Caracterização de variantes virais que acometem a carcinicultura

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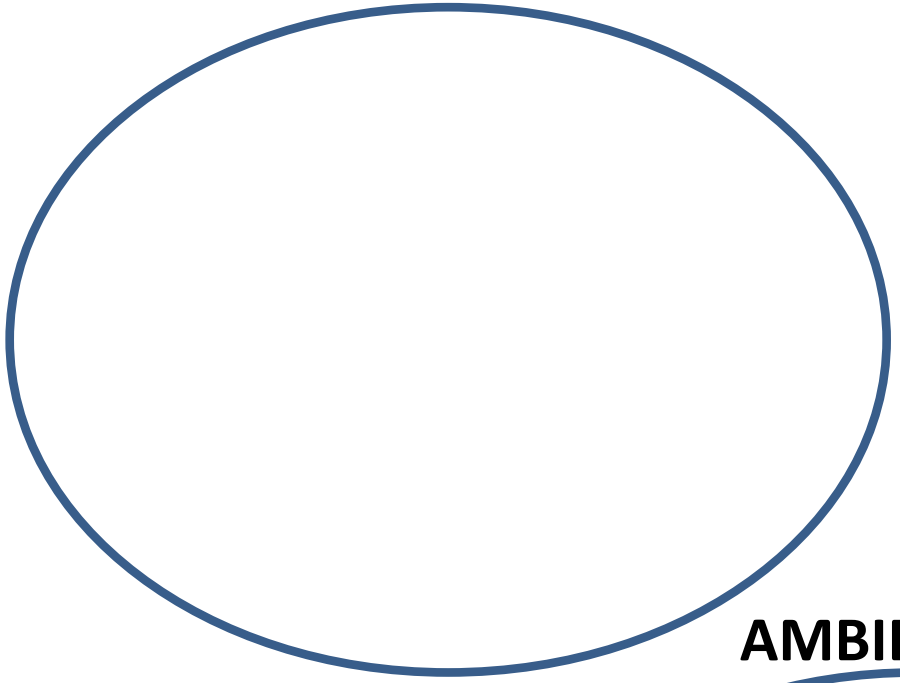
AMBIENTE - 1



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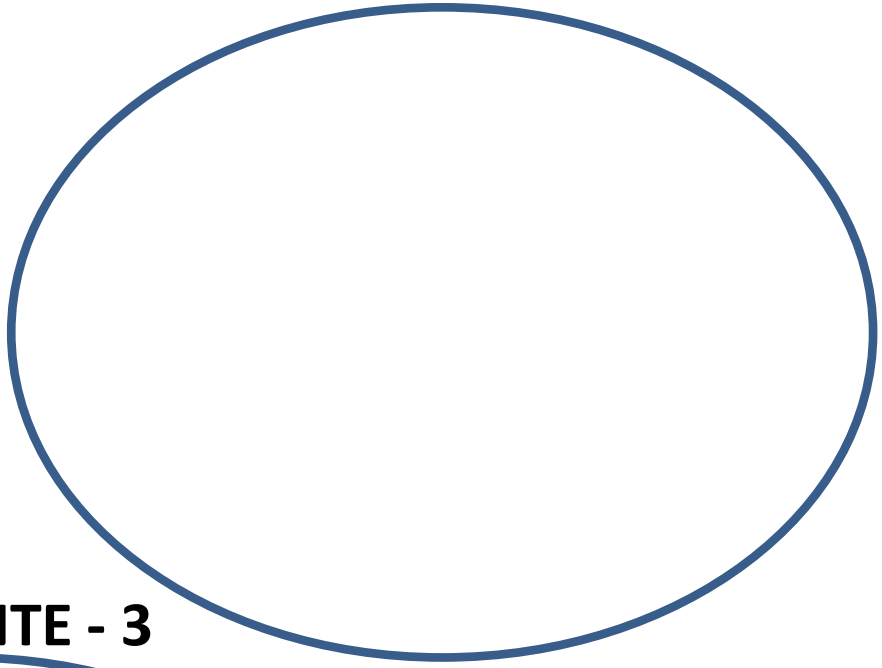

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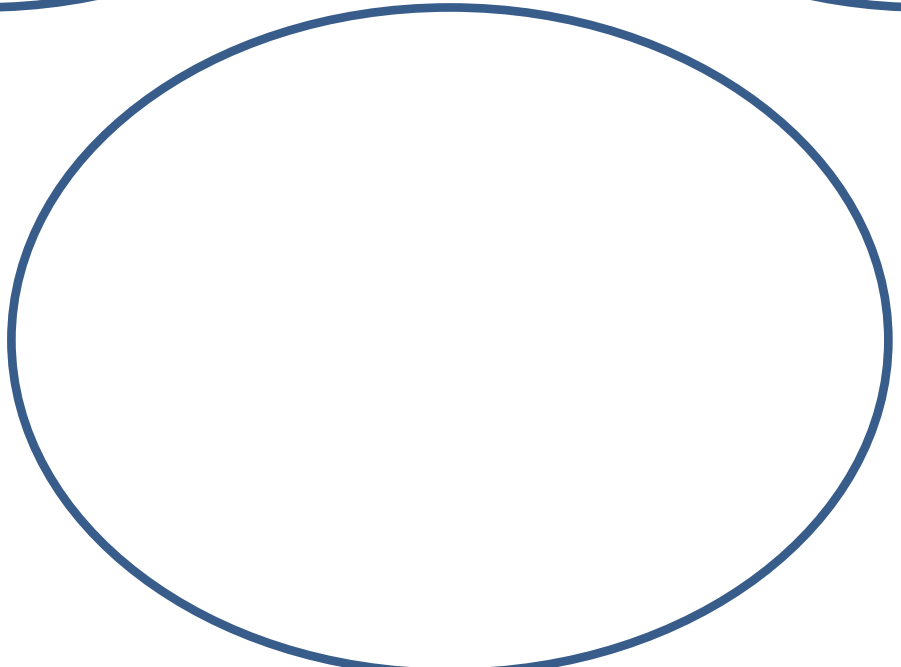

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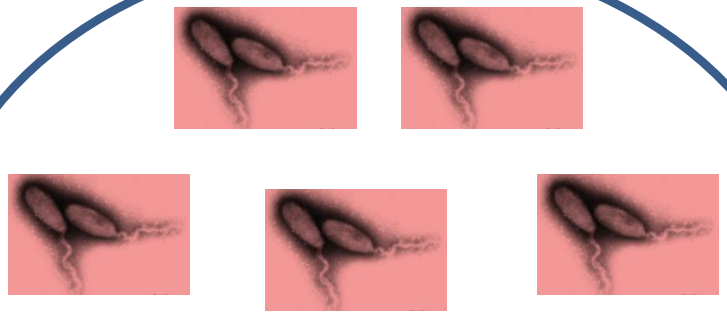

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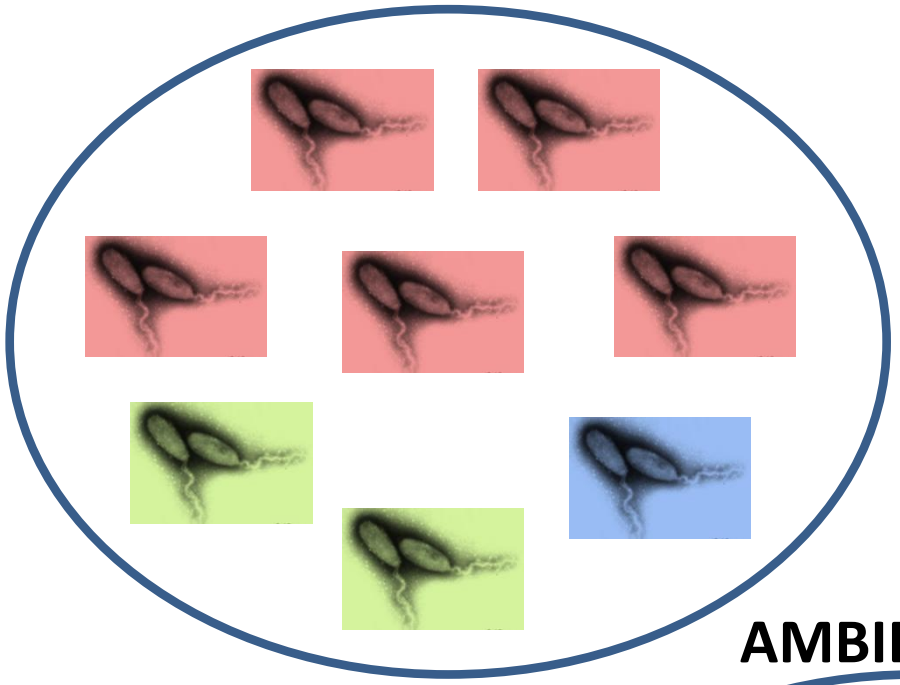

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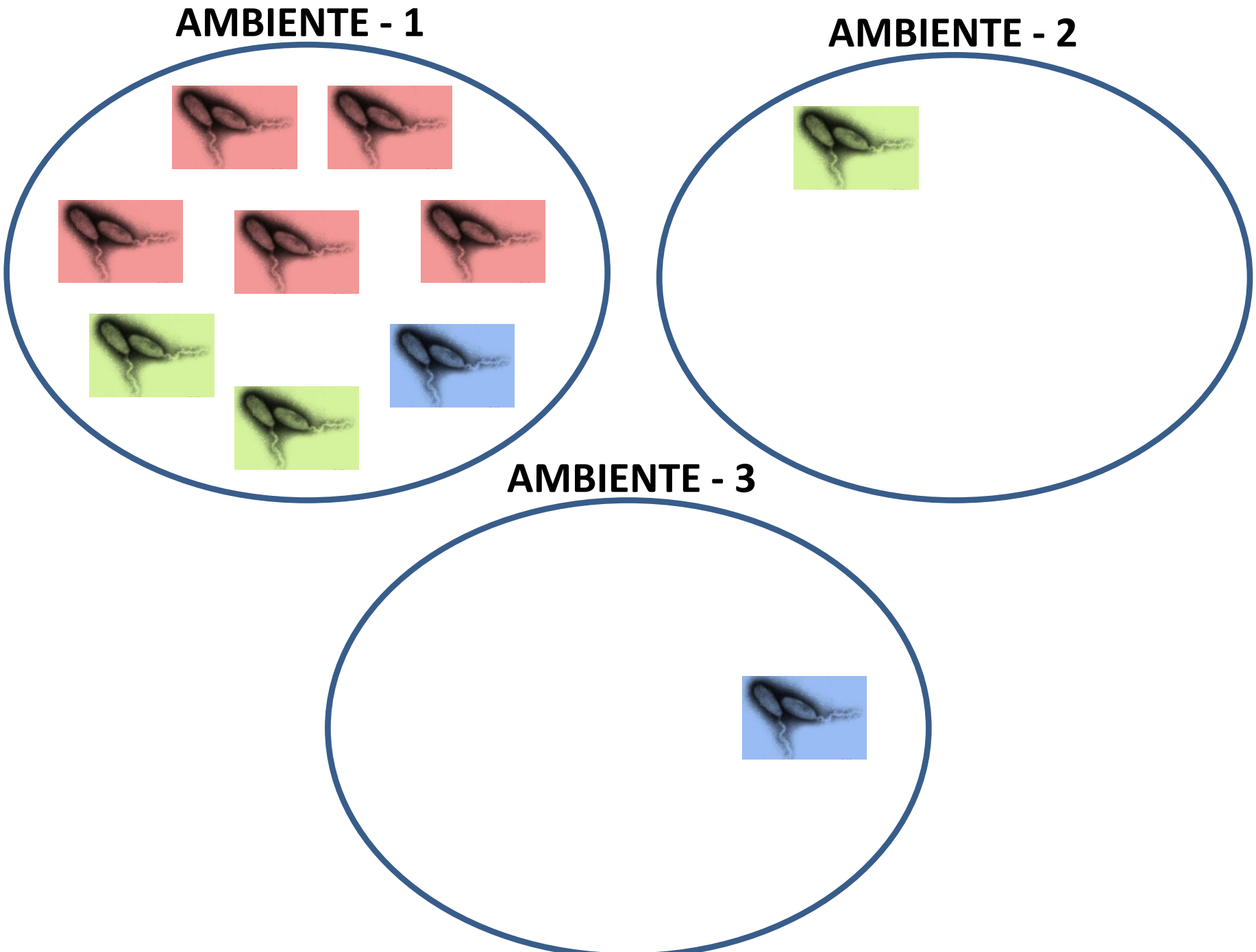
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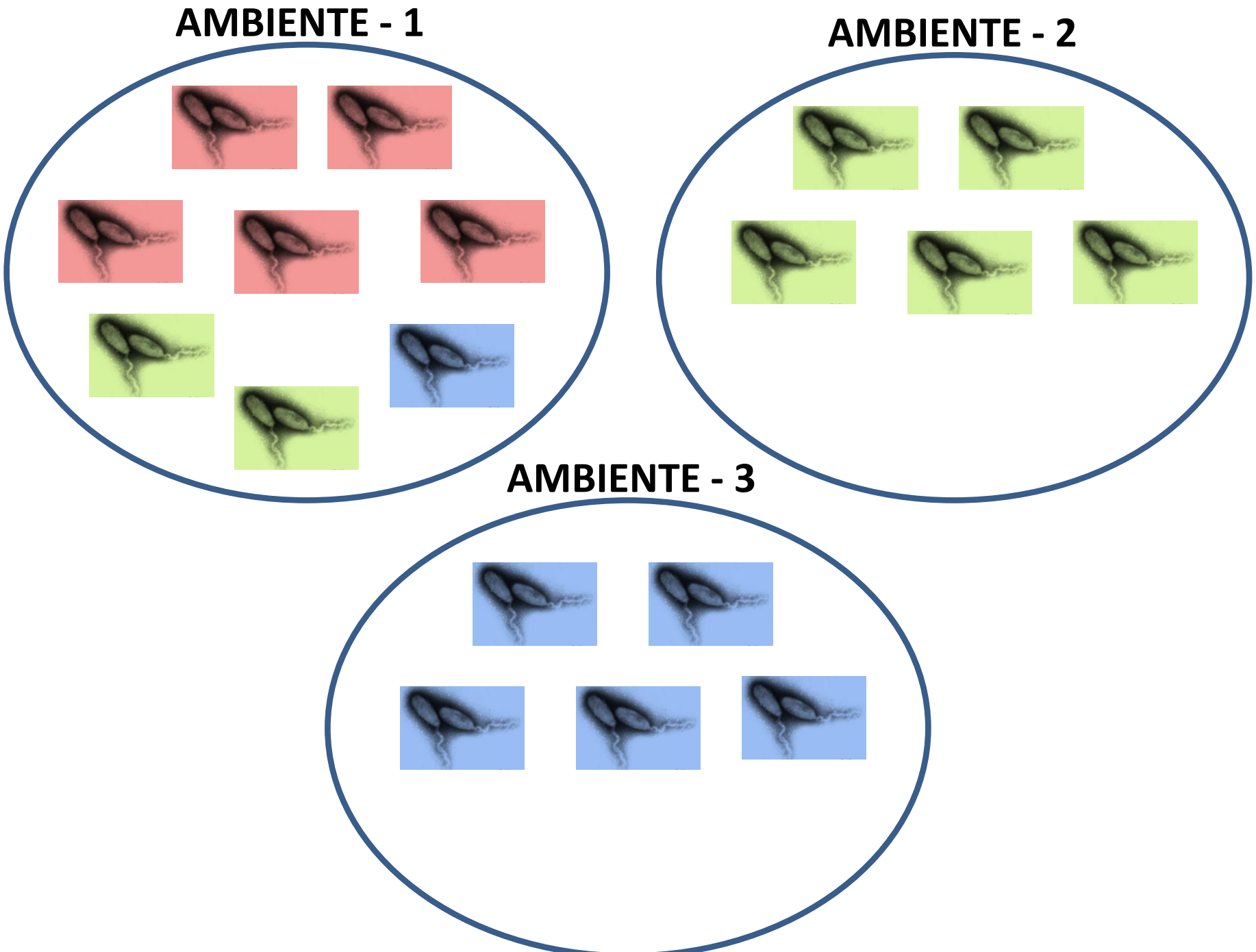
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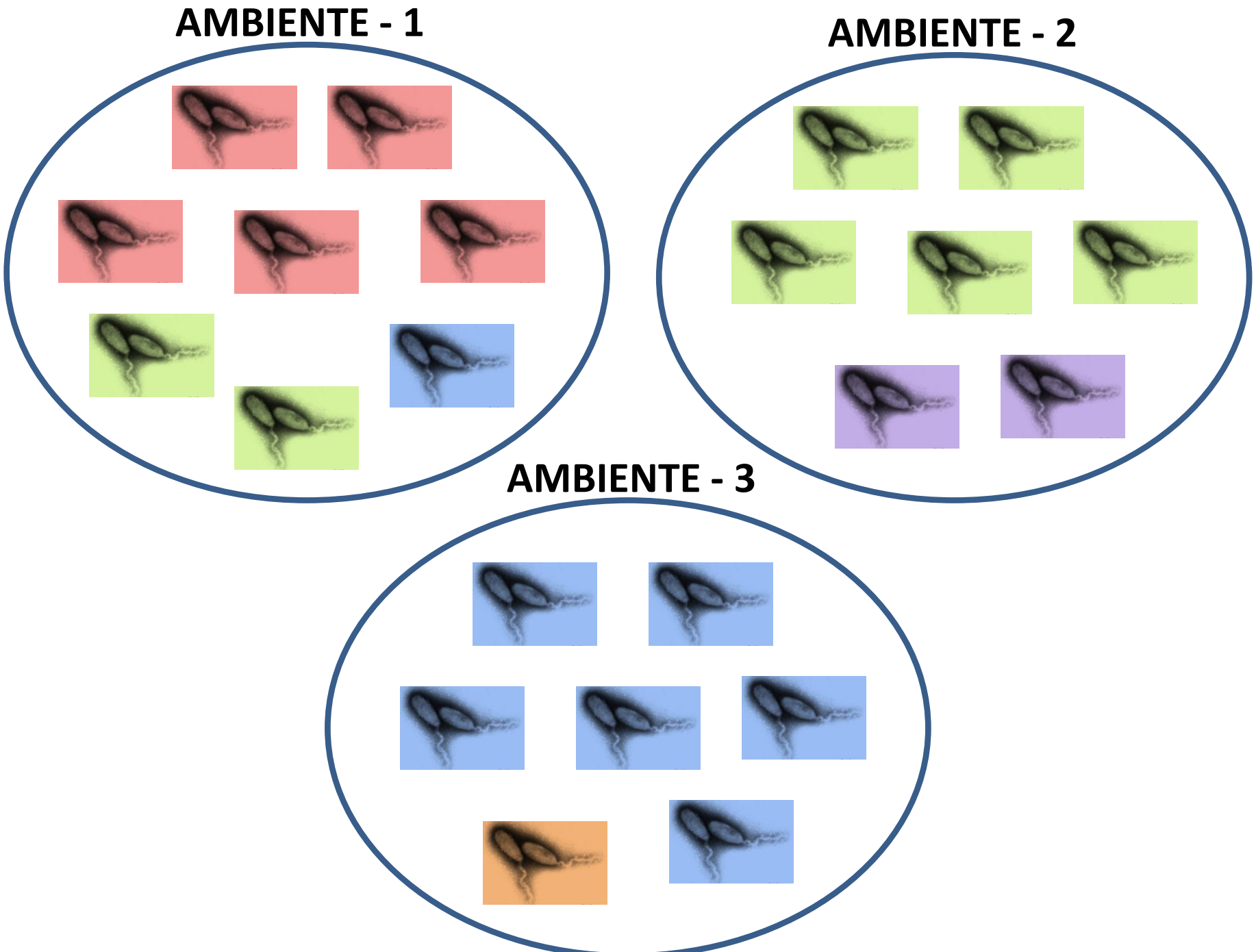
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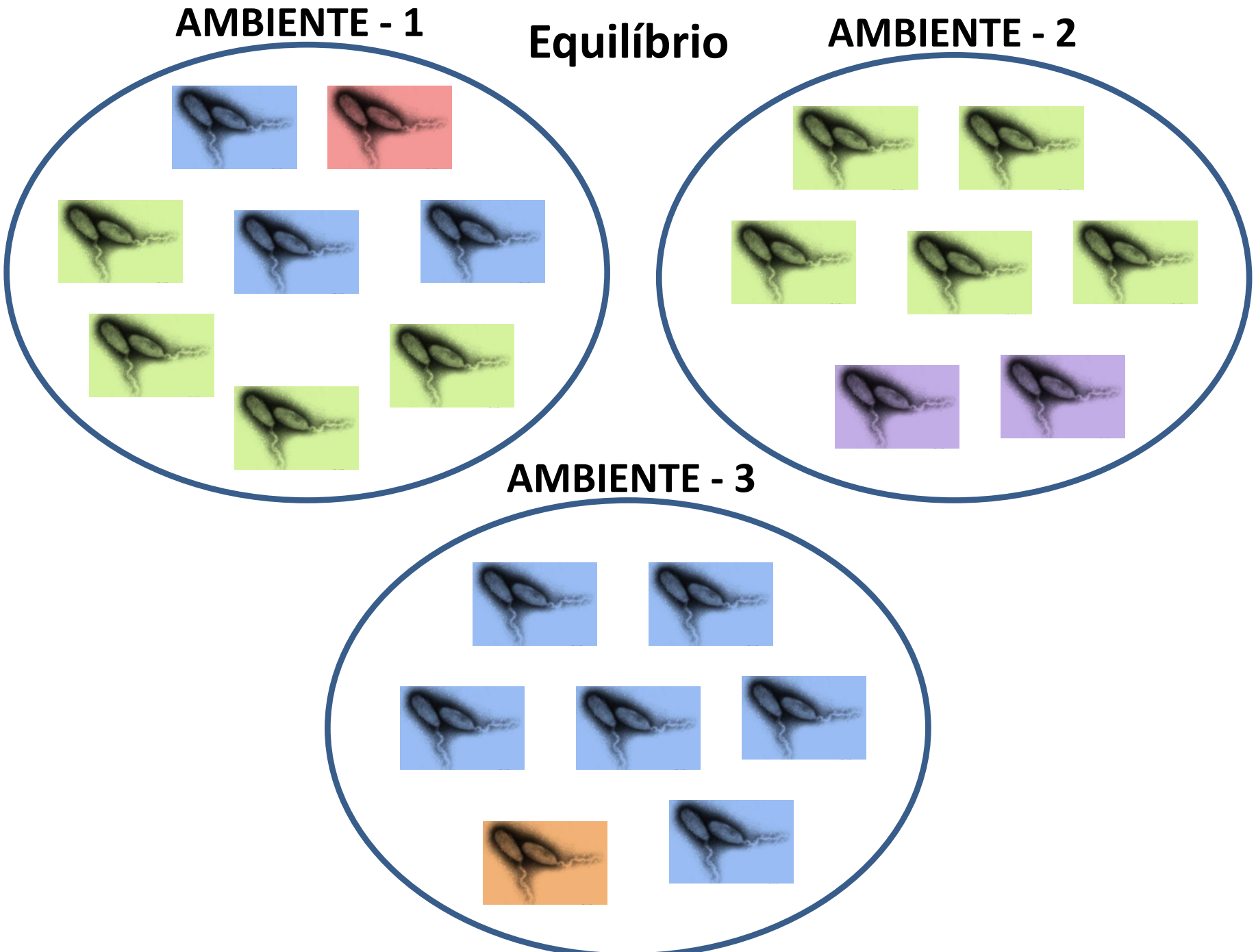
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Fitness and virulence of an ancestral White Spot Syndrome Virus isolate from shrimp

