Journal of Fish Diseases 2008, **31,** 415–422 doi:10.1111/j.1365-2761.2008.00921.x

Macrobrachium rosenbergii nodavirus infection in *M. rosenbergii* (de Man) with white tail disease cultured in Taiwan

C S Wang[1], J S Chang[1], C M Wen[1], H H Shih[2] and S N Chen[3]

1 Department of Life Sciences, National University of Kaohsiung, Kaohsiung, Taiwan
2 Department of Life Science, National Taiwan University, Taipei, Taiwan
3 Institute of Fisheries Science, National Taiwan University, Taipei, Taiwan

Abstract

White tail disease (WTD) is a serious problem in *Macrobrachium rosenbergii* hatcheries and nursery ponds in Asia. The causative agents have been identified as *M. rosenbergii* nodavirus (*Mr*NV) and its associated extra small virus. This is the first report demonstrating *Mr*NV virus in *M. rosenbergii* displaying WTD signs in Taiwan by reverse transcriptase-polymerase chain reaction (RT-PCR). Amplified fragments of 850 and 425 bp for RNA-1 and RNA-2 of *Mr*NV, respectively, were obtained by RT-PCR. RT-PCR products of about 850 and 1121 bp for RNA-1 and RNA-2 of *Mr*NV were also obtained using different primer pairs. The amplicons were individually cloned into pGEM-T vector and sequenced. Using this recombinant plasmid of *Mr*NV RNA-2 as DNA template, the non-radioactive DNA probes were prepared by PCR amplification with DIG–11-dUTP. The probes were used to successfully detect *Mr*NV infection in the striated muscle tissues of WTD-diseased prawns using *in situ* hybridization. The 1121 bp genomic fragment of RNA-2 of *Mr*NV consisted of a unique open reading frame with 1116 nucleotides, and it encoded a structural protein with 371 amino acids. The nucleotide sequence of the partial genome of *Mr*NV RNA-2 revealed a 97% identity with an Indian isolate. A phylogenetic tree constructed using the nucleotide sequence of the viral capsid gene from insect and fish nodaviruses revealed that the *Mr*NV Taiwan isolate could be interpreted as a new genus within the family *Nodaviridae*. However, its position showed more affinity with *Alphanodavirus* than with *Betanodavirus*. The study confirmed the presence of *Mr*NV infection in freshwater prawns cultured in Taiwan suffering from WTD.

Keywords: in situ hybridization, *Macrobrachium rosenbergii*, *Macrobrachium rosenbergii* nodavirus, reverse transcriptase-polymerase chain reaction, white tail disease.

Introduction

Viral diseases are a major problem for crustacean aquaculture worldwide. The giant freshwater prawn, *Macrobrachium rosenbergii* (de Man), is an economically important crustacean in Taiwan. A new viral disease designated as white tail disease (WTD) has been observed in freshwater prawn hatcheries and nursery ponds since 1992, causing high mortalities and huge economic losses. The affected prawns exhibit white, opaque muscle in the abdominal segments, commonly accompanied by progressive reduction in feeding and swimming. Mortalities may reach between 50% and 70% within 2 weeks after transfer of post-larvae (PL) to grow-out ponds (Tung, Wang & Chen 1999). WTD has recently been reported in the French West Indies (Arcier, Herman, Lightner, Redman, Mari & Bonami 1999), People's Republic of China (Qian, Shi, Zhang, Cao, Liu, Li, Xie, Cambournac & Bonami 2003), India (Sahul Hameed, Yoganandhan, Sri Widada & Bonami 2004; Shekhar,

Correspondence *C S Wang, Department of Life Sciences, National University of Kaohsiung, Kaohsiung 811, Taiwan (e-mail: cswang@nuk.edu.tw)*

Azad & Jithendran 2006) and Thailand (Yoganandhan, Leartvibhas, Sriwongpuk & Limsuwan 2006).

