



# **Considerações sobre a variabilidade genética dos vírus que acometem a carcinicultura: Os riscos inerentes à importação de camarões.**

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# Laboratório de biologia molecular aplicada – LAPLIC UFRN

- Foi criado em 2012 tendo como um dos principais objetivos estudar os principais vírus que acometem a carcinicultura;
- Trabalha atualmente com a caracterização de variantes virais, estudo da biologia dos vírus (virologia molecular) e desenvolvendo métodos para identificação molecular de vírus de interesse econômico;
- Foi o primeiro grupo brasileiro a sequenciar os genomas dos vírus IMNV e PstDENV (IHHNV) e WSSV;
- Atualmente conta com uma equipe de 10 pesquisadores que atuam diretamente nos estudos referentes aos vírus que acometem a carcinicultura.



ELSEVIER



# Infectious hypodermal and hematopoietic necrosis virus from Brazil: Sequencing, comparative analysis and PCR detection



Douglas C.D. Silva<sup>a</sup>, Allan R.D. Nunes<sup>a</sup>, Dárlío I.A. Teixeira<sup>c</sup>, João Paulo M.S. Lima<sup>b,d</sup>, Daniel C.F. Lanza<sup>a,\*</sup>

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                                CR1          CR2
1      GACGAGTGAAGAGGCTATTCCAAGTGACTAAGGACAATTTTGGAACGATACGAA 60
61     CGACCACCCATGGCAATCAATACCTAGTCCGTCATTATTTGGATCATCAAGTAACAGTGA 120
121    ACCAACAGAAGTCTTTCAAACGCTCTTCGGAGAAAGACAAACCCAAGGATACAAATGTAA 180
181    GTACAAGTACTGACTAAGTGACGATCCATTAATTCCTAATTGACGCAAGTGACGACGT 240
241    CATATGCGTCACTTACAAAAGACGTAACCGCTTTCGTCCATCACTCACATATATCTTTCT 300
301    CTACCTTTCAGACGACATACCCCAACAAATATCGCTGCGCTACTGCCAGATCACATTCT 360
361    ACCGTGGTGCTTCATAGGGAACAGACCCGTTCTCTACTGCCTCTGCAACGAGTGTTTTAT 420
                                CR3
421    AGACAATCTCAATGTCGACGGACAGTGTCAACACTGTCATCCCGACGACGAAGAATGGAC 480
        M S T D S V N T V I P T T K N G
481    AGAAAATATGGCCAAGGACATACTGCATACACGTCAGGGCGAACCAAGATCACTTAGTGA 540
        Q K I W P R T Y C I H V R A N Q N H L V
        M A K D I L H T R Q G E P E S L S E
                                CR4
541    ATTGCTTCGAGAACGCACGAACGAAACTCACTCCAGCCAGGGAATTTCTCCAAGCCTTCT 600
        N C F E N A R T K L T P A R E F L Q A F
        L L R E R T N E T H S S Q G I S P S L L
601    CACCCAGGTCCAATCAAGACCCTAAACCCACTACCGAACAACTTCTTAATATGTCTGA 660
        S P Q V Q I K T L N P L P N N F L I C L
        T P G P N Q D P K P T T E Q L L N M S E
                                CR5          CR6
661    AGAACTGTTCCAGTTTTCAGACGAGGAAGACAACTCTCAAACTCCTCCAAGAACTTCAAC 720
        K N C S S F Q T R K T T L K L L Q E L Q
        E L F Q F S D E E D N S Q T P P R T S T
                                CR7
721    ACCAGAACAAACTGATCCTAAGGTCTGCGTGGATAACCTGGGAATTCGAGAGGGAACAGG 780
        H Q N K L I L R S A W I T W E F E R E Q

