperature, salinity, DO, and pH. The temperature range was normal (28 °C–32 °C) for tropical coastal waters (Alongi *et al.,* 2009). The lowest salinity was recorded at site 3 which was a combination of freshwater and seawater downstream of the Setiu Wetlands during the monsoon season. The highest salinity was recorded at sampling site 6 which was located in the inshore area during the pre-monsoon. *Scylla* spp. are not known to be affected by elevated salinity because they can tolerate a wide range of salinities (Food and Agriculture Organization of the United Nation [FAO], 2011). The DO ranged from 3.58 mgL^{-1} to 5.74 mgL^{-1}. The lowest DO was due to aquaculture activities at the site. Aquaculture discharges contained high levels of organic matter such as feed and feces. The degradation of organic matter by microorganisms consume more oxygen, thereby lowering the DO content in the water (Hargrave, Duplisea, Pfeiffer, & Wildish, 1993). However, *Scylla* spp. can tolerate a low level of oxygen (FAO, 2011). The results for the water parameters were consistent with a previous study regarding water quality at Setiu Wetlands where aquaculture activities can affect the water quality parameters (Suratman, Hussein, Latif, & Weston, 2014).

A total of 33 samples of *Scylla* spp. (36%) that included *S. olivacea*, *S. Tranquebarica*, and *S. paramamosain* sampled from March to December 2015 demonstrated positive results for the presence of WSSV. The results from this study are consistent with several papers which showed positive results for WSSV infection in *Scylla* spp. using nested PCR (Chen *et al.,* 2000; Joseph, Roswin, Anbu Rajan, Surendran, & Lalitha, 2015; Liu *et al.,* 2011; Lo *et al.,* 1997; Otta *et al.,* 1999; Somboonna *et al.,* 2010; Vaseeharan, Jayakumar, & Ramasamy, 2003). Natural WSSV infections have been found in wild-caught and farmed mud crabs in various stages in many countries of the Asian region (Flegel, 1997, 2006; Kou, Peng, Chiu, & Lo, 1998; Lavilla-Pitogo, Marcial, Pedrajas, Quinitio, & Millamena, 2001; Lo *et al.,* 1996a; Otta *et al.,* 1999). Natural benthic larvae of wild *S. serrata* were found to be positive for WSSV infection at 60% prevalence (Lo *et al.,* 1997). WSSV infection (60% prevalence) was detected in benthic larvae of wild mud crab (*S. Serrata*) captured from Taiwan's coastal water (Chen *et al.,* 2000). WSSV was detected on diseased cultured mud crabs collected from Zhejiang Province of China during 2006–2008 which showed a prevalence of 34.82% (Liu *et al.,* 2011).

The percentages of WSSV positive *Scylla* spp. detected throughout the monsoon season in December, pre-monsoon season in August, and monsoon season in March were 14%, 14%, and 8%, respectively. A consistent prevalence for WSSV infections in mud crab populations captured in August and September was shown by Chen *et al.* (2000). The results of one-way ANOVA showed a significant difference between the three seasons and the prevalence of WSSV infection in wild mud crab (*Scylla* spp.). This result was consistent with a study that stated the infection rate of WSSV might be persistent throughout the year (Chen *et al.,* 2000). The high prevalence of WSSV infection during the monsoon season demonstrated that a WSSV outbreak is affected by the change of environmental factors which will depress the immune system of crustaceans (Gunalan, Soundarapandian, & Dinakaran, 2010). Temperature is the most important environmental factor because it has a profound effect on disease expression. Water temperatures between 18 °C to 30 °C can play an important role in inducing outbreaks of WSSV (Vidal, Granja, Aranguren, Brock, & Salazar, 2001). This study showed that the water temperature during the monsoon season was lower compared to post-monsoon and pre-monsoon seasons due to incoming freshwater during the rainy season (Sankar, Ramamoorthy, Sakkaravarthi, & Vanitha, 2011). Low water temperature has been known to influence the dispersal of WSSV (Gunalan *et al.,* 2010; Jiravanichpaisal, Soderhall, & Soderhall, 2004).