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Received 13 November 2004; received in revised form 29 December 2004; accepted 10 January 2005
Available online 5 February 2005

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_{50}) 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.

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

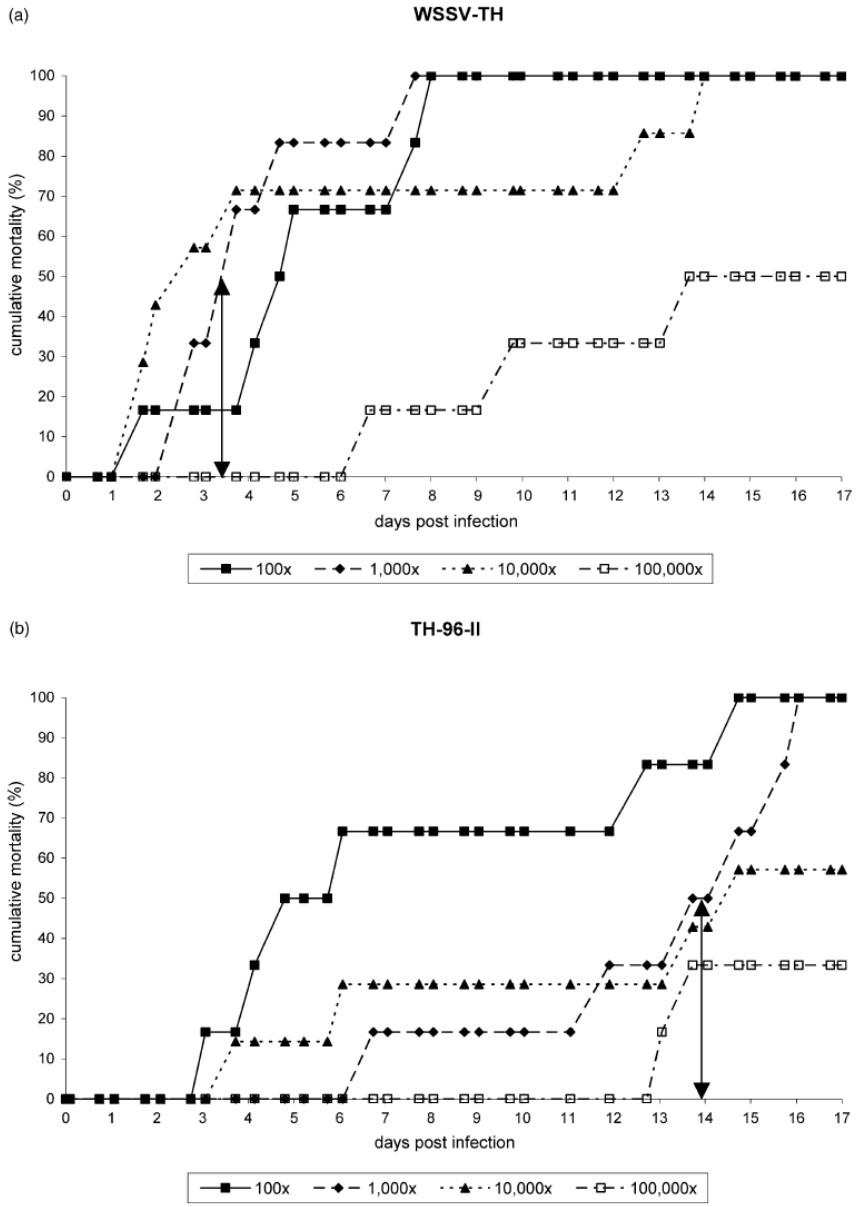

Fig. 3. Cumulative mortality rates of *P. monodon* by injection of WSSV using dilutions of (a) WSSV-TH and (b) TH-96-II. Cumulative mortality rates of shrimp from groups infected with different virus concentrations are plotted against the days after infection. The double sided arrows indicate the LT_{50} values for the 1000× dilutions.

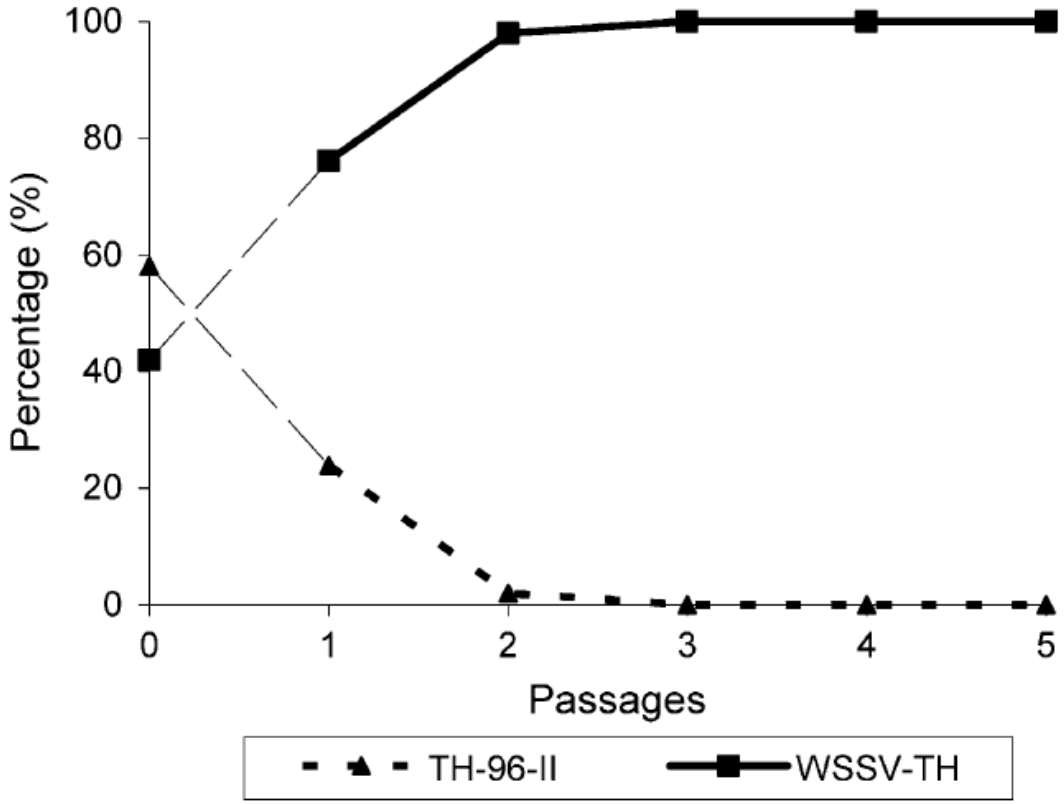

Fig. 4. Relative amount (%) of WSSV-TH DNA and TH-96-II DNA present during five consecutive passages of *P. monodon* injected with a 1:1 mix of both isolates (group C).

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Short Communication

Mixed-genotype white spot syndrome virus infections of shrimp are inversely correlated with disease outbreaks in ponds

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Outbreaks of white spot syndrome virus (WSSV) in shrimp culture and the relationship between the virus and virulence are not well understood. Here, we provide evidence showing that WSSV mixed-genotype infections correlate with lower outbreak incidence and that disease outbreaks correlate with single-genotype infections. We tested 573 shrimp samples from 81 shrimp ponds in the Mekong delta with outbreak or non-outbreak status. The variable number tandem repeat (VNTR) loci of WSSV were used as molecular markers for the characterization of single- and mixed-genotype infections. The overall prevalence of mixed-genotype WSSV infections was 25.7 %. Non-outbreak ponds had a significantly higher frequency of mixed-genotype infections than outbreak ponds for all VNTR loci, both at the individual shrimp as well as at the pond level. The genetic composition of WSSV populations appears to correlate with the health status of shrimp culture in ponds. The causal relationship between genotypic diversity and disease outbreaks can now be experimentally approached.

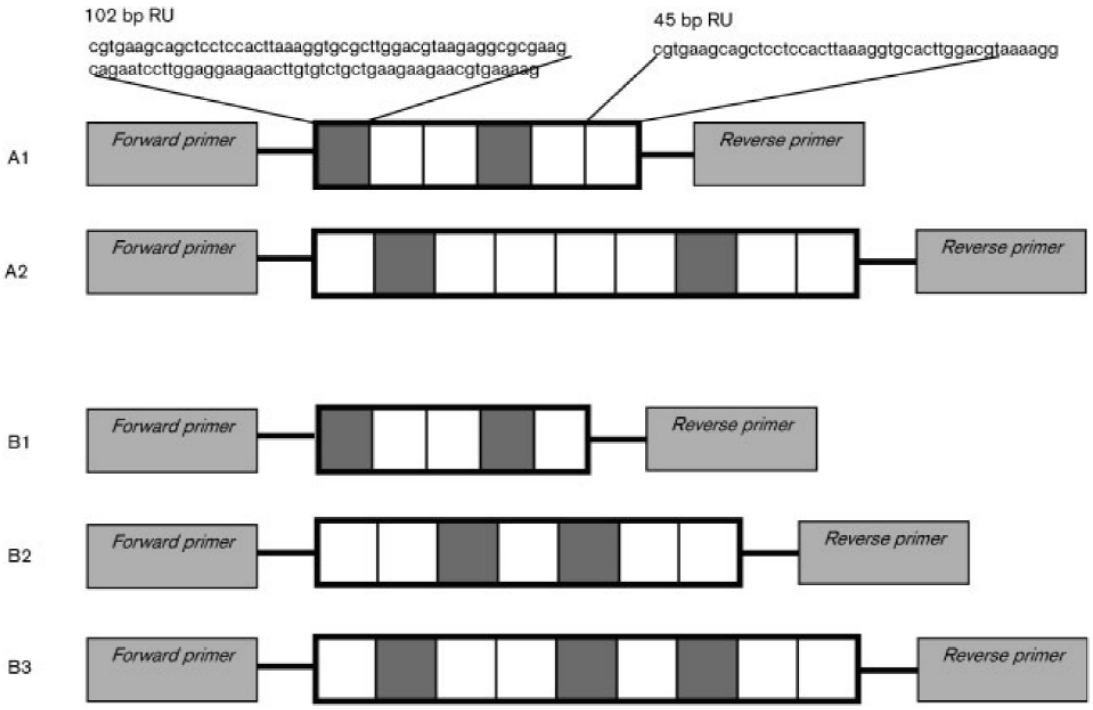

Fig. 2. Schematic representation of differences in the number of compound RUs among clones A1 and A2 (derived from PCR product of sample A), and clones B1, B2 and B3 (derived from PCR product of sample B) in ORF75. Plasmid clones of samples A and B, containing inserts of the correct size, were subjected to sequencing using universal T7 and/or Sp6 primers.

