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Dusky grouper massive die-off in a Mediterranean marine reserve

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Table S1. Primer pairs used for RT-qPCR.

| Gene | Forward primer (5' – 3') | Reverse primer (5' – 3') | Reference | Information |
|------|--------------------------|--------------------------|---------------------------------|---|
| RdRP | GAGGGTGCATTGCTATTGT | ACTGGCACCCAATTAAGCAC | (Valero <i>et al.</i> , 2015) | Amplifies RGNNV RdRP polymerase gene |
| Cp | AAATTGCACACCACCTGTGA | ACCCAGAATGGAATGTCAGC | (Valero <i>et al.</i> , 2015) | Amplifies RGNNV capsid protein gene |
| RNA2 | CAACTGACARCGAHCACAC | CCCACCAYTTGGCVAC | (Panzarin <i>et al.</i> , 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 <i>et al.</i> , 2011) |
| VNNV6 | ACCGGCGAACAGTATCTGAC | | 1150 - 1131 | RNA1 | (Bovo <i>et al.</i> , 2011) |
| VNNV1 | ACACTGGAGTTTGAAATTCA | 605 | 158 - 177 | RNA2 | (Bovo <i>et al.</i> , 2011) |
| VNNV2 | GTCTTGTTGAAGTTGTCCCA | | 762 - 743 | RNA2 | (Bovo <i>et al.</i> , 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 | <i>Mycteroperca marginatus</i> | Spain | 2023 | PP814714 | PP814716 |
| MmNNV2_2023 | RGNNV | <i>Mycteroperca marginatus</i> | Spain | 2023 | PP814715 | PP814717 |
| SpDI_IAusc168808 | RGNNV | <i>Dicentrarchus labrax</i> | Spain | 2008 | FJ803915.1 | - |
| 283.2009 | RGNNV | <i>Dicentrarchus labrax</i> | Italy | 2009 | JN189865 | - |
| SGWak97 | RGNNV | <i>Epinephelus septemfasciatus</i> | Japan | 1997 | AY324869 | AY324870 |
| RGNNVLCInd2016 | RGNNV | <i>Lates calcarifer</i> | India | 2016 | MH758753 | - |
| HN1 | RGNNV | <i>Epinephelus coioides</i> | China | 2016 | MG874757 | - |
| VNNV/ <i>S.aurata</i> /I/425-10/Sep2008 | RGNNV | <i>Sparus aurata</i> | Italy | 2008 | KY354681 | - |
| SG2001Nag | RGNNV | <i>Epinephelus septemfasciatus</i> | Japan | 2001 | - | AB373029 |
| KS1 | RGNNV | <i>Epinephelus coioides</i> | Taiwan | 2013 | - | MF144241 |
| G9508KS | RGNNV | <i>Epinephelus akaara</i> | Japan | 2002 | - | AY690596 |
| BVN2 | RGNNV | <i>Lates calcarifer</i> | India | 2008 | - | GU826692 |
| VNIN | RGNNV | <i>Lates calcarifer</i> | India | 2006 | - | HM485328 |
| PtSs_IAusc57304 | RGNNV/SJNNV | <i>Solea senegalensis</i> | Portugal | 2004 | FJ803914 | FJ803920 |
| 132.2005 | RGNNV/SJNNV | <i>Dicentrarchus labrax</i> | Italy | 2005 | JN189899 | JN189937 |
| PtSa_IAusc7405 | RGNNV/SJNNV | <i>Sparus aurata</i> | Portugal | 2005 | FJ803913 | - |
| VNNV/ <i>D.labrax</i> /ES/61-48/Feb2016 | RGNNV/SJNNV | <i>Dicentrarchus labrax</i> | Spain | 2016 | OL955902 | - |
| VNNV/ <i>S.aurata</i> /Farm2/165-6/Mar2016 | RGNNV/SJNNV | <i>Sparus aurata</i> | Italy | 2016 | KY354693 | - |
| 292.1.2.2009 | RGNNV/SJNNV | <i>Sparus aurata</i> | Greece | 2009 | JN189831 | JN189922 |
| SpSpIAusc939.20 | RGNNV/SJNNV | <i>Sardina pilchardus</i> | Spain | 2020 | - | ON745752 |
| SpSsIAusc160.03 | RGNNV/SJNNV | <i>Solea senegalensis</i> | Spain | 2003 | - | NC_024493 |
| 367.2.2005 | RGNNV/SJNNV | <i>Dicentrarchus labrax</i> | Italy | 2005 | - | JN189936 |
| VNNV/ <i>S.aurata</i> /Farm1/461-1/Nov2014 | RGNNV/SJNNV | <i>Sparus aurata</i> | Italy | 2014 | - | KY354702 |
| TPKag93 | TPNNV | <i>Takifugu rubripes</i> | Japan | 1993 | NC_013460 | NC_013461 |
| Ah95NorA | BFNNV | <i>Hippoglossus hippoglossus</i> | Norway | 1995 | MN245300 | - |
| BF93Hok | BFNNV | <i>Verasper moseri</i> | Japan | 1993 | EU826137 | EU826137 |
| SK-07 1324 | BFNNV | <i>Gadus morhua</i> | Norway | 2007 | MN245301 | - |
| GmN10/06 | BFNNV | <i>Gadus morhua</i> | Norway | 2006 | EF433473 | - |
| Ac06NorPpB | BFNNV | <i>Gadus morhua</i> | Norway | 2006 | EF617335 | - |
| GmSF15/07 | BFNNV | <i>Gadus morhua</i> | Norway | 2007 | EF577395 | - |
| BB09 | BFNNV | <i>Dicentrarchus labrax</i> | France | 2009 | - | AJ698094 |
| PCNNV | BFNNV | <i>Gadus macrocephalus</i> | China | 2014 | - | KM576685 |
| Ac06NorT | BFNNV | <i>Gadus morhua</i> | Norway | 2006 | - | EF617329 |
| GmN10/06 | BFNNV | <i>Gadus morhua</i> | Norway | 2006 | - | EF433465 |
| GmH14/06 | BFNNV | <i>Gadus morhua</i> | 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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