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Short Communication

White spot syndrome virus in wild penaeid shrimp caught in coastal and offshore waters in the southern Atlantic Ocean

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White spot syndrome virus (WSSV) is a double-stranded rod-shaped DNA virus, which belongs to the family *Nimaviridae*, genus *Whispovirus* (Mayo 2002). Since its first appearance in 1992 (Chou *et al.* 1995), the disease has rapidly spread to different regions of the world with an economic impact approaching US$ 10 billion (OIE 2006). The first notification of WSSV in Brazil occurred in 2005 in Laguna–Santa Catarina, southern Brazil where the disease affected more than 1400 ha of shrimp ponds causing a production decline from 4189 tonnes in 2004 to 300 tonnes in 2007 (Seiffert *et al.* 2006). Despite the lack of official records, it has been estimated that 90% of the shrimp industry in Santa Catarina collapsed in 2006 (Netto & Meurer 2006).

In coastal waters of Brazil, there are no reports regarding the presence of WSSV in native wild shrimp. We therefore conducted research to evaluate the presence of WSSV in wild shrimps in the Laguna estuarine complex. In addition, wild shrimps were collected from offshore waters to evaluate the possible extent of virus dissemination. Thus, in February 2008, 750 wild juveniles of *Farfantepenaeus paulensis* (Pérez-Farfante) were captured in five sampling stations along the Laguna estuarine system (Fig. 1). One hundred and fifty *Litopenaeus vannamei* (Boone) were collected in a shrimp farm that operates a shrimp–tilapia polyculture. In addition, 420 *F. paulensis* were collected in October 2007 on the Santa Catarina coast (26°55′ S, 48°11′ W), 42 km from the shoreline. Some of these animals died during the collecting trip and presented a white colour of the posterior somites. Two hundred and fifty *Farfantepenaeus brasiliensis* (Latreille) were caught further North in January 2009 (26°16′S 48°10′W) and *ca* 36 km from the coast.

Shrimps were fixed with ethanol 96 GL immediately after collection and sent to the laboratory for analysis. DNA extraction and diagnostic PCR for WSSV followed Lo *et al.* (1996a), as described in Cavalli *et al.* (2008). Partial WSSV DNA of PCR positive samples was sequenced using a MegaBACE

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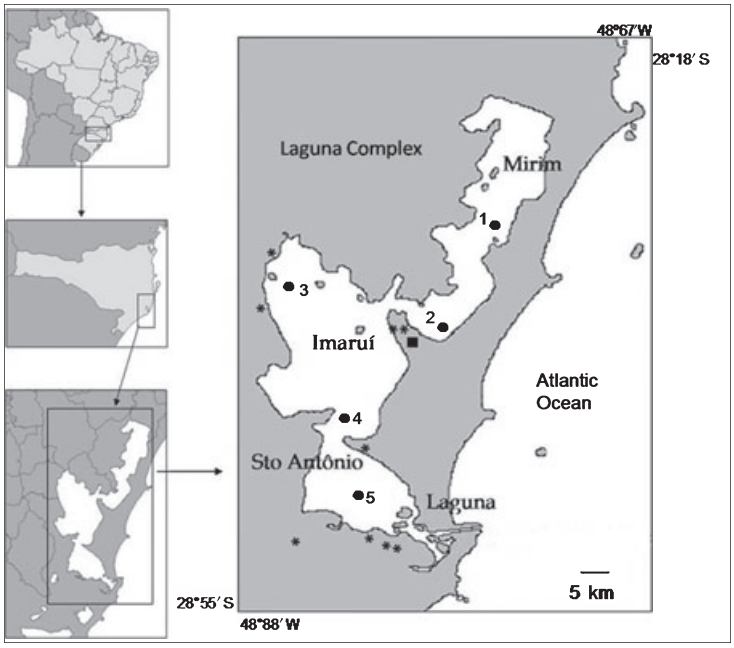

Figure 1 Laguna estuarine complex with the Mirim, Imaruí and Santo Antônio lagoons. Numbers indicate sampling stations where wild *Farfantepenaeus paulensis* were collected. Asterisks show the localization of four shrimp farms, and the solid square indicates the shrimp farm from where cultured *Litopenaeus vannamei* were sampled.

Table 1 Number of analysed shrimp, positive cases and estimated prevalence (%) of white spot syndrome virus (WSSV) in captured and cultured shrimp in the Laguna estuarine complex, Santa Catarina, Brazil

Locality	Analysed	Positive	Species	Prevalence (%)
Station 1 – Mirim	150	10	*Farfantepenaeus paulensis*	6
Station 2 – Mirim	150	5	*F. paulensis*	3
Station 3 – Imaruí	155	15	*F. paulensis*	9
Station 4 – Imaruí	150	0	*F. paulensis*	0
Station 5 – Santo Antonio	150	0	*F. paulensis*	0
Polyculture – Shrimp Farm	140	120	*Litopenaeus vannamei*	86

500 (GE Healthcare Life Sciences). The obtained sequences were submitted to the GenBank database through the BLASTn on-line tool (Altschul *et al.* 1997)to determine the levels of homology with the WSSV sequences available.