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Induced resistance to white spot syndrome virus infection in *Penaeus stylirostris* through pre-infection with infectious hypodermal and hematopoietic necrosis virus—a preliminary study

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Department of Veterinary Science and Microbiology, University of Arizona, Building 90 P.O. Box 210090, Tucson, AZ 85721 0090, USA

Received 25 June 2002

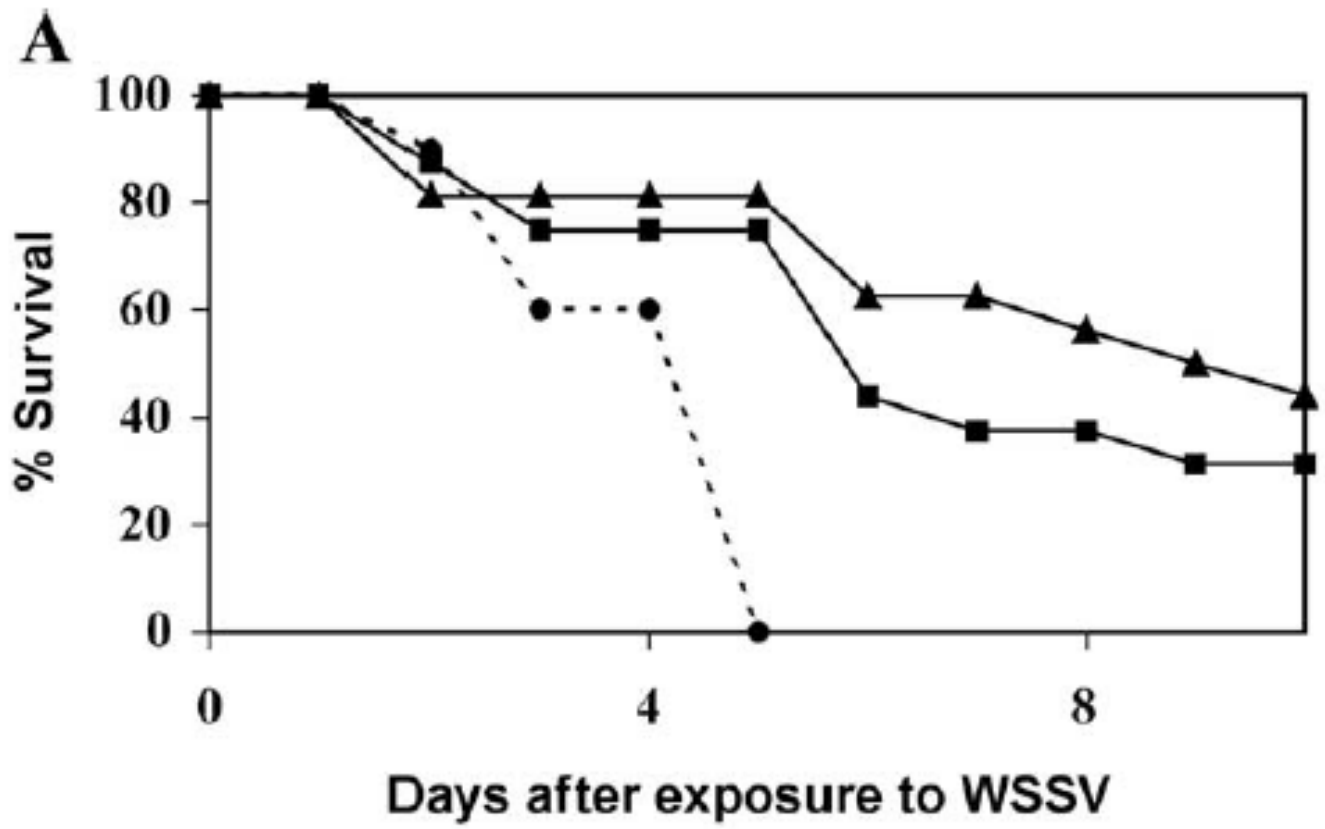

Fig. 1. Survival of *P. stylirostris* after exposure to WSSV. (A) *P. stylirostris* (a cross produced by High Health Aquaculture) in bioassay #1. WSSV tissue control tank (●): shrimp were not infected with IHHNV prior exposure to WSSV, replicate tanks of IHHNV pre-infected *P. stylirostris* (■) and (▲); (B) *P. stylirostris* (a cross

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NOTE

Viral interference between infectious hypodermal and hematopoietic necrosis virus and white spot syndrome virus in *Litopenaeus vannamei*

Valérie Bonnichon[1], Donald V. Lightner[2], Jean-Robert Bonami [1,*]

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[2]Department of Veterinary Science and Microbiology, University of Arizona, 1117 E. Lowell, Tucson, Arizona 85721, USA

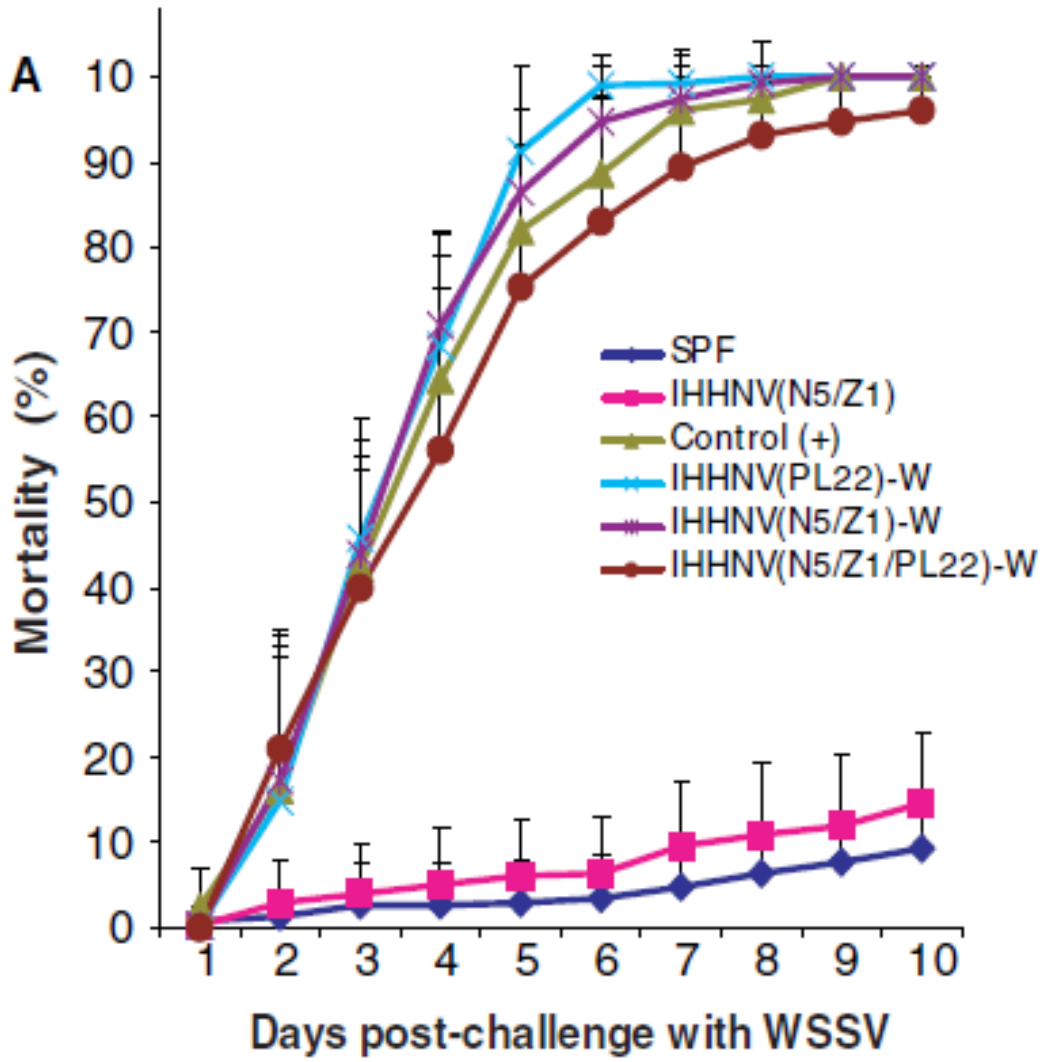
Table 1. *Litopenaeus vannamei*. Mortalities and mean (±SD) time to death (d) following WSSV challenge, either alone (positive control) or following primary infection and incubation with IHHNV. Mortality was determined using the total number of moribund and dead shrimp every day

Group	Daily mortality					Time to death
	Day 1	Day 2	Day 3	Day 4	Day 5	
Positive control	0	8	12	–	–	2.6 (± 0.5)
IHHNV Day 0	0	3	9	8	–	3.3 (± 0.7)
IHHNV Day 10	1	2	17	-	–	2.8 (± 0.5)
IHHNV Day 20	1	4	6	9	–	3.2 (± 0.9)
IHHNV Day 30	0	2	4	6	8	4.0 (± 1.0)
IHHNV Day 40	0	1	1	7	11	4.4 (± 0.8)
IHHNV Day 50	0	1	3	12	4	4.0 (± 0.7)

Pre-exposure to infectious hypodermal and haematopoietic necrosis virus or to inactivated white spot syndrome virus (WSSV) confers protection against WSSV in *Penaeus vannamei* (Boone) post-larvae

J Melena[1,4], B Bayot[1], I Betancourt[1], Y Amano[2], F Panchana[1], V Alday[3], J Calderón[1], S Stern[1], Ph Roch[4] and J-R Bonami[4]

1 Fundación CENAIM-ESPOL, Guayaquil, Ecuador
2 Instituto Nacional de Higiene, Leopoldo Izquieta Pérez, Guayaquil, Ecuador
3 INVE TECHNOLOGIES nv, Dendermonde, Belgium
4 Pathogens and Immunity, EcoLag, Université Montpellier 2, Montpellier cedex 5, France


A
Mortality (%)
0
10
20
30
40
50
60
70
80
90
10
1
2
3
4
5
6
7
8
9
10
Days post-challenge with WSSV
SPF
IHHNV(N5/Z1)
Control (+)
IHHNV(PL22)-W
IHHNV(N5/Z1)-W
IHHNV(N5/Z1/PL22)-W

PLOS | GENETICS

REVIEW

Exploiting Genetic Interference for Antiviral Therapy

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* leor.weinberger@gladstone.ucsf.edu

Abstract

Rapidly evolving viruses are a major threat to human health. Such viruses are often highly pathogenic (e.g., influenza virus, HIV, Ebola virus) and routinely circumvent therapeutic intervention through mutational escape. Error-prone genome replication generates heterogeneous viral populations that rapidly adapt to new selection pressures, leading to resistance that emerges with treatment. However, population heterogeneity bears a cost: when multiple viral variants replicate within a cell, they can potentially interfere with each other, lowering viral fitness. This genetic interference can be exploited for antiviral strategies, either by taking advantage of a virus's inherent genetic diversity or through generating de novo interference by engineering a competing genome. Here, we discuss two such antiviral strategies, dominant drug targeting and therapeutic interfering particles. Both strategies harness the power of genetic interference to surmount two particularly vexing obstacles—the evolution of drug resistance and targeting therapy to high-risk populations—both of which impede treatment in resource-poor settings.

Citation: Tanner EJ, Kirkegaard KA, Weinberger LS

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Effect of Associated Vaccines on the Interference between Newcastle Disease Virus and Infectious Bronchitis Virus in Broilers

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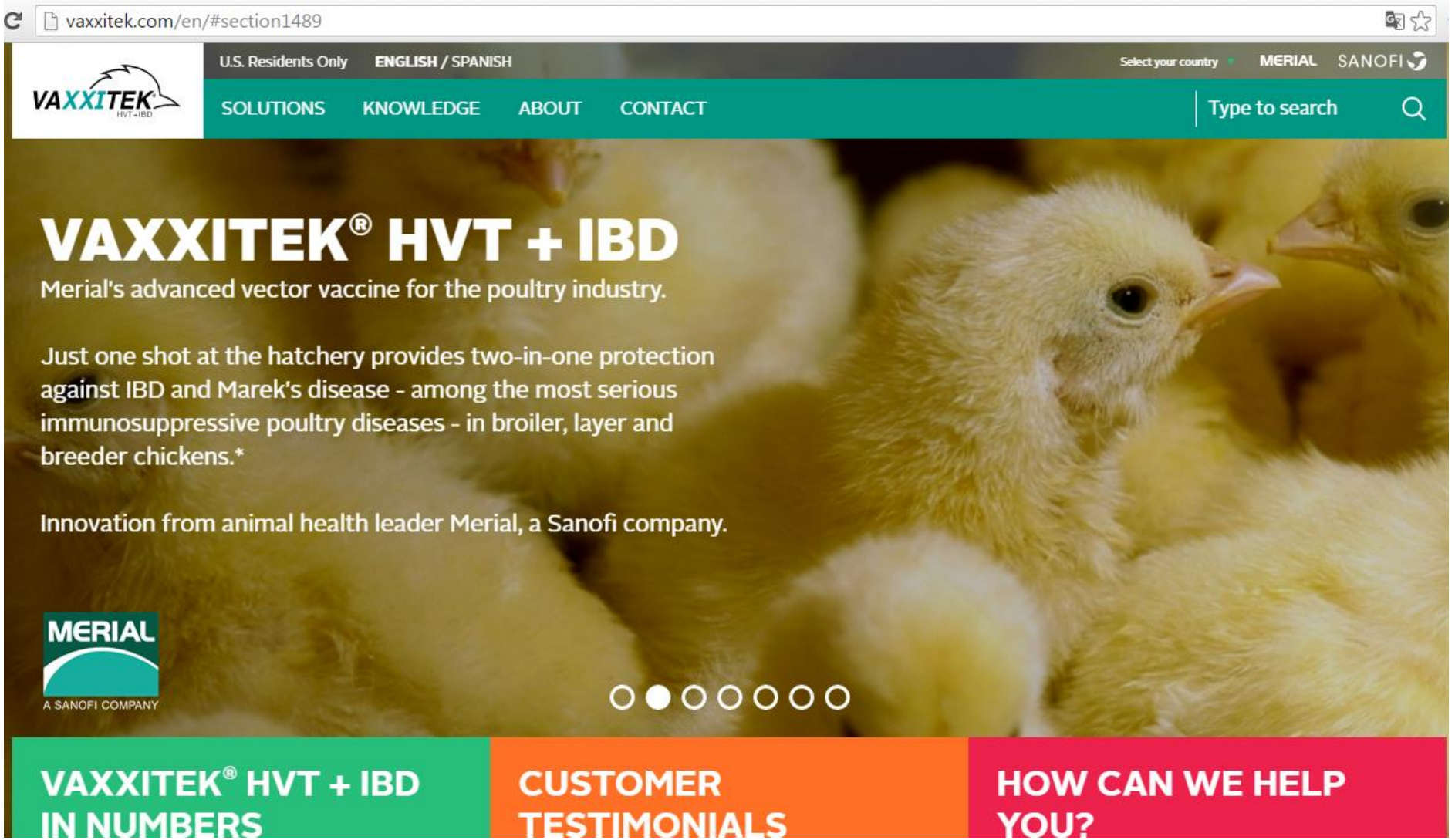
ABSTRACT

The phenomenon of viral interference between live vaccines against Newcastle Disease and infectious bronchitis has been reported since the 50's and many researchers have reported its prejudicial effects on avian immunization. Therefore, this study evaluated the effect of associated vaccines on the interference between Newcastle disease virus (NDV) and infectious bronchitis virus (IBV) in broilers. There were 400 broiler chicks divided into five groups. The groups were submitted to mono or polyvalent vaccinations against IBV and NDV, except for the non-vaccinated control group (CG). Sera were collected at 35 and 45 days of age and submitted to serologic tests to assess antibody levels. It was observed the occurrence of interference in the immune response against NDV by the use of associated vaccines to NDV and IBV; however, the group that was immunized with commercial combined vaccines (IBV+NDV) presented antibody titers to NDV similar to the group that was given only vaccine against NDV. We concluded based on these preliminary studies that the interference of IBV on the immune response against NDV depends also whether the association between the two vaccines is done just before vaccination or in the manufacturing laboratory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HOW CAN WE HELP YOU?