The causative agents of WTD have been identified as *Macrobrachium rosenbergii* nodavirus (*Mr*NV) associated with extra small virus (XSV) (Qian *et al.* 2003). *Mr*NV is a small icosahedral non-enveloped virus, 26–27 nm in diameter, observed in the cytoplasm of connective tissue cells in infected prawns. The viral genome is composed of two fragments of linear, single-stranded, positive-sense RNAs (RNA-1 and RNA-2), of about 2.9 and 1.3 kb, respectively. The viral capsid contains a single polypeptide of 43 kDa (Bonami, Shi, Qian & Sri Widada 2005). The XSV virus is icosahedral in shape and 15 nm in diameter. Its genome consists of a linear, single-stranded RNA of 796 nucleotides, encoding a single structural protein of 17 kDa (CP-17) (Sri Widada & Bonami 2004). Because the clinical signs of WTD are not specific, other methods are required to screen for the aetiological agents. A number of diagnostic methods have been developed for the detection of *Mr*NV, including dot-blot hybridization, *in situ* hybridization, reverse transcriptase-polymerase chain reaction (RT-PCR) and ELISA (Romestand & Bonami 2003; Sri Widada, Durand, Cambournac, Qian, Shi, Dejonghe, Richard & Bonami 2003). Dot-blot hybridization and RT-PCR have also been developed for the detection of XSV (Sri Widada, Richard, Shi, Qian & Bonami 2004). A single-tube, one-step multiplex RT-PCR has been developed for *Mr*NV and XSV (Yoganandhan, Sri Widada, Bonami & Sahul Hameed 2005).

In a previous study, XSV was detected in WTD-affected *M. rosenbergii* in Taiwan (Wang, Chang, Shi & Chen 2007). The present study is the first confirming *Mr*NV infection by RT-PCR in diseased freshwater prawns showing clinical signs of WTD. The RT-PCR amplified products of *Mr*NV were further cloned and sequenced. The Digoxigenin DNA probes for *Mr*NV were labelled by PCR amplification using the recombinant plasmid as template. Using *in situ* hybridization, the results verified *Mr*NV infection in the white muscle tissue of WTD-diseased prawns. The phylogenetic relationships of *Mr*NV with other nodavirus isolates were investigated by comparing the nucleotide sequences of viral coat protein.

Materials and methods

Collection of infected post-larvae

Infected PL of *M. rosenbergii* with WTD were collected from affected hatcheries located in Kaohsiung and Pington in southern Taiwan. More than 30 diseased giant freshwater prawn samples, each consisting of 10–30 individuals, were collected. Healthy animals were obtained from a hatchery with no record of WTD. The PL were washed in sterile distilled water and stored at −20 °C before processing. Some samples were placed in Davidson's fixative for *in situ* hybridization.

Total RNA extraction

Total RNA was extracted using TRIzol™ reagent (Invitrogen, Carlsbad, CA, USA) according to the procedure described by Wang *et al.* (2007). Total RNA was dissolved in 50 μL of diethyl pyrocarbonate-treated water and stored at −70 °C.

RT-PCR for *Mr*NV

Reverse transcriptase-polymerase chain reaction was performed using a Reverse-iT™ one-step RT-PCR kit (ABgene, Surrey, UK), allowing reverse transcription (RT) and amplification to be carried out in a single reaction tube. The details of primer sequences and amplified product sizes are given in Table 1. One primer pair (pair 1) designed from the RNA-1 sequence of *Mr*NV and another primer pair (pair 3) specific to the RNA-2 sequence of *Mr*NV were tested separately for the detection of *Mr*NV infection. The reaction was performed in 50 μL of RT-PCR buffer containing 10 pmol of each primer and RNA template, using the following steps: reverse transcription at 52 °C for 1 h; denaturation at 94 °C for 2 min followed by 35 cycles of denaturation at 94 °C for 40 s; annealing at 55 °C for 40 s and elongation at 72 °C for 1 min, ending with an additional elongation step of 10 min at 72 °C. The amplified product was analysed by electrophoresis on a 1.5% agarose gel.

Cloning

In order to determine the homology of nucleotide sequence of *Mr*NV isolated from Taiwan with other nodavirus isolates, RT-PCR was carried out with a different primer pair (pair 2) as well primer pair 1 used for the amplification of RNA-1. The RT-PCR

Table 1 Pairs of primers used in reverse transcriptase-polymerase chain reaction for the amplification of *Macrobrachium rosenbergii* nodavirus (*Mr*NV) RNA

