IHHNV infection from the wild shrimps of Andaman and Nicobar Islands, India

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The present study was intended to screen the wild shrimps of Andaman and Nicobar Islands (ANI) against infectious diseases. A total of 175 shrimp samples (35 pools) consisting of *Fenneropenaeus indicus*, *Penaeus monodon*, *Penaeus merguiensis* and *Metapenaeus monoceros* were collected from different landing centers across ANI. Out of 35 pools of samples analysed by PCR, a total of 10 pools of *Penaeus monodon* collected from Betapur (1 pool), Lohabarrack (4 pools) and Campbell Bay (5 pools) were found positive for Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV). Nucleotide sequence of IHHNV isolated from ANI showed 100% identity to the sequences of IHHNV reported from Vietnam, Taiwan, Australia, China, Egypt, USA, Ecuador, 99% identity to IHHNV reported from Brazil, Venezuela, Korea, 96% identity to IHHNV reported from Thailand and 95% identity to IHHNV reported from India. Based on phylogenetic tree analysis, IHHNV of ANI is closely related to IHHNV of Vietnam. Histopathological analysis revealed typical eosinophilic intranuclear cowdry type A inclusion bodies in gill lamellae which further confirmed the IHHNV infection. The present study provides a definitive evidence for the first report of infectious IHHNV in wild *P. monodon* from ANI.

Keywords: Andaman and Nicobar Islands, disease surveillance, IHHNV, *Penaeus monodon*, wild shrimp.

ANDAMAN AND NICOBAR group of Islands belonging to the union territory of India are situated between 6° to 14°N and 92° to 94°E in the Southeast of Bay of Bengal and consist of 572 islands coming under three districts namely, North and Middle Andaman, South Andaman and Nicobar. India ranks second in shrimp production next to China[1]. As India is one of the top ranked shrimp producers of the world, viral diseases poise a serious threat to Indian shrimp culture. Presently, the viral diseases detected in the mainland of India include White Spot Syndrome Virus (WSSV), IHHNV, Hepatopancreatic Parvo Virus (HPV), Monodon Baculo Virus (MBV) and Laem-Singh Virus (LSNV)[2–5]. At present, only freshwater carp farming is being practised in Andaman and Nicobar Islands (ANI), while brackishwater aquaculture, mainly shrimp farming and mariculture are the identified potential areas for development in aquaculture sector. When compared to mainland of India and neighbouring Southeast Asian countries, very few aquatic animal diseases, mainly shrimp diseases like vibriosis, LSNV and WSSV were reported from ANI[6–9]. ANI are believed to be free from many fish diseases as well as shrimp pathogens compared to the mainland of India and other neighbouring countries though it shares close proximity with Southeast Asian countries like Indonesia, Thailand and Malaysia where shrimp diseases like White Spot Disease (WSD), Infectious Hypodermal and Haematopoietic Necrosis (IHHN), Taura Syndrome (TS), Yellow Head Disease (YHD) and Monodon Baculo Virus Disease (MBVD) were reported[3,10–13]. The absence of many diseases in ANI may be due to geographical isolation of the Islands, absence of shrimp aquaculture at present or lack of intensified research on disease surveillance of aquatic animals. ANI are blessed with rich aquatic biodiversity and also well-known for quality shrimp broodstocks. Another point of concern is that Andaman Sea bounded by ANI in the West, Myanmar in the North, Thailand and Malaysia in the East, Indonesia in the South are considered as hotspots of intensified shrimp aquaculture with major threat of viral diseases. With this background, disease surveillance was carried out to check whether the wild shrimps of ANI are free from viral infections and confirmed the presence of IHHNV. Further, the extent of IHHNV infection in wild shrimps and its geographical range extension were also elucidated. This research may also be worthwhile in studying the transmission of shrimp viral diseases into the Island ecosystem.

IHHNV, being an Office International des Epizootics (OIE) listed disease, is caused by the smallest known penaeid shrimp virus[14]. IHHNV causes runt deformity syndrome[15] and the symptoms and clinical signs include slow mortality, abnormal physical defects, slow growth, small size and rostrum, antenna, thoracic and abdominal deformities. The current study offers concrete evidence for the occurrence of IHHNV in wild shrimps and its prevalence in these islands which may help establish precautionary measures for undertaking shrimp farming activity in the future.