A equipe do **Laboratório de Biologia Molecular Aplicada - LAPLIC** se dedica ao estudo de genomas de vírus que acometem a carcinicultura. A partir dos nossos estudos desenvolvemos ferramentas para detecção, classificação e controle desses patógenos.

1. Penaeus stylirostris densovirus - *Pst*DNV
2. Infectious Myonecrosis Virus - IMNV
3. White Spot Sindrome Virus - WSSV

Contents lists available at ScienceDirect

Virus Research

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

Douglas C.D. Silva[a], Allan R.D. Nunes[a], Dárlio I.A. Teixeira[c], João Paulo M.S. Lima[b,d], Daniel C.F. Lanza[a,*]

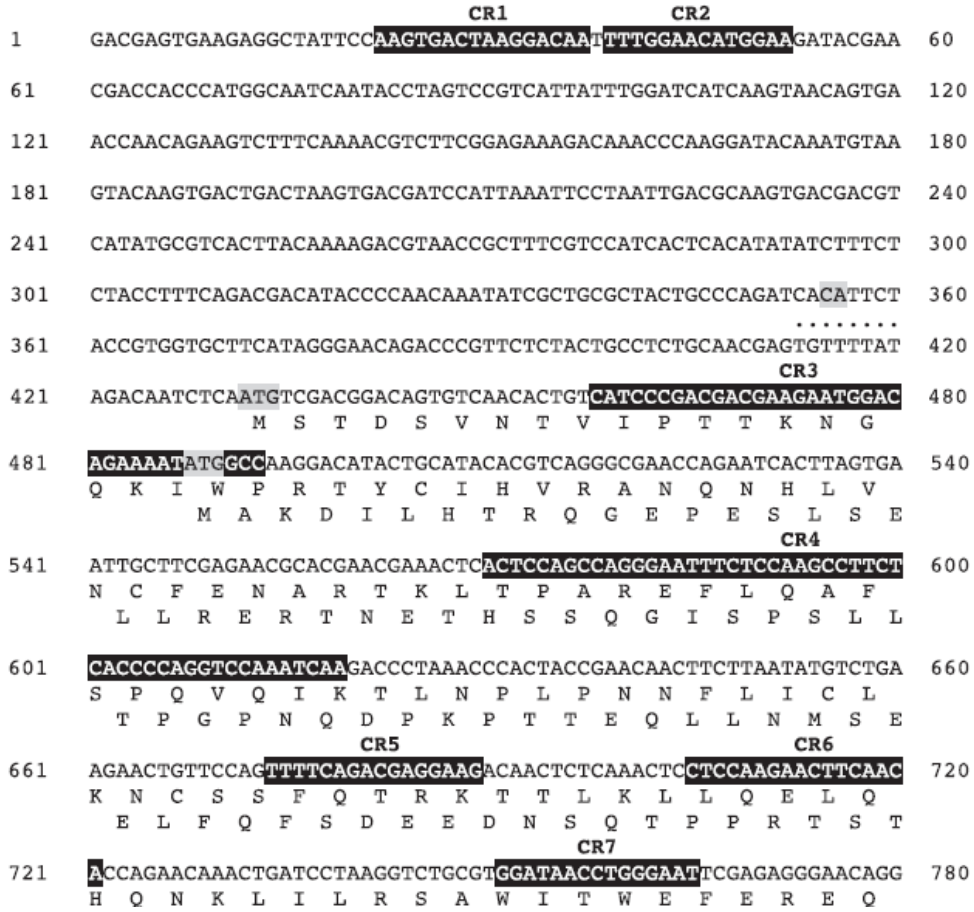


Table 2
Comparison of nucleotide and predicted amino acid sequences of Brazilian IHHNV and other IHHNV sequences available in GenBank.

Country/isolate	GenBank no	Genome identity (%)	Nucleotide identity (%)			Amino acid identity (%)		
			ORF1	ORF2	ORF3	ORF1	ORF2	ORF3
Hawaii[a]	AF218266	99.7	99.7	99.7	99.7	99.6	99.5	99.4
Taiwan A	AY355306	99.6	99.8	99.6	99.7	99.4	99.2	99.7
Taiwan C	AY355308	99.6	99.8	99.6	99.6	99.4	99.2	99.4
Ecuador	AY362548	99.6	99.8	99.7	99.4	99.4	99.5	98.8
China[a]	EF633688	99.5	99.6	99.5	99.7	99.3	99.2	99.7
México	AF273215	99.4	99.6	99.6	99.3	99.1	99.5	98.5
China[a]	JX258653	99.1	99.5	99.5	99.5	99.1	98.9	99.1
South Korea[a]	JN377975	99.3	99.4	99.4	99.3	99	98.9	98.5
China[a]	KF214742	99.3	99.3	99.1	99.1	98.4	97.8	98.5
Vietnam[a]	JX840067	98.6	99.4	99.1	99.2	98.4	98.6	98.2
Vietnam	KC513422	95.8	96.7	97.3	95.5	96.3	95.9	96.7
Thailand	AY362547	95.6	97	97.6	94.9	96.7	97	96.7
Taiwan B	AY355307	95.6	96.7	97.4	95.2	96.6	96.4	97
Thailand	AY102034	95.7	96.6	97.3	95.3	96.3	95.9	97
Vietnam	JN616415	95.6	94.6	96.8	97.2	84.2	88.6	98.8
Vietnam[a]	KF031144	95.6	96.3	96.8	95.2	95.7	95.3	96.7
Austrália	GQ475529	95.3	95.1	97.5	95.5	97	96.2	97.3
Índia[a]	GQ411199	93.5	96.2	97	93.5	87.6	95.3	95.8
East Africa	AY124937	91.3	92.5	–	91.1	91.8	–	96.7
Madagascar	DQ228358	86.0	87.0	–	87.3	84.3	–	93.3
Austrália	EU675312	85.7	87.5	89.3	87.2	69.5	86	93

[a] Complete genomes.

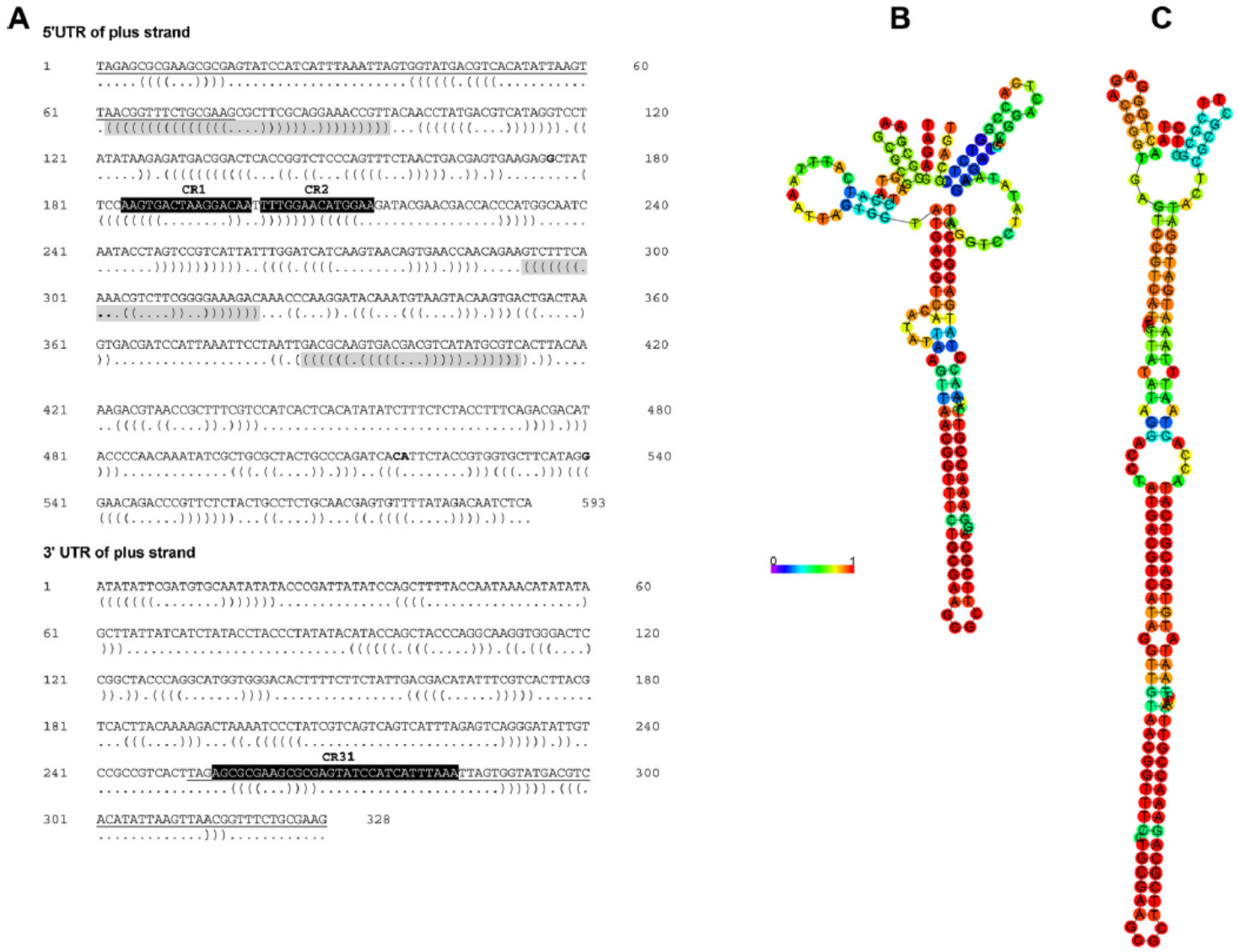


Fig. 3. Sequences of UTR regions, secondary structure bracket representation and minimum free energy (MFE) models of 5′ UTR hairpin. 5′ and 3′ UTR sequences of Hawaii IHHNV isolate (GenBank Accession No. AF218266.2) were analyzed using the software RNAfold. (A) The parentheses below the nucleotide sequences indicate base pairing and dots indicate unpaired regions in DNA secondary structure. Hairpins with the highest probability of occurrence are highlighted in gray and conserved regions are highlighted in black. The 77 nt sequence that is repeated in the 5′ and 3′ ends are underlined. (B) MFE model calculated using the first 154 nucleotides that correspond to 5′end of the plus strand of Hawaii isolate. (C) MFE model calculated using the correspondent 154 nucleotide sequence from minus strand of Hawaii isolate. The structures B and C are colored according to base-pairing probabilities. Red color denotes the high probability and purple denotes low probability of a given base is paired or not. For unpaired regions the color denotes the probability of being unpaired. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

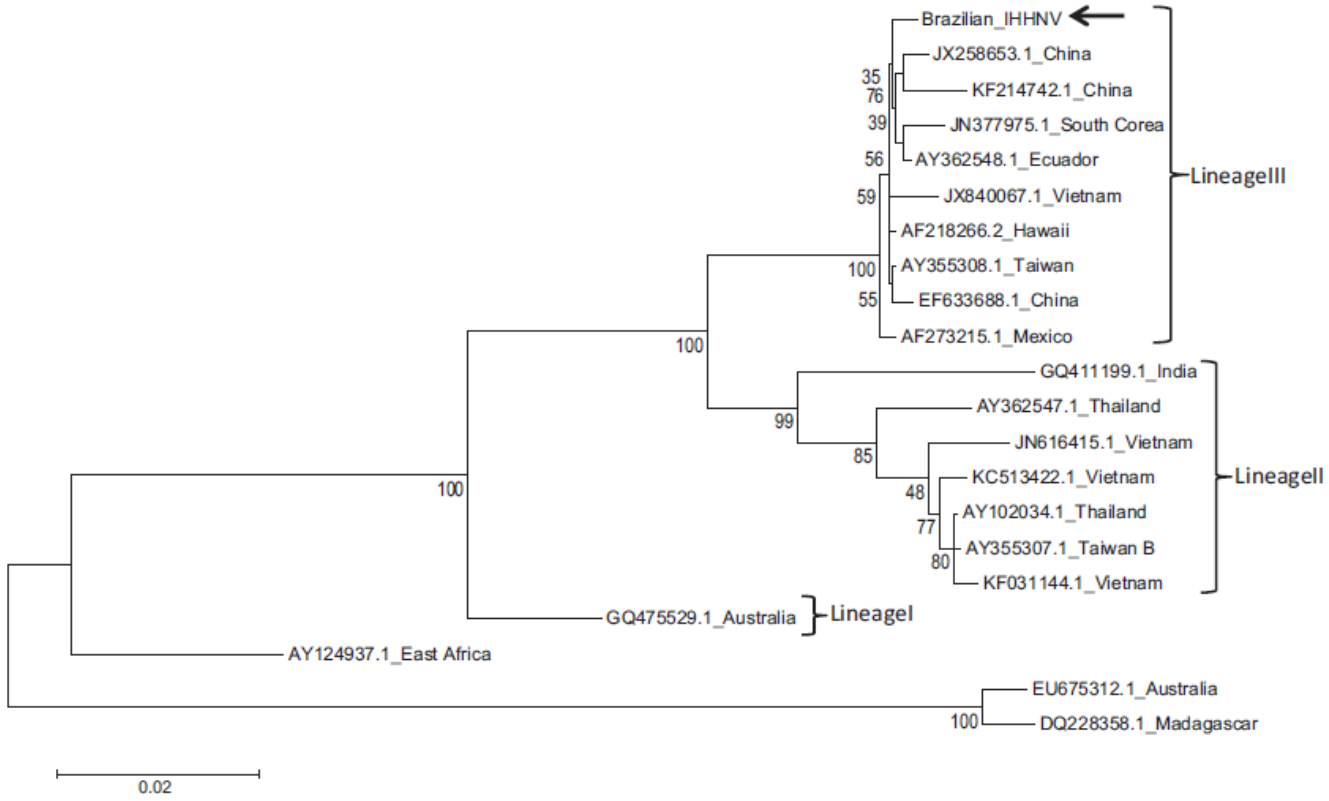


Fig. 4. Pylogenetic tree of IHHNV strains based on partial genomes comprising the ORF1, ORF2 and ORF3 coding regions. The phylogenetic relationship was estimated by maximum likelihood method using MEGA program. The infectious IHHNV strains were divided into three lineages, and the Brazilian IHHNV is clustered in lineage III (black arrow). Numbers indicate the percentages of bootstrap support from 1000 replicates.

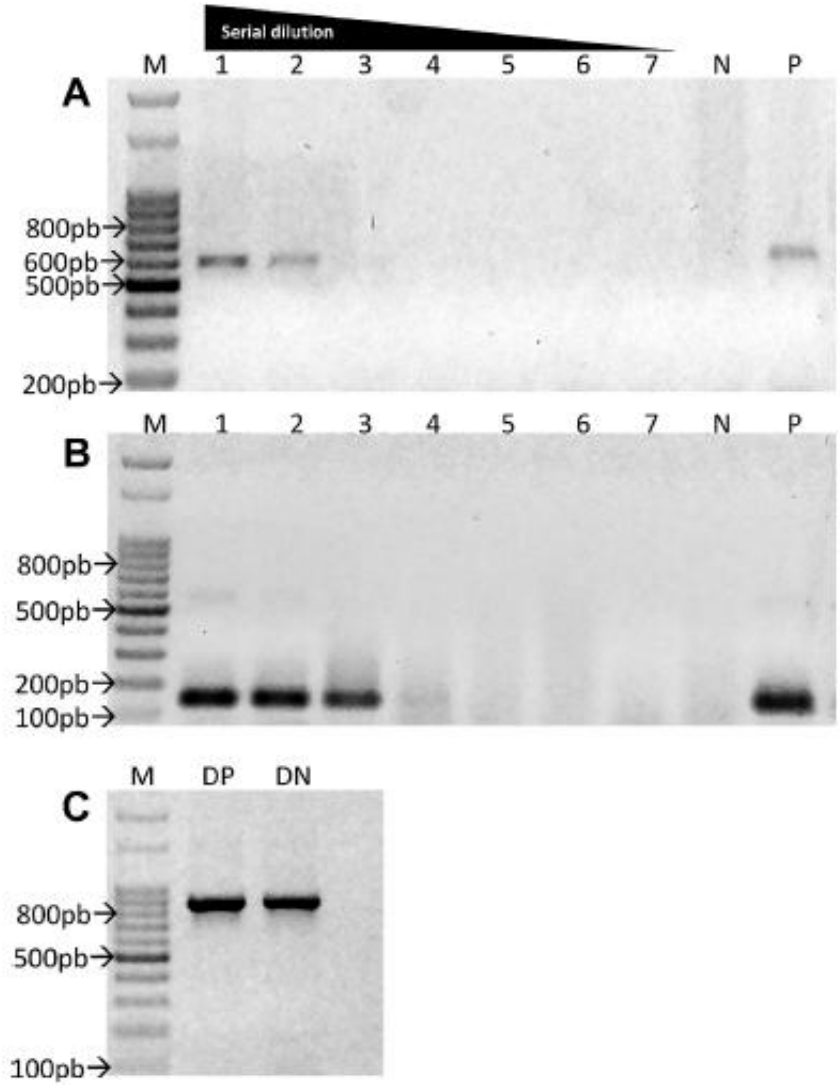


Fig. 5. Semi-nested PCR to IHHNV detection. Primers PF3/PR3 were used in the first PCR and PF3/PR2 in the nested PCR. (A) First PCR step, using the DNA templates submitted to a serial dilution by a factor of 5. Lane 1, reaction with undiluted template; lanes 2–7, reactions using templates submitted to a progressive serial dilution by a factor of 5 (lane 2 = lowest dilution; lane 7 = highest dilution). The expected amplicon of first step has 588 bp. (B) Nested reactions using first step reaction products as templates. Lane 1, nested PCR using the product of reaction 1 of first step as template; lanes 2–7, nested PCRs using the products of reactions 2–7 of first step as templates, respectively. The 153 bp fragment indicates the positive result. M, DNA ladder; N, negative control; P, positive control. (C) DNA quality control using decapod specific primers. DP, DNA shrimp sample used as positive control; DN, DNA shrimp sample used as negative control.