Pair	Name	Sequence	Size (bp)	Orientation	Reference
1 (RNA-1)	1A775	CCACGTTCTTAGTGGATCCT	850	Upstream primer	Sri Widada *et al.* (2003)
	1B690	CGTCCGCCTGGTAGTTCC		Downstream primer	
2 (RNA-2)	CapS	ATGGCTAGAGGTAAACAAAATTC	1121	Upstream primer	Sri Widada *et al.* (2003)
	CapC	ACAACCTAATTATTGCCGAC		Downstream primer	
3 (RNA-2)	*Mr*NV2aF	GCGTTATAGATGGCACAAGG	425	Upstream primer	Sahul Hameed *et al.* (2004)
	*Mr*NV2aR	AGCTGTGAAACTTCAACTGG		Downstream primer	

The primer pair specific to RNA-1 or RNA-2 of *Mr*NV is given in parentheses.

was performed under the same conditions as described previously; the amplified products were separately extracted from agarose gels, and purified using a PCR clean-up system (Promega Corporation, Madison, WI, USA). The DNA fragments were individually ligated into pGEM-T Easy Vector (Promega Corporation) and transformed into *Escherichia coli* DH-5α. The clones were designated as DMNV-1 and DMNV-2 for RNA-1 and RNA-2 of *Mr*NV, respectively. The recombinant plasmid DNA from the two clones were separately extracted, and automatic sequencing was performed commercially (Protech Technology Enterprise Co., Ltd, Tapei, Taiwan).

DNA probe labelling

The DIG-labelled probe for RNA-2 of *Mr*NV was generated with a PCR DIG Probe Synthesis Kit (Roche Molecular Biochemicals, Mannheim, Germany) following the manufacturer's instructions with slight modifications. Briefly, a 0.5-mL reaction tube was added to the following final concentration of reagents: 1X PCR buffer (10 mM Tris–HCl, 50 mM KCl, 2.5 mM $MgCl_2$), 200 μM PCR DIG probe synthesis mix, 0.5 μM of primer *Mr*NV2aF and *Mr*NV2aF, 1 unit *Taq* DNA polymerase, recombinant plasmid template derived from the clone DMNV-2 and distilled water to a final volume of 50 μL. The PCR was carried out in a thermal cycler (Thermo Hybaid, Needham Heights, MA, USA) with 35 cycles of 94 °C for 40 s, 55 °C for 40 s and 72 °C for 1 min. The resulting DIG-labelled DNA probe was extracted from agarose gel and purified using a PCR clean-up system (Promega Corporation).

In situ hybridization

All histological processing and staining followed standard procedures, as described by Bell & Lightner (1988). Sections, 5–7 μm thick, were used for *in situ* hybridization using DIG-labelled probe according to the manufacturer's manual (Roche Molecular Biochemicals). The sections were deparaffined and rehydrated in a series of graded ethanol, followed by digestion with proteinase K (100 μg mL^{-1}) and then fixed in cold 4% formaldehyde. The sections were heated to 95 °C for 10 min and allowed to hybridize for 12 h at 42 °C in the probe hybridization solution containing 50% formamide, 1 μg mL^{-1} DIG-labelled probe, 0.2 mg mL^{-1} sonicated salmon sperm DNA and 5% dextran sulphate, and 4X SSC (0.6 M sodium chloride, 60 mM sodium citrate, pH 7.0). The detection was performed using an alkaline phosphatase-conjugated anti-DIG antibody and substrate solution (nitroblue tetrazolium and bromo-4-chloro-3-indolyphosphate). After counterstaining with 0.1% eosin Y, the sections were dehydrated and mounted with Entellan (Merck KGaA, Darmstadt, Germany) and then examined under bright field microscopy (Olympus BX51, Tokyo, Japan).

Sequence analysis

Nucleotide sequence analysis for the partial genome of RNA-2 of *Mr*NV was performed with other nodavirus isolates including Indian *MrNV* (AY222840), black beetle virus (NC002037), Boolarra virus (NC004145), greasy grouper nervous necrosis virus (AF318942), Nodamura virus (AF174534), red spotted grouper nervous necrosis virus (RGNNV) (NC008041), Pariacoto virus (NC003692) and striped jack nervous necrosis virus (SJNNV) (AB056572) using BLAST (Altschul, Madden, Schäffer, Zhang, Zhang, Miller & Lipman 1997) and CLUSTAL W (Thompson, Higgins & Gibson 1994). The phylogenetic analysis was performed using the neighbour-joining method (Saitou & Nei 1987).

