

**Part 1:** **TITLE, AUTHORS, APPROVALS, etc**

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| **Code assigned:** | **2021.023M** |  |
| **Short title:** Create one new genus (*Sardinovirus*)including one new species (*Sardinovirus pilchardi*) (*Articulavirales*: *Orthomyxoviridae*) | | |
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**List the ICTV Study Group(s) that have seen this proposal**

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| ICTV *Orthomyxoviridae* Study Group |

**ICTV study group comments and response of proposer**

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**Submission dates**

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| Date first submitted to SC Chair | May 28, 2021 |
| Date of this revision (if different to above) |  |

**ICTV-EC comments and response of the proposer**

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**Part 3:** **TAXONOMIC PROPOSAL**

**Name of accompanying Excel module**

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| 2021.023M.R.Orthomyxoviridae\_1ngen\_1nsp\_Sardino |

**Abstract**

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| Pilchard orthomyxovirus (POMV) was first isolated in 1998 from healthy blue pilchards (*Sardinops sagax* (Jenyns, 1847)) collected from waters off the coast of South Australia and subsequently in Tasmania (Mohr et al.). The virus is pathogenic to Atlantic salmon (*Salmo salar* Linnaeus, 1758) in freshwater and marine environments and causes salmon orthomyxoviral necrosis (Godwin et al.). Transmission electron microscopy initially identified POMV isolates as belonging to the family *Orthomyxoviridae*. Recently the genomes of 11 POMV isolates were sequenced with each assembling into 8 viral genome segments that encode 10 putative proteins. The low homology of 6 POMV proteins with those of characterized orthomyxoviruses and the presence of an additional 4 proteins with no known homologue indicate that POMV is a unique virus within the *Orthomyxoviridae* family. We propose that POMV be considered as the type virus of a new species (*Sardinovirus pilchardi*) included in a new orthomyxovirus genus (*Sardinovirus*). |

