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A study on the occurrence of white spot syndrome virus (WSSV) in *Scylla seratta* (Forsskal, 1755) around Kasimedu, Vanagaram, Amjikarai

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Abstract
Scylla serrata is a highly exploited species in marine ecosystems. It has a complex life cycle with a dispersing larvae phase, and benthic juveniles and adults. The White Spot Syndrome Virus (WSSV) is one of the most serious diseases in the shrimp and crab farm. This virus disseminates very quickly and causes high mortality in culture pond, accompanied by great economic losses. Recently, high mortality of WSSV infected mud crabs were collected from Kasimedu, Vanagaram, Amjikarai, Tamil Nadu, South East Coast of India during 2019-2020. A total of 30 samples collected from the above mentioned places were tested for the presence of WSSV. WSSV has been found across different shrimp species in Asian continents. *Scylla seratta* implicated the virus as the cause of death in most crabs. The aim of the study is to examine WSSV in shrimp with PCR detection and to identify the disease which spreads from crabs to humans. *Scylla seratta* was prominently spotted in estuaries and seas of Asian continents which was consumed by humans. The disease can create wider impact by affecting progeny leading to stunt production. Our examinations on the crab confirmed the occurrence of White Spot Syndrome Virus on *Scylla serrata.*

Keywords: marine ecosystem, asia, *Scylla serrata*, WSSV, PCR

Introduction

Scylla seratta belongs to the family *Portunidae*, is an economically important crab. In 1980, mud crab fattening technology was introduced by Philippines as one of the exotic aquatic species (Surtida, 1997) and has been successfully cultured in Indonesia, China, and Vietnam in coastal mud flats. Since 1940, studies on *Scylla seratta* has been reported (Keenan *et al*., 1998).

WSSV is one of the most prevalent virus which causes the diseases that are responsible for the mass (Bonami and Zhang, 2011) mortality of cultured shrimp and crab in India. White Spot Syndrome is one the world's most serious diseases in the crabs (Rajendran *et al*., 1999). WSSV is a double stranded DNA, rod shaped virus under the family *Nimaviridae*, genus Whispovirus (Van Hulten and Vlak, 2001; Mayo, 2002). White Spot Disease (WSD) is one of the major high mortality causing diseases in the crustacean's species. The clinical signs of this WSSV disease include appearance (Gopalakrishnan *et al*., 2011) of white spots in the inner surface of the crab and sluggish movement. The histological changes in the gills, hepatopancreas and heart showed the cause of WSSV disease.

Electron microscopic study based diagnosis has been reported for WSSV (Wang *et al*., 1995). Polymerase Chain Reaction (PCR) based molecular diagnostic method has been done for early diagnosis (Kimura *et al.*, 1996; Takahashi *et al.*, 1996; Umesha *et al*., 2006) [20]. The high mortality rate (80-100%) has been reported from infected crab farming due to WSSV infection at Xinghua city of Jiangsu Province, Eastern China (Ding *et al*., 2015). The *Scylla serrata* can be consumed by humans as it consist of essential nutrients such as protein, amino acids, fatty acids, vitamins, and minerals. It is widely distributed in the Indian Coastal Regions. This species has both ecological and economic (Kathirvel *et al*., 2004) [19] importance in the marine environment. The sign of the disease associated with this virus is the presence of white spots in the exoskeleton and epidermis of the diseased crab. The causative virus has an envelope, rod shaped nucleocapsid. It has a wide host range and (Erlich *et al*., 1988) [14] targets various tissues pathogens. PCR detection of WSSV in *Scylla seratta* implicated the virus as the cause of death in most crabs. Seawater crabs belong to sub-phylum crustacean, where the group includes lobster, shrimp, and krill.

The uniqueness of *Scylla seratta* exclusively varies from its deep, mottled green on its surface. The marine environment serves as the earth's largest living space for all the organisms and maintains balanced (Gonzalez Castro *et al.*,

2010**)** biodiversity. Marine resources provide the nutritional food security and the blue economy for various countries including India. Changes in genetic diversity, leads to loss of species in the biodiversity. This loss mainly occurs when microorganisms interfere and make them infectious.

The present study is to identify and collect *Scylla seratta* in various locations around Kasimedu, Vanagaram, Amjikarai and to determine WSSV molecular characters using PCR.

Materials and Methods

The crab samples were collected in the month of October – November 2019 to 2020. Mature crabs was collected from various locations such as (Table.1). Samples were labeled and stored under -20ºC. They were first morphologically identified based on the colour, size, shape, length, external and internal clinical signs were observed.

In the extraction of muscles the gill tissue was collected aseptically washed thoroughly in sterile water, 70% ethanol and saline. The tissue (100mg) was homogenized with 1ml of lysis buffer in a sterile motor and pestle.

DNA isolation and amplification was carried out using physical and chemical methods. A 16 positive result in the first step of this standard protocol implies a serious WSSV infection, the only positive (Lo *et al*., 2004) result is obtained in the second amplification step and a latent infection is indicated as well. PCR commercial kits are available for WSSV diagnosis.