A BLAST search of the WSSV_S5-5 (*S. paramamosain*) segments showed 98% sequence identity with MX08 (KU216744.1), CN01 (KT995472.1), CN02 (KT995470.1), CN03 (KT995471.1), EG3 (KR083866.1), K-LV1 (JX515788.1), Taiwan WSSV 419 (AY850066.1), CM-VN1 (JX564899.1), WSSV with partial sequence (AF361 753.1), and WSSV complete genome (AF369029.1). The published sequences compared to S5-5 (*S. paramamosain*) were mostly sequences from cultured white shrimp (*L. vannamei*). WSSV_S5-6 (*S. olivacea*) and WSSV_S3-1 (*S. tranquebarica*) were found to be 99% similar with the above published sequences. S5-5 (*S. paramamosain*) showed 98% similarity with the whole genome of a WSSV Mexican strain (WSSV-MX08), which was the first WSSV isolated from cultured *L. vannamei* from Mexican farms (Rodriguez-Anaya *et al.,* 2016). WSSV was first discovered in 1992 from Taiwanese cultured *Penaeus japonicus* (Flegel, 2006). Since then, WSSV has spread across Asian countries including Vietnam, Thailand, Korea, India, China, and Malaysia (Flegel, 1997; Mohan, Shankar, Kulkarni, & Sudha, 1998; Nakano *et al.,* 1994; Wang, Nunan, & Lightner, 2000; Zhan *et al.,* 1998). The sequence similarity of S5-5 (*S. paramamosain*) to *L. vannamei* indicated that the WSSV infections could have been transmitted to the wild environment from farm or vice versa. WSSV can be transmitted horizontally and vertically (Chou, Huang, Wang, Lo, & Kou, 1998; Lo & Kou, 1998; Otta *et al.,* 1999). Horizontal transmission of WSSV was shown to be via ingestion and waterborne routes (Chou *et al.,* 1998). Water disposal from shrimp farms was shown to transmit the WSSV infectious agent into the natural enzootic areas (Esparza-Leal *et al.,* 2009; Lo & Kou, 1998). A cannibalistic behavior of mud crab also contributes to the transmission of WSSV (Lo *et al.,* 1997). Consequently, the virus can be transmitted to the whole population of the cultured or wild crab and shrimp (Office International des Epizooties [OIE], 2015).

However, a phylogenetic tree analysis showed that sample WSSV_S5-6 (*S. olivacea*) shared a common ancestry relationship with the Taiwan WSSV 419 strain (GenBank accession no. AY850066.1), while sample WSSV_S3-1 (*S. tranquebarica*) was the WSSV complete genome strain (GenBank accession no. AF369029.2). Based on the published sequences of the WSSV Mexican strain (WSSV-MX08) (Rodriguez-Anaya *et al.,* 2016), Taiwan WSSV 419 strain (Reville *et al.,* 2005), and WSSV complete genome (van Hulten *et al.,* 2001), it is evident that the WSSV was originally found in *L. vannamei* and *Penaeus monodon*. Both published sequences of the Taiwan WSSV 419 strain (Reville *et al.,* 2005) and WSSV complete genome (van Hulten *et al.,* 2001) originated from Thailand. Since Thailand is a neighboring country of Malaysia, WSSV could be transmitted

to the crustacean population via the horizontal route of infection through the release of ship ballast water and illegal importation of live post-larvae (Lo *et al.,* 1997; Muller, Andrade, Tang-Nelson, Marques, & Lightner, 2010; Sanchez-Martinez, Aguirre-Guzman, & Mejia-Ruiz, 2007).

Genetic variations were observed among the WSSV detected from the 3 different host species of *Scylla* spp. The host species played a role in the selection of a mutant within a viral population which led to a genetic variation of WSSV (Wang *et al.,* 2000). The genetic variation of WSSV in different host species can be explained by the passage of the virus through different hosts which can induce a genomic alteration and alter the pathogenicity of the virus (Waikhom, John, George, & Jeyaseelan, 2006). The non-static properties of WSSV genome enable itself to adapt to any environmental condition (Sablok *et al.,* 2010). A previous study reported that the occurrence of genomic mutations resulted from the adaptation of the virus to different environmental conditions (Waikhom *et al.,* 2006) due to mutations in the viral genome of the WSSV after its introduction into different countries (Muller *et al.,* 2010).

5. Conclusions

A total of 90 samples of wild *Scylla* spp. including *S. olivacea*, *S. tranquebarica*, and *S. paramamosain* were obtained from the Setiu Wetlands, Terengganu during the post-monsoon, pre-monsoon, and monsoon seasons. The water parameters taken from the sampling sites showed a normal range for the *Scylla* spp. habitat. A total of 33 samples during post-monsoon, pre-monsoon, and monsoon seasons were found to be positive for the presence of WSSV. The sequenced sample of the S5-6 (S. *olivacea*) and S3-1 (S. *tranquebarica*) showed high similarity (99%) while the S5-5 (S. *paramamosain*) showed 98% to the WSSV Mexican Strain (WSSV-MX08). Our findings are the first detection of WSSV at a new location from wild mud crabs (*Scylla* spp.) at the Setiu Wetlands, Terengganu, Malaysia.

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