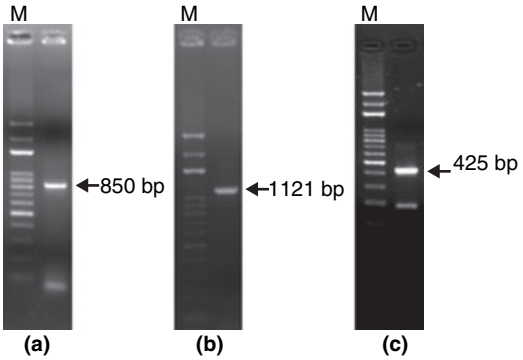

Figure 1 Agarose gels showing reverse transcriptase-polymerase chain reaction products of *Macrobrachium rosenbergii* nodavirus (*Mr*NV) extracted from post-larvae of white tail disease infected *M. rosenbergii* using different primer pairs. (a) 850 bp amplified product using primer pair 1 for RNA-1 of *Mr*NV; (b) 1121 bp amplified product using primer pair 2 for RNA-2 of *Mr*NV; (c) 425 bp amplified product using primer pair 3 for RNA-2 of *Mr*NV. M: 100-bp DNA ladder marker.

Results

Diseased 24 to 32 day old post-larvae with whitish muscle were collected from different hatcheries in southern Taiwan. All WTD-affected cases were confirmed with *Mr*NV virus infection by RT-PCR analysis. As shown in Fig. 1a & c, the 850 and 425 bp amplified products were obtained using primer pairs 1 and 3 specific to the genome of the RNA-1 and RNA-2 of *Mr*NV virus, respectively. Samples obtained from the unaffected hatchery were negative for *Mr*NV. Using different primers (pairs 1 and 2), 850 and 1121 bp amplified products were obtained by RT-PCR (Fig. 1a & b). The nucleotide sequence for the partial viral genome have been deposited in GenBank (GenBank accession no. DQ521574 for RNA-1 of *Mr*NV; DQ521575 for RNA-2 of *Mr*NV). The DIG DNA probes for *Mr*NV were labelled by PCR amplification using the recombinant plasmid as template. Using *in situ* hybridization, the results showed that the *Mr*NV genome could be successfully detected in the striated muscle of WTD-affected prawns. Positive reactions were recognized by a blue purple precipitate in the myofibrils but not in the nuclei of the muscle tissues (Fig. 2). However, no labelling was observed in the gill, hepatopancreas, nerve cord or stomach. No *Mr*NV infection was found in the muscle tissue of healthy prawns using *in situ* hybridization.

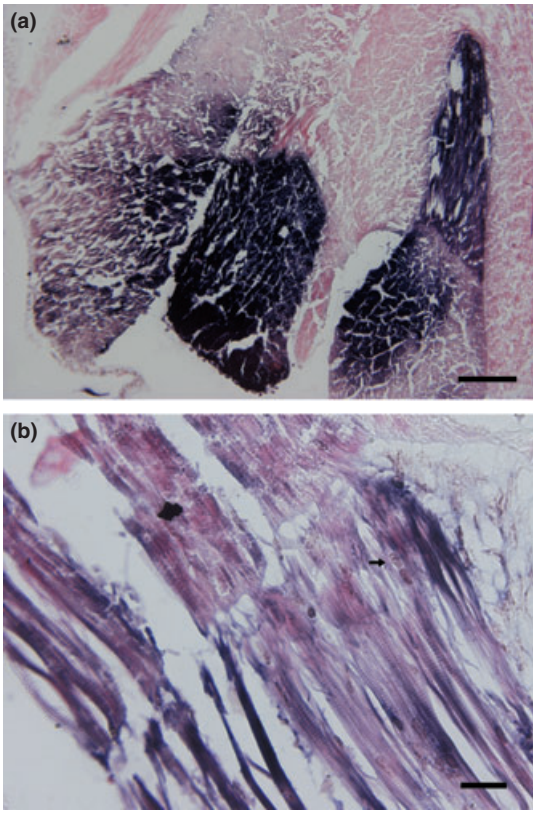

Figure 2 *In situ* hybridization of *M. rosenbergii* nodavirus (*Mr*NV)-infected *M. rosenbergii* with white tail disease using a DIG-labelled probe. (a) The striated muscle of an *Mr*NV-infected prawn showing a positive reaction in the affected muscle tissue (eosin Y as counterstain; bar = 100 μm). (b) A higher magnification of the necrotic muscle showing dark purple colouration in the myofibrils; the nuclei are negative (arrow) (eosin Y as counterstain; bar = 25 μm).