Table 3
Some primers used to IHHNV identification available so far.

Primer name	Primer sequence 5′- 3′[a]	References
IHHNV392F	GGGCGAACCAGAATCACTTA	Tang et al. (2000)
IHHNV392R	ATCCGGAGGAATCTGATGTG	
77012F	ATCGGTGCACTACTCGGA	OIE (2000)
77353R	TCGTACTGGCTGTTCATC	
389F	CGGAACACAACCCGACTTTA	OIE (2003)
389R	GGCCAAGACCAAAATACGAA	
IHHNV309F	TCCAACACTTAGTCAAAACCAA	Tang et al. (2007)
IHHNV309R	TGTCTGCTACGATGATTATCCA	
IHHNV648F	GAACGGCTTTCGTATTTTGG	Rai et al. (2009)
IHHNV648R	AGCGTAGGACTTGCCGATTA	
IHHNVF	ATGTGCGCCGATTCAACAAG	Tang and Lightner (2002)
IHHNVR1	CTAAGTGACGGCGGACAATA	
IHHNV721F	CTACTGCCTCTGCAACGAG	Tang and Lightner (2002)
IHHNV2860R	GTGGGTCTGGTCCACTTGAT	
IHHN3065F	GACGACGAAGAATGGACAGA	Tang et al. (2003)
IHHN3065R	TGCCTGGGTAGCTGGTATGTATA	
77012F	ATCGGTGCACTACTGGGA	Nunan et al. (2000)
2553R	CGGACAATATCCCTGACT	
I2814F	TAATGAAGACGAAGAACACGCCGAAGG	Yang et al. (2007)
I3516R	TGGGTAGACTAGGTTTCCAAGGGATGGTT	

[a] Underlined bases indicate the polymorphic sites.

Contents lists available at ScienceDirect

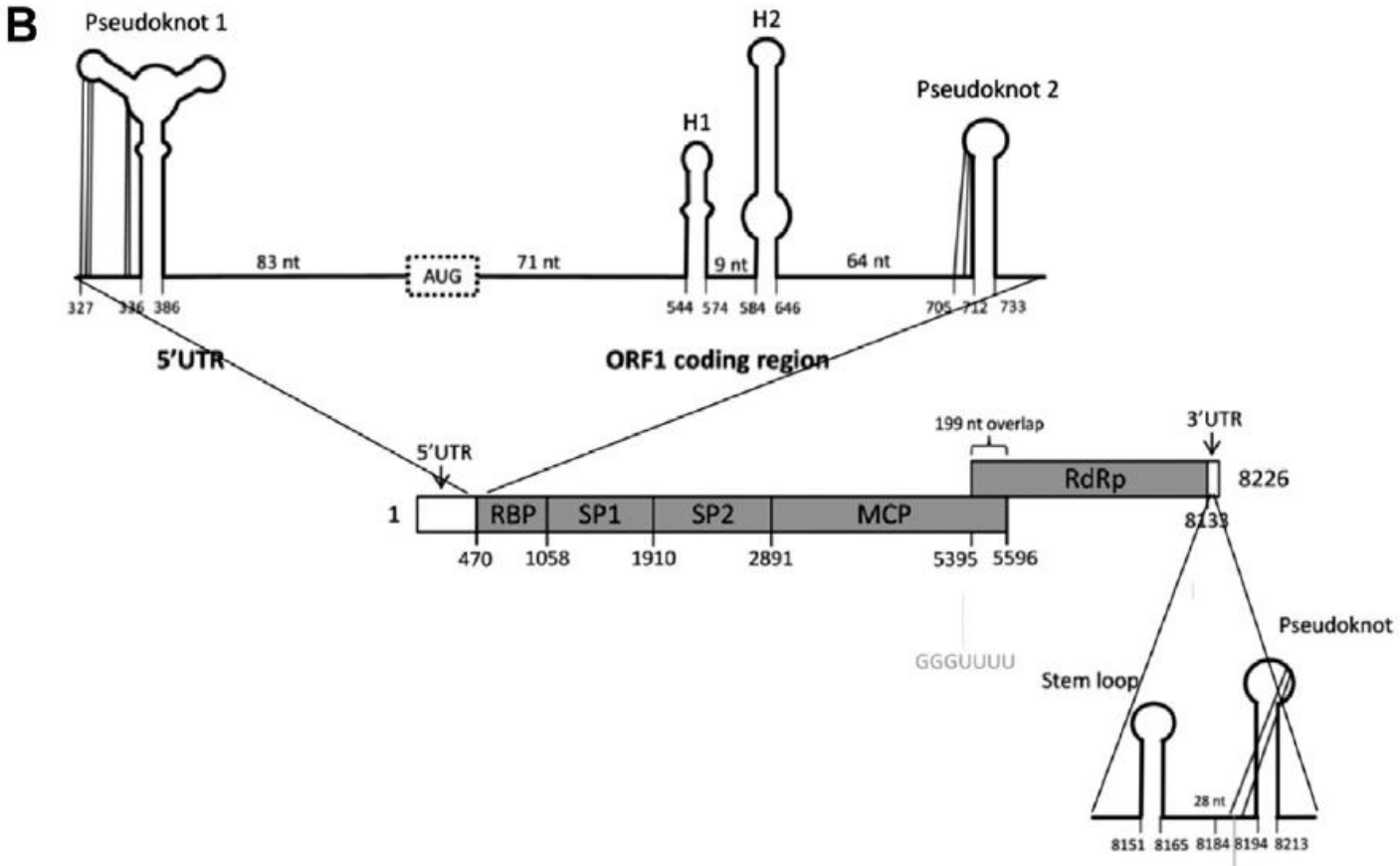
Virus Research

journal homepage: www.elsevier.com/locate/virusres

Short communication

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

Márcia Danielle A. Dantas[a,b], Suely F. Chavante[b], Dárlio Inácio A. Teixeira[c], João Paulo M.S. Lima[b,d], Daniel C.F. Lanza[a,b,*]



Contents lists available at ScienceDirect

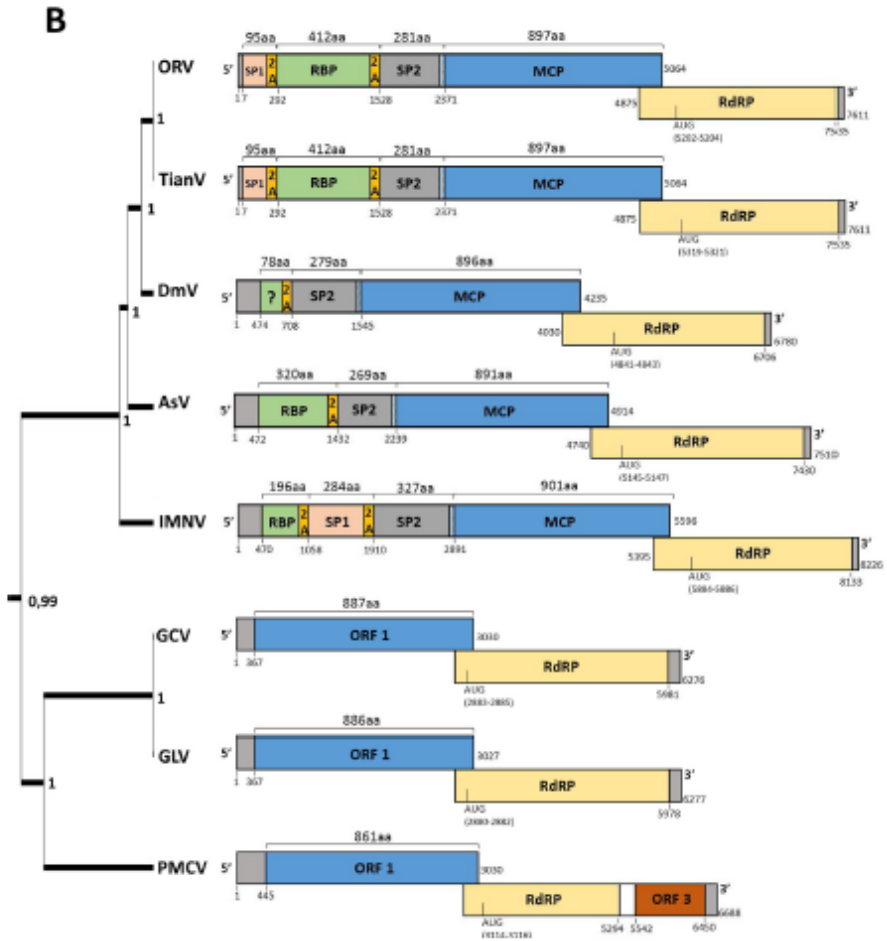
Virus Research

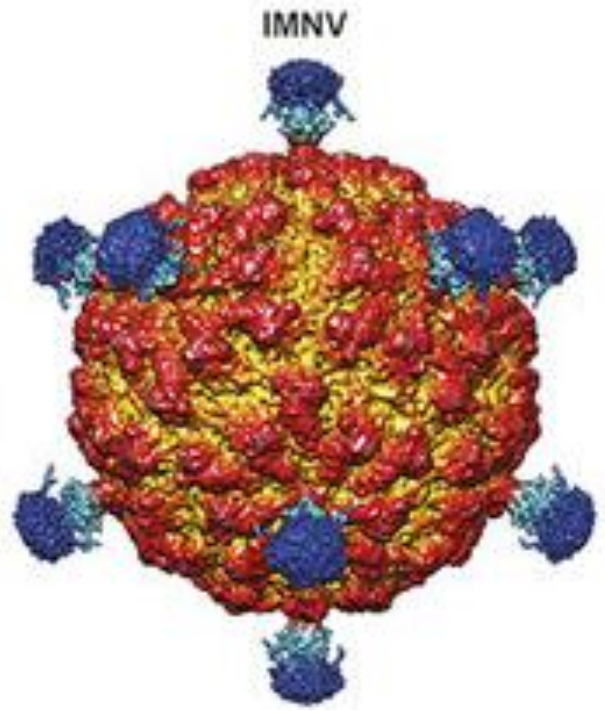
journal homepage: www.elsevier.com/locate/virusres

Short communication

New insights about ORF1 coding regions support the proposition of a new genus comprising arthropod viruses in the family *Totiviridae*

Márcia Danielle A. Dantas[a,b,1], Gustavo Henrique O. Cavalcante[a,1], Raffael A.C. Oliveira[b], Daniel C.F. Lanza[a,b,*]





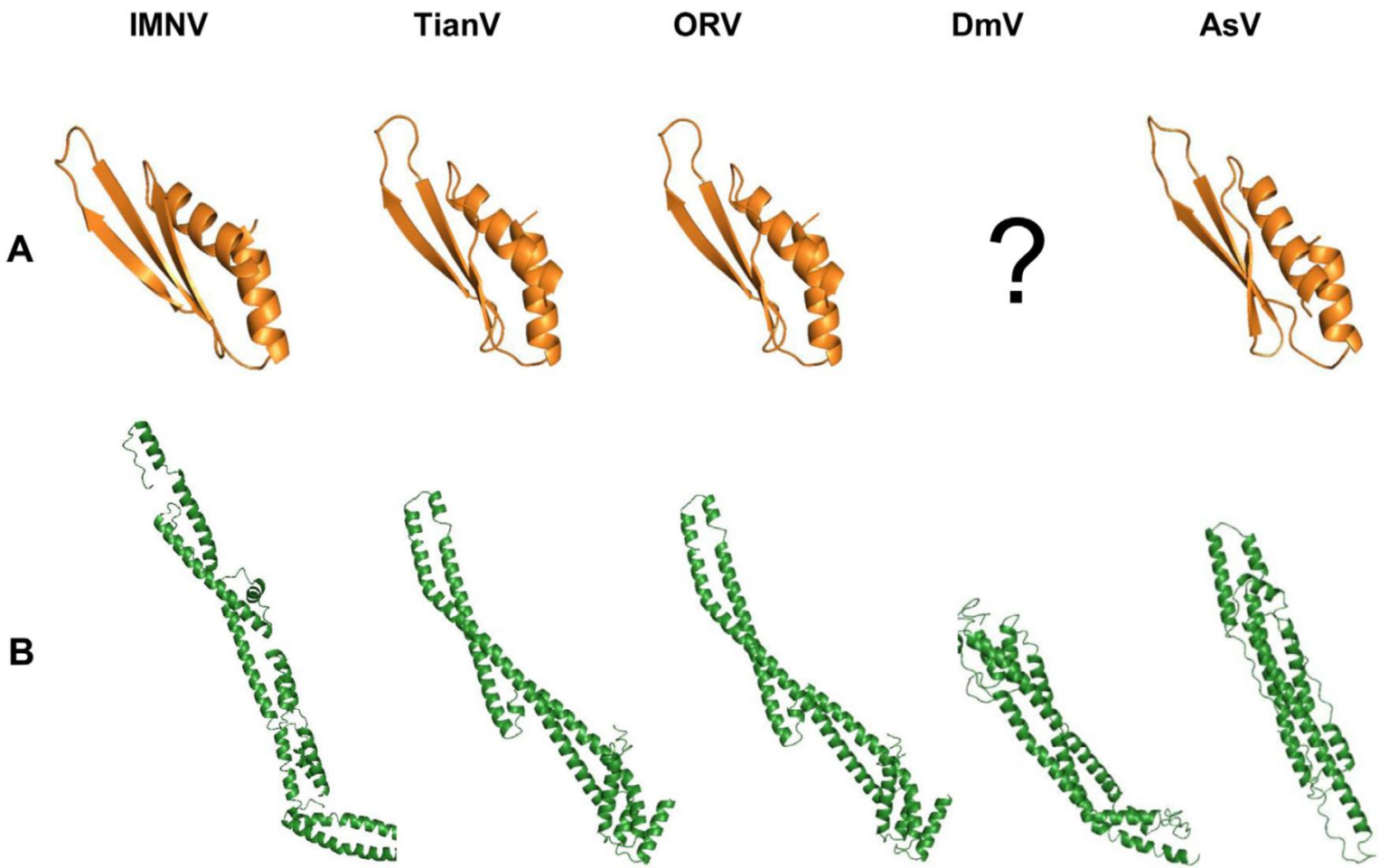
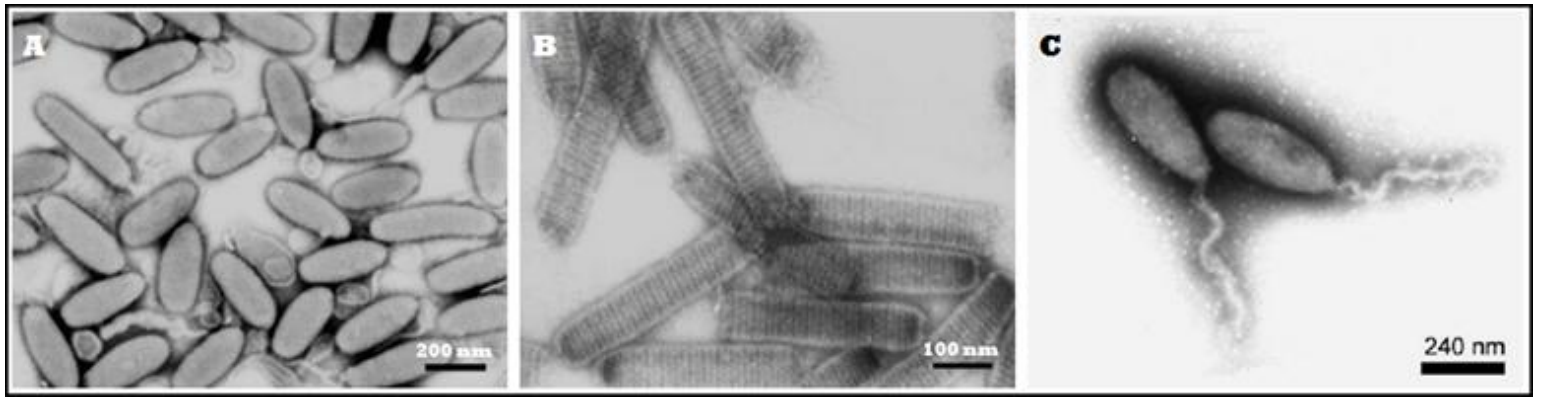