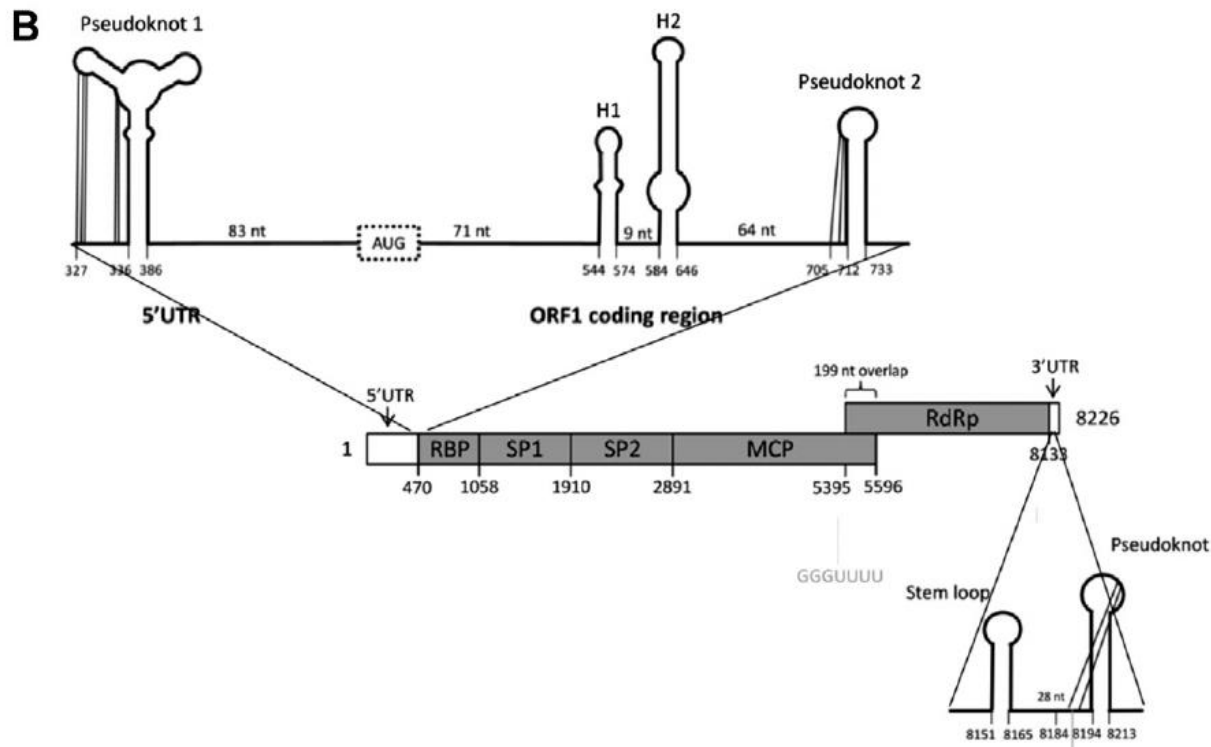
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Short communication

## Analysis of new isolates reveals new genome organization and a hypervariable region in infectious myonecrosis virus (IMNV)



