

Erratum to: Genomic classification of betanodavirus by molecular phylogenetic analysis of the coat protein gene

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Genomic classification of betanodavirus by molecular phylogenetic analysis of the coat protein gene

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Abstract The classification of betanodavirus into four species was reviewed including newer and well-characterised isolates. Six major clusters were identified, four of which were similar to the classic species. Two single isolate clusters were worth consideration as new species.

Betanodaviruses are small (37 nm average) icosahedral viruses with two positive-stranded non-polyadenylated RNAs as its genome. RNA1 (3.1 kb) codes for the viral RNA-dependent RNA polymerase (protein A), while RNA2 (1.4 kb) codes for the coat protein [16]. A subgenomic RNA3 synthesized from RNA1 during early viral replication codes for protein B2 [17], which functions against the host RNA interference system [3, 8]. These viruses cause viral nervous necrosis (VNN) or viral encephalopathy and retinopathy (VER) in fin fishes. They were first identified as picorna-like virus particles [5] and later described as members of the family *Nodaviridae* [12]. Taxonomically, these viruses belong to the genus *Betanodavirus* of family *Nodaviridae*, with four species recognised so far [16] based on similarities in the variable region of the viral coat protein (CP) gene (nt 604–1030) [14]. The currently recognized species are *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), with SJNNV as the type species. The preferred temperature ranges of members of these species are 25–30 °C, 20–25 °C, 20 °C and 15–20 °C, respectively.

Over 40 fish species have been reported to be susceptible to betanodaviruses, and the number is steadily increasing [10, 13]. Since the first description 15 years ago, the taxonomy of betanodavirus has remained more or less the same irrespective of the huge number of virus isolates being reported from different geographical environments and from different fish hosts. Some modifications were proposed to the system [4, 11, 15, 19], but these have been largely restricted to the geographical area of the researcher. The study presented here is an attempt to analyze the global phylogenetic relationships among the viruses by comparing all isolates for which full-length coat protein (CP) gene ORF sequence information is available in the NCBI GenBank database. A short variable region in the CP gene is shown to be sufficient for determining the phylogenetics of these viruses [14], but the full-length CP gene ORF is used in this study to obtain more reliable results.

Sequences of the full-length coat protein gene ORF of betanodaviruses were retrieved from the NCBI GenBank database with due care to include members of the species described in Eighth Report of the International Committee on Taxonomy of Viruses [16]. A total of 78 sequences were retrieved from the database. The sequences were labelled with their geographical location of isolation for ease of compilation for analysing the distribution pattern of the isolates included in this study. Betanodaviruses show high temperature specificity [6] and low host specificity [19], and this is expected to be reflected in their geographical distribution. The nucleotide and deduced amino acid sequences were aligned using the MUSCLE program [2] in MEGA software [18]. Neighbour-joining trees were