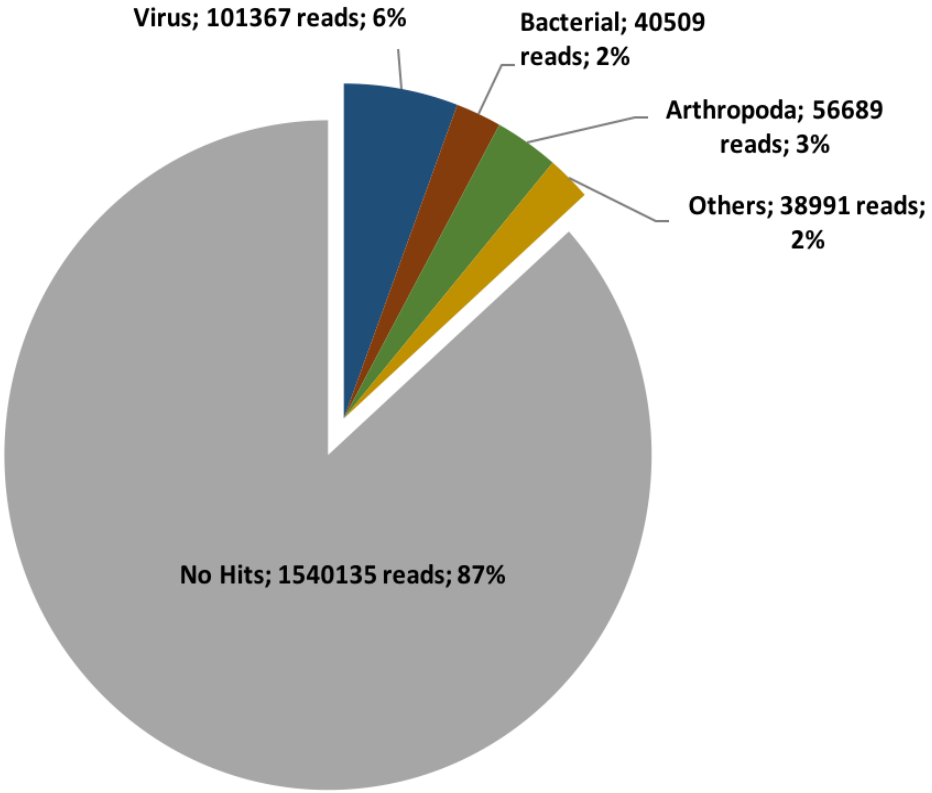
Fig. 3. DSRM and SP2 structural models. The models were calculated using the software I-TASSER that combines *ab initio* and threading approaches. The reliability scores of each model are shown in Supplementary Table 2. (A) DSRM tertiary structures for each of the four sequences analysed. The conserved structure formed by two α-helices and three β-sheets, typical of dsRNA-binding motifs, is clear in all predicted structures. (B) SP2 predicted structures for all arthropod totiviruses. In all cases the tertiary structure is rich in alpha helix regions and ressembles a fiber shape.

Estudo do vírus causador da síndrome da mancha branca em camarões - WSSV

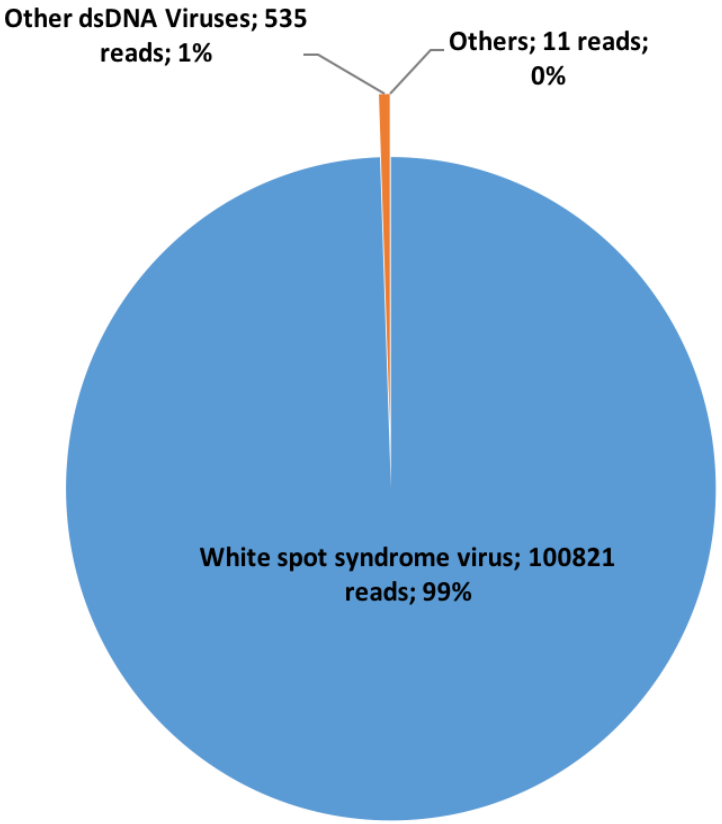


Sequenciamento do genoma do WSSV

A



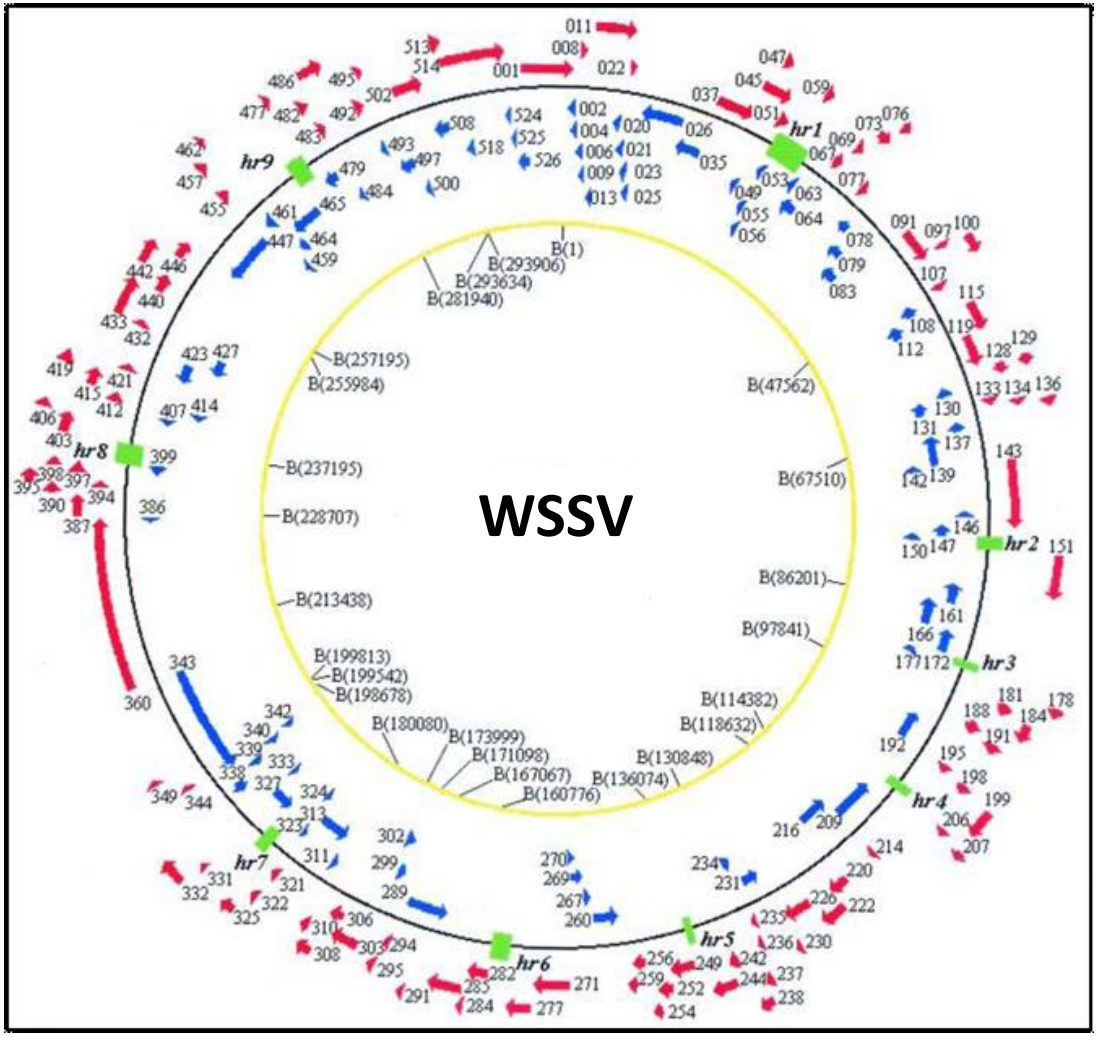
B



→ Total de *reads* da biblioteca: 1.777.691

→ Total de *reads* utilizados na montagem do genoma:120.795

Sequenciamento do genoma do WSSV



→ Tamanho do genoma do WSSV brasileiro: ~294.561 pb

Table 1: WSSV complete/partial genomes available and major features.

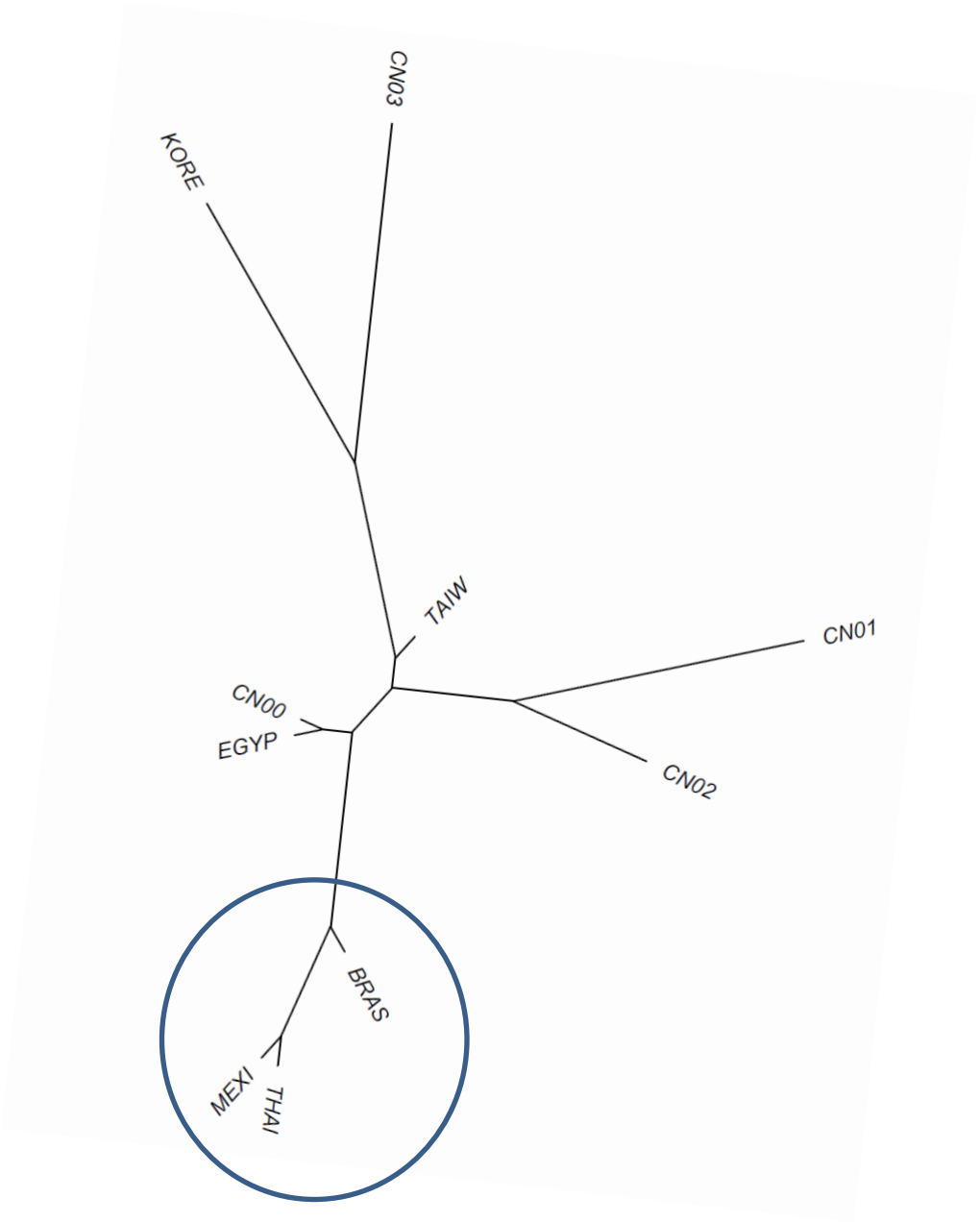
Accession number	Country	Collection year	Host	Genome size (bp)	G+C content (%)
AF440570.1	Taiwan	1994	*Penaeus monodon*	307.287	41.0
AF332093.3	China	1996	*Penaeus japonicus*	305.107	41.0
AF369029.2	Thailand	1996	*Penaeus monodon*	292.967	41.1
JX515788.1	South Korea	2011	*Litopenaeus vannamei*	295.884	40.9
KR083866.1	Egypt	2014	?	305.119	41.0
KT995472.1	China CN01	1994	*Marsupenaeus japonicus*	309.286	40.9
KT995470.1	China CN02	2010	*Procambarus clarkii*	294.261	41.0
KT995471.1	China CN03	2010	*Litopenaeus vannamei*	284.148	41.0
KU216744.1	Mexico	2008	*Litopenaeus vannamei*	293.183	41.1
Unpublished	Brazil	2015	*Litopenaeus vannamei*	292.912	41.1

Análise de identidade - WSSV

	WSSV_KR	WSSV_TAI	WSSV_CN01	WSSV_CN02	WSSV_EG	WSSV_CN	WSSV_CN03	WSSV_MEX	WSSV_THAI	WSSV-BR FI...
WSSV_KR		96.17%	94.56%	96.67%	96.87%	96.87%	93.26%	95.57%	95.75%	95.79%
WSSV_TAI	96.17%		97.10%	95.38%	98.75%	98.75%	90.83%	94.21%	94.43%	94.64%
WSSV_CN01	94.56%	97.10%		94.28%	97.27%	97.27%	89.66%	92.62%	92.79%	93.01%
WSSV_CN02	96.67%	95.38%	94.28%		96.22%	96.22%	93.55%	94.57%	94.77%	94.93%
WSSV_EG	96.87%	98.75%	97.27%	96.22%		100.00%	91.48%	94.87%	95.10%	95.45%
WSSV_CN	96.87%	98.75%	97.27%	96.22%	100.00%		91.48%	94.87%	95.10%	95.45%
WSSV_CN03	93.26%	90.83%	89.66%	93.55%	91.48%	91.48%		93.57%	93.71%	93.99%
WSSV_MEX	95.57%	94.21%	92.62%	94.57%	94.87%	94.87%	93.57%		99.58%	98.19%
WSSV_THAI	95.75%	94.43%	92.79%	94.77%	95.10%	95.10%	93.71%	99.58%		98.51%
WSSV-BR FINAL 050416	95.79%	94.64%	93.01%	94.93%	95.45%	95.45%	93.99%	98.19%	98.51%	

→ **4.418** nucleotídeos diferentes em relação ao genoma mais semelhante.