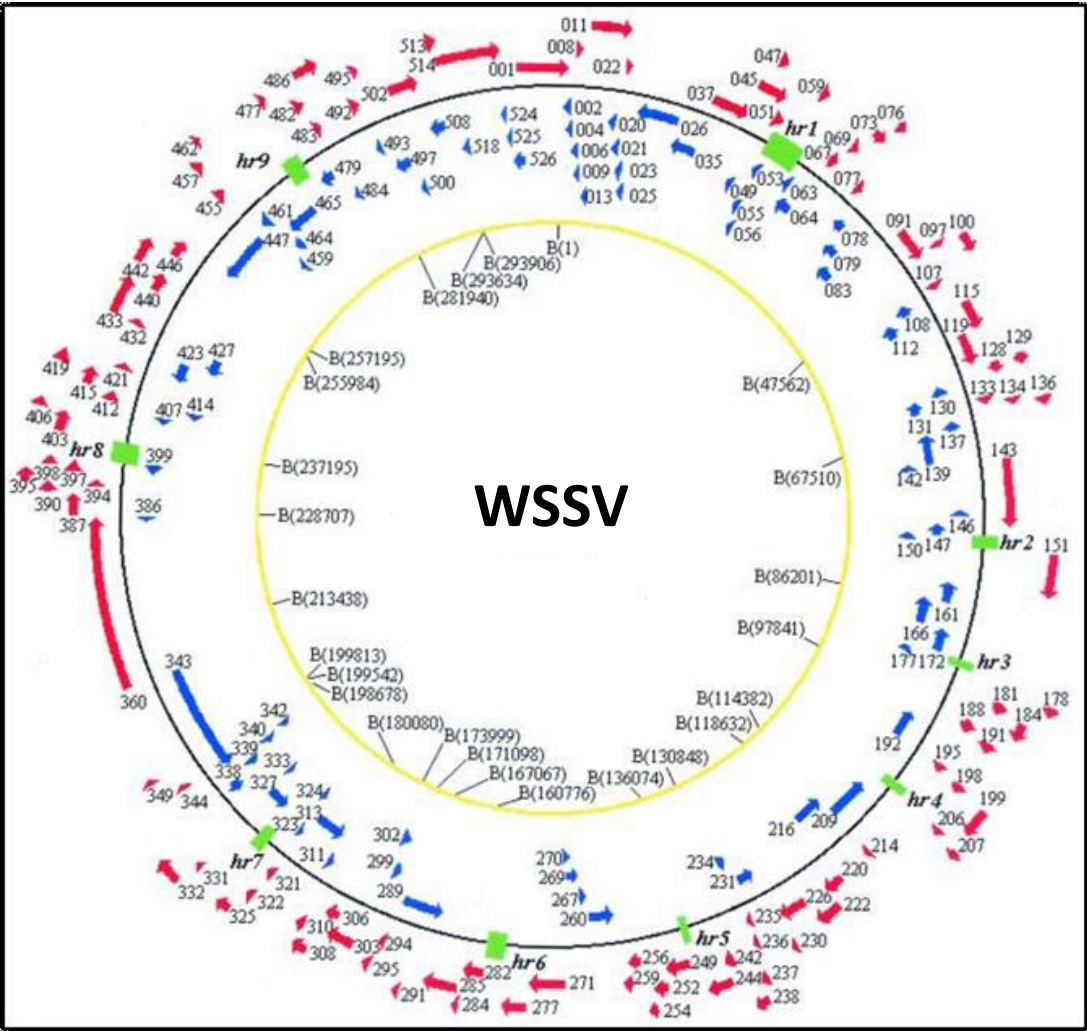
Márcia Danielle A. Dantas<sup>a,b</sup>, Suely F. Chavante<sup>b</sup>, Dárlío Inácio A. Teixeira<sup>c</sup>,  
João Paulo M.S. Lima<sup>b,d</sup>, Daniel C.F. Lanza<sup>a,b,\*</sup>



# Manifestações clínicas em camarões infectados pelo vírus causador da síndrome da mancha branca



# Sequenciamento do genoma do WSSV



**Genoma extremamente complexo!!!!**

Fonte: [J Virol.](#) 2001 Dec;75(23):11811-20. **Complete genome sequence of the shrimp white spot bacilliform virus.** [Yang F<sup>1</sup>](#), [He J](#), [Lin X](#), [Li Q](#), [Pan D](#), [Zhang X](#), [Xu X](#).

# Genomas completos/parciais do WSSV disponíveis atualmente nos bancos de dados públicos

Accession number	Country	Collection year	Host	Genome size (bp)
AF440570.1	Taiwan	1994	<i>Penaeus monodon</i>	307.287
AF332093.3	China	1996	<i>Penaeus japonicus</i>	305.107
AF369029.2	Thailand	1996	<i>Penaeus monodon</i>	292.967
JX515788.1	South Korea	2011	<i>Litopenaeus vannamei</i>	295.884
KR083866.1	Egypt	2014	?	305.119
KT995472.1	China CN01	1994	<i>Marsupenaeus japonicus</i>	309.286
KT995470.1	China CN02	2010	<i>Procambarus clarkii</i>	294.261
KT995471.1	China CN03	2010	<i>Litopenaeus vannamei</i>	284.148
KU216744.1	Mexico	2008	<i>Litopenaeus vannamei</i>	293.183

# Análise de identidade - WSSV

	WSSV_CN01	WSSV_CN02	WSSV_CN03	WSSV_CN	WSSV_EG	WSSV_KR	WSSV_TW	WSSV_MEX	WSSV_THAI	WSSV_BR
WSSV_CN01		94.28%	89.59%	97.27%	97.27%	94.53%	97.07%	92.65%	92.82%	92.67%
WSSV_CN02	94.28%		93.67%	96.21%	96.21%	96.64%	95.35%	94.62%	94.82%	94.67%
WSSV_CN03	89.59%	93.67%		91.25%	91.25%	92.58%	90.58%	93.31%	93.44%	93.71%
WSSV_CN	97.27%	96.21%	91.25%		100.00%	96.85%	98.71%	94.91%	95.14%	95.09%
WSSV_EG	97.27%	96.21%	91.25%	100.00%		96.85%	98.71%	94.91%	95.14%	95.09%
WSSV_KR	94.53%	96.64%	92.58%	96.85%	96.85%		96.17%	95.57%	95.75%	95.62%
WSSV_TW	97.07%	95.35%	90.58%	98.71%	98.71%	96.17%		94.18%	94.40%	94.22%
WSSV_MEX	92.65%	94.62%	93.31%	94.91%	94.91%	95.57%	94.18%		99.58%	97.93%
WSSV_THAI	92.82%	94.82%	93.44%	95.14%	95.14%	95.75%	94.40%	99.58%		98.27%
WSSV_BR	92.67%	94.67%	93.71%	95.09%	95.09%	95.62%	94.22%	97.93%	98.27%	