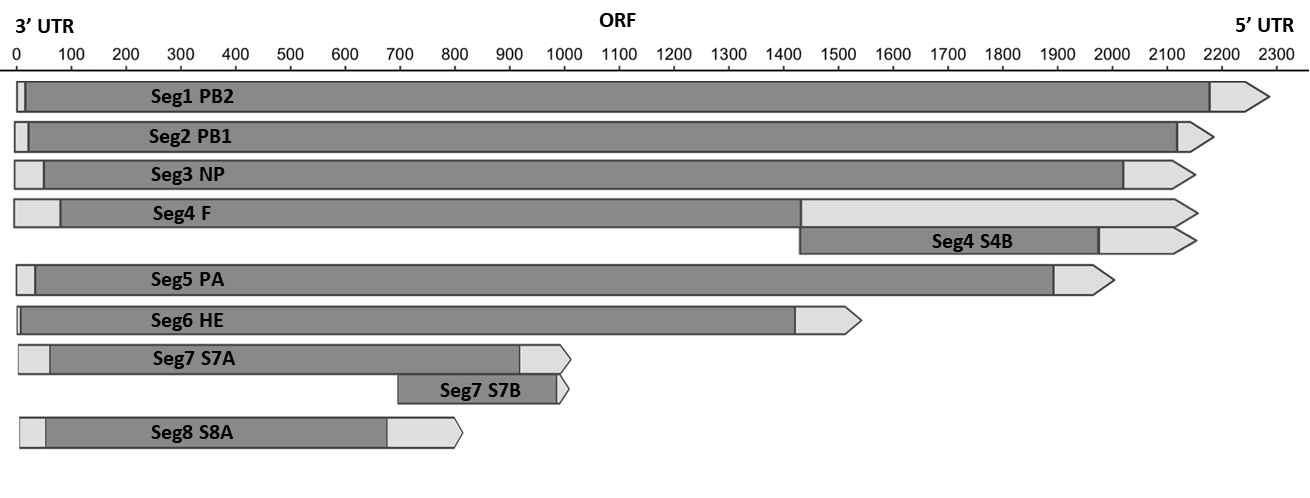
**Text of proposal**

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| |  | | --- | | We propose that pilchard orthomyxovirus (POMV) represents the type virus of a new species (*Sardinovirus pilchardi*) within a new genus, *Sardinovirus,* within the existing *Orthomyxoviridae* family. The virus is named pilchard orthomyxovirus (POMV) because it was first isolated from pilchards and has ultrastructural and genomic characteristics most similar to viruses of the *Orthomyxoviridae* family. The proposed genus name is *Sardinovirus* as POMV was first isolated from pilchards, which are sardines*.* POMV is the only virus to be characterised within the *Sardinovirus* genus and therefore species demarcation criteria are not proposed for the new species *Sardinovirus pilchardi*. The species epithet is the singular genitive in Latin pilchardus, included in the host species name *Sardinus pilchardus.*  The reasons for proposing the establishment of genus *Sardinovirus* within family *Orthomyxoviridae* are:    1. POMV genomes consist of 8 viral RNA segments that putatively encode 10 proteins. This is a similar genome structure to infectious salmon anaemia virus (ISAV; genus *Isavirus*) and rainbow trout orthomyxovirus (RbtOV; proposed new genus “*Mykissvirus*” [Batts et al.]). ISAV was isolated from Atlantic salmon, whereas RbtOV was isolated from rainbow trout (*Oncorhynchus mykiss* (Walbaum, 1792)) (Mohr et al.).  2. POMV encodes 6 putative proteins that have highest homology with orthomyxoviruses also isolated from fish, ISAV and RbtOV (Mohr et al.).  3. POMV encodes 4 putative proteins that have no homologues among currently available GenBank nucleotide and amino acid sequences. Both S4B and S8A proteins are expressable in *in vitro* culture (Mohr et al.).  4. Genomes of POMV isolates share high sequence identity with each other but are distantly related to genomes of other orthomyxoviruses, such as ISAV and RbtOV, based on *PB1* phylogenetic analysis (Mohr et al.). | |

**Supporting evidence**

***1. POMV genomes consist of 8 viral RNA segments that putatively encode 10 proteins similar to genomes of ISAV* and *RbtOV.* Evidence = Figure 1 and Table 1.**

**Figure 1.** Pilchard orthomyxovirus (POMV) 8 genomic segments (untranslated regions [UTR] and open reading frames [ORF]) from RNA-sequencing data (Modified from Mohr et al., an open access publication).



**Table 1.** Comparison of POMV, ISAV and RbtOV genome open reading frames (ORFs) and proteins. nt: nucleotides; aa: amino acids (Mohr et al.)

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **POMV** | | | **ISAVa** | | | **RbtOVb** | | |
| **Segment** | **Protein** | **ORF**  **(nt)** | **Protein**  **(aa)** | **Protein** | **ORF**  **(nt)** | **Protein**  **(aa)** | **Protein** | **ORF**  **(nt)** | **Protein**  **(aa)** |
| 1 | PB2 | 2199 | 732 | PB2 | 2166 | 722 | PB2 | 2184 | 727 |
| 2 | PB1 | 2121 | 706 | PB1 | 2127 | 709 | PB1 | 2106 | 701 |
| 3 | NP | 1971 | 656 | NP | 1851 | 616 | NP | 1917 | 638 |
| 4 | F  S4B | 1350  546 | 449  181 | PA | 1737 | 579 | PA | 1767 | 588 |
| 5 | PA | 1872 | 623 | F | 1332 | 444 | F | 1443 | 480 |
| 6 | HE | 1398 | 465 | HE | 1167 | 389 | HE | 1395 | 464 |
| 7 | S7A  S7B | 885  339 | 294  112 | NS1  NEP | 903  477 | 301  159 | NS1  NEP | 921  432 | 306  143 |
| 8 | S8A | 621 | 206 | M1  M2 | 591  726 | 197  242 | M1  M2 | 624  708 | 207  235 |

a Cottet et al.

b Batts et al.

***2. POMV encodes 6 putative proteins that have highest homology with orthomyxoviruses also isolated from fish; ISAV and RbtOV.* Evidence = Tables 2 and 3.**

