# Is appendage deformity syndrome caused by *Macrobrachium rosenbergii* nodavirus?

The giant freshwater prawn *Macrobrachium rosenbergii*, popularly known as scampi, is an economically important, farmed crustacean species. Its farming has dramatically increased within the last five years and the current production is recorded to the tune of 30,450 mt during the year 2002–03 in India, being ranked third in the world. Andhra Pradesh is the major contributing state in India, with 21,580 ha of culture (62% of the total scampi culture area) producing 27,020 mt (89% of India's total production)[1]. Diseases are becoming the major constraints to the spread of this industry. Since 2002 onwards, a major viral disease, caused by *Macrobrachium rosenbergii* nodavirus (*Mr*NV), has affected the hatcheries and nursery tanks in the southern states of India, bringing about massive loss to the industry[2,3]. Recently, a new disease with unusual clinical signs has been reported in the major scampi culture area, Nellore district, Andhra Pradesh, which has been named as appendage deformity syndrome (ADS)[4]. ADS has affected more than 80% of the area under culture in Nellore district, bringing down culturable area to ~20,000 acres from more than one lakh acres of scampi ponds. The ADS-affected prawns revealed bent or deformed rostrum, antennae cut, beaded or corrugated appearance of antennules, more prone to breakage, corrugated appearance of the carapace, poor growth and varied mortality. It mostly starts after one to two months of stocking of juveniles in the culture ponds and is more pronounced during 4–5 months of culture. This type of disease or clinical sign has not been reported from any other country. The area under investigation has also recorded poor rainfall during the last three years.

A previous study revealed that carotenoid supplementation (1 g/kg) in the diet significantly reduces the ADS in prawns, both in laboratory condition and in a few ponds tested[4]. The same study also ruled out the possibility of involvement of any bacterial or viral (white spot syndrome virus, infectious hypodermal haematopoietic necrosis virus or monodon baculo virus) agents responsible for causing ADS. The disease is also not contagious in nature as has been seen from co-habitational studies.

Recently, the Central Institute of Freshwater Aquaculture (CIFA), Bhubaneswar has developed a RT–PCR based diagnostic tool to diagnose *Mr*NV; the sensitivity of the method is to detect as low as four viral particles[3]. It seems to be the most sensitive PCR method developed so far for detection of this virus, among all the published protocols[2,5]. The intensive survey conducted in Nellore scampi farms revealed that the aquafarmers believe that, the virus responsible for causing white tail disease (WTD) in scampi, is associated with or responsible for ADS in prawns. Although WTD has been reported from the West Indies since 1995, and Taiwan and China since 2002 onwards[5], no ADS-related clinical signs have been described. Herein, attempts have been made to identify the association of virus *Mr*NV using CIFA-developed diagnostic tool, or any other causal agents with ADS prawns.

ADS-affected prawns (5–100 g) were collected from culture ponds of Nellore district from 12 different locations over a period of one year and brought to the laboratory on ice. Apparently healthy, early juvenile sample from a nursery tank in one of the above locations was also collected. The water samples from the affected ponds as well as from the bore well sources feeding to the ponds were collected for physico-chemical analysis following standard methods[6]. Haemolymph samples were collected with the help of sterile syringes from the ADS-affected prawns.

On arrival in the laboratory, one portion of the muscle tissue, pleopods and hepatopancreas of ADS prawns from each location, was processed for isolation of RNA using TRI reagent (Sigma). Samples were analysed for the presence of *Mr*NV using CIFA-standardized RT–PCR technique.

To determine whether bacteria were the causal agents of ADS, the collected haemolymph and hepatopancreas of affected prawns were processed aseptically for bacterial isolation after inoculating into tryptone soya broth or brain heart infusion broth (Hi Media) with or without supplementation of 3% sodium chloride. After incubation for 24–48 h at 37°C, the broth cultures were streaked on tryptone soya agar plates to obtain any bacterial colony.

Remaining portions of hepatopancreas and muscles of ADS prawns were preserved in 10% neutral buffered formalin and processed subsequently for light microscopy using standard protocol.

The ADS-affected prawns did not reveal the presence of *Mr*NV in all the samples collected. However, the juvenile sample collected from nursery pond, which did not show any clinical signs of WTD, was found to be positive on nested PCR (Figure 1). The obtained amplified segment was also further confirmed to be the sequence of *Mr*NV by restriction analysis with *Kpn*I (Genei, India), by observing the expected product sizes of 313 and 176 bp on 1% agarose gel (Figure 2).

Attempts to isolate any bacteria from haemolymph or hepatopancreas failed; even a single colony could not be obtained on agar. The broth did not show any turbidity after three days of incubation. Simi-

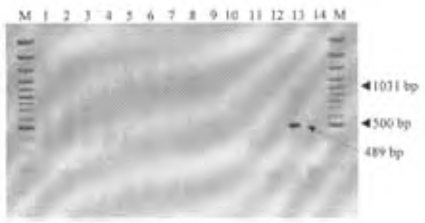


**Figure 1.** Agarose gel showing RT–PCR amplicon for detection of *Mr*NV from ADS-affected prawns and one apparently healthy early juvernile. M, DNA marker; lanes 1–12, ADS samples; lane 13, Apparently healthy early juvenile from nursery; lane 14, Known negative control.