→ A diferença pode corresponder a **milhares** de nucleotídeos entre os isolados de diferentes regiões, em decorrência de mutações e recombinações.





## Fitness and virulence of an ancestral White Spot Syndrome Virus isolate from shrimp

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Available online 5 February 2005

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### Abstract

White Spot Syndrome Virus, the type species of the virus family *Nimaviridae*, is a large dsDNA virus infecting shrimp and other crustaceans. Genomic analysis of three completely sequenced WSSV isolates identified two major polymorphic loci, “variable region ORF14/15” and “variable region ORF23/24”. Here, we characterize a WSSV isolate originating from shrimp collected in Thailand in 1996 (TH-96-II). This isolate contains the largest WSSV genome (~312 kb) identified so far, mainly because of its sequences in both major polymorphic loci. Analysis of “variable region ORF14/15” suggests that TH-96-II may be ancestral to the WSSV isolates described to date. A comparison for virulence was made between TH-96-II and WSSV-TH, a well characterized isolate containing the smallest genome (~293 kb) identified at present. After injection of the isolates into *Penaeus monodon* the mortality rates showed that the median lethal time (LT<sub>50</sub>) of TH-96-II was approximately 14 days, compared to 3.5 days for WSSV-TH. When both isolates were mixed in equal amounts and serially passaged in shrimp, WSSV-TH outcompeted TH-96-II within four passages. These data suggest a higher virulence of WSSV-TH compared to TH-96-II. The molecular basis for the difference in virulence remains unclear, but a replication advantage of the 19 kb smaller WSSV-TH genome could play a role.

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**Keywords:** White Spot Syndrome Virus; WSSV common ancestor; Polymorphic loci; Competitive fitness; Virulence