For histopathological analysis, the shrimps were fixed with Bouin's solution for 24 h. Tissues were processed for standard histological analyses (Ulrika & Mikel 1994) and stained with haematoxylin and eosin (H & E) and Periodic Acid Shiff's (PAS).

The PCR results showed that WSSV was present in native shrimp of the Laguna estuarine system at three stations and in *L. vannamei* from the farm (Table 1). Similarly, *F. paulensis* from the Atlantic Ocean also showed the presence of WSSV. All positive cases were amplified at least twice to confirm the results. All sequenced PCR positive samples showed high nucleotide similarity with WSSV sequences available at GenBank (*E value* = 0).

The histological analysis confirmed the presence of the virus in *F. paulensis* and *F. brasiliensis* collected in the Atlantic Ocean as shown by the presence of intranuclear inclusion bodies densely stained with H & E, characteristic of WSSV (Fig. 2a,b). To our knowledge, this is the first report of WSSV in wild shrimp collected from offshore waters of the southern Atlantic Ocean.

Cavalli *et al.* (2008) conducted the first study to determine possible WSSV infection in wild shrimp in the Laguna complex, but no positive results were obtained. The small sample number (114 wild shrimp) could have contributed to the underesti-

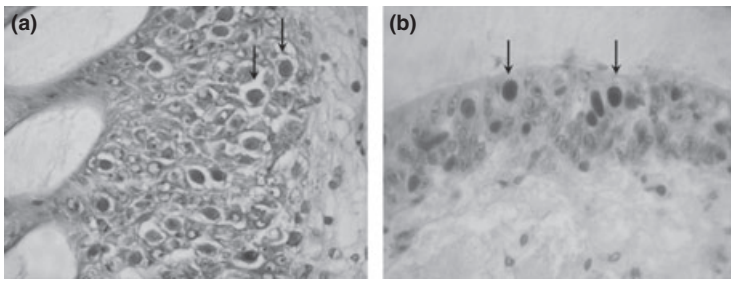

Figure 2 Stomach epithelial cells of *Farfantepenaeus paulensis* (a) and *F. brasiliensis* (b) captured off the Santa Catarina coast. Arrows indicate basophilic intranuclear inclusion bodies (H & E, X40).

mation of infected shrimp. Although the sampling number was increased in this study, only a low prevalence was detected at three sampling stations in the inner lagoon. In contrast, *L. vannamei* collected in the polyculture system were highly contaminated. The presence of virus in large numbers of farmed shrimp indicates that effluents from shrimp production are an important source of contamination, although few farms are still active in the region. A higher prevalence of infected wild animals was reported in areas close to shrimp cultures by Vaseeharan *et al.* (2003).

In this study, stations #4 and #5 did not present any infected wild shrimp. This could be related to their proximity to the lagoons' connection with the Atlantic Ocean, which possibly contributed to a dilution of virus particles. In contrast, infected shrimps were found in the Mirim lagoon (stations #1 and #2), which is a more enclosed system. In this case, the longer water residence time probably helped to maintain the virus in the system. Thus, the hydrology of the Laguna complex may play an important role in the dissemination and infection of wild shrimp.

Infected wild *Farfantepenaeus paulensis* and *F. brasiliensis* were also found in offshore waters of the Atlantic Ocean. It is very unlikely that effluents of shrimp farms from Laguna infected wild shrimp collected 30–40 km from the coast, mainly because of the very high dilution of outflows from the Laguna complex as they enter the Atlantic Ocean. In addition, the Brazil current flows southwards, away from the Laguna system, although virus could originate from shrimp farms from coastal regions of northern Brazil.

WSSV-infected wild shrimp was found in coastal waters of South Carolina, in the northern Atlantic Ocean (Chapman *et al.* 2004). Other studies have also reported virus infections of wild animals captured in coastal waters of Taiwan (Lo *et al.* 1996b), of the southeastern (Uma *et al.* 2005) and eastern coasts of India (Vaseeharan *et al.* 2003) and also in coastal waters of Panama (Nunan *et al.* 2001). Thus, the reports cited here and our results suggest that the presence of WSSV in coastal waters is more common than previously thought and that the virus is already widespread in most of the world's oceans.

Infected but asymptomatic wild shrimp from coastal regions may function as vectors of WSSV and other diseases. This is of great concern especially because most penaeid shrimp found in coastal lagoons enter these systems as larvae and remain in the lagoons until reaching the juvenile phase.

In addition, the use of wild larvae or broodstock for farming is also possible ways in which pathogens are introduced into shrimp farms (Fegan & Clifford 2001; Lightner 2005).

However, Laguna, as well as other coastal ecosystems in Brazil where *L. vannamei* is cultured, presents several risk factors for the dissemination of WSSV and other diseases, such as industry internationalization, bad culture practices and almost no health management (Fegan & Clifford 2001; McClennen 2004). Thus, the outbreak of WSSV disease cannot be related only to possible introduction of the virus from wild shrimp.