Shrimp samples were collected from landing centres of ANI covering North and Middle Andaman, South Andaman and Nicobar districts. A total of 175 shrimp samples consisting of *Fenneropenaeus indicus* (Milne Edwards), *Penaeus monodon* (Fabricius), *Penaeus merguiensis* (De

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Man) and *Metapenaeus monoceros* (Fabricius) were collected from 9 landing centers namely Durgapur (13°16′45.7″N; 93°2′9.1″E), Laxmipur (13°17′33.03″N; 92°57′29.16″E), Mayabunder (12°54′35.3″N; 92°54′ 29.1″E), Betapur (12°36′1.3″N; 92°57′22.3″E), Yerrata (12°27′36.06″N; 92°53′47.54″E), Junglighat (11°39′25. 26″N; 92°43′30.23″E), Lohabarrack (11°37′21.32″N; 92°38′49.03″E), Wandoor (11°35′44.66″N; 92°36′ 28.81″E) and Campbell Bay (6°54′07.30″N; 93°53′44. 20″E) across ANI from August 2015 to March, 2016 (Figure 1). Shrimp samples with mean length and mean weight of 14 cm and 63 g respectively were collected for disease screening. Out of 175 shrimp samples, a total of 35 pools of samples were made by pooling 5 numbers of shrimp samples in each pool for disease screening (Table 1). Tissues like pleopod, gill and muscle were dissected out and preserved in 90% ethanol for DNA isolation.

Modified CTAB (cetyl trimethyl ammonium bromide) method[16] was used to extract DNA. PCR was performed following the OIE protocol[17]. The primer set[18] of forward 309F, 5′ TCCAACACTTAGTCAAAACCAA 3′ and reverse 309R, 5′ TGTCTGCTACGATGATTATCCA 3′ were used giving 309 bp of amplicon size. A 25 µl of PCR mix contained 2.5 µl of 10X PCR buffer, 2 µl of 25 mM $MgCl_2$, 0.5 µl of 2 mM dNTP, 0.3 µl of 50 pmol forward and reverse primers, 0.125 µl of 5 units μl^{-1} Taq polymerase, 1 µl template DNA with concentration of 1 µg and 18.575 µl of nuclease free water. Amplification reactions consisted of 95°C for 5 min, 35 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 1 min and finally 72°C for 7 min in a thermal cycler (Bio-Rad, USA). The PCR products were resolved in 1.5% agarose gel containing ethidium bromide and analysed using a gel documentation system (Bio-Rad, USA).

The positive PCR products were sequenced using 309F and 309R primers in ABI 3500 DNA analyser (Shrimpex Biotech, Chennai). The generated sequences were analysed using the Basic Local Alignment Search Tool (BLAST) program at the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov/blast/) GenBank nucleotide database for finding homology with other sequences. A neighbour-joining phylogenetic tree was constructed based on the nucleotide sequence of IHHNV isolated from ANI with other sequences retrieved from the GenBank using MEGA6 software[19], employing pairwise deletion and Kimura-2 method[20]. For histopathological analysis, tissue samples were fixed in Davidson's fixative for 48 h and processed using routine histological techniques[21].

In this present study, the primer IHHNV 309F/R was used which could amplify only IHHNV and did not amplify the integrated virus-related sequences in shrimp genome[18]. Out of 35 pools of samples analysed by PCR, a total of 10 pools of samples of *Penaeus monodon* collected from Betapur (1 pool), Lohabarrack (4 pools) and Campbell Bay (5 pools) were found positive for IHHNV (Figure 2). Likewise in India, 67.4% prevalence of IHHNV infections in post larval samples and 34% prevalence in adult shrimps were reported from cultured *P. monodon* by using IHHNV 309F/R primers[22]. IHHNV infection was reported only from the tiger shrimp, *P. monodon* which supports the fact that IHHNV affects mainly *P. monodon*, *P. vannamei* and *P. stylirostris*[23] and also IHHNV is an endemic virus in the geographical range of *P. monodon*[13]. Higher rate of IHHNV infection was recorded from Nicobar which may be due to the reason that the sample collection station, i.e. Campbell Bay is very near Southeast Asian countries like Indonesia and Thailand where high prevalence of IHHNV was reported from wild and cultured *P. monodon*[24–26]. It was also supported by earlier reports that the occurrence of IHHNV in Southeast Asian (Singapore, Malaysia, Indonesia, Philippines) shrimp culture facilities using only wild *P. monodon* broodstock suggests that this region is within the virus' natural geographic range, and that *P. monodon* may be among its natural host species[10].

Nucleotide sequence of IHHNV isolated from ANI (GenBank Accession number KU992382) showed 100% identity to the sequences of IHHNV reported from Vietnam, Taiwan, Australia, China, Egypt, USA, Ecuador; 99% identity to the sequences of IHHNV reported from Brazil, Venezuela, Korea and 96% identity to the sequence of IHHNV reported from Thailand. On the other hand, nucleotide sequence of IHHNV isolated from ANI showed 95% identity to the sequence of IHHNV reported from mainland of India. Based on phylogenetic tree analysis, IHHNV of ANI is closely related to IHHNV of Vietnam (Figure 3). It is corroborated that IHHNV of ANI is closely related to the IHHNV of Southeast Asian countries like Vietnam and Thailand than mainland of India. Further, histology of IHHNV infected shrimp gill lamellae sections unveiled the presence of eosinophilic cowdry type A intra-nuclear inclusions in the hypertrophied nuclei of epithelial cells that are pathognomonic for IHHNV infection (Figure 4).

