

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

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| **Code assigned:** | **2020.017D** |  |
| **Short title:** Create one new species in the genus *Ranavirus* (*Pimascovirales*: *Iridoviridae*) | | |
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**List the ICTV Study Group(s) that have seen this proposal**

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| *Iridoviridae* SG |

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

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| Ten of 10 members of the SG have seen and support this proposal. There are no dissenting votes, and three non-responders. |

**Authority to use the name of a living person**

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| **Taxon name** | **Person from whom the name is derived** | **Permission attached (Y/N)** |
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**Submission dates**

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| Date first submitted to SC Chair | July 29, 2020 |
| Date of this revision (if different to above) | August 16, 2020 |

**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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| 2020.017D.R.Ranavirus\_1nsp |

**Abstract**

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| We propose to establish a seventh species within the genus *Ranavirus*. This species, designated *European North Atlantic ranavirus*, will include isolates from lumpfish (*Cyclopterus lump*us, LfRV), cod (*Gadus morhua,* CoIV), and turbot (*Scophthalmus maximus*, Rmax). Isolates from these three fish species were gained between 1979 and 2016. Twelve isolates from lumpfish collected in Iceland, Scotland, Ireland, and the Faroe Isles displayed 99.7–100% identity based on a partial sequence (473 bp) of the major capsid protein gene. Similar to CoIV (114,865 bp) and Rmax (115,510 bp), the genomes of three lumpfish isolates were sequenced and ranged in length from 115,616–115,971 nucleotides, with GC contents of 54–55%. Phylogenetic analysis showed that lumpfish isolates formed a well-supported clade with other fish ranaviruses. Moreover, the clade formed by the marine fish ranaviruses CoIV and Rmax was supported as the sister group to that of the lumpfish ranaviruses. |

**Text of proposal**

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| |  | | --- | | Currently there are six recognized species within the genus *Ranavirus* [3, 4]. We propose here to establish a seventh species that contains viruses isolated from lumpfish, cod, and turbot.  Lumpfish are currently being used as cleaner fish to remove sea lice from Atlantic salmon in fish farms. During routine pathogen screening of wild brood lumpfish, 12 unknown viruses were isolated in fish from the Faroe Islands, Iceland, Scotland, and Ireland. All isolations, except for one, were made from apparently healthy fish. Characterization of these isolates showed them to be members of the genus *Ranavirus* within the family *Iridoviridae*. These isolates are markedly similar to earlier isolates from cod (cod iridovirus, CoIV) and turbot (ranavirus maximus, Rmax) and are proposed to represent a new species designated *European North Atlantic ranavirus* [1, 2, 8, 9].  Twelve isolates were gained from lumpfish collected at various locations in the North Atlantic ocean (Iceland, Faroe Islands, and Scotland) and from a hatchery in Ireland. [9]. One of the Icelandic isolates was tested on five different cell lines (BF-2, CHSE-214, EPC, FHM, RTG-2) at three different temperatures (10, 15, 20oC). Cytopathic effect was seen on all cell lines except FHM; best growth was seen on BF-2 cells where yields were two or more logs higher.  Transmission electron microscopy of infected BF-2 cells detected icosahedral viral particles with a diameter of 141 nm (face-to-face) within a large, electron-lucent viral assembly site. Virions were also seen acquiring an envelope by budding through the plasma membrane (Fig. 1).  As seen with other members of the genus and consistent with high sequence identity within the MCP, the Icelandic isolate F24/15 cross-reacted by immunofluorescence antibody test (IFAT) with rabbit anti-Bohle iridovirus and rabbit anti-EHNV sera. These sera target members of different viral species within the genus *Ranavirus*.  Sequence analysis of a 473 bp fragment from the MCP gene showed that all 12 isolates were identical except for a single non-synonymous substitution in the Irish isolate. Complete genomic sequences were obtained from three Icelandic isolates (F24/15, V4955, and F140-16). Similar to CoIV and Rmax (114,865 bp and 115,510 bp, respectively), lumpfish genomes ranged in length from 115,616 to 115971 bp with a G+C value of 54–55%. The lumpfish ranavirus genomes are predicted to encode 97 ORFs and are identical to each other except for the presence of one gene (ORF18) and the absence of another (ORF56) in V4955 and F24/15. Genome size, G+C content, gene number, and gene order are very similar among lumpfish ranavirus, CoIV, and Rmax [9] (Fig.2). Moreover, this European North Atlantic ranavirus (ENARV) genome is an intermediate in size between the amphibian iridoviruses (FV3, CMTV, and ATV) and fish iridoviruses (EHNV, SGIV) (Table 1).  Phylogenetic analysis of representative ranavirus sequences based on a concatenated set of 26 iridovirus core genes [6, 7] shows that the three Icelandic isolates form a sister clade with Rmax and CoIV supporting their status as isolates within the same species. Furthermore this clade is distinct from other clades within the genus [9] (Fig. 3).  In addition to sequence and phylogenetic data, host range (marine fish) and geographic sites of isolation (various locations within the North Atlantic ocean) support establishment of *European North Atlantic ranavirus* as a unique species within the genus.  **Species demarcation:** Although high amino acid sequence similarities exist among most species within the genus *Ranavirus* (except for Santee-Cooper ranavirus and Singapore grouper iridovirus), six species have been established based on principle host species infected, genome size, gene order, and phylogenetic relationships. While ENARV gene order is similar to that seen with ATV and EHNV, genome size is intermediate between these two species, i.e., ATV, 106 kbp, ENARV, 116 kbp, and EHNV, 127 kbp. Moreover, ENARV is distinguished from ATV (caudate amphibians) and EHNV (freshwater fish) by the principle species infected by ENARV (marine fish).  **Origin of the species name:** The species designation reflects the geographic region from which all current isolates were gained, i.e., the North Atlantic ocean around Europe. | |