The nucleotide and deduced amino acid sequences of *Mr*NV RNA-2 partial genome are shown in Fig. 3. It contained a unique open reading frame with 1116 nucleotides and encoded a structural protein with 371 amino acids. A comparison of the sequence of the RNA-2 of the *Mr*NV Taiwan isolate showed a 97% identity BLAST analysis with the *Mr*NV Indian isolate (AY222840). In addition, the deduced amino acid sequences of the *Mr*NV Taiwan isolate showed strong homology with the Indian isolate with five deduced amino acid substitutions at positions 84 (T → I), 248 (T → S), 291 (V → I), 321(F → S) and 333 (S → L). In order to more precisely establish the systemic position of *Mr*NV, a phylogenetic tree was constructed using the amino acid sequences deduced from the viral capsid gene of insect and fish nodaviruses. The results showed that the *Mr*NV Taiwan isolate had more affinity with

```
1    atggctagaggtaaacaaaattctaatcagattcaaaataatagtaacgcaaacggcaag
      M  A  R  G  K  Q  N  S  N  Q  I  Q  N  N  S  N  A  N  G  K   20
60   cgccgtaagcgtaatcgaaggaatcgtaatccgcagacggttcccaactttaacccc att
      R  R  K  R  N  R  R  N  R  N  P  Q  T  V  P  N  F  N  P  I   40
120  gtcgcaaagccgacggttgccccacttcaaactaacattagaagtgctaggagtgac gtt
      V  A  K  P  T  V  A  P  L  Q  T  N  I  R  S  A  R  S  D  V   60
180  aatgccatcaccgttttaaatggcagcgatttccttacaactgtcaaagtccgaggt tct
      N  A  I  T  V  L  N  G  S  D  F  L  T  T  V  K  V  R  G  S   80
240  aataacttaattgattccaagtctagaatcttggttaagcaaccaatttctgcgagt tct
      N  N  L  I  D  S  K  S  R  I  L  V  K  Q  P  I  S  A  S  S  100
300  tttcttggtaccagaatttctggtctatcgcaattttgggagcgttatagatggcac aag
      F  L  G  T  R  I  S  G  L  S  Q  F  W  E  R  Y  R  W  H  K  120
360  gctgcagtcagatatgttcctgcagtacccaatactttagcttgccaacttattggt tac
      A  A  V  R  Y  V  P  A  V  P  N  T  L  A  C  Q  L  I  G  Y  140
420  atcgatacagatccactagatgaccctaacgttatcctcgatgttgatcagttactt agg
      I  D  T  D  P  L  D  D  P  N  V  I  L  D  V  D  Q  L  L  R  160
480  caggccacgtcacaagtgggtgcgcggcagtggaatttttctgatacaacaactatt cca
      Q  A  T  S  Q  V  G  A  R  Q  W  N  F  S  D  T  T  T  I  P  180
540  ttgattgtcaggcgtgatgatcaattgtactatactggccaagataaggagaacgtt cgt
      L  I  V  R  R  D  D  Q  L  Y  Y  T  G  Q  D  K  E  N  V  R  200
600  ttctctcaacagggtgtattttacctcttgcaagtgactacactactcaatattagt ggt
      F  S  Q  Q  G  V  F  Y  L  L  Q  V  T  T  L  L  N  I  S  G  220
660  gaagccattacaaatgatttgatttcaggttcactatatttagattgggtctgtgga ttt
      E  A  I  T  N  D  L  I  S  G  S  L  Y  L  D  W  V  C  G  F  240
720  tccatgccacaaattaatccttcaccagtggaagtttcacagctaacttataatgcg gat
      S  M  P  Q  I  N  P  S  P  V  E  V  S  Q  L  T  Y  N  A  D  260
780  actattggcaattgggttccaccaacagaactcaagcaaacttatactcaagatatt act
      T  I  G  N  W  V  P  P  T  E  L  K  Q  T  Y  T  Q  D  I  T  280
840  ggtttgaagccaaattctaaatttattattataccttatatggatagagtaagttct gaa
      G  L  K  P  N  S  K  F  I  I  I  P  Y  M  D  R  V  S  S  E  300
900  gtactgcagaagtgcacaattacttgtaatgaggttgacgccgttggttcaatctca tat
      V  L  Q  K  C  T  I  T  C  N  E  V  D  A  V  G  S  I  S  Y  320
960  tccgatactagcgctatcaaatgtgatgggtacatattatttcaggccaatagcatt ggt
      S  D  T  S  A  I  K  C  D  G  Y  I  L  F  Q  A  N  S  I  G  340
1020 gaagcaaccttcaccttagtgaccgattatcagggtgcggttgaccctaaaccctat cag
      E  A  T  F  T  L  V  T  D  Y  Q  G  A  V  D  P  K  P  Y  Q  360
1080 tataggattatcagagctatcgtcggcaataattaggttgt
      Y  R  I  I  R  A  I  V  G  N  N  *                        371
```