**Table 2.** Nucleotide pair-wise comparison of the open reading frames encoding 6 putative proteins (PB2, PB1, NP, F, PA and HE) from ISAV (Glesvaer/2/90) with those from POMV isolates (Modified from Mohr et al.).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **ISAV - Glesvaer/2/90 (% identity)** | | | | | | |
| **POMV Isolate** | ***PB2*** | ***PB1*** | ***NP*** | ***F*** | ***PA*** | ***HE*** |
| 98-01382 | 52.7 | 55.8 | 50.3 | 48.9 | 49.0 | 48.9 |
| 06-04216 | 52.7 | 55.8 | 50.2 | 48.5 | 48.8 | 48.8 |
| 07-01002 | 53.0 | 55.8 | 50.1 | 49.3 | 49.1 | 48.7 |
| 12-01390 | 53.0 | 55.9 | 50.0 | 49.2 | 48.9 | 48.9 |
| 12-02055 | 53.0 | 55.9 | 50.0 | 49.2 | 48.9 | 48.9 |
| 12-02935 | 53.0 | 55.9 | 50.1 | 49.3 | 48.9 | 48.9 |
| 13-01407 | 53.0 | 56.0 | 50.1 | 49.3 | 48.9 | 48.9 |
| 13-02097 | 52.7 | 55.9 | 50.1 | 48.8 | 48.9 | 48.9 |
| 13-03566 | 52.6 | 55.9 | 50.1 | 48.5 | 48.9 | 48.9 |
| 13-03672 | 52.9 | 55.8 | 50.2 | 49.3 | 48.9 | 48.8 |
| 14-01514 | 52.6 | 56.0 | 50.2 | 48.5 | 48.9 | 48.9 |

**Table 3.** Nucleotide pair-wise comparison of the open reading frames encoding 6 putative proteins (PB2, PB1, NP, F, PA and HE) from RbtOV (Rainbow/Idaho/347/1997) with those from POMV isolates (modified from Mohr et al.).

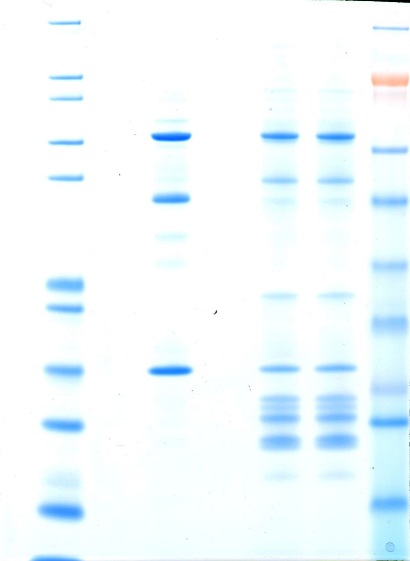
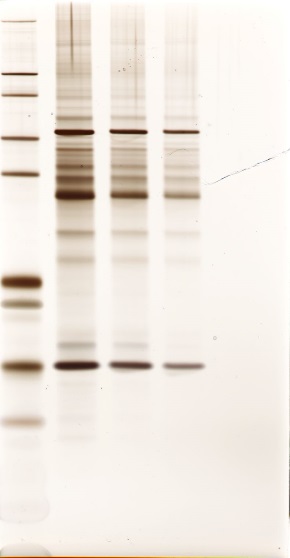
|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **RbtOV - Rainbow/Idaho/347/1997 (% identity)** | | | | | | |
| **POMV Isolate** | ***PB2*** | ***PB1*** | ***NP*** | ***F*** | ***PA*** | ***HE*** |
| 98-01382 | 52.2 | 56.8 | 50.7 | 48.6 | 49.8 | 49.1 |
| 06-04216 | 52.3 | 56.9 | 50.3 | 48.6 | 49.9 | 49.1 |
| 07-01002 | 52.4 | 57.2 | 50.6 | 48.5 | 49.6 | 49.0 |
| 12-01390 | 52.6 | 57.2 | 50.8 | 48.6 | 49.9 | 49.4 |
| 12-02055 | 52.6 | 57.2 | 50.7 | 48.5 | 49.9 | 49.4 |
| 12-02935 | 52.6 | 57.1 | 50.7 | 48.6 | 49.9 | 49.4 |
| 13-01407 | 52.5 | 57.3 | 50.7 | 48.7 | 50.1 | 49.4 |
| 13-02097 | 52.3 | 57.1 | 50.3 | 48.6 | 50.1 | 49.4 |
| 13-03566 | 52.4 | 57.2 | 50.4 | 48.6 | 50.1 | 49.0 |
| 13-03672 | 52.5 | 57.1 | 50.8 | 48.6 | 50.1 | 49.4 |
| 14-01514 | 52.3 | 57.3 | 50.2 | 48.6 | 49.9 | 49.4 |

***3. POMV encodes 4 putative proteins that have no homologues within currently available GenBank nucleotide and amino acid sequences. Both S4B and S8A proteins are expressed during* in vitro *culture.* Evidence = Table 4, Figure 2 and Table 5.**

**Table 4.** NCBI BLAST nucleotide and protein search results for four POMV isolate 14-01514 proteins without known homologues in GenBank (except POMV accessions). Searches last performed 2nd July 2020.