Estudos filogenéticos - WSSV



Variantes virais no estado do RN - WSSV

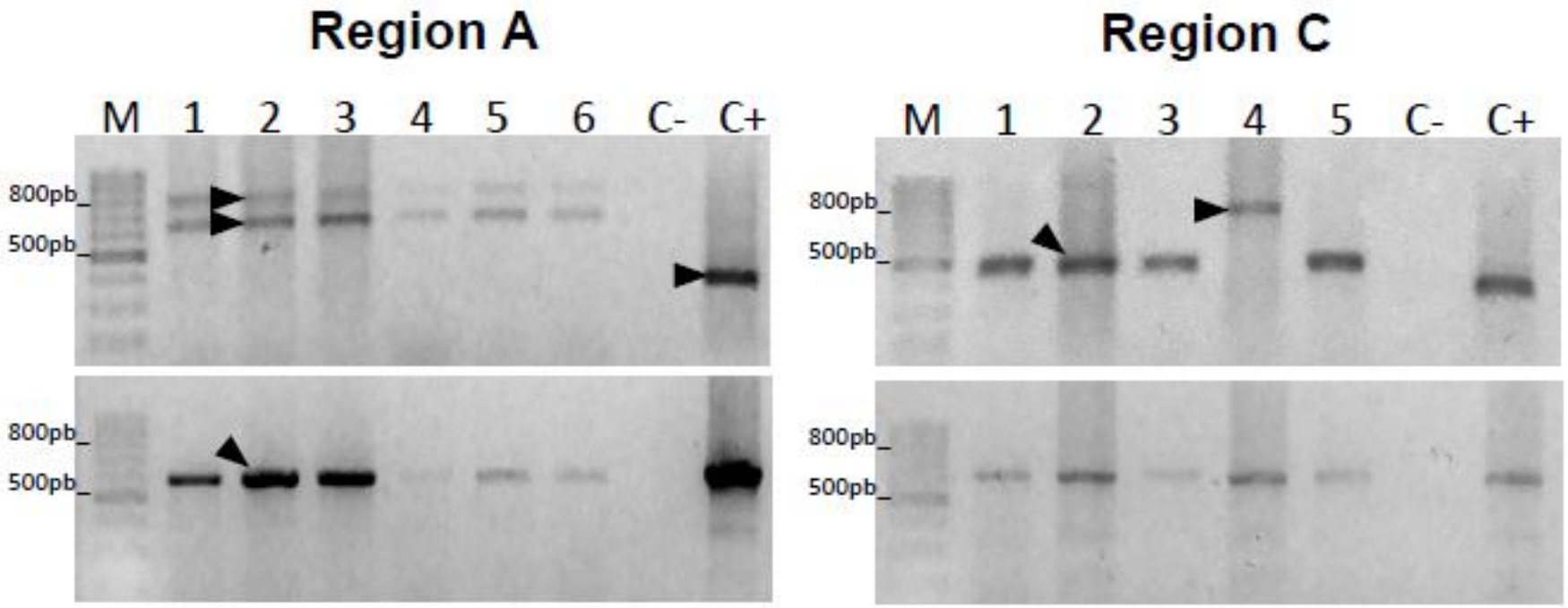
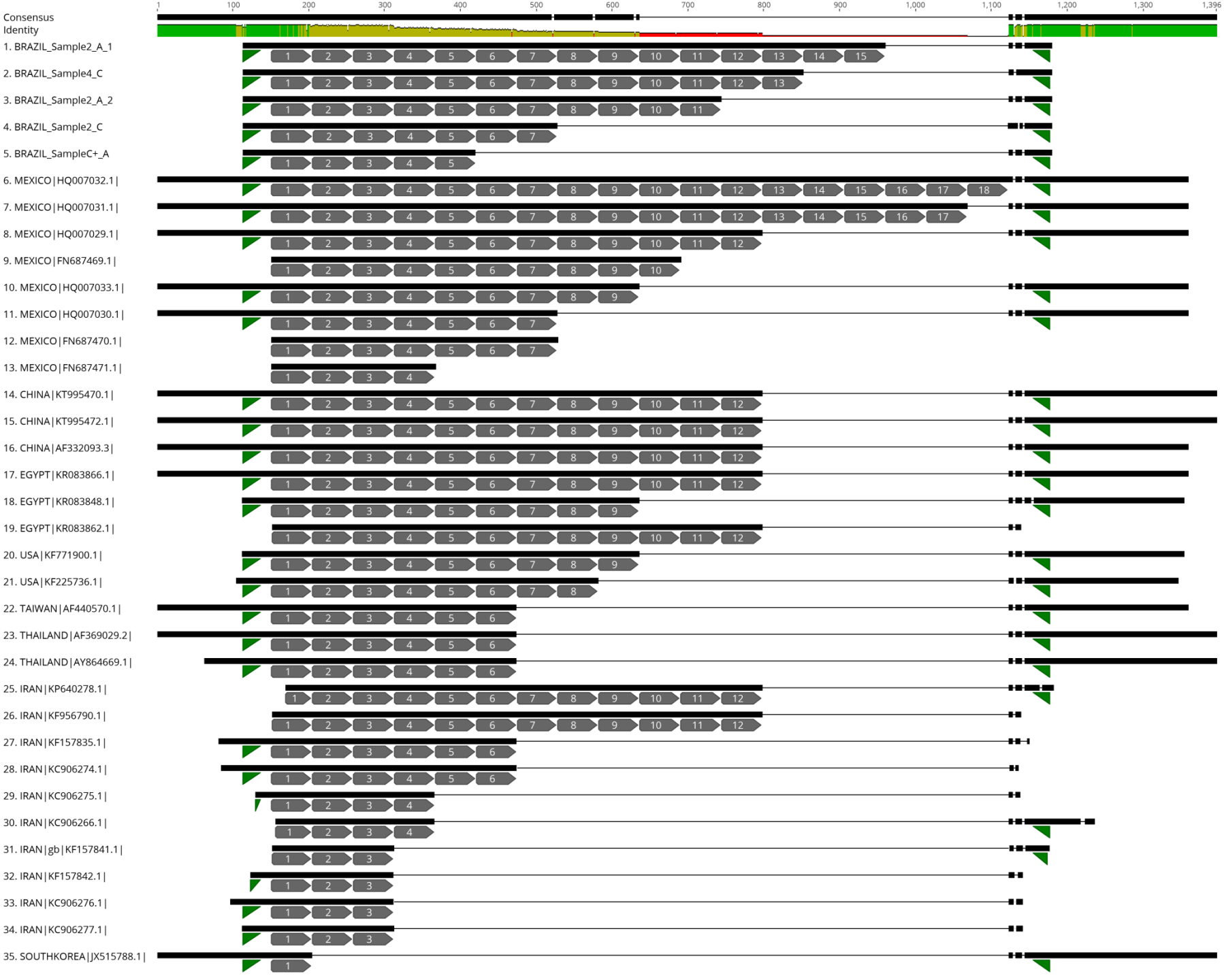


Figure 2


Consensus
Identity
1
100
200
300
400
500
600
700
800
900
1,000
1,100
1,200
1,300
1,396
1. BRAZIL_Sample2_A_1
2. BRAZIL_Sample4_C
3. BRAZIL_Sample2_A_2
4. BRAZIL_Sample2_C
5. BRAZIL_SampleC+_A
6. MEXICO|HQ007032.1|
7. MEXICO|HQ007031.1|
8. MEXICO|HQ007029.1|
9. MEXICO|FN687469.1|
10. MEXICO|HQ007033.1|
11. MEXICO|HQ007030.1|
12. MEXICO|FN687470.1|
13. MEXICO|FN687471.1|
14. CHINA|KT995470.1|
15. CHINA|KT995472.1|
16. CHINA|AF332093.3|
17. EGYPT|KR083866.1|
18. EGYPT|KR083848.1|
19. EGYPT|KR083862.1|
20. USA|KF771900.1|
21. USA|KF225736.1|
22. TAIWAN|AF440570.1|
23. THAILAND|AF369029.2|
24. THAILAND|AY864669.1|
25. IRAN|KP640278.1|
26. IRAN|KF956790.1|
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33. IRAN|KC906276.1|
34. IRAN|KC906277.1|
35. SOUTHKOREA|JX515788.1|

Perspectivas

Contents lists available at ScienceDirect

Developmental and Comparative Immunology

journal homepage: www.elsevier.com/locate/dci

Review

Evolution of specific immunity in shrimp – A vaccination perspective against white spot syndrome virus

Syed Khader Syed Musthaq [a], Jimmy Kwang [a,b,*]

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Specific immunity
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WSSV

ABSTRACT

Invertebrates lack true adaptive immunity and it solely depends on the primitive immunity called innate immunity. However, various innate immune molecules and mechanisms are identified in shrimp that plays potential role against invading bacterial, fungal and viral pathogens. Perceiving the shrimp innate immune mechanisms will contribute in developing effective vaccine strategies against major shrimp pathogens. Hence this review intends to explore the innate immune molecules of shrimp with suitable experimental evidences together with the evolution of "specific immune priming" of invertebrates. In addition, we have emphasized on the development of an effective vaccine strategy against major shrimp pathogen, white spot syndrome virus (WSSV). The baculovirus displayed rVP28 (Bac-VP28), a major envelope protein of WSSV was utilized to study its vaccine efficacy by oral route. A significant advantage of this baculovirus expression cassette is the use of WSSV-immediate early 1 (ie1) promoter that derived the abundant expression of rVP28 protein at the early stage of the infection in insect cell. The orally vaccinated shrimp with Bac-VP28 transduced successfully in the shrimp cells as well as provided highest survival rate. In support to our vaccine efficacy we analysed Pattern Recognition Proteins (PRPs) β-1,3 glucan lipopolysaccharides (LGBP) and STAT gene profiles in the experimental shrimp. Indeed, the vaccination of shrimp with Bac-VP28 demonstrated some degree of specificity with enhanced survival rate when compared to control vaccination with Bac-wt. Hence it is presumed that the concept of "specific immune priming" in relevant to shrimp immunity is possible but may not be common to all shrimp pathogens.

Table 1
Different vaccine strategies studied against WSSV.

Vaccines	Details of vaccine	References
1. Inactivated	Inactivated WSSV	Namikoshi et al. (2004)
	Inactivated WSSV	Singh et al. (2005)
2. Recombinant protein	VP19, VP28 expressed in bacteria	Witteveldt et al. (2004a,b)
	VP19, VP28 expressed in bacteria	Jha et al. (2006)
	VP28 expressed in *Bombyx mori*	Wei and Xu (2005)
	VP28 expressed in *Bacillus subtilis*	Fu et al. (2010)
	VP28 expressed in baculovirus	Syed Musthaq et al. (2009), Syed Musthaq and Kwang (2011)
3. DNA vaccine	VP15, VP28, VP35 and VP281 DNA constructs	Rout et al. (2007)
	VP28 DNA construct with chitosan nanoparticle	RajeshKumar et al. (2009)
	VP28 DNA construct in *Salmonella typhimurium*	Ning et al. (2009)
	VP28 DNA construct	Li et al. (2010)
4. double stranded RNA	Non-specific dsRNA	Robalino et al. (2004)
	VP28, VP281 specific dsRNAs	Kim et al. (2007)
	VP28 specific dsRNA	Sarathi et al. (2008)
	VP28, VP26 specific dsRNAs	Mejía-Ruíz et al. (2011)

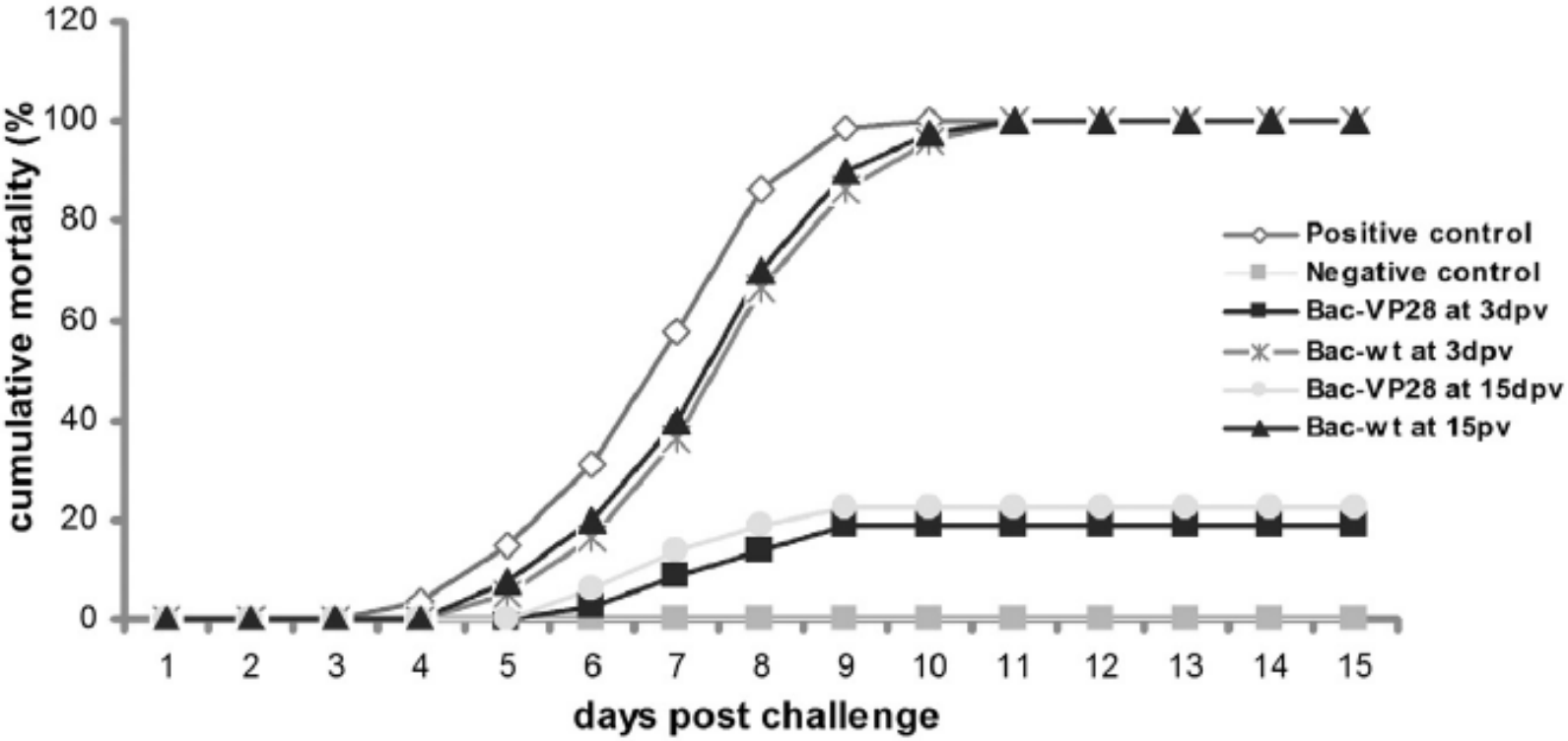

Fig. 4. Time-mortality relationship of oral vaccine and followed by WSSV challenge experiment. Shrimp were administrated orally with Bac-VP28, Bac-wt or PBS (positive control) coated feed for 7 days continuously. Third day after final vaccination, the above shrimp groups were immersion challenge with WSSV containing sea water for 2 h, then the shrimp were transferred to normal seawater, whereas negative control shrimp was challenged with PBS containing seawater.

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DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published November 9

REVIEW

RNA interference-based therapeutics for shrimp viral diseases

P. Krishnan, P. Gireesh-Babu*, K. V. Rajendran, Aparna Chaudhari

Central Institute of Fisheries Education, Indian Council of Agricultural Research, Seven Bungalows, Andheri West, Versova, Mumbai-400061, India

ABSTRACT: RNA interference (RNAi) has emerged as a powerful tool to manipulate gene expression in the laboratory. The presence of a double-stranded RNA (dsRNA) in eukaryotic cells triggers this post-transcriptional gene-silencing mechanism, leading to a sequence-specific degradation of the target mRNA. Among its many potential biomedical applications, silencing of viral genes stands out as a promising therapeutic strategy. Marine shrimp viral diseases, especially white spot disease (WSD), represents one of the most attractive targets for the development of therapeutic RNAi owing to its widespread economic impact. This review summarizes the current knowledge in the therapeutic application of RNAi for combating viral diseases in shrimp. The basic principles of RNAi are described, focusing on features important for its therapeutic manipulation. Subsequently, a stepwise strategy for the development of therapeutic RNAi is presented.

