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ERRATUM

Erratum to: Genetic characterization of betanodavirus isolates from Asian seabass *Lates calcarifer* (Bloch) in India

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Unfortunately, in the original publication, an author in the author group was missed and the acknowledgements are wrongly published. The correct author group and acknowledgements are given below:

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BRIEF REPORT

Genetic characterization of betanodavirus isolates from Asian seabass *Lates calcarifer* (Bloch) in India

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Abstract Betanodavirus has been detected in Asian seabass in India. Molecular characterization of the isolates on the basis of the full-length viral RNA2 sequence was performed. Subsequent phylogenetic analysis with sequences from members of the four species in the genus *Betanodavirus* revealed that the present isolates are closely related to members of the species *Redspotted grouper nervous necrosis virus*. The analysis also revealed that the RNA2 sequence was not responsible for acute symptoms in seabass. This is the first attempt to characterize Indian isolates of fish nodaviruses, and the result will be useful for devising specific control and health-management strategies for this virus.

Viral nervous necrosis (VNN) or viral encephalopathy and retinopathy (VER) is a globally emerging disease reported in over 40 fish species from marine, brackish-water and freshwater environments [13, 17]. In India, the disease has been reported in Asian seabass [1, 18], some freshwater ornamental fish [10], and clown fish (in press). The disease affects the neuronal tissues of brain, spinal cord and eye. Specific symptoms include erratic and corkscrew-like swimming behavior and lethargy. Mortality of the fish stock may reach 100 % within 2-3 days of disease onset. The disease is caused by betanodaviruses, of the family *Nodaviridae*, which are small, icosahedral (25 nm) RNA viruses [14]. Betanodaviruses cause acute as well as latent infections in fish [5, 6, 11]. Latent infection may develop to an acute phase with biological and environmental stress factors [15] or facilitate vertical or horizontal transmission of the virus [2, 12]. Genetically, the virus genome contains two positive-sense RNA strands, of which RNA1 (3.1 kb) codes for the viral RNA-dependent RNA polymerase (protein A), while RNA2 (1.4 kb) codes for the coat protein [14]. A subgenomic RNA, RNA3, which is synthesized from RNA1 during early viral replication, codes for protein B2 [20], which is involved in anti-host RNA interference [4, 9]. Four distinct species of betanodaviruses have been recognized so far [19] based on previously observed similarities in the variable region of the viral coat protein (CP) gene (nt 604-1030) [16]. These viruses have remarkable temperature specificity: redspotted grouper nervous necrosis virus (RGNNV), striped jack nervous necrosis virus (SJNNV), tiger puffer nervous necrosis virus (TPNNV) and barfin flounder nervous necrosis virus (BFNNV) favour temperatures of 25-30 °C, 20-25 °C, 20 °C, and 15-20 °C, respectively.

Samples of Asian seabass (*Lates calcarifer*) were collected from farms and the wild environment along the coastline of southern India (Table 1). The wild environments from where samples were collected had no commercial fish culture activity in the vicinity. Latently infected (n = 11) as well as moribund fish with acute VNN symptoms (n = 3) were used in this study. Samples from acutely ill fish were collected from commercial farms in the summer when the water temperature was around 28 °C. Live and moribund fish were euthanized using an excess concentration of AQUI-S® (AQUI-S New Zealand Ltd.), and brain and retinal tissues of the eye were removed surgically. Total RNA was extracted using TRIzol™ Reagent (Invitrogen, USA) following the manufacturer's

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Table 1 Details of samples analyzed in this study

