24. Tinni, T. B. R., Ali, M. A., Mehraj, H., Mutahera, S. and Jamal-Uddin, A. F. M., Effect of pruning technique on growth and yield of Brinjal. *J. Exp. Biosci*., 2014, **5**(1), 55–60.
25. Tongumpai, P., Charnwichit, S., Srisuchon, S. and Subhadrabandhu, S., Effect of thiourea on terminal bud break of mango. *Acta Hortic*., 1997, **455**, 71–75; doi.10.17660/ActaHortic. 1997.455.10.
26. Rane, J., Lakkineni, K. C., Kumar, P. and Abrol, Y. P., Salicylic acid protects nitrate reductase activity of wheat (*Triticum aestivum* L.) leaves. *Plant Physiol. Biochem*., 1995, **22**(2), 119–121.
27. Lakkineni, K. C., Rane, J., Kumar, P. A. and Abrol, Y. P., Thiol compounds support nitrate reductase activity *in vivo* in the leaves of *Brassica campestris*. *Indian J. Exp. Biol.*, 1995, **34**, 387–389.
28. Sivakumar, M. V. K., Motha, R. P. and Das, H. P., Natural Disaster and Extreme Events in Agriculture: Impacts and Mitigation. Springers Science and Business Media, 2005, p. 367.
29. Chaum, S., Siringam, K., Juntawong, N. and Kirdmanee, C., Water relations, pigment stabilization, photosynthetic abilities and growth improvement in salt stressed rice plants treated with exogenous potassium nitrate application. *Int. J. Plant Prod.*, 2012, **4**(3), 187–198.
30. Garg, B. K., Burman, U. and Kathju, S., Influence of Thiourea on photosynthesis, nitrogen metabolism and yield of clusterbean (Cyamopsistetragonoloba (L.) Taub.) under rainfed conditions of Indian Arid Zone. *Plant Growth Regul.*, 2006, **48**(3), 237–245.
31. Sivasankar, A., Lakkineni. K. C., Rane, J., Kumar, P. A., Nair, T. V. R. and Abrol, Y. P., Photosynthetic characteristics of urea-treated wheat (*Triticum aestivum* L.). *J. Plant Nutr.*, 1995, **18**, 2213–2217.

Received 26 February 2017; accepted 16 June 2017

doi: 10.18520/cs/v113/i10/2021-2027

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

K. Saravanan[1,*], P. Puneeth Kumar[1], Arunjyoti Baruah[1], J. Praveenraj[1], T. Sathish Kumar[2], S. Pramod Kumar[1], T. Sivaramakrishnan[1], A. Anuraj[1], J. Raymond Jani Angel[1], R. Kiruba Sankar[1] and S. Dam Roy[1]

[1]ICAR-Central Island Agricultural Research Institute, Port Blair 744 105, India
[2]ICAR-Central Institute of Brackishwater Aquaculture, 75 Santhome High Road, Raja Annamalai Puram, Chennai 600 028, India

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 centres across ANI. Out of 35 pools of samples analysed by polymerase chain reaction (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°–14°N and 92°–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 pose a serious threat to Indian shrimp culture. Presently, the viral diseases detected in the mainland of India include White Spot Syndrome Virus (WSSV), Infectious Hypodermal and Hematopoietic Necrosis Virus (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

*For correspondence. (e-mail: sarocife@gmail.com)

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 Man) and *Metapenaeus monoceros* (Fabricius) were collected from 9 landing centres 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 sec, 55°C for 30 sec, 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