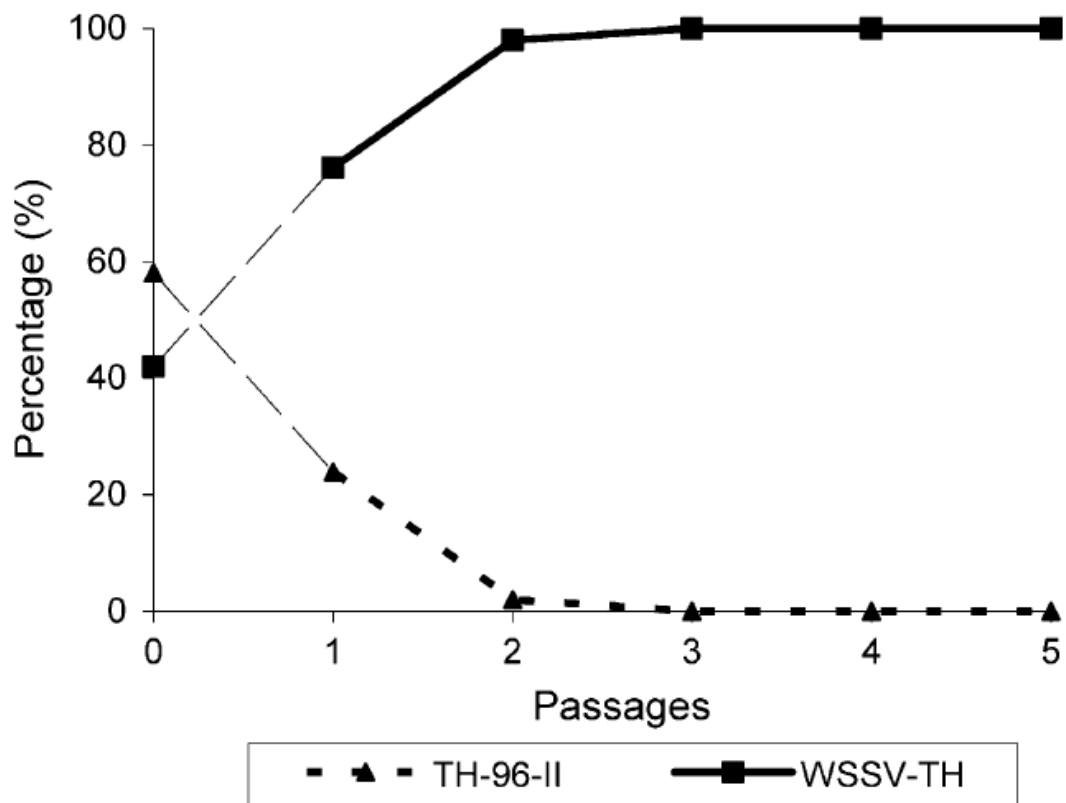


Fig. 4. Relative amount (%) of WSSV-TH DNA and TH-96-II DNA present during five consecutive passages of *P. monodon* injected with a 1:1 mix of both isolates (group C).

# Virulence and genotypes of white spot syndrome virus infecting Pacific white shrimp *Litopenaeus vannamei* in north-western Mexico

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were evident. Using mortality data, the four WSSV strains grouped into three virulence levels. The Mx-F strain (intermediate virulence) and the Mx-C strain (high virulence) showed more genetic differences than those observed between the Mx-G (low-virulence) and Mx-H (high-virulence) strains, in ORF94 and ORF125. The application of high-viral-load inocula proved useful in determining the different virulence phenotypes of the WSSV strains from the Eastern Pacific.

# Novel, closely related, white spot syndrome virus (WSSV) genotypes from Madagascar, Mozambique and the Kingdom of Saudi Arabia

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<sup>2</sup>AQUALMA, Aquaculture de la Mahajamba, Mahajanga 401, Madagascar

**ABSTRACT:** White spot syndrome virus (WSSV) is highly pathogenic to penaeid shrimp and has caused significant economic losses in the aquaculture industry around the world. During 2010 to 2012, WSSV caused severe mortalities in cultured penaeid shrimp in Saudi Arabia, Mozambique and Madagascar. To investigate the origins of these WSSV, we performed genotyping analyses at 5 loci: the 3 open reading frames (ORFs) 125, 94 and 75, each containing a variable number of tandem repeats (VNTR), and deletions in the 2 variable regions, VR14/15 and VR23/24. We categorized the WSSV genotype as (N<sub>125</sub>-N<sub>94</sub>-N<sub>75</sub>-ΔX<sub>14/15</sub>-ΔX<sub>23/24</sub>) where N is the number of repeat units

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## GENOTYPING OF WHITE SPOT SYNDROME VIRUS (WSSV) AND INFECTIOUS HYPODERMAL AND HEMATOPOIETIC NECROSIS VIRUS (IHHNV) IN ECUADORIAN CULTURED SHRIMP

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The phylogenetic analysis of the Ecuadorian IHHNV samples and viral isolates from other shrimp producer countries showed that the Ecuadorian isolates were classified in the infectious IHHNV group and suggesting the presence of different genotypes circulating within the country. On the other hand, variable number of tandem repeat (VNTR) analysis of ORF94 for the WSSV genome also showed high levels of genetic variation with distinct numbers of repeat units (RUs) of VNTRs (Table 1). The comparison of the variable region ORF14/15 showed variable length for the presence of indels in the Ecuadorian samples. We found novel strains of WSSV with a unique insertion in comparison with WSSV isolates from other shrimp producer countries.

# Genotyping of white spot syndrome virus (WSSV) geographical isolates from Brazil and comparison to other isolates from the Americas