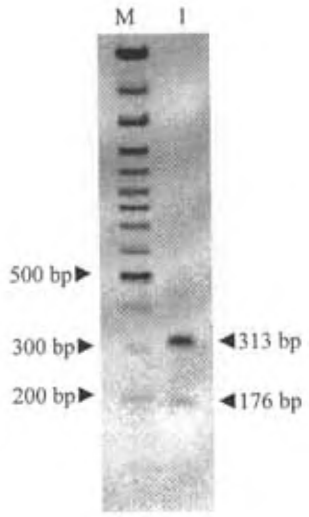


**Figure 2.** Restriction enzyme (*Kpn*I) profile of second-step PCR product of the detected *Mr*NV sample run on 1% agarose gel. M, Marker; lane 1, Second-step product after digestion.

**Table 1.** Water quality parameters of ADS-affected ponds

| Parameter | Range | Average (mean ± SE) |
|---|---|---|
| pH | 7.4–8.4 | 7.79 ± 0.11 |
| Total alkalinity (mg/l, as $CaCO_3$) | 50–400 | 270 ± 35.68 |
| Total hardness (mg/l, as $CaCO_3$) | 40–245 | 155.9 ± 19.29 |
| $NH_4$-N (mg/l) | 0.01–1.70 | 0.64 ± 0.22 |
| $NO_3$-N (mg/l) | 0.005–0.60 | 0.24 ± 0.08 |
| Dissolved phosphate (mg/l) | 0.02–0.08 | 0.05 ± 0.01 |
| Conductivity (mmho/cm) | 0.73–2.47 | 1.61 ± 0.31 |

Data are mean of 12 samples collected from different ponds/borewells of ADS-affected regions of Nellore.

larly, the histology of muscle tissue or hepatopancreas did not reveal any significant pathology.

On the other hand, major alterations in the physico-chemical parameters of the water samples were observed, which may not be suitable for scampi culture on long-term basis (Table 1). The observed high alkalinity, hardness and ammonia content in the cultured water, where the culture is mostly practised on a continuous basis without intermittent drying of the pond, might have occurred due to high organic load in the pond. Most of these parameters observed in the culture ponds are beyond the recommended levels of freshwater prawn farming. Ideal levels of transparency (25–40 cm); alkalinity ($CaCO_3$; 20–60 mg/l); hardness ($CaCO_3$; 30–150 mg/l), and ammonia (0.1–0.3 mg/l) in *M. rosenbergii* culture ponds are essential[7]. The role of water quality parameters in the sampled ponds with relation to the occurrence of ADS needs to be further investigated.

Similar type of culture technology is also practised in other parts of Andhra Pradesh, particularly Kakinada and Vijayawada areas, using the same seed sources and feed materials. However, occurrence of ADS has not been reported so far. The low rainfall in Nellore region, forcing farmers to exploit underground source of water and continous culture in the same pond, might be playing roles as predisposing factors or causal factors for ADS. However, further in-depth study is required to confirm involvement of any pathogen or carotenoid deficiency or water quality as the possible cause(s) of ADS.

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# Reproductive biology of *Withania somnifera* (L.) Dunal

*Withania somnifera* (family Solanaceae) is an important medicinal plant known classically for its rejuvenating properties, and hence called Indian Ginseng[1]. In view of its varied therapeutic potential, it is the subject of considerable modern scientific attention. A perusal of the literature shows that little information exists on floral biology and reproductive behaviour, and there is emerging need to select high-yielding plants and genetically improve them for cultivation to meet the demand of the pharmaceutical industries. Some crosses between two cultivated lines (WS20 and WS22) with wild type were attempted for increase in root yield and seed yield over their parents[2–4].

In our institutional experimental farm, we have a wide genetic resource base of the species constituting 50 accessions collected from all over India. Morphological and chemical characterization of these accessions resulted in identification of some elite types, which will eventually help in evolving varieties of commercial significance. The present investigation was undertaken to study the morphological and functional characteristics of the flowers contributing towards the reproductive success of the best performing morphotype – AGB002 (a selectant originated from wild populations in Rajasthan region) growing in experimental plots of our institute.

Pollen transfer experiments were carried out to investigate the effect of pollen source on fruit set and number of seeds/fruit (seed set) as well as seed germination. The treatments tried were: (i) autogamy – pollination with pollen from the same flower ($n = 20$ flowers on 20 plants); (ii) open pollination – 20 plants were tagged without receiving any treatment and (iii) xenogamy – pollination with pollen from another accession AGB025 (cultivated variety) followed by bagging ($n = 20$). Pollination was manually undertaken during 0900 to 1100 h (period of high stigma receptivity) during April to May 2003. In all the cases, once the necessary time had elapsed, fruit set and seed set