|  |  |  |
| --- | --- | --- |
| **POMV isolate (14-01514)** | **BLASTN POMV Query nucleotide sequence /Result** | **BLASTP POMV Query protein sequence**  **/Result** |
| S4B | MN241394 (1350 to 1895)  No significant match | QJQ28673.1  No significant match |
| S7A | MN241397 (1 to 885)  No significant match | QJQ28676.1  20% coverage, 36.23% identity WP\_116739814.1RagB/SusD family nutrient uptake outer membrane protein [Filimonas sp. YR581] |
| S7B | MN241397 (617 to 955)  No significant match | QJQ28677.1  No significant match |
| S8A | MN241398  No significant match | QJQ28678.1  No significant match |

**Figure 2.** Identification of POMV proteins. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) separation of proteins derived from purified POMV particles visualized using (A) Coomassie Brilliant Blue stain and (B) silver stain. Eleven protein bands excised from gels for mass spectrometry analysis are indicated. Lanes 1 and 3: Mark12 marker (Invitrogen); Lanes 2 and 4: 5 μg POMV. (Mohr et al.)



Band

number

**1**

**2**

**3**

**8**

**9**

**4-6**

**7**

**10**

**11**

200

97

55

36

31

66

21

14

6

kDa

116

B

A

1 2

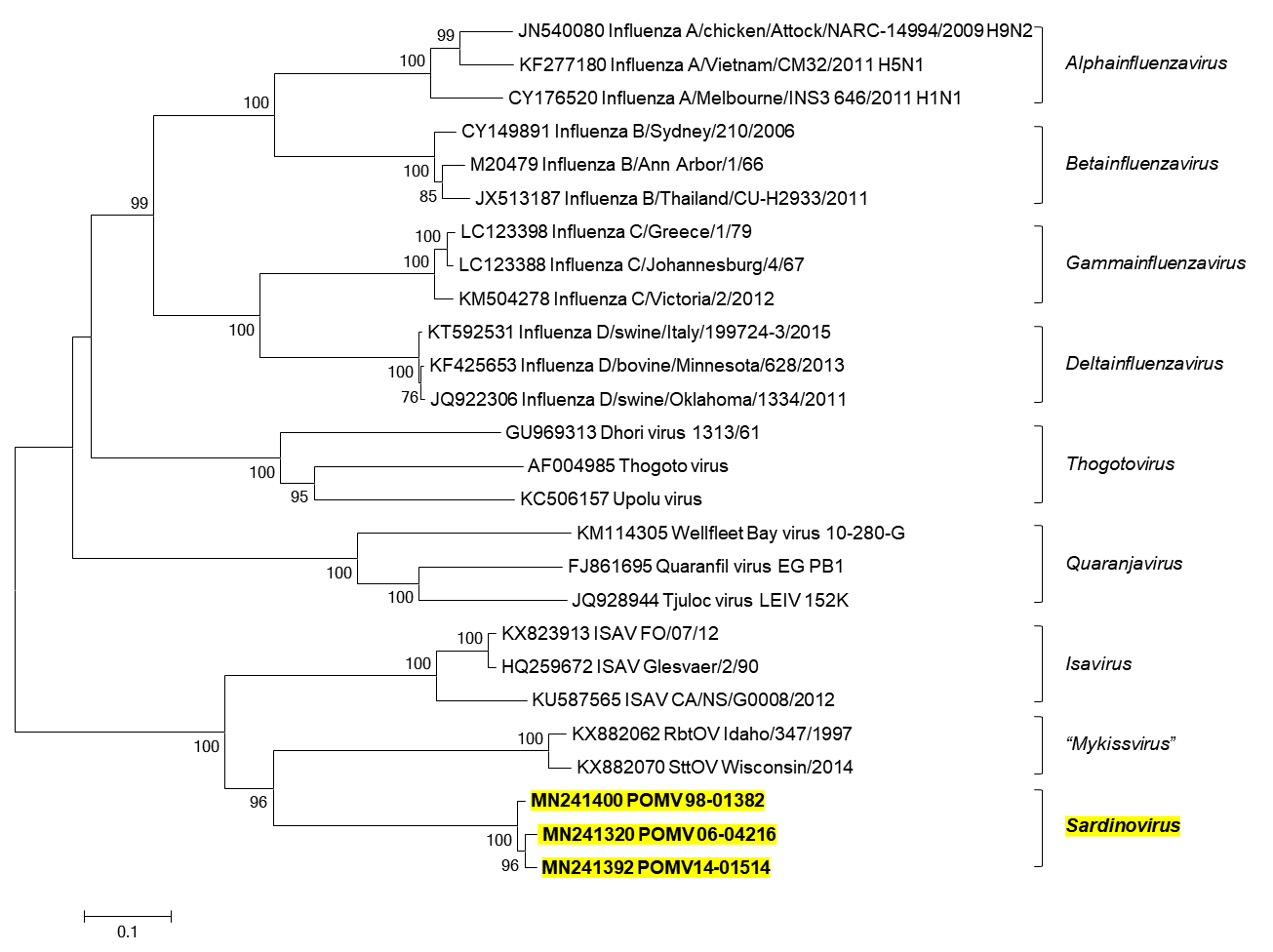
3 4

**Table 5.** Pilchard orthomyxovirus (POMV) proteins from excised gel bands identified by mass spectrometry with corresponding amino acid coverage attained as a percentage of full length protein (Mohr et al.).

|  |  |  |
| --- | --- | --- |
| **POMV protein** | **Band number** | **Amino acid sequence coverage (%)** |
| PB1 | 1  2 | 43  25 |
| PB2 | 1  2 | 44  30 |
| NP | 3  4  5 | 78  51  55 |
| F | 7  8  9 | 39  38  36 |
| S4B | 10 | 23 |
| PA | 3 | 49 |
| HE | 6  7 | 57  79 |
| S8A | 10  11 | 43  91 |

***4. Genomes of POMV isolates share high sequence identity with each other but are distantly related to genomes of viruses such as ISAV or RbtOV based on PB1 phylogenetic analysis.* Evidence = Figure 3 and Tables 6 and 7.**

**Figure** **3.** Phylogenetic relationships of the *PB1* gene of pilchard orthomyxovirus (POMV) and other orthomyxoviruses inferred using the neighbor-joining method with 1558 nucleotides. Three representative members of orthomyxovirusgenera, *Alphainfluenzavirus*, *Betainfluenzavirus*, *Gammainfluenzavirus*, *Deltainfluenzavirus*, *Quaranjavirus*, *Thogotovirus*,and *Isavirus* as well as recently characterized orthomyxoviruses of fish (RbtOV and steelhead trout orthomyxovirus [SttOV; proposed new