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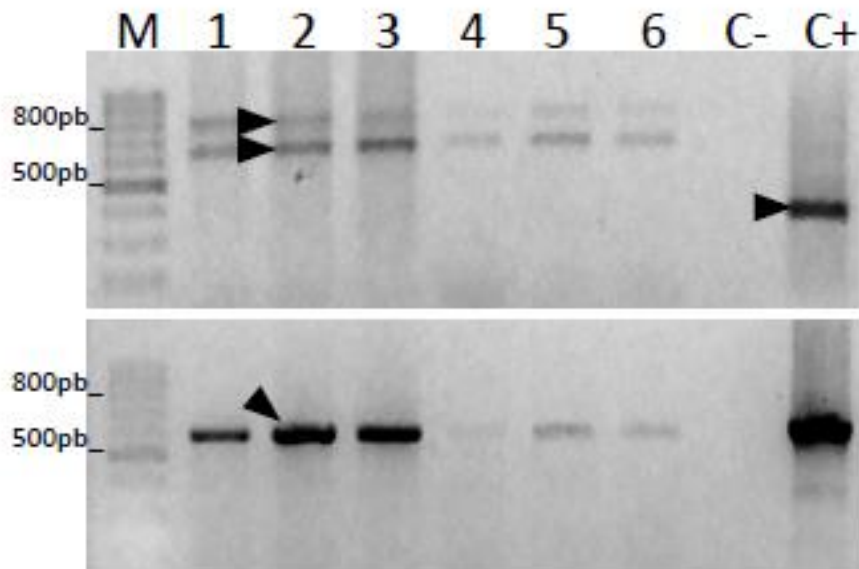
<sup>2</sup>Departamento de Bioquímica, CCB, Universidade Federal de Santa Catarina, Campus Universitário, Trindade, Florianópolis, Santa Catarina 88049-970, Brazil

markers for genotyping. WSSV-infected shrimp *Litopenaeus vannamei* were collected in 2 Brazilian regions (Santa Catarina and Bahia) from 2005 to 2008. DNA was extracted and PCR of the variable regions was performed, followed by sequencing. All Santa Catarina samples showed the same number of repeats for the minisatellites analyzed. Bahia samples showed a different pattern for the regions, indicating that there are at least 2 different WSSV genotypes in Brazil. Both Brazilian isolates have an 11 453 bp deletion in ORF 23/24 when compared with WSSV-TW (Taiwan), which has the full sequence for this locus. The Brazilian WSSV isolates were compared with WSSV isolates from

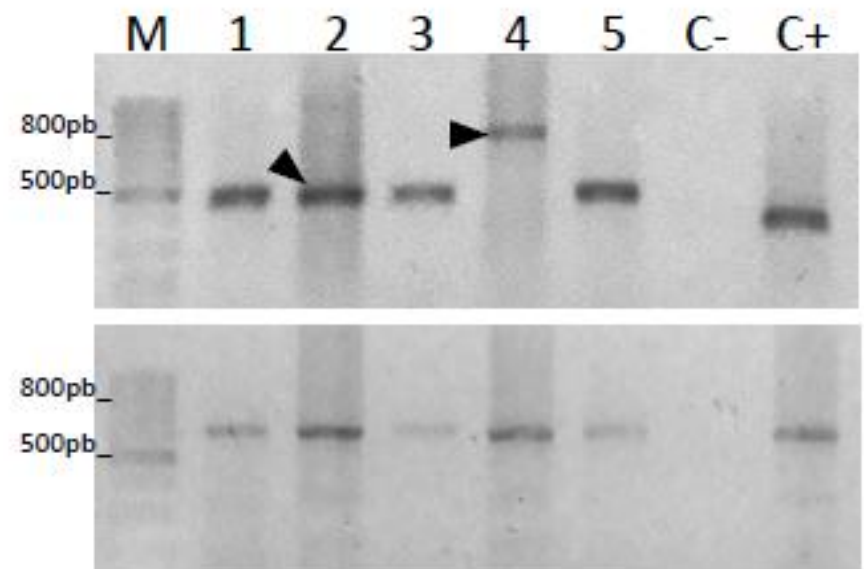
# Variantes virais no estado do RN – WSSV