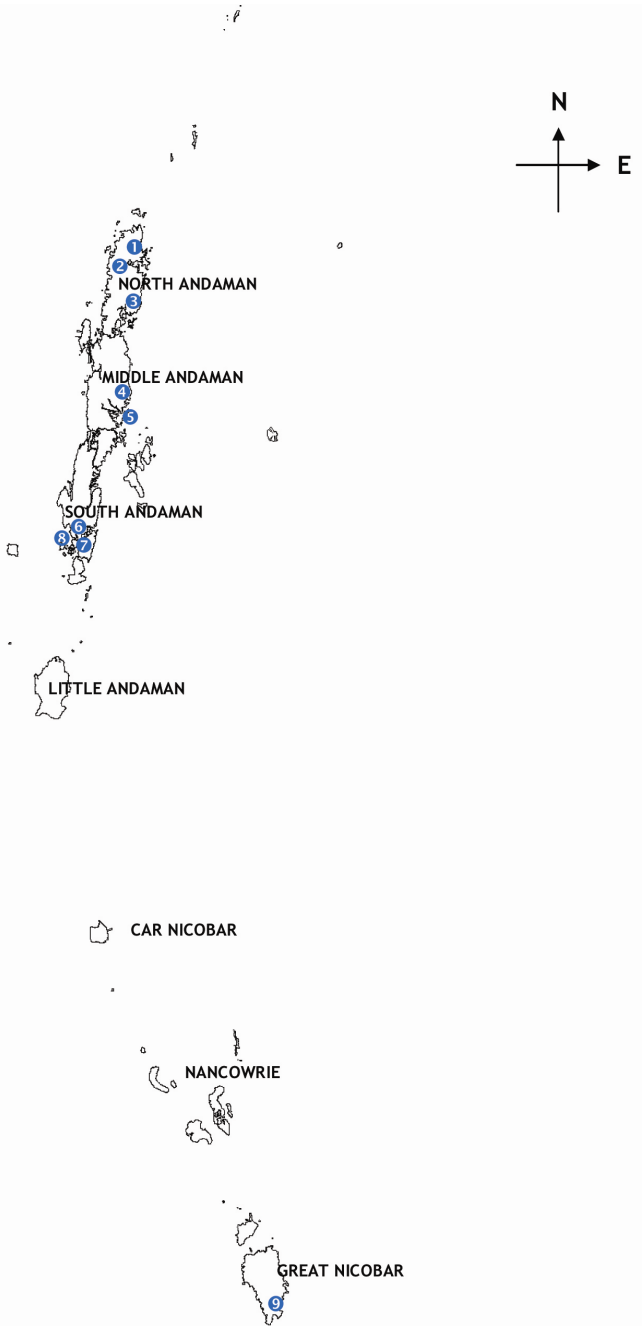

Figure 1. Map showing sample collection sites in Andaman and Nicobar Islands. 1, Durgapur; 2, Laxmipur; 3, Mayabunder; 4, Betapur; 5, Yerrata; 6, Junglighat; 7, Lohabarrack; 8, Wandoor and 9, Campbell Bay.

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

District	Landing centre	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

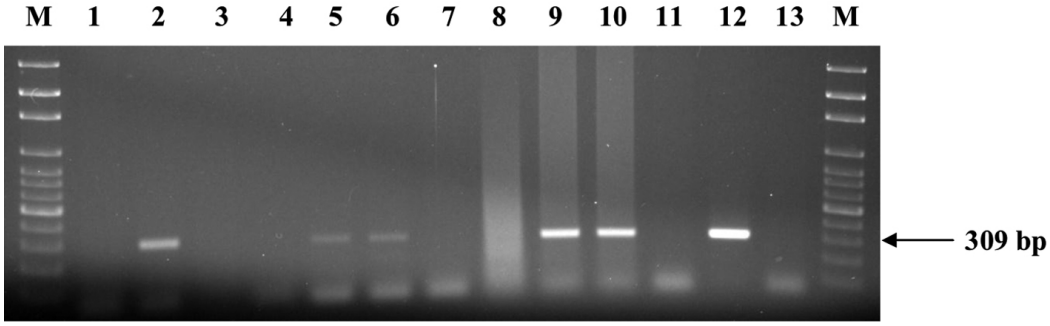

Figure 2. PCR amplification using IHHNV 309F/R primers. Lane M: Molecular weight marker (100 bp); Lane 1, Durgapur sample; Lane 2, Betapur sample; Lane 3, Mayabunder sample; Lane 4, Yerrata sample; Lanes 5 and 6, Lohabarrack samples; Lanes 7 and 8, Junglighat samples; Lanes 9 and 10, Campbell Bay samples;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

