



### Glimpses On White Spot Disease in Shrimps and its Significance in e-Resource Development

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## Abstract

The worldwide distribution of White Spot Disease (WSD) instigated by White Spot Syndrome Virus (WSSV) is one of the most pathogenic and devastating viruses in shrimps and crustaceans. WSSV categorized into a new family named Nimaviridae causes the disease that has been considered as havoc as it severely affects the shrimp population and in its most fatal form due to its high virulence, it has the ability to wipe out all the shrimp population within a week. Surprisingly till date, no effective drugs are available to prevent the disease because of incomplete understanding on the disbandment of the disease mechanism and further protein of this virus has not shown any homology with already existing viral proteins. Earlier, many attempts were made to understand the mechanism of entry, propagation and spread of this virus within the host, but till now, the information and knowledge generated from these studies have not been compiled with an aim to construct systematic and comprehensive e-resource covering information relating to genes, proteins and protein interactions of the disease. To accomplish this, it is imperative to have the understanding about the available resources that contains information and knowledge about this disease so as to provide the complete understanding about the disease and the related phenomena. Such publically available resources can be of extreme importance in developing an e-resource in the form of an online database that might be of much useful to the shrimp and allied researchers and in future of tremendous benefit as the shrimp researchers might perform many operations from the 'omics' data stored in the database. The present paper discusses about WSD, its resources and utility in accomplishing database in a nutshell.

**Keywords:** White Spot Disease; White Spot Syndrome Virus; Shrimps, e-resource; Database

## Introduction

The importance of marine viruses was remained underestimated till 1980s due to insufficient information on their ecological significance and impact over globe. With the elapse of time, their significance at global level was recognised due to their momentous role in diverse biogeochemical cycles. Further, they began to be considered as most copious “life forms” in the oceans acting as reservoir for the extraordinary genetic diversity on earth. Among viruses, Herpesviruses, Reoviruses, Nodaviruses, Birnaviruses, and Rhabdoviruses are the most studied viruses that infect commercially important aquatic organisms. White Spot Syndrome Virus (WSSV), Yellow Head Virus (YHV), Monodon Baculo Virus (MBV), Hepatopancreatic Parvo Virus (HPV), Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV), and Taura Syndrome Virus (TSV) are the most potent lethal viruses that have caused deteriorating and shattering effects worldwide on the aquatic population especially in shrimps and other crustaceans [1].

White Spot Disease (WSD), after its first appearance in early 1990s has become one of the greatest dangers for the crustacean aquaculture industry across the globe [2]. WSD first reported from China in 1991 [3] spread rapidly in other major aquaculture regions like America, India, Middle East [3-8] and Europe [9]. After its emergence in different aquaculture regions, the cumulative economic damage caused to the shrimp aquaculture industry has been evaluated to be \$8-\$15 billion

[10], and it has been estimated to aggravate by \$1 billion annually [1,11]. Thus, the white spot syndrome virus (WSSV) because of its high virulence

remains one of the most harmful pathogens causing great economic losses to the shrimp industry. To wipe out the disease completely, a well systematic and comprehensive road map is essential providing the insight into the disease and its mechanism into the host. Although many recent attempts have been made to understand the molecular mechanism of infection of the White Spot Syndrome Virus (WSSV) into shrimps [12-13] yet there is a gap of a centralised single resource covering details about the different host species, different WSSV strains, viral/host genes, viral/host proteins, protein interactions. Such a resource covering genomic, transcriptomic, proteomic data relevant to the disease can be useful to the shrimp aquaculture researchers to take up the further targeted research in preventing and controlling the disease outbreak and minimising its effect through drug target identification, formulation and implementation.

## Current resources on WSD

The progress in the contemporary biological research is crucially determined by the accumulation of diversified data pertaining to genetic, genomic, transcriptomic, proteomic, molecular, and cell biological information stored in the databases. These biological databases act as repositories and supported with data management includes ability to store, manage and disseminate the data in a systematic way. Such databases facilitate research in myriad of ways. Till date, there

is not even a single platform accommodated with information related to genomic, proteomic and protein interaction study for WSD in shrimps. Fish Site (<https://thefishsite.com>), Food and Agriculture Organization of the United Nations (<http://www.fao.org>), National pest and disease outbreaks (<http://www.outbreak.gov.au>) are the few open access sites that provide information related to WSD. Apart from these, the information regarding morphology and ultrastructure of WSSV, WSSV transmission and host range, WSSV virulence, tissue tropism, strategies for control of the virus etc are accessible from the published literatures only [14]. The random data mining from National Centre for Biotechnology Information (<https://www.ncbi.nlm.nih.gov>) reveals 81,968 nucleotide (DNA and RNA) sequences, 16,990 expressed sequence tag (EST) sequences, 718 gene sequences, 4162 protein sequences, 8 protein structures of WSSV.