KEY WORDS: RNAi therapy · Shrimp diseases · Small interfering RNA · siRNA · Long hairpin RNA · lhRNA

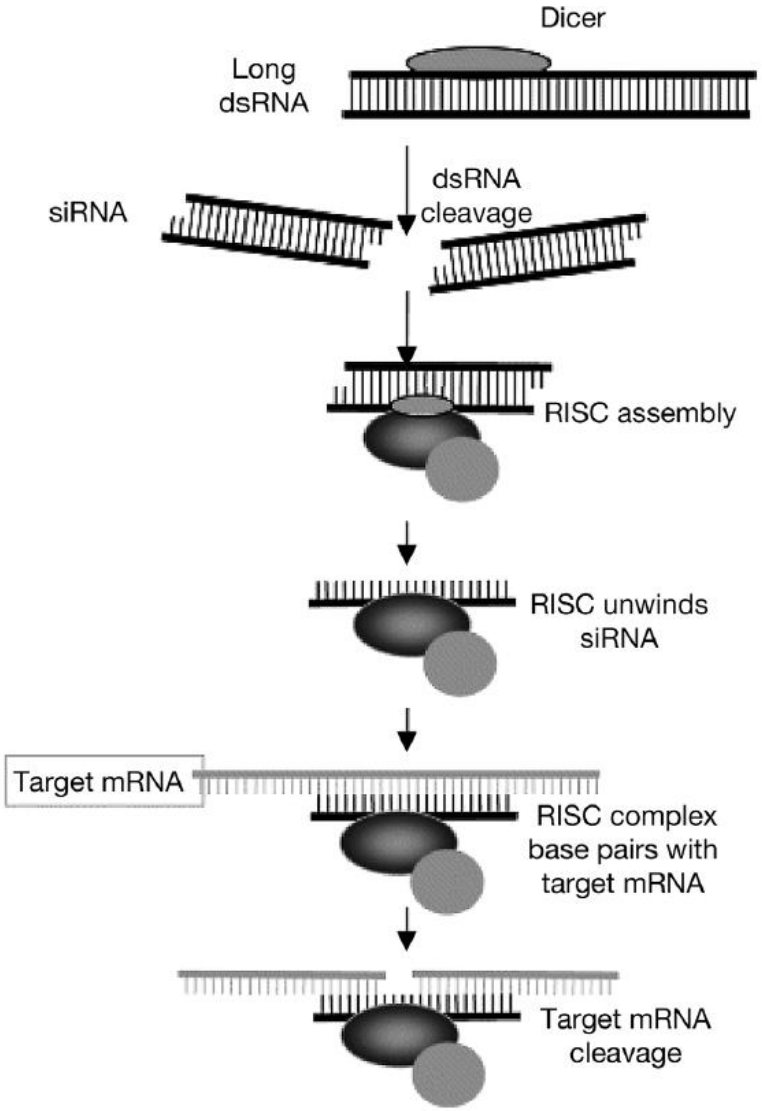

Fig. 1. Simple schematic of the RNA interference mechanism. Dicer: multidomain ribonuclease III enzyme; dsRNA: double-stranded RNA; siRNA: small interfering RNA; RISC: RNA-induced silencing complex

Contents lists available at ScienceDirect

Aquaculture Reports

journal homepage: www.elsevier.com/locate/aqrep

Use of microalgae *Chlamydomonas reinhardtii* for production of double-stranded RNA against shrimp virus

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[b] Department of Biotechnology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand
[c] Department of Biology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand
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Chlamydomonas reinhardtii
Double-stranded RNA
Shrimp
Yellow head virus
Nuclear transformation
qRT-PCR

ABSTRACT

RNA interference has been proposed to be a promising tool for combating shrimp viruses. Antiviral double-stranded (ds)RNA has been mostly produced in *Escherichia coli*-expression system because of its high efficiency and inexpensive operations. However, overusing the bacteria may raise concerns regarding public health and environmental contamination, and seeking for a new dsRNA production platform would be alternative for future molecular farming. In this study, we exploited the green microalgae *Chlamydomonas reinhardtii* to produce dsRNA targeting the lethal shrimp yellow head virus (YHV). The expression plasmid pSL18 for *C. reinhardtii* was constructed to contain YHV-specific hairpin RNA expression cassette, and the successful assembly of pSL18-YHV was confirmed by PCR and enzymatic digestions. Glass bead method was employed for transformation of *C. reinhardtii* nuclear genome with pSL18-YHV. Microalgal expression of dsRNA-YHV, approximately 45 ng from 100-mL culture, was detected by qRT-PCR. Oral feeding experiment on postlarval shrimp revealed that the formulated feed with *C. reinhardtii* expressing dsRNA-YHV, at the ratio of 1×10^8 transformants per gram feed, improved 22% survival rate after YHV challenge. The present study suggests that *C. reinhardtii* can be bioengineered to produce viral-

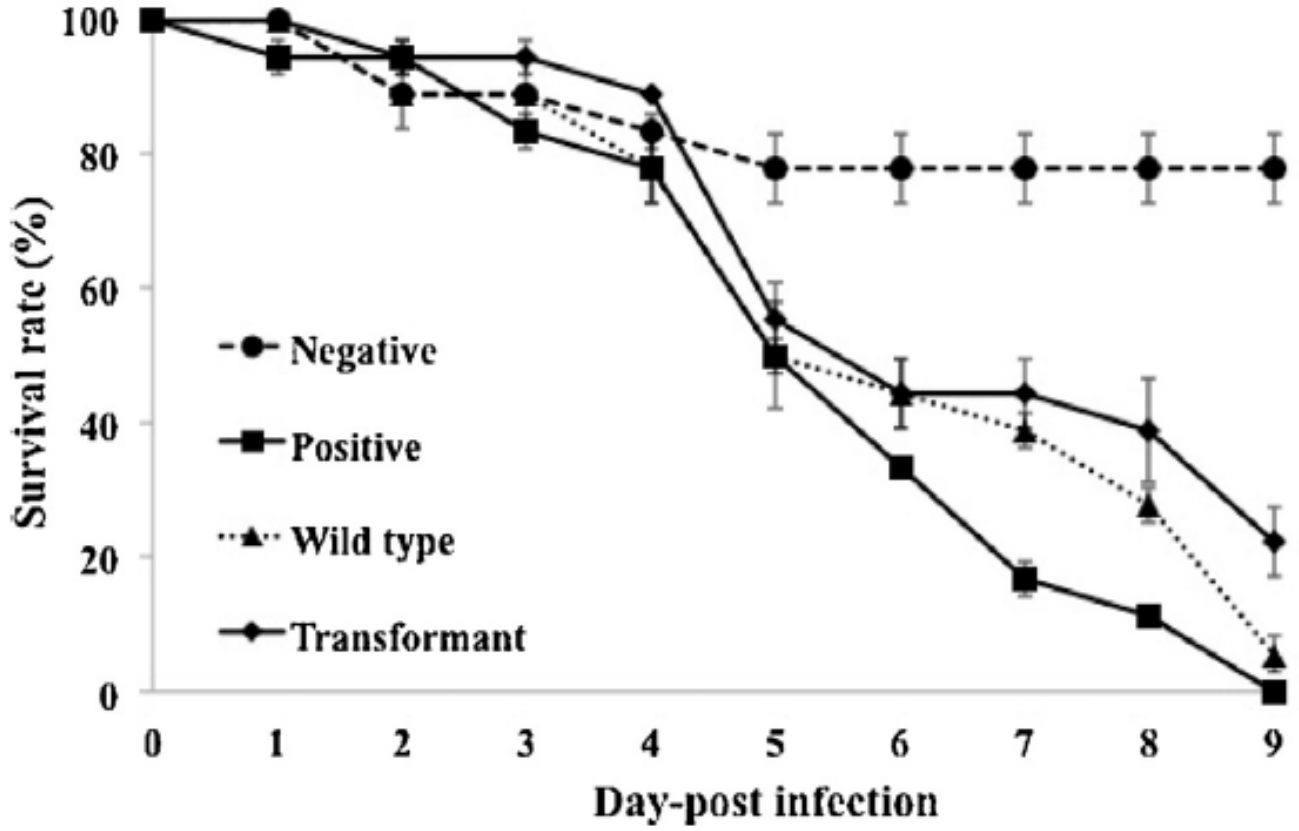

Fig. 5. Percentage of shrimp survival as a function of time after YHV challenge. Bars represent the means ± standard errors. Negative = normal feed and no YHV infection; positive = normal feed; wild-type = non-transformed algae; transformant = algae expressing dsRNA-YHV.

Potipora & LAPLIC Laboratório de Biologia Molecular Aplicada

1. Consultoria em análises moleculares;
2. Identificação das variantes virais que ocorrem nas fazendas;
3. Estudos de virulência x variabilidade genética;
4. Desenvolvimento de estratégias para o controle de doenças;
5. Adaptação de tecnologias para inserção no sistema produtivo;
6. Auxílio ao programa de melhoramento genético.

Desenvolvimento de ferramentas de análise molecular

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Buscar

Dashboard

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08/04/16 - 10:01

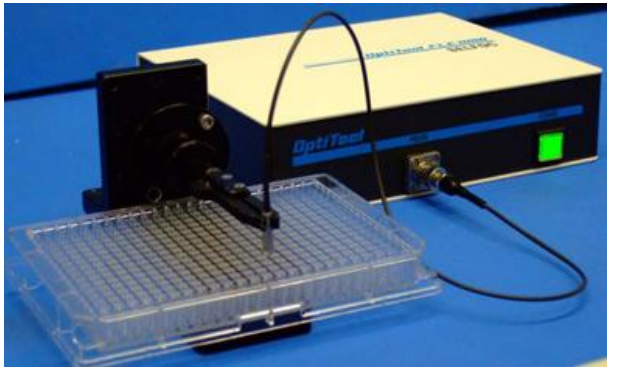
- Dashboard
- Configurações
- Análise Molecular
- Sair

Resultado

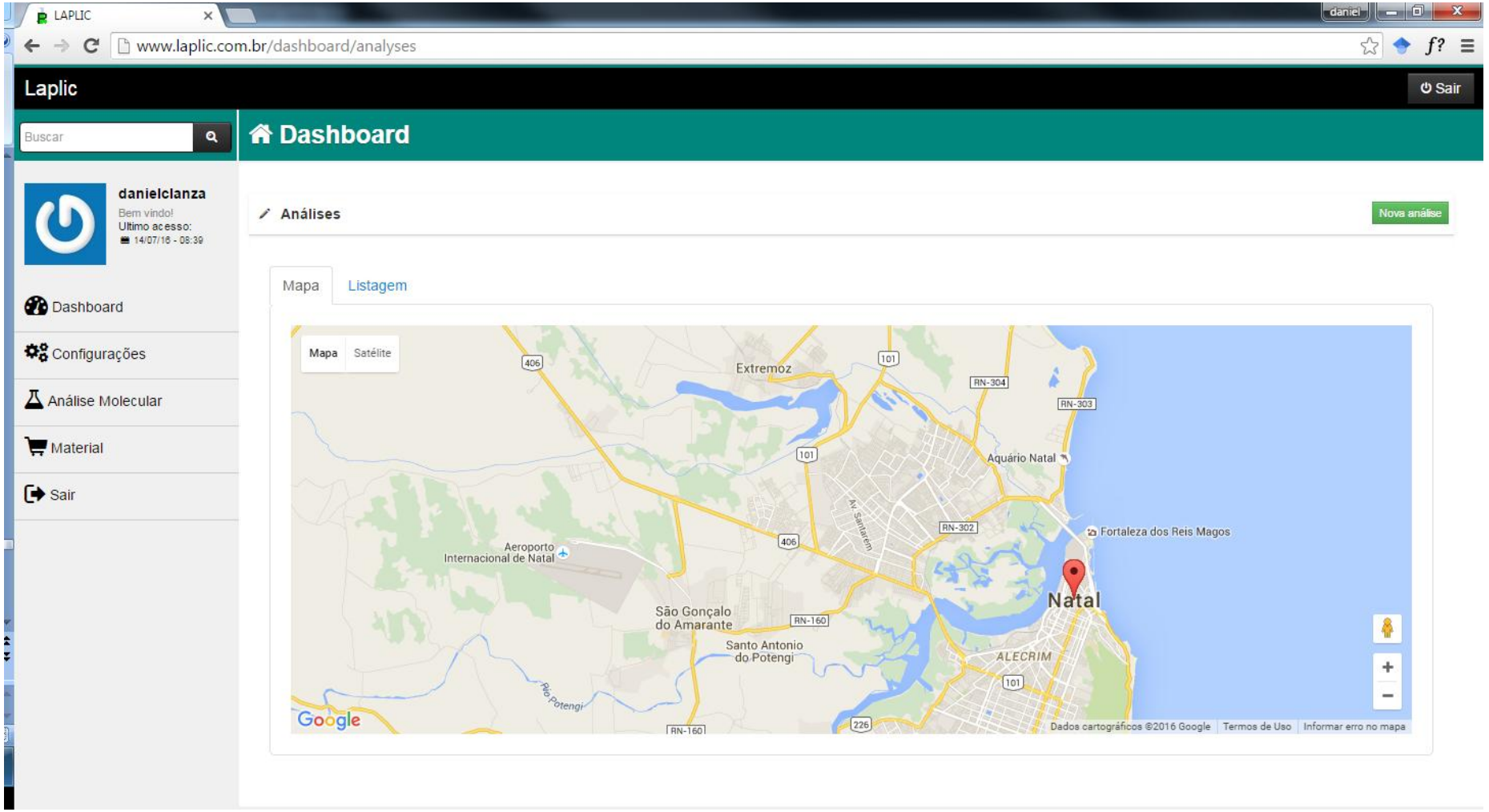
Resultados | Alinhamento | Proteínas | Sequências

Resultados:

Strains	Gra7	ROP5	ROP18
Type I	65.0%	42.86%	82.35%
Type II	85.0%	42.86%	52.94%
Type III	40.0%	50.0%	11.76%
P89	35.0%	50.0%	11.76%
CAST	50.0%	42.86%	82.35%
GUYKOE	100.0%	100.0%	82.35%



Epidemiologia Molecular



LAPLIC INÍCIO • INTERESSES • PROJETOS • EQUIPE • PUBLICAÇÕES • CONTATO

Laboratório de Biologia Molecular Aplicada

Identificação molecular de patógenos, bioinformática e inovação

Prof. Dárlio Inácio (EAJ)
Prof. João Paulo Matos (DBQ)
Profa. Karina Ribeiro (EAJ)
Prof. Rodrigo Carvalho (EAJ)
Prof. Valter Andrade Neto (DMP)
Prof. Lucymara Agnez (DBG)
Prof. Hugo Alexandre Rocha (DBQ)
Prof. José Veríssimo Fernandes (DMP)
Prof. Josélio Araújo (DMP)

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- Programa de Pós Graduação em Bioquímica
- Programa de Pós Graduação em Bioinformática
- Programa de Pós Graduação em Biologia Parasitária
- Mestrado Profissional em Inovação Tecnológica

Criação do cartão para fornecimento dos dados genéticos de aves.

CÓDIGO DE IDENTIDADE GENÉTICA

Unigen BIOLOGIA PELO DNA

CIG Modelo Fictício

O Laboratório Unigen, CRBio: 071-01-1, realizou exame de DNA e encontrou o seguinte padrão de marcadores genético de "STRs" na a amostra biológica abaixo identificada

Loco:	Oa2	Oa7	Oa26	Oa35	Un5	Un7	Un10	Un13	Un14	Un15	Un19	Un21	Un30	Un34	Un38
Alelos:	33 / 38	13 / 28	18 / 23	3 / 8	5 / 15	20 / -	15 / 35	5 / 40	15 / 25	10 / 20	5 / 20	5 / 30	20 / 40	30 / 35	15 / 25

As informações abaixo são de responsabilidade do solicitante:

(a coleta e identificação da amostra são de responsabilidade do solicitante)

Solicitante do Exame Antonio Francisco Ferreira Neto

Espécie *Saltator similis*

ID (anilha) 213 131 FCB BR 04

Nome CIG Modelo Fictício **Data de Nasc.** 11/7/1967

Imagem ilustrativa da espécie

Informações da Solicitação:

Cód. da Solicitação	Data da solicitação	Nº da Amostra	Cliente Unigen
AFFN081204	8/12/2004	U00000	AFFN

WWW.UNIGEN.COM.BR 55-011-29791528

Sequenciamento do genoma do WSSV

Alinhamento dos genomas completos/parciais disponíveis no banco de dados;

Construção de um “genoma quimera” a partir do alinhamento (314.232 pb);

Montagem do utilizando o WSSV quimera como sequência referência;

Total de *reads* da biblioteca*: 1.777.691

Total de *reads* usados na montagem: 120.795

Inspeção visual cuidadosa do genoma montado

Tamanho do genoma do WSSV brasileiro: 294.561 pb