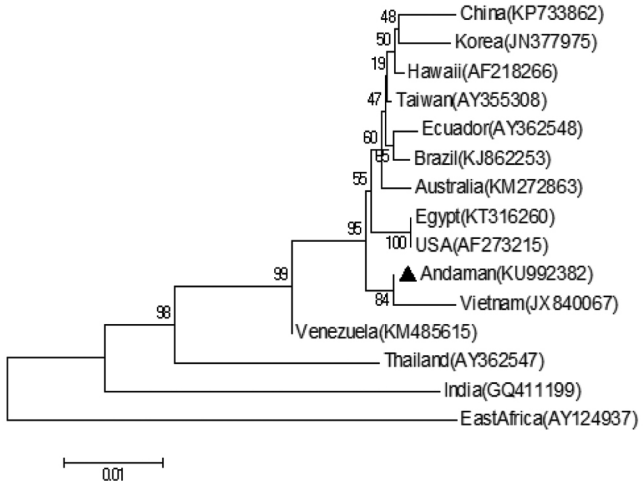

Figure 3. Phylogenetic analysis of IHHNV of Andaman and Nicobar Islands (Andaman) with IHHNV of other countries retrieved from GenBank. Values within parentheses represent GenBank accession numbers. The phylogenetic tree was generated using neighbour-joining method of MEGA. Numbers indicate the percentage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

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 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].

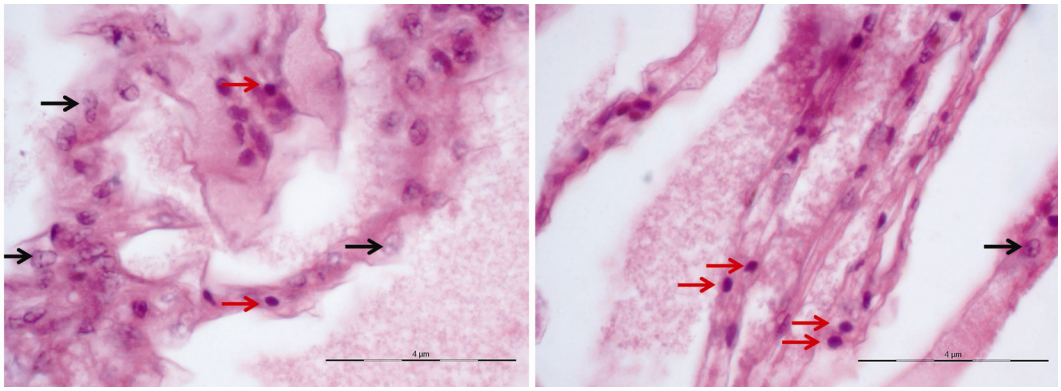

Figure 4. Gill sections of infected *Penaeus monodon* showing epithelial cells with diagnostic IHHN eosinophilic intranuclear cowdry type A inclusion bodies in their hypertrophied nuclei (red arrows) of IHHNV infection and normal cells (black arrows) (100× magnification).

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*[15] 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*[23–25]. 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[15,26]. 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.