In addition to these resources, the three databases namely, Marine Genomics Database [15-16], and the *Penaeus* Genome database [17] designed in 2005, 2006 and 2009 respectively enrich the shrimp transcriptomic data. All these three databases basically contain ESTs, contigs from various marine organisms. The Marine Genomics Database includes 314,766 ESTs and 46,421 contigs from Elkhorn Coral, Mallard Duck, Atlantic Bay Scallop, *Calanus finmarchicus* (Zooplankton), Blue Crab, Pacific Oyster, Eastern Oyster, Atlantic Stingray, North Atlantic Right Whale, Brown Shrimp, Fundulus Genus, American Lobster, *Karenia brevis* (toxic dinoflagellate), Little Skate, Atlantic White Shrimp, Blue Shrimp, Pacific White Shrimp, Lobed Star Coral, *Montastrea faveolata* (Coral), Ivory Tree Coral, Mozambique Mouthbrooder, Grass Shrimp, Red King Crab, Tiger Prawn, Mustard hill coral, Finger Coral, Spiny

Dogfish, Bottlenose Dolphin. It additionally plans to enrich the database by including microarray data of marine organisms in future [15]. The *Penaeus monodon* EST Project database contained 40001 ESTs and 10536 contigs on its release from multiple libraries and tissues of *P. monodon*. The database was created with the rational of gene discovery in the black tiger shrimp *P. monodon*. Tissues from different organs viz. eyestalk, hepatopancrease, haematopoietic tissue, haemocyte, lymphoid organ, and ovary were taken to create such a database [16]. The *Penaeus* Genome database provides the ESTs and contigs for penaeid shrimp species (196248 ESTs and 42332 contigs) and additionally includes information on genetic linkage map and fosmid library end sequences of *P. monodon*.

Another important database created recently is ShrimpGPAT (Shrimp Gene and Protein Annotation Tool) [18]. ShrimpGPAT serves as a common platform for the shrimp research community for extensive collection of shrimp molecular sequences for their functional annotation, curation and annotate in the form of tags and comments. It accommodates much diversified form of data such as EST sequences, NGS short reads, cDNAs, protein sequences, contigs, protein records for *in-silico* functional prediction, and putative protein-protein interactions. ShrimpGPAT initially contained with a set of ESTs for six decapod species, including four penaeid shrimp currently encompasses 107315 Black tiger shrimp, 299052 Pacific white leg shrimp, 1330 White shrimp, 13895 Fleshy prawn, 1344 Indian prawn, 5550 Kuruma prawn, 14001 Giant freshwater prawn, 795 Cray fish, 2003 Signal crayfish, 43079 American lobster, 404 Orange mud crab, 5446 Green mud crab, 13001 Blue crab, 23779 Green crab recodes. It further houses 343880 EST records, 101035 contig records, 82015 nucleotides, and 4394 protein records in shrimps and other crustacean species. The

following section of the manuscript elucidates information related to different host, different viral strains, genomic, proteomic, protein interaction that has significance for developing e-resource on WSD in the form of a database.

### WSD host range

WSSV affects an extensive range of hosts that comprised of all cultured and wild marine shrimps, crabs, crayfishes, lobsters, copepods [19-24]. WSSV is potentially fatal to almost all the commercially important species of penaeid shrimps including *P. monodon*, *P. vannamei*, *P. indicus*, *P. japonicus*, *P. chinensis*, *P. penicillatus*, *P. azteus*, *P. merguensis*, *F. duorarum*, *P. stylirostris*. (Table 1) lists the most common host species for WSSV. The susceptibility to WSSV differs significantly among the hosts. In some hosts, WSSV causes potential threat leading to mortality while in some species WSSV causes only latent infections and makes the species as a potential virus reservoir. Among the entire available hosts, the genome sequence of *Penaeus monodon* became available recently [25]. Fosmid library end sequencing was performed to understand the genome sequence of *P. monodon*.

**Table (1):** Different host range for WSD

Sr.No	Host
1.	Artemia sp.
2.	A. franciscana
3.	Schmackeria dubia
4.	Alpheus brevicristatus

5.	A. lobidens
6.	Astacus leptodactylus
7.	Pacifastacus leniusculus
8.	Calappa lophos
9.	C. philargius
10.	Callianassa sp.
11.	Cancer pagurus
12.	Orconectes limosus
13.	O. punctimanus
14.	Procambarus clarkii
15.	Paradorippe granulata
16.	Menippe rumphii
17.	Grapsus albolineatus
18.	Metopograpsus messor
19.	Philyra syndactyla

