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Table S1. Primer pairs used for RT-qPCR.

Gene	Forward primer (5' – 3')	Reverse primer (5' – 3')	Reference	Information
RdRP	GAGGGTGCGATTGCTATTGT	ACTGGCACCCAATTAAGCAC	(Valero *et al.*, 2015)	Amplifies RGNNV RdRP polymerase gene
Cp	AAATTGCACACCACCTGTGA	ACCCAGAATGGAATGTCAGC	(Valero *et al.*, 2015)	Amplifies RGNNV capsid protein gene
RNA2	CAACTGACARCGAHCACAC	CCCACCAYTTGGCVAC	(Panzarin *et al.*, 2010)	Amplifies segment 2 of the NNV viral genome

Table S2. Primer pairs used for RT-PCR and sequencing. The table shows the size of the resulting amplicons, the fragment of the viral genome they amplify and the position in the genome.

Primer	Sequence (5' – 3')	Amplicon size	Nucleotide position	Genome fragment	Reference
FOR 521	ACGTGGACATGCATGAGTTG	630	521 - 540	RNA1	(Bovo *et al.*, 2011)
VNNV6	ACCGGCGAACAGTATCTGAC		1150 - 1131	RNA1	(Bovo *et al.*, 2011)
VNNV1	ACACTGGAGTTTGAAATTCA	605	158 - 177	RNA2	(Bovo *et al.*, 2011)
VNNV2	GTCTTGTTGAAGTTGTCCCA		762 - 743	RNA2	(Bovo *et al.*, 2011)

Table S3. Betanodavirus isolates used for phylogenetic analysis, genotype, host species, country and year of isolation, GenBank accession number for RNA1 and RNA2 segments.

Betanodavirus isolate	Genotype	Isolation			GenBank accession no.	
		Host species	Country	Year	RNA1	RNA2
MmNNV1_2023	RGNNV	*Mycteroperca marginatus*	Spain	2023	PP814714	PP814716
MmNNV2_2023	RGNNV	*Mycteroperca marginatus*	Spain	2023	PP814715	PP814717
SpDl_IAusc168808	RGNNV	*Dicentrarchus labrax*	Spain	2008	FJ803915.1	-
283.2009	RGNNV	*Dicentrarchus labrax*	Italy	2009	JN189865	-
SGWak97	RGNNV	*Epinephelus septemfasciatus*	Japan	1997	AY324869	AY324870
RGNNVLCInd2016	RGNNV	*Lates calcarifer*	India	2016	MH758753	-
HN1	RGNNV	*Epinephelus coioides*	China	2016	MG874757	-
VNNV/*S.aurata*/I/425-10/Sep2008	RGNNV	*Sparus aurata*	Italy	2008	KY354681	-
SG2001Nag	RGNNV	*Epinephelus septemfasciatus*	Japan	2001	-	AB373029
KS1	RGNNV	*Epinephelus coioides*	Taiwan	2013	-	MF144241
G9508KS	RGNNV	*Epinephelus akaara*	Japan	2002	-	AY690596
BVN2	RGNNV	*Lates calcarifer*	India	2008	-	GU826692
VNIN	RGNNV	*Lates calcarifer*	India	2006	-	HM485328
PtSs_IAusc57304	RGNNV/SJNNV	*Solea senegalensis*	Portugal	2004	FJ803914	FJ803920
132.2005	RGNNV/SJNNV	*Dicentrarchus labrax*	Italy	2005	JN189899	JN189937
PtSa_IAusc7405	RGNNV/SJNNV	*Sparus aurata*	Portugal	2005	FJ803913	-
VNNV/*D.labrax*/ES/61-48/Feb2016	RGNNV/SJNNV	*Dicentrarchus labrax*	Spain	2016	OL955902	-
VNNV/*S.aurata*/Farm2/165-6/Mar2016	RGNNV/SJNNV	*Sparus aurata*	Italy	2016	KY354693	-
292.1.2.2009	RGNNV/SJNNV	*Sparus aurata*	Greece	2009	JN189831	JN189922
SpSpIAusc939.20	RGNNV/SJNNV	*Sardina pilchardus*	Spain	2020	-	ON745752
SpSsIAusc160.03	RGNNV/SJNNV	*Solea senegalensis*	Spain	2003	-	NC_024493
367.2.2005	RGNNV/SJNNV	*Dicentrarchus labrax*	Italy	2005	-	JN189936
VNNV/*S.aurata*/Farm1/461-1/Nov2014	RGNNV/SJNNV	*Sparus aurata*	Italy	2014	-	KY354702
TPKag93	TPNNV	*Takifugu rubripes*	Japan	1993	NC_013460	NC_013461
Ah95NorA	BFNNV	*Hippoglossus hippoglossus*	Norway	1995	MN245300	-
BF93Hok	BFNNV	*Verasper moseri*	Japan	1993	EU826137	EU826137
SK-07 1324	BFNNV	*Gadus morhua*	Norway	2007	MN245301	-
GmN10/06	BFNNV	*Gadus morhua*	Norway	2006	EF433473	-
Ac06NorPpB	BFNNV	*Gadus morhua*	Norway	2006	EF617335	-
GmSF15/07	BFNNV	*Gadus morhua*	Norway	2007	EF577395	-
BB09	BFNNV	*Dicentrarchus labrax*	France	2009	-	AJ698094
PCNNV	BFNNV	*Gadus macrocephalus*	China	2014	-	KM576685
Ac06NorT	BFNNV	*Gadus morhua*	Norway	2006	-	EF617329
GmN10/06	BFNNV	*Gadus morhua*	Norway	2006	-	EF433465
GmH14/06	BFNNV	*Gadus morhua*	Norway	2006	-	EF433467

Table S4. RT-qPCR results showing the Ct value obtained for the samples analyzed.

	C +	Sample 1, brain	Sample 1, retina	Sample 2, brain	Sample 2, retina
Cp	7,138	8,192	16,198	15,1055	13,2335
RdRP	7,332	8,218	15,529	15,113	13,633
RNA2	7,7	9,25	16,64	15,79	15,06

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