1. Ravuru, D. B. and Mude, J. N., Growth of cultured white leg shrimp *Litopenaeus vannamei* (Boone, 1931) of brackishwater culture system in summer season with artificial diet. *Pelagia Res. Lib.*, 2014, **5**, 25–28.
2. Bondad-Reantaso, M. *et al.*, Disease and health management in Asian aquaculture. *Vet. Parasitol.*, 2005, **132**, 249–272.
3. Flegel, T. W., Detection of major penaeid shrimp viruses in Asia, a historical perspective with emphasis on Thailand. *Aquaculture*, 2006, **258**, 1–33.
4. Prakasha, B. K., Ramakrishna, R. P., Karunasagar, I. and Karunasagar, I., Detection of Laem-Singh virus (LSNV) in cultured *Penaeus monodon* from India. *Dis. Aquat. Org.*, 2007, **77**, 83–86.
5. Kalaimani, N., Ravisankar, T., Chakravarthy, N., Raja, S., Santiago, T. C. and Ponniah, A. G., Economic losses due to disease incidences in shrimp farms of India. *Fish. Technol.*, 2013, **50**, 80–86.
6. Shome, R., Shome, B. R. and Soundararajan, R., Studies on luminous *Vibrio harveyi* isolated from *Penaeus monodon* larvae reared in hatcheries in Andamans. *Indian J. Fish.*, 1999, **46**(2), 141–147.
7. Kumar, T. S., Krishnan, P., Makesh, M., Chaudhari, A., Purushothaman, C. S. and Rajendran, K. V., Natural host-range and experimental transmission of Laem-Singh virus (LSNV). *Dis. Aquat. Org.*, 2011, **96**, 21–27.
8. Sethi, S. N., Mahendran, V., Nivas, K., Krishnan, P., Roy, S. D., Ram, N. and Sethi, S., Detection of white spot syndrome (WSSV)

in broodstock of tiger shrimp, *Penaeus monodon* and other crustaceans of Andaman waters. *Indian J. Marine Sci*., 2011, **40**(3), 403–406.
9. Saravanan, K. *et al*., Overview of aquatic animal diseases in Andaman and Nicobar Islands. *J. Immunol. Immunopathol*., 2015, **17**(1), 17–24.
10. Lightner, D. V., Bell, T. A. and Redman, R. M., A review of the known hosts, geographical range and current diagnostic procedures for the virus diseases of cultured penaeid shrimp. *Adv. Trop. Aquacul.*, 1989, 113–126.
11. Walker, P. J., Cowley, J. A., Spann, K. M., Hodgson, R. A. J., Hall, M. R. and Withychumnarnkul, B., Yellow head complex viruses: transmission cycles and topographical distribution in the Asia-Pacific region. In The New Wave: Proceedings of the Special Session on Sustainable Shrimp Culture, Aquaculture 2001 (eds Browdy, C. L. and Jory, D. E.), The World Aquaculture Society, Baton Rouge, Louisiana, 2001, pp. 227–237.
12. Nielsen, L., Sang-oum, W., Cheevadhanarak, S. and Flegel, T. W., Taura syndrome virus (TSV) in Thailand and its relationship to TSV in China and the Americas. *Dis. Aquat. Org*., 2005, **63**, 101–106.
13. Flegel, T. W., Current status of viral diseases in Asian shrimp aquaculture. *Isr. J. Aquacul. – Bamidgeh*, 2009, **61**, 229–239.
14. Bonami, J. R. and Lightner, D. V., Unclassified viruses of Crustacea. In *Atlas of Invertebrate Viruses* (eds Adams, J. R. and Bonami, J. R.), CRC Press, Boca Raton, Florida, 1991, pp. 597–622.
15. Vega-Heredia, S., Mendoza-Cano, F. and Sanchez-Paz, A., The infectious hypodermal and haematopoietic necrosis virus: a brief review of what we do and do not know. *Transbound. Emerg. Dis*., 2012, **59**, 95–105.
16. Bruce, L. D., Trumper, B. B. and Lightner, D. V., Methods for viral isolation and DNA extraction for a penaeid shrimp baculovirus. *J. Virol. Methods*, 1993, **34**, 245–254.
17. OIE, Manual of diagnostic tests for aquatic animals, Office International des Epizootics, Paris, 2015.
18. Tang, K. F. J., Navarro, S. A. and Lightner, D. V., A PCR assay for discriminating between infectious hypodermal and hematopoietic necrosis virus (IHHNV) and the virus-related sequences in the genome of *Penaeus monodon*. *Dis. Aquat. Org*., 2007, **74**, 165–170.
19. Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S., MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.*, 2013, **30**, 2725–2729.
20. Kimura, M., A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evolut*., 1980, **16**, 111–120.
21. Bell, T. A. and Lightner, D. V., A handbook of normal penaeid shrimp histology. Special Publication no. 1, World Aquaculture Society, Baton Rouge, 1988.
22. Rai, P., Pradeep, B., Safeena, M. P., Karunasagar, I. and Karunasagar, I., Simultaneous presence of infectious hypodermal and hematopoietic necrosis virus (IHHNV) and type A virus-related sequence in *Penaeus monodon* from India. *Aquaculture*, 2009, **295**, 168–174.
23. Flegel, T. W., Major viral diseases of the black tiger prawn (*Penaeus monodon*) in Thailand. *World J. Microbiol. Biotechnol*., 1997, **13**, 433–442.
24. Primavera, J. H. and Quinitio, E. T., Runt-deformity syndrome in cultured giant tiger prawn *Penaeus monodon*. *J. Crustacean Biol*., 2000, **20**, 796–802.
25. Tang, K. F. J., Poulos, B. T., Wang, J., Redman, R. M., Shih, H. H. and Lightner, D. V., Geographic variations among infectious hypodermal and hematopoietic necrosis virus (IHHNV) isolates and characteristics of their infection. *Dis. Aquat. Org*., 2003, **53**, 91–99.
26. Rai, P., Safeena, M. P., Krabsetsve, K., La Fauce, K., Owens, L. and Karunasagar, I., Genomics, molecular epidemiology and diagnostics of infectious hypodermal and hematopoietic necrosis virus. *Indian J. Virol.*, 2012, **23**, 203–214.