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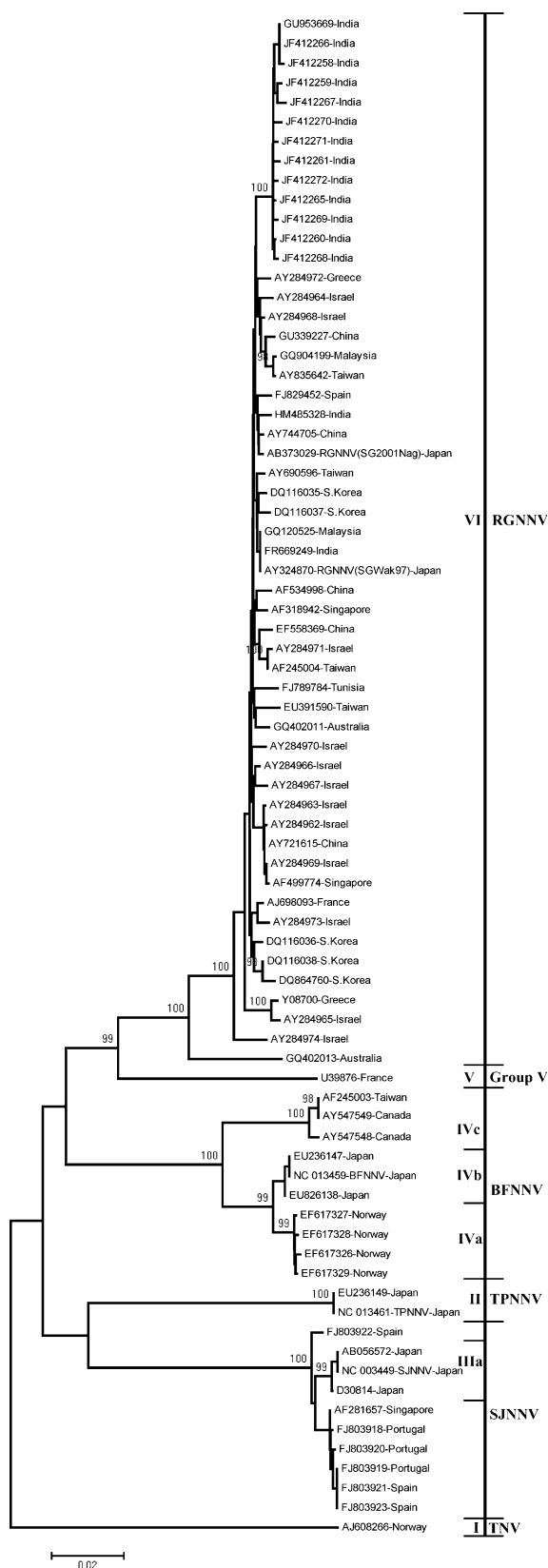


Fig. 1 Phylogenetic tree of betanodaviruses constructed in this study based on nucleotide sequence similarity of the CP gene ORF. Note that the isolates are grouped into six distinct clusters