20.	<i>Lithodes maja</i>
21.	<i>Doclea hybrida</i>
22.	<i>Matuta miersi</i>
23.	<i>M. planipes</i>
24.	<i>Gelasimus marionis nitidu</i>
25.	<i>Macrophthalmus sulcatus</i>
26.	<i>Uca pugilator</i>
27.	<i>Exopalaemon orientis</i>
28.	<i>Macrobrachium idella</i>
29.	<i>M. lamarrei</i>
30.	<i>Palaemon adspersus</i>
31.	<i>Panulirus homarus</i>
32.	<i>P. longipes</i>
33.	<i>P. ornatus</i>
34.	<i>P. penicillatus</i>
35.	<i>P. polyphagus</i>

36.	<i>P. versicolor</i>
37.	<i>Cherax destructor albidus</i>
38.	<i>C. quadricarinatus</i>
39.	<i>Parathelphusa hydrodomous</i>
40.	<i>P. pulvinata</i>
41.	<i>Parthenope prensor</i>
42.	<i>Metapenaeus brevicornis</i>
43.	<i>M. dobsoni</i>
44.	<i>M. ensis</i>
45.	<i>M. lysianassa</i>
46.	<i>M. monoceros</i>
47.	<i>Parapeneopsis stylifera</i>
48.	<i>Penaeus aztecus</i>
49.	<i>P. chinensis</i>

50	<i>P. duorarum</i>
51	<i>P. indicus</i>
52	<i>P. japonicas</i>
53	<i>P. merguensis</i>
54	<i>P. monodon</i>
55	<i>P. penicillatus</i>
56	<i>P. schmitti</i>
57	<i>P. semisulcatus</i>
58	<i>P. setiferus</i>
59	<i>P. stylirostris</i>
60	<i>P. vannamei</i>
61	<i>Trachypenaeus curvirostris</i>
62	<i>Callinectes arcuatus</i>
63	<i>C. sapidus</i>
64	<i>Carcinus maenas</i>

65	<i>Charybdis annulata</i>
66	<i>Ch. Cruciata</i>
67	<i>Ch. Granulate</i>
68	<i>Ch. Feriatus</i>
69	<i>Ch. Japonica</i>
70	<i>Ch. Lucifera</i>
71	<i>Ch. Natator</i>
72	<i>Liocarcinus depurator</i>
73	<i>Lio. Puber</i>
74	<i>Podophthalmus vigil</i>
75	<i>Portunus pelagicus</i>
76	<i>P. sanguinolentus</i>
77	<i>Scylla serrata</i>
78	<i>S. tranquebarica</i>
79	<i>Thalamita danae</i>
80	<i>Scyllarus arctus</i>
81	<i>Acetes sp.</i>

82	Sesarma oceanica
83	Solenocera indica
84	Helice tridens
85	Pseudograpsus intermedius
86	Atergatis integerrimus
87	Demanina splendida
89	Halimede ochtodes
90	Liagore rubromaculata
91	Ephydrida sp
92	Squilla mantis
93	Marphysa graveleyi
94	Brachionus urceus

### WSSV strains

Till date the four different strains of WSSV viz. Chinese strain (WSSV-CN), Thailand strain (WSSV-TH), Taiwan strain (WSSV-TW), Korean strain (WSSV-KR) have been sequenced. WSSV-CN was isolated from *Penaeus japonicus* in Oct 1996 from Xiamen, China. The sequence of the isolated WSSV-CN of 305,107 base pairs long was submitted under accession AF332093 in GenBank.

Again, WSSV-TH was isolated from *Penaeus monodon* in May 1996 from Suratthan, Thailand. The sequence of this virus of 292,967 base pairs long was submitted under accession AF369029 in GenBank. Further, WSSV-TW was isolated from *Penaeus monodon* in Nov 1994 from Southern Taiwan and the sequence of this virus of 307,287 base pairs long was submitted under accession AF440570 in GenBank. Similarly, WSSV-KR isolated from *Litopenaeus vannamei* in Aug 2011 from Jeollanam-Do, Korea, whose sequence was 295,884 base pairs long and this sequence was submitted under accession JX515788 in GenBank. The availability of complete genomic sequence of these strains have opened up the opportunities to get the insight into the molecular mechanism of virus causing pathogenesis. Table 2 lists the different WSSV strains that cause WSD in various shrimp species.