ACKNOWLEDGEMENTS. This work was carried out under the National Surveillance Programme for Aquatic Animal Diseases (NSPAAD), coordinated by the ICAR-National Bureau of Fish Genetic Resources (NBFGR), Lucknow. The authors thank the Indian Council of Agricultural Research (ICAR) and National Fisheries Development Board (NFDB), Govt. of India, for financial support to carry out this work. The authors are grateful to the Referral Laboratory at ICAR-CIBA, Chennai for validating the IHHNV positive samples.

Received 13 June 2016; revised accepted 12 May 2017

doi: 10.18520/cs/v113/i10/2027-2031

How $NaCl$, Na_2SO_4, $MgCl_2$ and $CaCl_2$ salts affect the germinability of *Pinus halepensis* Mill.

Bouzid Nedjimi*

Laboratory of Exploration and Valorization of Steppe Ecosystem, Faculty of Science of Nature and Life, University of Djelfa, Cité Aîn Chih, P.O. Box 3117 Djelfa 17000, Algeria

In the Mediterranean forests, *Pinus halepensis* Mill. (Aleppo pine) plays an important role against desertification, reforestation of degraded lands and soil rehabilitation. Therefore, knowledge of its seed germinability requirements is necessary for its propagation in field conditions to colonize new territories habitually not conventional for other species. The study was carried out to assess the effects of different soluble salts ($NaCl$, Na_2SO_4, $MgCl_2$ and $CaCl_2$) on seed germination characteristics [germination percentage (GP) and rate of germination (RG)] of this conifer. Data show that all soluble salts decreased both parameters GP and RG. The highest GP was obtained in conditions without salinity. The maximum values of germination were obtained by low concentrations of $MgCl_2$. Comparatively, $NaCl$ was generally the most toxic salt followed by $CaCl_2$ and Na_2SO_4. The present findings could be useful in the design of future projects for reforestation of degraded arid lands.

Keywords: Aleppo pine, rate of germination, reforestation, saline soils.

ECO-PHYSIOLOGICAL studies about regeneration of endemic conifers species grown in arid and semi-arid areas and the factors influencing them are important for the

*e-mail: bnedjimi@yahoo.fr