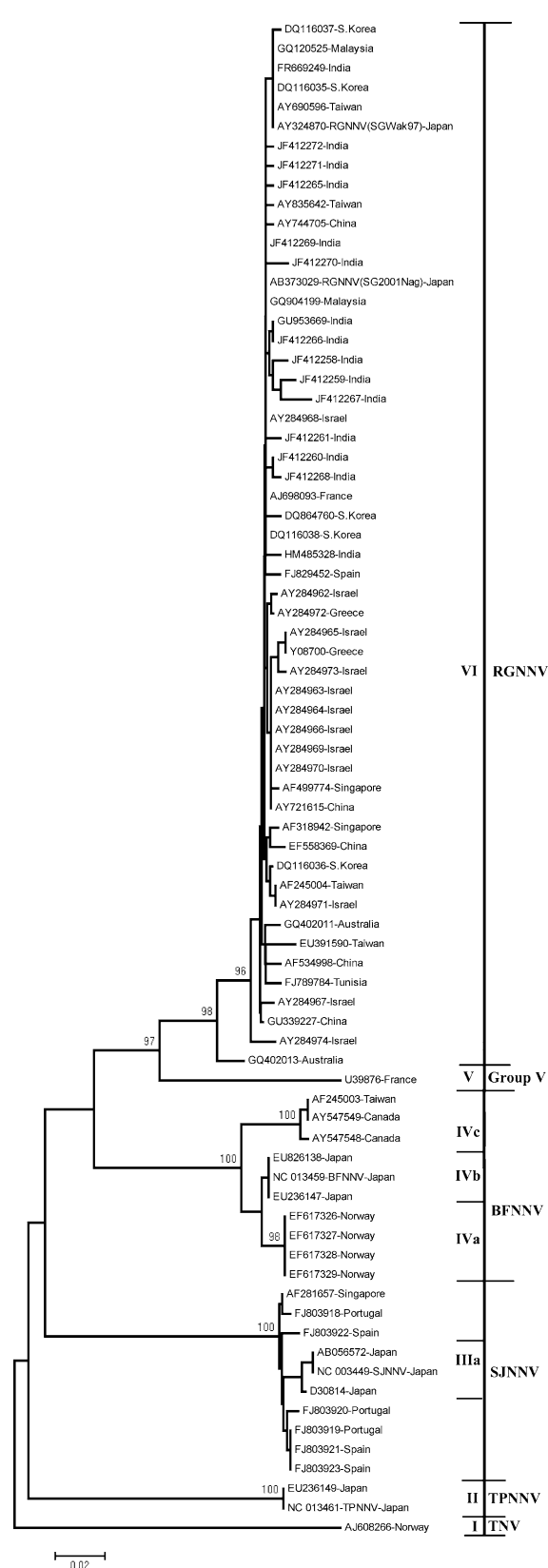


Fig. 2 Phylogenetic tree of betanodaviruses constructed in this study based on deduced amino acid sequence similarity of the CP gene ORF. Note that the isolates are grouped into six distinct clusters

constructed using the maximum composite likelihood substitution model for nucleotides and the Poisson model for amino acid sequences. Bootstrap tests were conducted, and values were obtained from 1000 re-samplings. Bootstrap values less than 95 were hidden in the tree for ease of interpretation. Details of the sequences used are given in Table 1 in Appendix. Phylogenetic trees based on nucleotide and deduced amino acid sequences are shown in Fig. 1 and 2, respectively.

In the phylogenetic trees, the sequences were grouped into six major clusters with subgroups within them. Four of the clusters each included a member or two of the four classical species: group II (TPNNV), group III (SJNNV), group IV (BFNNV) and group VI (RGNNV). Group II included two isolates from tiger puffer in Japan. Group III included 10 isolates from striped jack, gilthead seabream, sole fish and, greasy grouper, and the three isolates from striped jack formed a consistent subgroup in the nucleotide and amino acid trees. Group IV had nine isolates in three subgroups. Group IVa had four isolates from Atlantic cod, group IVb had three isolates from barfin flounder, and group IVc had one isolate each from Atlantic cod and Malabar grouper. Group VI was represented by 55 isolates from 14 fish species with very different geographical locations in Asia, Europe and Australia. Groups I and V were single-isolate clusters whose members originated from colder regions (France and Norway, respectively). They included virus isolates from turbot and European seabass, respectively. The group I isolate was proposed previously to represent a fifth species in the genus *Betanodavirus* [9]. In this study, it was placed at the root of the tree, imparting to it the status of an ancestor of all of the betanodavirus groups represented in this study.

All of the major clusters in the phylogenetic trees were consistent and supported by high bootstrap values. The branch length and bootstrap values confirmed that of the major clusters corresponding to distinct species. The four classical species were also well preserved in the trees in distinct major clusters. The inconsistency between the nucleotide and amino acid sequence trees in some subgroups can be attributed to silent and point mutations that were observed in the selected sequences. The isolate

AJ608266 was originally denoted as TNV (turbot nodavirus) apart from the four species [9]. In this study, this isolate clustered separately as group I, forming the root of the tree. Group III was represented by SJNNV and had isolates from striped jack and sole fish, as observed previously [19]. In this study, this group contained isolates from gilthead seabream and greasy grouper as well. Group IV had three consistent subgroups, similar to what has been observed previously [19] even though the sequences used in the two studies were different. The isolate U39876 was originally classified as a member of the family *Nodaviridae* that differed considerably from other nodaviruses [1]. In this study, this isolate was placed in between the BFNNV and RGNNV clusters. The isolate GQ402013 was also placed distinctly but was previously included in the RGNNV cluster [7].

Strict host specificity was observed among the isolates in the TNV, TPNNV and group V clusters. However, a conclusion cannot be made in this study due to the limited information available about the isolates. For the isolates from other clusters, however, multiple host species were observed, as expected from previous studies [19].