genus “*Mykissvirus*” [Batts et al.]). Three POMV sequences were included in the analysis, 98-01382 isolated from pilchards off the coast of South Australia in 1998, and 06-04216 and 14-01514 isolated from Atlantic salmon from northern and southern Tasmania in 2006 and 2014, respectively. The percentage of replicate trees in which the associated taxa clustered together >70% in the bootstrap test (10 000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. All positions containing gaps and missing data were eliminated. (Modified from Mohr et al. 2020)

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**Table 6.** Nucleotide pair-wise comparison of POMV 14-01514 and other orthomyxovirus *PB1* ORFs in Figure 3. (Modified from Mohr et al. 2020)

|  |  |
| --- | --- |
| **Representative virus from *Orthomyxoviridae* genera** | **POMV 14-01514**  **(MN241392)**  **(% identity)** |
| ***Sardinovirus*** (this TaxoProp to ICTV) | |
| MN241400 POMV 98-01382 | 95.8 |
| MN241320 POMV 06-04216 | 97.1 |
| ***Isavirus*** | |
| KU587565 ISAV CA/NS/G0008/2012 | 50.3 |
| KX823913 ISAV FO/07/12 | 49.9 |
| HQ259672 ISAV Glesvaer/2/90 | 49.8 |
| ***“Mykissvirus*” [proposed in a separate TaxoProp]** | |
| KX882062 RbtOV Idaho/347/1997 | 51.3 |
| KX882070 SttOV Wisconsin/2014 | 51.9 |
| ***Alphainfluenzavirus*** | |
| JN540080 influenza A virus A/chicken/Attock/NARC-14994/2009 H9N2 | 35.2 |
| CY176520 influenza A virus A/Melbourne/INS3\_646/2011 H1N1 | 34.6 |
| KF277180 influenza A virus A/Vietnam/CM32/2011 H5N1 | 34.7 |
| ***Betainfluenzavirus*** | |
| M20479 influenza B virus B/Ann Arbor/1/66 | 36.5 |
| CY149891 influenza B virus B/Sydney/210/2006 | 36.7 |
| JX513187 influenza B virus B/Thailand/CU-H2933/2011 | 36.5 |
| ***Gammainfluenzavirus*** | |
| LC123398 influenza C virus C/Greece/1/79 | 37.8 |
| LC123388 influenza C virus C/Johannesburg/4/67 | 38.0 |
| KM504278 influenza C virus C/Victoria/2/2012 | 38.1 |
| ***Deltainfluenzavirus*** | |
| KF425653 influenza D virus D/bovine/Minnesota/628/2013 | 39.5 |
| KT592531 influenza D virus D/swine/Italy/199724-3/2015 | 39.7 |
| JQ922306 influenza D virus D/swine/Oklahoma/1334/2011 | 39.5 |
| ***Quaranjavirus*** | |
| FJ861695 Quaranfil virus EG PB1 | 32.9 |
| JQ928944 Tjuloc virus LEIV 152K | 32.9 |
| KM114305 Wellfleet Bay virus 10-280-G | 32.8 |
| ***Thogotovirus*** | |
| AF004985 Thogoto virus | 32.4 |
| GU969313 Dhori virus 1313/61 | 34.4 |
| KC506157 Upolu virus | 33.9 |