Figure 3 Nucleotide sequences and deduced amino acid sequences of the partial genome of *Macrobrachium rosenbergii* nodavirus RNA-2. The numbering for the nucleotide sequences is given on the left and the numbering for the sequences of deduced amino acid is on the right.

Alphanodavirus than with *Betanodavirus*, although its position could be interpreted as a new genus within the family *Nodaviridae* (Fig. 4).

Discussion

Outbreaks of WTD in PL of *M. rosenbergii* had previously been reported in Taiwan (Tung *et al.* 1999). That histopathological study showed progressive segmental myofibre degeneration of muscles and necrotic myopathy. Dense viral inclusion bodies were observed in ultra-thin sections of affected muscle. Non-enveloped virions with a mean size of 23 nm were found in the cytoplasm of infected cells in the prawns, and were tentatively named as *Macrobrachium* muscle virus (MMV). Although MMV was not isolated and characterized, in its morphology, cytoplasmic location and striated muscle affinity, and in the clinical gross signs displayed by infected animals, it appears very similar to *Mr*NV. The present study is the first report confirming *Mr*NV virus infection associated with WTD of *M. rosenbergii* cultured in Taiwan by RT-PCR and *in situ* hybridization.

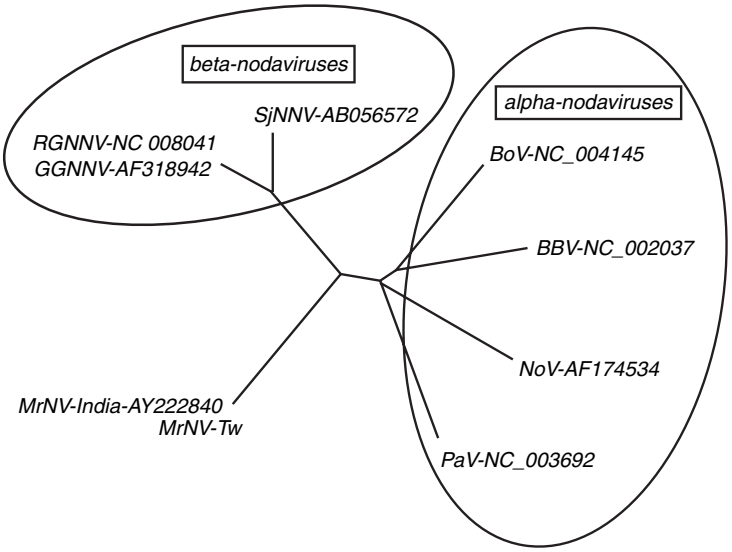

Figure 4 Phylogenetic un-rooted neighbour-joining tree deduced from analysis of nucleotide sequences of the complete gene of *Macrobrachium rosenbergii* nodavirus (*Mr*NV) capsid protein (Taiwan isolates) compared with other nodavirus isolates; Indian *Mr*NV strain (AY222840), black beetle virus (BBV) (NC002037), Boolarra virus (BoV) (NC004145), greasy grouper nervous necrosis virus (GGNNV) (AF318942), Nodamura virus (NoV) (AF174534), red spotted grouper nervous necrosis virus (RGNNV) (NC008041), Pariacoto virus (PaV) (NC003692), striped jack nervous necrosis virus (SJNNV) (AB056572).

Genome-based detection methods have been developed to detect *Mr*NV, among which RT-PCR is considered one of the most sensitive diagnostic methods in routine health monitoring (Sri Widada *et al.* 2003). In the present study, two primer pairs specific to *Mr*NV were used; 850 and 425 bp amplified products were obtained with primer pairs specific to RNA-1 and RNA-2, respectively, confirming that *Mr*NV was associated with WTD of *M. rosenbergii* cultured in Taiwan. In addition, the striated muscle of WTD-affected prawns was shown to be positive for *Mr*NV by *in situ* hybridization. Precipitation was only observed in the cytoplasm of necrotic muscle. The absence of any precipitate in tissues not infected by *Mr*NV suggested that this DIG probe does not cross-react with prawn tissue. *In situ* hybridization can accurately provide the precise location of the nuclei acids present in tissue sections as a result of the highly specific interaction between the probe and target sequence of viral nuclei acids. The techniques can be used to investigate the transmission route of *Mr*NV and help produce specific pathogen-free larvae of freshwater prawns in the future.