A lot of emphasis has been given to evaluate the IHHNV infection in wild populations of shrimps[23,27]. The present study provides definitive evidence for the occurrence of infectious IHHNV in wild *P. monodon* from ANI. At present, shrimp culture is not intensified as commercial venture in ANI. However, the local administration is trying to promote brackishwater aquaculture and mariculture in future. Shrimp broodstocks collected from ANI cannot be presumed to be disease-free and hence a strong specific pathogen-free (SPF)-based monitoring process should be put in place before promoting wide-scale aquaculture in these Islands.

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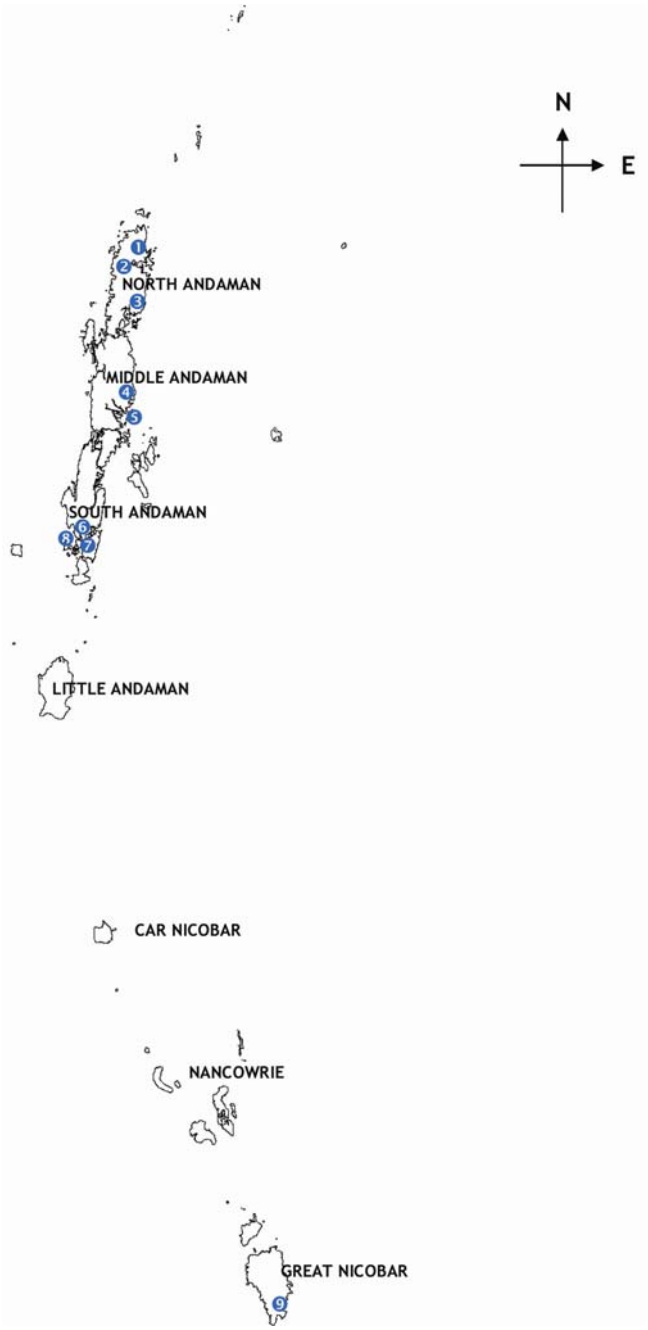

Figure 1. Map showing sample collection sites in Andaman and Nicobar Islands. 1, Durgapur; 2, Laxmipur; 3, Mayabunder; 4, Betapur; 5, Yerrata; 6, Junglighat; 7, Lohabarrack; 8, Wandoor and 9, Campbell Bay.