**Table 7.** Pair-wise comparison of *PB1* from 11 POMV isolates, nucleotide (upper right) and amino acid (lower left). (Modified from Mohr et al.)

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **98-01382** | **06-04216** | **07-01002** | **12-01390** | **12-02055** | **12-02935** | **13-01407** | **13-02097** | **13-03566** | **13-03672** | **14-01514** |
| **98-01382** |  | 97.2 | 97.2 | 97.1 | 97.1 | 97 | 97 | 96.9 | 97 | 96.6 | 96.6 |
| **06-04216** | 100 |  | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.4 | 97.5 | 97.3 | 97.3 |
| **07-01002** | 100 | 100 |  | 99.5 | 99.5 | 99.5 | 99.2 | 99.5 | 99.6 | 99.2 | 99.2 |
| **12-01390** | 100 | 100 | 100 |  | 100 | 100 | 99.6 | 99.8 | 100 | 99.5 | 99.5 |
| **12-02055** | 100 | 100 | 100 | 100 |  | 100 | 99.6 | 99.8 | 100 | 99.5 | 99.5 |
| **12-02935** | 100 | 100 | 100 | 100 | 100 |  | 99.6 | 99.7 | 99.9 | 99.5 | 99.5 |
| **13-01407** | 100 | 100 | 100 | 100 | 100 | 100 |  | 99.5 | 99.7 | 99.4 | 99.4 |
| **13-02097** | 100 | 100 | 100 | 100 | 100 | 100 | 100 |  | 99.8 | 99.4 | 99.4 |
| **13-03566** | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |  | 99.6 | 99.6 |
| **13-03672** | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 |  | 99.6 |
| **14-01514** | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 99.7 |  |

**References**

Batts WN, LaPatra SE, Katona R, Leis E, Ng TFF, Brieuc MSO, Breyta RB, Purcell MK, Conway CM, Waltzek TB, Delwart E, Winton JR (2017) Molecular characterization of a novel orthomyxovirus from rainbow and steelhead trout (*Oncorhynchus mykiss*). Virus Res 230: 38−49

https://doi.org/10.1016/j.virusres.2017.01.005

Cottet L, Rivas-Aravena A, Cortez-San Martin M, Sandinoa AM, Spencer E (2011) Infectious salmon anemia virus - genetics and pathogenesis. Virus Res 155: 10−19

<https://doi.org/10.1016/j.virusres.2010.10.021>

Godwin SE, Morrison RN, Knowles G, Cornish MC, Hayes D, Carson J (2020) Pilchard orthomyxovirus (POMV). II. Causative agent of salmon orthomyxoviral necrosis, a new disease of farmed Atlantic salmon *Salmo salar*. Dis Aquat Org 39:51–68.

<https://doi.org/10.3354/dao03469>

Mohr PG, Crane MStJ, Hoad J, Williams LM, Cummins D, Neave MJ, Shiell B, Beddome G,

Michalski WP, Peck GR, Samsing F, Wynne JW, Crameri SG, Hyatt AD, Moody NJG (2020) Pilchard orthomyxovirus (POMV). I. Characterisation of an emerging virus isolated from pilchards *Sardinops sagax* and Atlantic salmon *Salmo salar*. Dis Aquat Org 139:35–50.

<https://doi.org/10.3354/dao03470>