Macrobrachium rosenbergii nodavirus is classified as a member of the family *Nodaviridae* by its morphological and biochemical characteristics (Qian *et al.* 2003). Nodaviruses were first isolated from insects (Garzon & Charpentier 1991) and recently have been found in the larvae or juveniles of some marine fish (Munday, Kwang & Moody 2002). Because a highly conserved region was observed on the coat protein genes of fish nodaviruses, comparison with the variable region of the gene sequences could elucidate their evolutionary relationships. Based on the partial sequences of coat proteins from 25 isolates, fish nodaviruses are classified into four genotypes: barfin flounder nervous necrosis virus, tiger puffer nervous necrosis virus, SJNNV and RGNNV (Nishizawa, Furuhashi, Nagai, Nakai & Muroga 1997). In the present study, RT-PCR amplified products of the viral capsid gene of *Mr*NV were sequenced and compared using BLAST analysis with the nucleotide sequence of insect and fish nodaviruses. The results showed that the *Mr*NV Taiwan isolate did not belong to any genus within the family *Nodaviridae*, but has more affinity with the genus *Alphanodavirus* than with *Betanodavirus*. The phylogenetic relationships between *Mr*NV and other nodaviruses were in agreement with previous reports which compared the amino acid sequences deduced from the partial gene of RNA-dependent RNA polymerase (Bonami *et al.* 2005).

Sequence analysis of the partial genome of *Mr*NV RNA-2 demonstrated that it contained a unique open reading frame with 1116 nucleotides and encoded a structural protein with 371 amino acids. These results are similar to previous reports for an Indian isolate. The nucleotide sequence of RNA-2 of the *Mr*NV Taiwan isolate showed 97% homology with an Indian isolate by BLAST analysis. In addition, the deduced amino acid sequence of

the *Mr*NV Taiwan isolate showed similar homology. Only five deduced amino acid substitutions were observed compared with the Indian isolate. The conserved sequences indicate that *Mr*NV from different geographical regions are closely related.

White tail disease has been associated with *Mr*NV and its associated XSV. Although the role of the two viruses in the development of WTD is unknown, it is hypothesized that XSV represents a new species of satellite virus (Sri Widada & Bonami 2004). Using real-time RT-PCR, these authors concluded that the higher the infection dose of *Mr*NV, the higher the yield of both *Mr*NV and XSV. Furthermore, the linear correlation between *Mr*NV and XSV genome copies in infected prawns demonstrated that XSV is a satellite virus dependent on *Mr*NV (Zhang, Wang, Yuan, Li, Zhang, Bonami & Shi 2006). The present study demonstrated *Mr*NV infection in WTD-infected PL of freshwater prawns in Taiwan. The same specimens also showed XSV co-infection as demonstrated by RT-PCR and *in situ* hybridization (data not shown). The pathogenicity of *Mr*NV and the associated XSV virus and their relationship to WTD merit further study.

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.

Arcier J.M., Herman F., Lightner D.V., Redman R.M., Mari J. & Bonami J.R. (1999) A viral disease associated with mortalities in hatchery-reared postlarvae of the giant freshwater prawn *Macrobrachium rosenbergii*. *Diseases of Aquatic Organisms* **38,** 177–181.

Bell T.A. & Lightner D.V. (1988) *A Handbook of Normal Penaeid Shrimp Histology*. World Aquaculture Society, Baton Rouge, LA.

Bonami J.R., Shi Z., Qian D. & Sri Widada J. (2005) White tail disease of the giant freshwater prawn, *Macrobrachium rosenbergii*: separation of the associated virions and characterization of *Mr*NV as a new type of nodavirus. *Journal of Fish Diseases* **28,** 23–31.

Garzon S. & Charpentier G. (1991) Nodaviridae. In: *Atlas of Invertebrate Viruses* (ed. by J.R. Adams & J.R. Bonami), pp. 351–370. CRC Press, Boca Raton, FL.