Sl no.	Virus isolate	GenBank accession number	Host species	Age of fish/ location	Salinity (ppt)	Temperature (°C)	Disease state
1	BVN4	GU953669	*Lates calcarifer*	Fingerling/wild	35	27	Latent infection
2	BVN101	JF412258	*Lates calcarifer*	Yearling/wild	34	26	Latent infection
3	BVN102	JF412259	*Lates calcarifer*	Fingerling/wild	35	26	Latent infection
4	BVN103	JF412260	*Lates calcarifer*	Larva/farm	32	28	Acute infection
5	BVN104	JF412261	*Lates calcarifer*	Fingerling/wild	28	25	Latent infection
6	BVN105	JF412265	*Lates calcarifer*	Fingerling/wild	28	25	Latent infection
7	BVN106	JF412266	*Lates calcarifer*	Fingerling/wild	14	25	Latent infection
8	BVN107	JF412267	*Lates calcarifer*	Yearling/farm	26	28	Acute infection
9	BVN108	JF412268	*Lates calcarifer*	Fingerling/farm	4	28	Acute infection
10	BVN109	JF412269	*Lates calcarifer*	Fingerling/wild	6	25	Latent infection
11	BVN110	JF412270	*Lates calcarifer*	Fingerling/wild	6	26	Latent infection
12	BVN111	JF412271	*Lates calcarifer*	Larva/farm	14	26	Latent infection
13	BVN112	JF412272	*Lates calcarifer*	Fingerling/wild	14	27	Latent infection
14	BVN113	JF412273	*Lates calcarifer*	Fingerling/wild	13	26	Latent infection

protocol. Complete betanodavirus RNA2 was amplified by reverse transcription PCR using primers described previously [8]. The PCR products were resolved in a 1.5 % TBE agarose gel stained with 10 mg ml^{-1} ethidium bromide. Specific positive bands were excised, and the DNA was eluted using QIAquick Gel Extraction Kit (QIAGEN, USA) and cloned into pTZ57R/T vector using InsTA^{TM} PCR Cloning Kit (Fermentas Life Sciences, Canada) following the manufacturer's instructions. Positive clones were confirmed by blue-white selection on an IPTG-X-gal plate and standard colony PCR with M13 universal primers. Plasmids were extracted using HiYield^{TM} Plasmid Mini Kit (Real Biotech Corporation, Taiwan), their quality was analyzed by spectrophotometry, and the DNA was sequenced. Nucleotide sequence data were analyzed using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and deposited in the NCBI GenBank database. Full-length viral RNA2 sequences of members of the four species of betanodavirus, RGNNV (AY324870 [8], AB373029 [21]), SJNNV (NC_003449 [7]), TPNNV (NC_013461: Okinaka, 2011) and BFNNV (NC_013459: Okinaka, 2009), were retrieved from NCBI GenBank, and their similarity to the present isolates was assessed at the nucleotide level using MatGat [3]. The nucleotide sequences were aligned using ClustalW program in MEGA4 [22]. A phylogenetic tree was inferred by the neighbor-joining module in the program with 1000 re-samplings.

The full-length viral RNA2 sequences of the present isolates contained 1433-1434 bases. They made BLAST hits with up to 98 % similarity to other isolates reported globally, with E values equal to 0.0. The isolates also showed 99-100 % similarity among themselves and 77.8-98.3 % similarity to members of the four species of betanodavirus when analyzed with MatGat tool. In the phylogenetic tree, the present isolates were distinctly clustered and closely aligned with the RGNNV species (Fig. 1).

Viral nervous necrosis is a significant problem to fish culture, especially in the highly sought-after Asian seabass in India, yet the genetic identity of the Indian isolate is not known. As such, this study is the first attempt to analyze full-length nucleotide sequences of RNA2 of betanodavirus isolates in India, by which their taxonomic and phylogenetic position were determined. Betanodaviruses isolated from samples from fish with acute as well as latent