**Table (2):** Different viral strains that cause WSD across the globe

Sr.No.	Strain	GenBank Accession Number	Ref
1.	Chinese isolate (WSSV-CN)	AF332093	Yang 2001
2.	Thailand isolate (WSSV-TH)	AF369029	Van 2001
3.	Taiwanese isolate (WSSV-TW)	AF440570	Tsai 2000
4.	Korean isolate (WSSV-KR)	JX515788	Chai 2013

## Protein interactions involved in WSD

Generally, the DNA viruses utilize multi-protein complexes to get entry into their host. Similarly WSSV also utilizes multi-protein complexes for entry into shrimp. Till date it is not very certain how WSSV enters into shrimp cells. On the contrary, some candidate receptor proteins have been anticipated that have concerns in directing WSSV into *P. monodon*. These shrimp receptors proteins include viral attachment proteins (VAP) [26],  $\beta$ -integrin [27] PmCPB [28] and PmRab7 [29]. On the other hand, the viral envelop and its structural proteins form the first and most important component of the virus to directly come in contact with the shrimp. The structural proteins often play primitive roles in cell targeting, virus entry, assembly and budding. About 60% of this viral envelop is constituted by the structural proteins; VP26, VP28 and VP24. VP28 is a major structural envelope protein of WSSV, which is highly accountable for causing the systemic infection in shrimps. Further, it also forms a significant part of “infectome” crucial in cell recognition, attachment and guiding the virus in the shrimp cell. Thus, in an attempt to identify the envelope proteins involved in WSSV infection to shrimp, the neutralization experiments of antibodies against six envelope proteins (VP22/VP26, VP28, VP68, VP281, VP292 and VP466), as well as WSSV virions, were also done and the result suggested that four envelope proteins (VP28, VP68, VP281 and VP466) might play key roles in the initial steps of WSSV infection in shrimp [30]. In this way, a holistic approach for understanding the interaction between shrimp and viral proteins might provide a road map for understanding the mechanism of entry of WSSV into shrimps. Table 3 lists various important shrimp protein-WSSV protein interactions.

**Table (3):** Various shrimp-WSSV protein interactions

Sr No	Shrimp protein with host name	Viral protein	Reference
1	Chitin-binding protein (PmCBP) <i>Penaeus monodon</i>	VP24, VP32, VP39B, VP41A, VP51B, VP53A, VP53B, VP60A, VP110, VP124, VP337	Chen 2007, Chen 2009
2	Glu1 <i>P. monodon</i>	VP53A	Huang 2012
3	C-type lectin (LvCTL1) <i>Litopenaeus vannamei</i>	VP95, VP28, VP26, VP24, VP19, VP14	Zhao 2009
4	C-type lectin (FcLec3) <i>Fenneropenaeus chinensis</i>	VP28	Wang 2009
5	C-type lectins (MjLecA, MjLecB, MjLecC) <i>Marsupenaeus japonicus</i>	VP26, VP28	Song 2010
6	C-type lectins (MjsvCL) <i>M. japonicus</i>	VP28	Wang 2014
7	$\beta$ -Integrin <i>P. japonicus</i> / <i>P. clarkii</i>	VP187	Li 2007
8	$\beta$ -integrin, Syndecan	WSSV-CLP	Sun 2014

	F. chinensis		
9	Rab7 (PmRab7) <i>P. monodon</i>	VP28	Sritunyaluck sana 2006
10	Histones <i>P. monodon</i>	ICP11	Wang 2008
11.	RACK1 (PmRACK1) <i>P. monodon</i>	VP9	Tongnunt 2009
12	FKBP46 (PmFKBP46) <i>P. monodon</i>	VP15	Sangsuriya 2011
13	Arginine kinase (LvAK) <i>L. vannamei</i>	VP14	Ma 2014
14	Actin <i>Procambarus clarkii</i> VP26		Xie 2005
15	PPs <i>L. vannamei</i>	ORF427	Lu 2004
16	TATA box-binding protein (PmTBP) <i>P. monodon</i>	WSSV IE1	Liu 2011
17	WSV056 Retinoblastoma protein (Lv-RBL) <i>L. vannamei</i>	WSSV IE1,	Ran 2013
18	Prohibitin (PcPHB1) <i>Procambarus clarkii</i>	VP28, VP26, and VP24	Lan 2013
19	Peritrophin-Like Protein (LvPT) <i>Litopenaeus. Vannamei</i>	VP32, VP38A, VP39B, and VP41A.	Xie 2015

## Conclusion

The present paper highlights on the host species, viral strains, genomic, proteomic data related to WSD in shrimps. Such a mini review provides a bird's eye view on the present disease that can be helpful to the shrimp research community for getting the information related to the disease. Further, it might lay the foundation for developing the open access centralised single database by utilising these resources that might be of extreme utility to the shrimp and allied researchers. The database to be designed in future may be of tremendous benefit since the shrimp researchers may perform many operations from the 'omics' data stored in the database. Some of the applications for which the futuristic database may be used are: (i) performing inter-species comparative genomics and phylogenetic studies among all the viral strains (ii) performing subtractive genomics approach between viral strains and shrimp species in order to identify non-homologous genes and further searching for essential viral genes among these non-homologous genes to further target them for drug designing (iii) comparing the individual transcriptome to derive meaningful information from RNAs and (iv) studying protein-protein interactions between specific host and viral proteins to have deeper insight into the overall interactome the governs and dictates the molecular interaction involved in WSD.

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