## Dados preliminares do nosso grupo

### Sul do RN



### Norte do RN

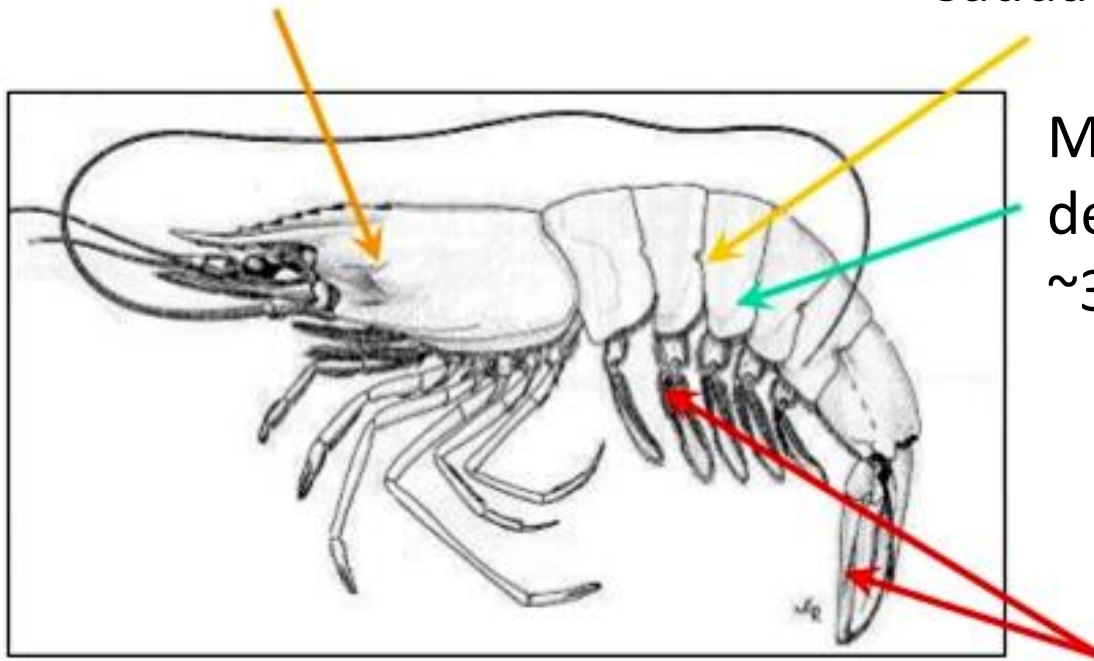


**A variabilidade genética no estado do RN é menor do que a observada em outras regiões do mundo.**

# Número de cópias do WSSV/ $\mu\text{g}$ DNA em um camarão na fase aguda da infecção viral

Cabeça inteira:  $\sim 2.5 \times 10^7$  cópias

Cauda inteira:  $\sim 1.2 \times 10^7$  cópias

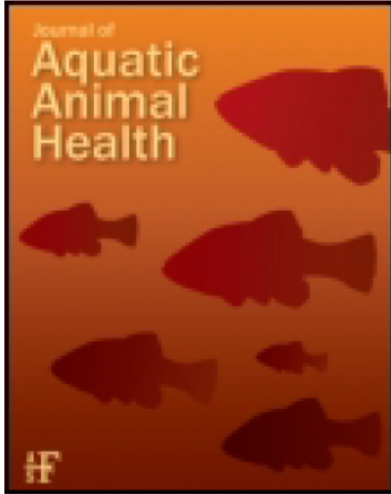


Músculo da cauda  
descascada mais intestino:  
 $\sim 3.4 \times 10^7$  cópias

Casca e pleópodos  $\sim 4.8 \times 10^8$  cópias



# Riscos referentes à importação de camarões.



## Journal of Aquatic Animal Health

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<http://www.tandfonline.com/loi/uahh20>

### Frozen Commodity Shrimp: Potential Avenue for Introduction of White Spot Syndrome Virus and Yellow Head Virus

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# Doença causada por RNA vírus

## CHAPTER 2.2.4.

### TAURA SYNDROME

ssRNA vírus!!!  
Altas taxas de  
mutação!

#### 1. Scope

Taura syndrome (TS) is a virus disease of penaeid shrimp caused by infection with Taura syndrome virus (TSV) (3, 15, 27, 42). The principal host species in which TSV can cause significant disease outbreaks and mortalities are

*Penae*  
pathog  
and ch  
for TSV  
Vertica

#### 2.3.2. Prevalence

In regions where the virus is enzootic in farmed stocks, the prevalence of TSV has been found in various surveys to range from 0 to 100% (5, 24, 25).

#### 2. D

2

#### 2.3.3. Geographical distribution

TS is widely distributed in the shrimp-farming regions of the Americas and South-East Asia (4, 5, 8, 19, 27, 28, 37, 45, 56, 58, 63).

*The Americas:* following its recognition in 1992 as a distinct disease of cultured *P. vannamei* in Ecuador (6, 23), TS spread rapidly throughout many of the shrimp-farming regions of the Americas through shipments of infected PL and broodstock (5, 7, 19, 27, 28). Within the Western Hemisphere, TS and TSV have been reported from virtually every penaeid shrimp-growing region in the Americas and Hawaii (1, 5, 53). TSV is enzootic in cultured penaeid shrimp stocks on the Pacific coast of the Americas from Peru to Mexico, and it has been occasionally found in some wild stocks of *P. vannamei* from the same region (31, 34). TSV has also been reported in farmed penaeid stocks from the Atlantic, Caribbean, and Gulf of Mexico coasts of the Americas, but it has not been reported in wild stocks from these regions (19, 27, 28, 30).