Table 1: Samples collected from various locations around Chennai

S.No	Locations	Number of sample collected
1	Kasimedu	8
2	Vanagaram	8
3	Amjikarai	8

Fig 1: *Scylla Seratta* sample collected from Kasimedu, Vanagaram, Amjikarai

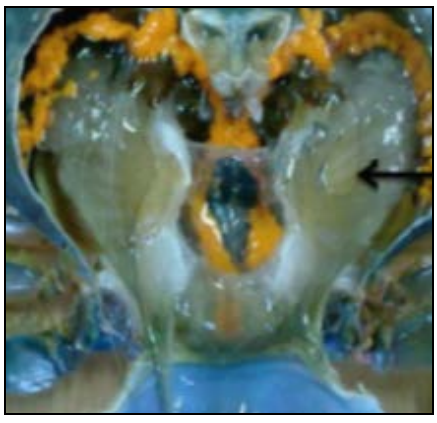

Fig 2: Internal structure of *Scylla Seratta*

Result and Discussion

Among 30 *Scylla Seratta* samples only 24 were tested and 14 samples were found to be positive for WSSV by PCR assay (Table 2). The observation of an amplified product (1023 bp and 601bp first step & nested PCR respectively). However, following PCR amplification with WSSV detection primers clear products were revealed, indicating the presence of latent infection. A 16 positive result in the first step of this standard protocol implies a serious WSSV infection, the only positive (Lo *et al*., 2004) result is obtained in the second amplification step and a latent infection is indicated as well. The total DNA that was extracted from the samples were investigated using conventional PCR specific to genes specific for WSSV primers which generated amplicons of 1023 and 601 bp, respectively.

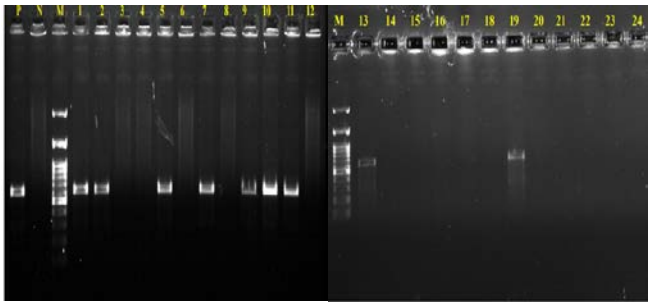

Fig 3: PCR Detection of White Spot Syndrome Virus DNA in *Scylla Seratta*

Lane 1: Positive control
Lane 2: Negative control
Lane 3: Molecular marker (1-kb ladder)
Lane 4: 12 samples
Lane 1: 1kb Marker
Lane 13: 24 Samples

Table 2

S.No	Locations	Sample Count	WSSV	
			WSSV^{+}	WSSV^{-}
1.	Vanagaram	8		+
2.				+
3.			-	
4.			-	
5.			-	
6.				+
7.			-	+
8.			-	
9.	Kasimedu	8		+
10.				+
11.				+
12.			-	
13.				+
14.			-	
15.			-	
16.			-	
17.	Amjikarai	8		-
18.			-	
19.			+	
20.			-	
21.			-	
22.			-	
23.			-	
24.			-	

SEM (Scanning Electron Microscope) Analysis

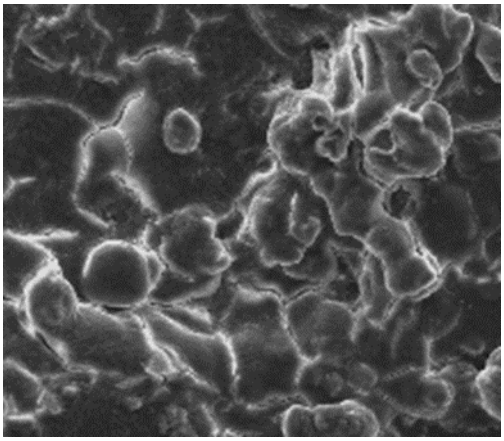

Fig 4: SEM image of crab tissue sample with White Spot Syndrome

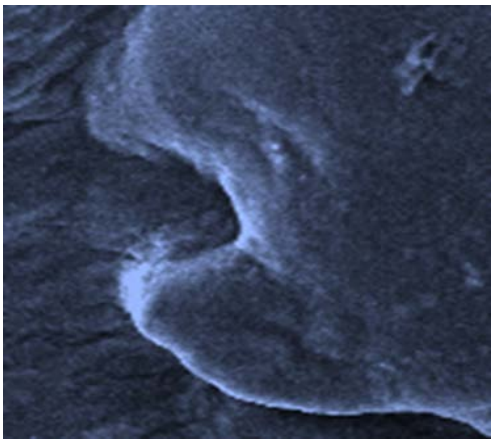

Fig 5: Normal crab tissue

Conclusion

In this study, Polymerase Chain Reaction (PCR) was used for analyzing WSSV. Among 30 mud crab samples of which around 14-16 were found to be positive for WSSV by PCR assay. The observation of an amplified product (1023 bp and 601bp first step & nested PCR respectively) confirmed WSSV infection in mud crab samples. The WSSV, has emerged globally as one of the most prevalent, widespread and lethal for crab populations. WSSV can be prevented and controlled using de-chlorinated water, observation of biosecurity in the rearing system, including natural food and feeds.

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