Munday B.L., Kwang J. & Moody N. (2002) Betanodavirus infections of teleost fish: a review. *Journal of Fish Diseases* **25,** 127–142.

Nishizawa T., Furuhashi M., Nagai T., Nakai T. & Muroga K. (1997) Genomic classification of fish nodaviruses by molecular phylogenetic analysis of the coat protein gene. *Applied and Environmental Microbiology* **63,** 1633–1636.

Qian D., Shi Z., Zhang S., Cao Z., Liu W., Li L., Xie Y., Cambournac I. & Bonami J.R. (2003) Extra small virus-like particles (XSV) and nodavirus associated with whitish muscle disease in the giant freshwater prawn, *Macrobrachium rosenbergii*. *Journal of Fish Diseases* **26,** 521–527.

Romestand B. & Bonami J.R. (2003) A sandwich enzyme linked immunosorbent assay (S-ELISA) for detection of *Mr*NV in the giant freshwater prawn, *Macrobrachium rosenbergii* (de Man). *Journal of Fish Diseases* **26,** 71–75.

Sahul Hameed A.S., Yoganandhan K., Sri Widada J. & Bonami J.R. (2004) Studies on the occurrence of *Macrobrachium rosenbergii* nodavirus and extra small virus-like particles associated with white tail disease of *M. rosenbergii* in India by RT-PCR detection. *Aquaculture* **238,** 127–133.

Saitou N. & Nei M. (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4,** 406–425.

Shekhar M.S., Azad I.S. & Jithendran K.P. (2006) RT-PCR and sequence analysis of *Macrobrachium rosenbergii* nodavirus: Indian isolate. *Aquaculture* **252,** 128–132.

Sri Widada J. & Bonami J.R. (2004) Characteristics of the monocistronic genome of extra small virus, a virus-like particle associated with *Macrobrachium rosenbergii* nodavirus: possible candidate for a new species of satellite virus. *Journal of General Virology* **85,** 643–646.

Sri Widada J., Durand S., Cambournac I., Qian D., Shi Z., Dejonghe E., Richard V. & Bonami J.R. (2003) Genome-based detection methods of *Macrobrachium rosenbergii* nodavirus, a pathogen of the giant freshwater prawn, *Macrobrachium rosenbergii*: dot-blot, *in situ* hybridization and RT-PCR. *Journal of Fish Diseases* **26,** 583–590.

Sri Widada J., Richard V., Shi Z., Qian D. & Bonami J.R. (2004) Dot-blot hybridization and RT-PCR detection of extra small virus (XSV) associated with white tail disease of prawn *Macrobrachium rosenbergii*. *Diseases of Aquatic Organisms* **58,** 83–87.

Thompson J.D., Higgins D.G. & Gibson T.J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22,** 4673–4680.

Tung C.W., Wang C.S. & Chen S.N. (1999) Histological and electron microscopic study on *Macrobrachium* muscle virus (MMV) infection in the giant freshwater prawn, *Macrobrachium rosenbergii* (de Man), cultured in Taiwan. *Journal of Fish Diseases* **22,** 319–323.

Wang C.S., Chang J.S., Shi H.H & Chen S.N. (2007) RT-PCR amplification and sequence analysis of extra small virus associated with white tail disease of *Macrobrachium rosenbergii* (de Man) cultured in Taiwan. *Journal of Fish Diseases* **30,** 127–132.

Yoganandhan K., Sri Widada J., Bonami J.R. & Sahul Hameed A.S. (2005) Simultaneous detection of *Macrobrachium rosenbergii* nodavirus and extra small virus by a single tube, one-step multiplex RT-PCR assay. *Journal of Fish Diseases* **28,** 65–69.

Yoganandhan K., Leartvibhas M., Sriwongpuk S & Limsuwan C. (2006) White tail disease of the giant freshwater prawn *Macrobrachium rosenbergii* in Thailand. *Diseases of Aquatic Organisms* **69,** 255–258.

Zhang H., Wang J., Yuan J., Li L., Zhang J., Bonami J.R. & Shi Z. (2006) Quantitative relationship of two viruses (*Mr*NV and XSV) in white-tail disease of *Macrobrachium rosenbergii*. *Diseases of Aquatic Organisms* **71,** 11–17.

Received: 30 November 2006
Revision received: 13 July 2007
Accepted: 2 August 2007