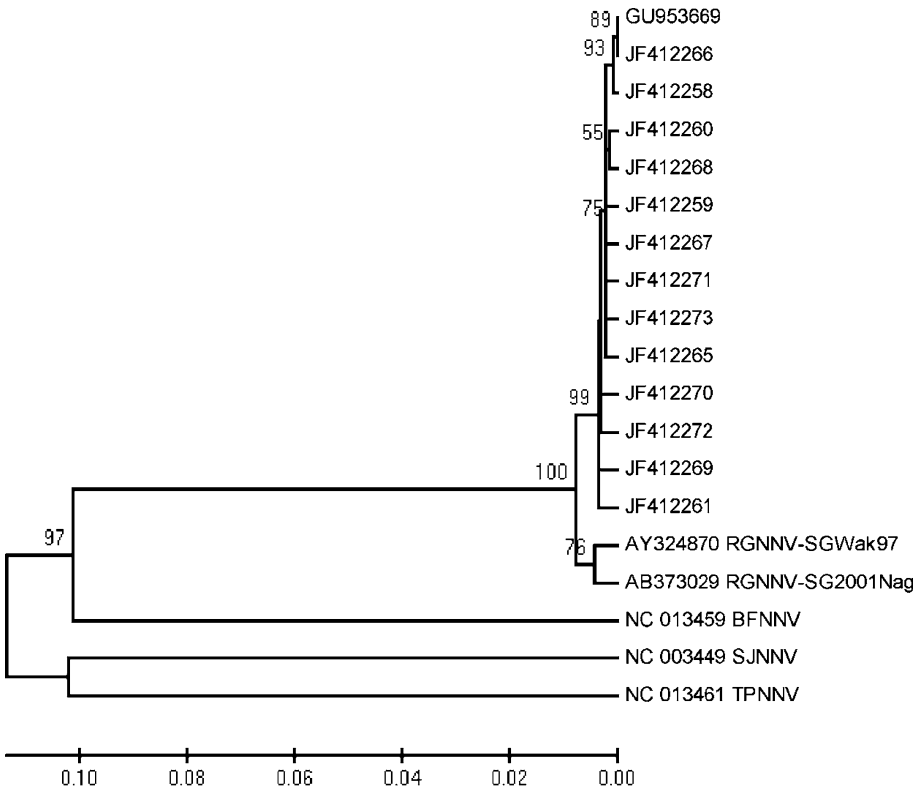

Fig. 1 Phylogenetic tree of betanodaviruses sequenced in this study based on nucleotide sequence similarity of full length-RNA2. Note that the isolates from India in this study are grouped along with the RGNNV cluster

infection used in this study seem to exhibit high genetic homology and belong to a single genotype, as is evident from the phylogenetic tree (Fig. 1). They were grouped in to a single cluster together with isolates from the RGNNV species. It is currently believed that RGNNV is the only species whose members prefer the tropical temperature prevailing in India. From these observations, it was concluded that the isolates from this study belong to the RGNNV species of betanodavirus.

The environmental and physical conditions to which the fish are subjected are known to affect the severity of betanodavirus infection. Generally, disease is observed more often in marine fish at earlier stages of life. However, such inferences cannot be drawn from the present data. Acute infection was observed in all age groups from larvae to yearlings at 4-32 ppt salinity. A salinity preference of the virus was not apparent in the present study, as both latent and acute isolates were seen in low- as well as high-saline environments. The nucleotide sequence data also show that isolates from the two environments did not significantly differ at the molecular level. Acute infection was observed at higher water temperature (28 °C), but an association in this regard can be drawn only after conducting experiments under controlled conditions.

The observation that the betanodaviruses in India belong to the RGNNV species raises concerns to the booming aquaculture industry in this country. Members of this species are known to have the widest host range of all the fish nodaviruses, and latently infected fish pose more of a threat to aquaculture as asymptomatic carriers, which can spread the virus to a naïve environment. Acute infection was observed in farms, whereas latent infection was observed in wild environments as well. However, more study is needed to determine the local transmission route of the virus resulting in acute infection, as the seabass culture in India is sustained by both hatchery production and wild collection of seeds. The occurrence of betanodaviruses in the wild environment where no commercial fish culture activities have been launched suggests a wider distribution of the virus in nature. Horizontal transmission from carrier fish in the wild or vertical transmission from parents, or both, may have contributed to the spread of betanodavirus in farms.

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