*Asia:* TSV was introduced into Chinese Taipei in 1999 with infected imported Pacific white shrimp, *P. vannamei*, from Central and South American sources (59, 60). Since that initial introduction, the disease

## **Mecanismos identificados para a transferência potencial de vírus em produtos congelados importados para populações domésticas de estoques de camarão cultivado ou selvagem**

- A liberação de resíduos líquidos ou sólidos não tratados de plantas de importação e processamento de camarão diretamente para as águas costeiras,
- Eliminação inadequada de resíduos sólidos de camarão Importando e processando em aterros, nos quais o lixo está acessível para gaivotas e outras aves marinhas,
- O uso de camarão importado como isca por pescadores esportivos.

# Efeito do cozimento e/ou do congelamento na viabilidade do WSSV



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## WHITE SPOT SYNDROME VIRUS (WSSV) TRANSMISSION RISK THROUGH INFECTED COOKED SHRIMP PRODUCTS ASSESSED BY POLYMERASE CHAIN REACTION (PCR) AND BIO-INOCULATION STUDIES

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primers IK1 & IK2 – IK3 & IK4 from the cooked shrimp samples. The cooked shrimps, which gave positive results for WSSV by PCR, were further confirmed for the viability of WSSV by conducting the bio-inoculation studies. Mortality (100%) was observed within 123 h of intra-muscular post injection (P.I) into the live healthy WSSV-free shrimps (*P. monodon*). These results show that the WSSV survive the cooking process and even infected cooked shrimp products may pose a transmission risk for WSSV to the native shrimp farming systems.

# Efeito do cozimento e/ou do congelamento na viabilidade do WSSV

Journal of Fish Diseases 2006, 29, 569–572

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Short communication

## **Studies on the inactivation of white spot syndrome virus of shrimp by physical and chemical treatments, and seaweed extracts tested in marine and freshwater animal models**

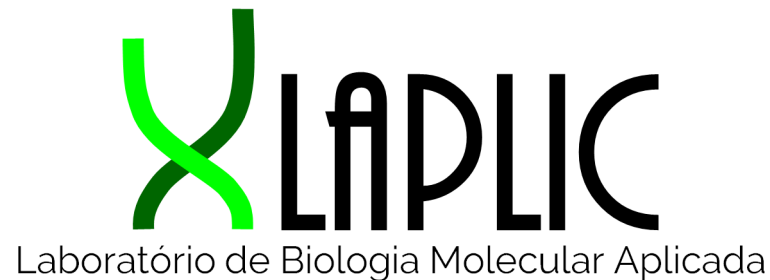
**G Balasubramanian, R Sudhakaran, S Syed Musthaq, M Sarathi and A S Sahul Hameed**

Aquaculture Biotechnology Division, Department of Zoology, C. Abdul Hakeem College, Melvisharam, Tamil Nadu, India

**Table 1** Effect of various physical treatments tested on white spot syndrome virus infectivity in *Penaeus indicus* and *Paratetaphusa hydrodomous*

Physical treatment	Number of shrimp dead <sup>a</sup> / number of shrimp examined	
	<i>P. indicus</i>	<i>P. hydrodomous</i>
Temperature		
RT (28 °C)	20/20	20/20
50 °C/20 min	20/20	20/20
50 °C/30 min	20/20	18/20
60 °C/10 min	20/20	18/20
60 °C/20 min	0/20	0/20
70 °C/10 min	0/20	0/20
UV irradiation exposure time (min) and dosage ( $\mu\text{W s cm}^{-2}$ )		
0	20/20	20/20
5 ( $7.6 \times 10^4$ )	20/20	20/20
10 ( $1.5 \times 10^5$ )	20/20	16/20
20 ( $3.1 \times 10^5$ )	0/20	0/20
30 ( $4.6 \times 10^5$ )	0/20	0/20
pH		
12	0/20	0/20
7	20/20	20/20
3	20/20	18/20
1	20/20	0/20

<sup>a</sup> Dead shrimp were white spot syndrome virus-positive by PCR and Western blot.



**Obrigado.**

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