According to this phylogenetic study, betanodaviruses can be distinctly grouped into six major clusters. Four of these clusters are synonymous with the four established species, and their geographical distribution confirmed specific temperature preferences, as observed previously. One of the two new clusters (TNV, group I) observed in this study, was proposed previously as a fifth species of betanodavirus [9]. Three clusters (groups I, II and V) were strictly host specific, while others displayed low host specificity. The subgroups in the RGNNV cluster were the least consistent between the nucleotide and amino acid sequence trees. The BFNNV cluster had three distinct subgroups, indicative of its fast divergence, while the subgroups observed in the nucleotide and amino acid sequence trees were largely inconsistent in the other clusters.

Appendix

See Table 1.

Table 1 Details of betanodavirus RNA2 sequences retrieved from NCBI GenBank database for phylogenetic analysis in this study

Sl no.	Group	Subgroup	Isolate name	Accession number	Country
1	Group I (TNV)		Turbot nodavirus	AJ608266	Norway
2	Group II (TPNNV)		Tiger puffer nervous necrosis virus isolate TPKag93	EU236149	Japan
3			Tiger Puffer nervous necrosis virus	NC_013461	Japan

Table 1 continued

Sl no.	Group	Subgroup	Isolate name	Accession number	Country
4	Group III (SJNNV)	IIIa	Striped Jack nervous necrosis virus	NC_003449	Japan
5			Striped jack nervous necrosis virus, strain SJ-G91	D30814	Japan
6			Striped Jack nervous necrosis virus	AB056572	Japan
7			Gilthead sea bream betanodavirus isolate PtSa_IAusc6105	FJ803918	Portugal
8			Gilthead sea bream betanodavirus isolate PtSa_IAusc7405	FJ803919	Portugal
9			Gilthead sea bream betanodavirus isolate SpSa_IAusc15603	FJ803921	Spain
10			Senegalese sole Iberian betanodavirus isolate SpSs_IAusc197408	FJ803922	Spain
11			Senegalese sole Iberian betanodavirus isolate SpSsIAusc16003	FJ803923	Spain
12			Senegalese sole Iberian betanodavirus isolate PtSs_IAusc57304	FJ803920	Portugal
13			Epinephelus tauvina nervous necrosis virus	AF281657	Singapore
14	Group IV (BFNNV)	IVa	Atlantic cod betanodavirus Ac06NorE	EF617328	Norway
15			Atlantic cod betanodavirus Ac06NorPm	EF617326	Norway
16			Atlantic cod betanodavirus Ac06NorPp	EF617327	Norway
17			Atlantic cod betanodavirus Ac06NorT	EF617329	Norway
18		IVb	Barfin flounder nervous necrosis virus, JFIwa98	EU236147	Japan
19			Barfin flounder virus BF93Hok	EU826138	Japan
20		IVc	Barfin Flounder nervous necrosis virus	NC_013459	Japan
21			Atlantic cod nervous necrosis virus isolate Cod02ac	AY547548	Canada
22			<i>Melanogrammus aeglefinus</i> nervous necrosis virus isolate Had02ac2	AY547549	Canada
23			Malabaricus nervous necrosis virus	AF245003	Taiwan
24	Group V		Dicentrarchus labrax encephalitis virus	U39876	France
25	Group VI (RGNNV)		Redspotted grouper nervous necrosis virus isolate MnNNV_12/06	GQ402013	Australia
26			Dicentrarchus labrax encephalitis virus	Y08700	Greece
27			Dicentrarchus labrax encephalitis virus	AY284969	Israel
28			Dicentrarchus labrax encephalitis virus isolate DL-120799-IL	AY284970	Israel
29			Dicentrarchus labrax encephalitis virus DL-040700-IL	AY284971	Israel
30			Dicentrarchus labrax encephalitis virus DL-060900-GR	AY284972	Greece
31			Dicentrarchus labrax encephalitis virus isolate v26	AJ698093	France
32			Dragon grouper nervous necrosis virus	AF245004	Taiwan
33			Dragon grouper nervous necrosis virus	AY721615	China
34			Epinephelus aeneus encephalitis virus isolate EA-040799-IL	AY284963	Israel
35			Epinephelus aeneus encephalitis virus isolate EA-080899-IL	AY284964	Israel
36			Epinephelus aeneus encephalitis virus isolate EA-221199-IL	AY284965	Israel
37			Epinephelus aeneus encephalitis virus isolate EA-030601-IL	AY284966	Israel
38			Epinephelus aeneus encephalitis virus isolate EA-150102-IL	AY284967	Israel
39			Epinephelus aeneus encephalitis virus isolate EA-061002-IL	AY284968	Israel
40			Epinephelus coioides nervous necrosis virus	AF534998	China
41			Epinephelus tauvina nervous necrosis virus	AF318942	Singapore
42			Golden pompano nervous necrosis virus	GQ904199	Malaysia
43			Guppy nervous necrosis virus	AF499774	Singapore
44			Lates calcarifer encephalitis virus isolate LC-220800-IL	AY284973	Israel
45			Lates calcarifer encephalitis virus isolate LC-121100-IL	AY284974	Israel
46			Lates calcarifer nervous necrosis virus	GQ120525	Malaysia
47			Lates calcarifer nervous necrosis virus	FR669249	India