In conclusion, this article reports the first record of WSSV-infected wild shrimp in the southern Atlantic Ocean. The low prevalence of WSSV in wild shrimp in the Laguna complex suggests that the severe WSSV outbreak in shrimp farms did not significantly affect wild shrimp. Indeed, the industrial shrimp fishery in the Santa Catarina coastal region showed a slight increase in recent years (IBAMA 2008).

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References

Altschul S.F., Madden T.L., Schäffer A.A., Zhang J., Zhang Z., Miller W. & Lipman D.J. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* **25,** 3389–3402.

Cavalli L.S., Marins L.F., Netto S. & Abreu P.C. (2008) Evaluation of white spot syndrome virus (WSSV) in wild shrimp after a major outbreak in shrimp farms at Laguna, southern Brazil. *Atlântica* **30,** 45–52.

Chapman R.W., Browdy C.L., Savin S., Prior S. & Wenner E. (2004) Sampling and evaluation of white spot syndrome virus in commercially important Atlantic penaeid shrimp stocks. *Diseases of Aquatic Organisms* **59,** 179–185.

Chou H.Y., Huang C.Y., Wang C.H., Chiang H.C. & Lo C.F. (1995) Pathogenicity of a baculovirus infection causing white spot syndrome vírus in cultured penaeid shrimp in Taiwan. *Diseases of Aquatic Organisms* **23,** 165–173.

Fegan D.F. & Clifford H.C. (2001) Health management for viral diseases in shrimp farms. In: *The New Wave, Proceedings of the Special Session on Sustainable Shrimp Culture, Aquaculture* (ed. by C.L. Browdy & D.E. Jory), pp. 105–135. The World Aquaculture Society, Baton Rouge, LA.

IBAMA (2008) *Estatística da Pesca 2006 – Brasil: grandes regiões e unidades da federação*. Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis, Brasília.

Lightner D.V. (2005) Biosecurity in shrimp farming: pathogen exclusion through use of SPF stock and routine surveillance. *Journal of the World Aquaculture Society* **36,** 229–248.

Lo C.H., Leu J.H., Ho C.H., Chen C.H., Peng S.E., Chen Y.T., Chou C.M., Yeh P.Y., Huang C.J., Chou H.Y., Wang C.H. & Kou G.H. (1996a) Detection of baculovirus associated with white spot syndrome (WSBV) in penaeid shrimps using polymerase chain reaction. *Diseases of Aquatic Organisms* **25,** 133–141.

Lo C.F., Ho C.H., Peng S.E., Chen C.H., Hsu H.C., Chiu Y.L., Chang C.F., Liu K.F., Su M.S., Wang C.H. & Kou G.H. (1996b) White spot syndrome baculovirus (WSBV) detected in cultured and captured shrimp, crabs and other arthropods. *Diseases of Aquatic Organisms* **27,** 215–225.

Mayo M.A. (2002) A summary of taxonomic changes recently approved by ICTV. *Archives of Virology* **147,** 1655–1656.

McClennen C. (2004) *White spot syndrome virus – The economic, environmental and technical implications on the development of Latin American shrimp farming*. Master of Arts in Law and Diplomacy Thesis, The Fletcher School, Tufts University, Medford, MA.

Netto S.A. & Meurer A.Z. (2006) Influência de efluentes de cultivo de camarão na estrutura das comunidades bênticas do sistema estuarino de Laguna (SC, Brasil). In: *Sistemas de cultivos aqüícolas costeiros no Brasil: Recursos, tecnologias e aspectos ambientais e sócio-econômicos* (ed. by G.F. Barroso, L.H.S. Poersch, R.O. Cavalli & A.O. Galvez), pp. 139–150. Rio de Janeiro, Brazil.

Nunan L.M., Arce S.M., Staha R.J. & Lightner D.V. (2001) Prevalence of infectious hypodermal and hematopoietic necrosis virus (IHHNV) and white spot syndrome virus (WSSV) in *Litopenaeus vannamei* in the Pacific Ocean off the coast of Panama. *Journal of the World Aquaculture Society* **32,** 330–334.

OIE (2006) *Manual of Diagnostic Tests for Aquatic Animals*. Office International des Epizooties, Paris, France.

Seiffert W.Q., Beltrame E., Andreatta E.R. & Maggioni D.S. (2006) Enfermidades, uma oportunidade para repensar o cultivo de camarões marinhos. *Panorama da Aquicultura* **97,** 32–38.

Ulrika V. & Mikel B.S. (1994) *Advanced Laboratory Methods in Histology and Pathology*. Armed Forces Institute of Pathology, Washington, DC.

Uma A., Koteeswaran A., Karunasagar I. & Karasunagar I. (2005) Prevalence of white spot syndrome virus and monodon baculovirus in *Penaeus monodon* broodstock and postlarvae from hatcheries in southeast coast of India. *Current Science* **89,** 1619–1622.

Vaseeharan B., Jayakumar R. & Ramasamy P. (2003) PCR-based detection of white spot syndrome virus in cultured and captured crustaceans in India. *Letters in Applied Microbiology* **37,** 443–447.

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