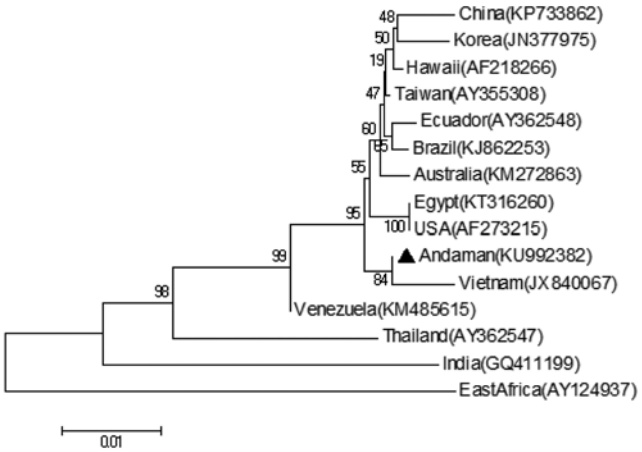

Figure 3. Phylogenetic analysis of Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV) of Andaman and Nicobar Islands (Andaman) with IHHNV of other countries retrieved from GenBank. Values within parentheses represent GenBank accession numbers. The phylogenetic tree was generated using neighbor-joining method of MEGA. Numbers indicate the percentage of bootstrap support from 1000 replicates.

Table 1. Details of sample collection. Each pool contains five shrimps

Name of the District	Landing center	Shrimp species	Number of pools	Number of pools positive for IHHNV
North and Middle Andaman	Durgapur	*P. monodon*	1	
		P. merguiensis	1	
	Laxmipur	*F. indicus*	1	
		P. merguiensis	1	
	Mayabunder	*F. indicus*	2	
	Betapur	*P. monodon*	2	1
		P. merguiensis	1	
	Yerrata	*F. indicus*	1	
		P. merguiensis	2	
South Andaman	Junglighat	*P. merguiensis*	4	
	Lohabarrack	*P. monodon*	13	4
	Wandoor	*M. monoceros*	1	
Nicobar	Campbell Bay	*P. monodon*	5	5
		Total	35	10

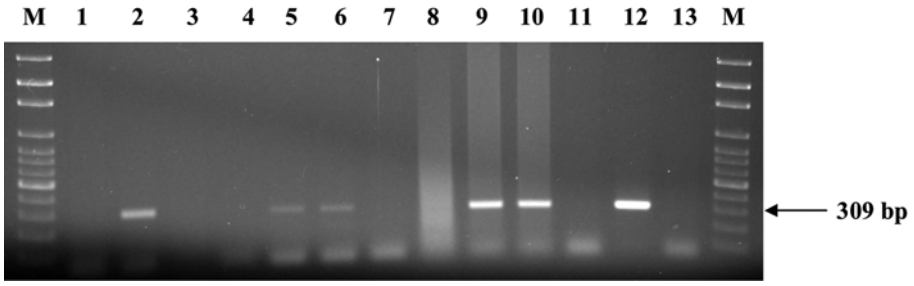

Figure 2. PCR amplification using IHHNV 309F/R primers. Lane M: Molecular weight marker (100 bp); Lane 1, Durgapur sample; Lane 2, Betapur sample; Lane 3, Mayabunder sample; Lane 4, Yerrata sample; Lanes 5 and 6, Lohabarrack samples; Lanes 7 and 8, Junglighat samples; Lanes 9 and 10, Campbell Bay samples; Lane 11, Wandoor sample; Lane 12, Positive control; Lane 13, Negative control.

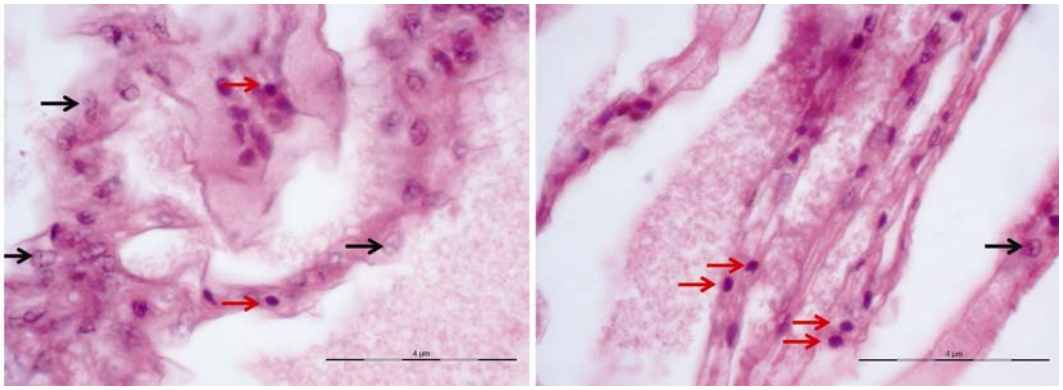

Figure 4. Gill sections of infected *Penaeus monodon* showing epithelial cells with diagnostic IHHN eosinophilic intranuclear cowdry type A inclusion bodies in their hypertrophied nuclei (red arrows) of IHHNV infection and normal cells (black arrows) (100× magnification).