Table 1 continued

Sl no.	Group	Subgroup	Isolate name	Accession number	Country
48			Lates calcarifer nervous necrosis virus strain VNIN	HM485328	India
49			Lates calcarifer nervous necrosis virus isolate BVN4	GU953669	India
50			Lates calcarifer nervous necrosis virus isolate BVN101	JF412258	India
51			Lates calcarifer nervous necrosis virus isolate BVN102	JF412259	India
52			Lates calcarifer nervous necrosis virus isolate BVN103	JF412260	India
53			Lates calcarifer nervous necrosis virus isolate BVN104	JF412261	India
54			Lates calcarifer nervous necrosis virus isolate BVN105	JF412265	India
55			Lates calcarifer nervous necrosis virus isolate BVN106	JF412266	India
56			Lates calcarifer nervous necrosis virus isolate BVN107	JF412267	India
57			Lates calcarifer nervous necrosis virus isolate BVN108	JF412268	India
58			Lates calcarifer nervous necrosis virus isolate BVN109	JF412269	India
59			Lates calcarifer nervous necrosis virus isolate BVN110	JF412270	India
60			Lates calcarifer nervous necrosis virus isolate BVN111	JF412271	India
61			Lates calcarifer nervous necrosis virus isolate BVN112	JF412272	India
62			Mugil cephalus encephalitis virus isolate MC-061198-IL	AY284962	Israel
63			Redspotted grouper nervous necrosis virus	AY744705	China
64			Redspotted grouper nervous necrosis virus isolate G9508KS	AY690596	Taiwan
65			Redspotted grouper nervous necrosis virus isolate RBNNV-Korea	DQ116035	S.Korea
66			Redspotted grouper nervous necrosis virus isolate SGNNV-Korea	DQ116036	S.Korea
67			Redspotted grouper nervous necrosis virus isolate JFNNV-Korea	DQ116037	S.Korea
68			Redspotted grouper nervous necrosis virus isolate GMNNV-Korea	DQ116038	S.Korea
69			Redspotted grouper nervous necrosis virus	DQ864760	S.Korea
70			Redspotted grouper nervous necrosis virus isolate China 1	EF558369	China
71			Redspotted grouper nervous necrosis virus strain TN1	EU391590	Taiwan
72			Redspotted grouper nervous necrosis virus	FJ789784	Tunisia
73			Redspotted grouper nervous necrosis virus isolate LcNNV_09/07	GQ402011	Australia
74			Sea bass Iberian betanodavirus isolate SpDI_IAusc168808	FJ829452	Spain
75			Sevenband grouper nervous necrosis virus strain SGWak97	AY324870	Japan
76			Sevenband grouper nervous necrosis virus strain SG2001Nag	AB373029	Japan
77			Trachinotus ovatus nervous necrosis virus	GU339227	China
78			White star snapper nervous necrosis virus	AY835642	Taiwan

References

- Delsert C, Morin N, Comps M (1997) A fish encephalitis virus that differs from other nodaviruses by its capsid protein processing. *Arch Virol* 142:2359–2371
- Edgar Robert C (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 32:1792–1797
- Fenner BJ, Thiagarajan R, Chua HK, Kwang J (2006) Betanodavirus B2 is an RNA interference antagonist that facilitates intracellular viral RNA accumulation. *J Virol* 80:85–94
- Gagne N, Johnson SC, Cook-Versloot M, MacKinnon AM, Olivier G (2004) Molecular detection and characterization of nodavirus in several marine fish species from the northeastern Atlantic. *Dis Aquat Org* 62:181–189
- Glazebrook JS, Heasman MP, De Beer SW (1990) Picorna-like viral particles associated with mass mortalities in larval barramundi, *Lates calcarifer* (Bloch). *J Fish Dis* 13:245–249
- Hata N, Okinaka Y, Iwamoto T, Kawato Y, Mori K, Nakai T (2010) Identification of RNA regions that determine temperature sensitivities in betanodaviruses. *Arch Virol* 155: 1597–1606
- Hick P, Whittington RJ (2010) Optimisation and validation of a real-time reverse transcriptase-polymerase chain reaction assay for detection of betanodavirus. *J Virol Methods* 163: 368–377
- Iwamoto T, Mise K, Takeda A, Okinaka Y, Mori KI, Arimoto M, Okuno T, Nakai T (2005) Characterization of *Striped jack nervous necrosis virus* subgenomic RNA3 and biological activities of its encoded protein B2. *J Gen Virol* 86:2807–2816
- Johansen R, Sommerset I, Torud B, Korsnes K, Hjortaa MJ, Nilsen F, Nerland AH, Dannevig BH (2004) Characterization of nodavirus and viral encephalopathy and retinopathy in farmed turbot, *Scophthalmus maximus* (L.). *J Fish Dis* 27:591–601
- Maltese C, Bovo G (2007) Monografie. Viral encephalopathy and retinopathy. *Ittiopatologia* 4:93–146

11. Moody NJG, Horwood PF, Reynolds A, Mahony TJ, Anderson IG, Oakey HJ (2009) Phylogenetic analysis of betanodavirus isolates from Australian finfish. *Dis Aquat Org* 87:151–160
12. Mori K, Nakai T, Muroga K, Arimoto M, Mushiake K, Furusawa I (1992) Properties of a new virus belonging to nodaviridae found in larval striped jack (*Pseudocaranx dentex*) with nervous necrosis. *Virology* 187:368–371
13. Munday BL, Kwang J, Moody N (2002) Betanodavirus infections of teleost fish: a review. *J Fish Dis* 25:127–142
14. 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. *Appl Environ Microbiol* 63:1633–1636
15. Nylund A, Karlsbakk E, Nylund S, Isaksen TE, Karlsen M, Korsnes K, Handeland S, Martinsen R, Pedersen TM, Ottem KF (2008) New clade of betanodaviruses detected in wild and farmed cod (*Gadus morhua*) in Norway. *Arch Virol* 153:541–547
16. Schneemann A, Ball LA, Delsert C, Johnson JE, Nishizawa T (2005) Family Nodaviridae. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA (eds) *Virus taxonomy. Classification and nomenclature of viruses*. Elsevier Academic Press, Eighth report of the international committee on the taxonomy of viruses, pp 869–872
17. Sommerset I, Nerland AH (2004) Complete sequence of RNA1 and subgenomic RNA3 of Atlantic halibut nodavirus (AHNV). *Dis Aquat Org* 58:117–125
18. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 28:2731–2739
19. Thiery R, Cozien J, de Boisseson C, Kerbart-Boscher S, Nevarez L (2004) Genomic classification of new betanodavirus isolates by phylogenetic analysis of the coat protein gene suggests a low host-fish species specificity. *J Gen Virol* 85:3079–3087