**Supporting evidence**

Table 1:Characteristics of the members of the different species within the genus *Ranavirus*

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| **Virus name** (abbreviation) species | **Genome size (kbp)** | **G+C** | **Number of ORFs** | **Gene orderb** | **Principle host species** | **Genbank acc. No.** |
| frog virus 3(FV3)  *Frog virus 3* | 106 | 55% | 98 | A | Amphibians | AY548484 |
| common midwife toad virus(CMTV)  *Common midwife toad virus* | 107 | 55% | 104 | B | Amphibians | JQ231222 |
| Ambystoma tigrinum virus(ATV) *Ambystoma tigrinum virus* | 106 | 54% | 96 | C | Caudate amphibians | AY150217 |
| epizootic haematopoietic necrosis virus (EHNV) *Epizootic haematopoietic necrosis virus* | 127 | 54% | 100 | C | Freshwater fish | FJ433873 |
| lumpfish isolate F24/15 (F24/15) *European North Atlantic ranavirus* | 116 | 55% | 97 | C | Marine fish (cod, turbot, lumpfish) | MH665358 |
| Santee-Cooper ranavirus(SCRV) *Santee-Cooper ranavirus* | NDa | ND | ND | ND | Freshwater fish (e.g., largemouth bass) | MK681855 |
| Singapore grouper iridovirus(SGIV) *Singapore grouper iridovirus* | 140 | 48% | 162 | D | Marine fish (grouper) | AY521625 |

a. The full genomic sequence of Santee-Cooper ranavirus has not yet been published. A phylogenetic tree based on the sequence of a concatenated set of 26 cores genes supports the designation of SCRV as a separate species. Furthermore, where comparisons have been performed, amino acid similarity within the major capsid protein and other gene products lies between FV3, CMTV, ATV and EHNV (which all show similarity levels of >90%) and SGIV (which displays ~70% amino acid similarities among the tested gene products).

b. Gene order is based on DotPlot analysis. Currently four different patterns (A through D) reflective of viral gene order are known among ranaviruses. For profiles A though C, although marked conservation of gene order involving large blocks of genes is present, characteristic inversions and deletions have been noted. Profile D (seen with SGIV) shows marked gene rearrangements and little evidence of collinearity.

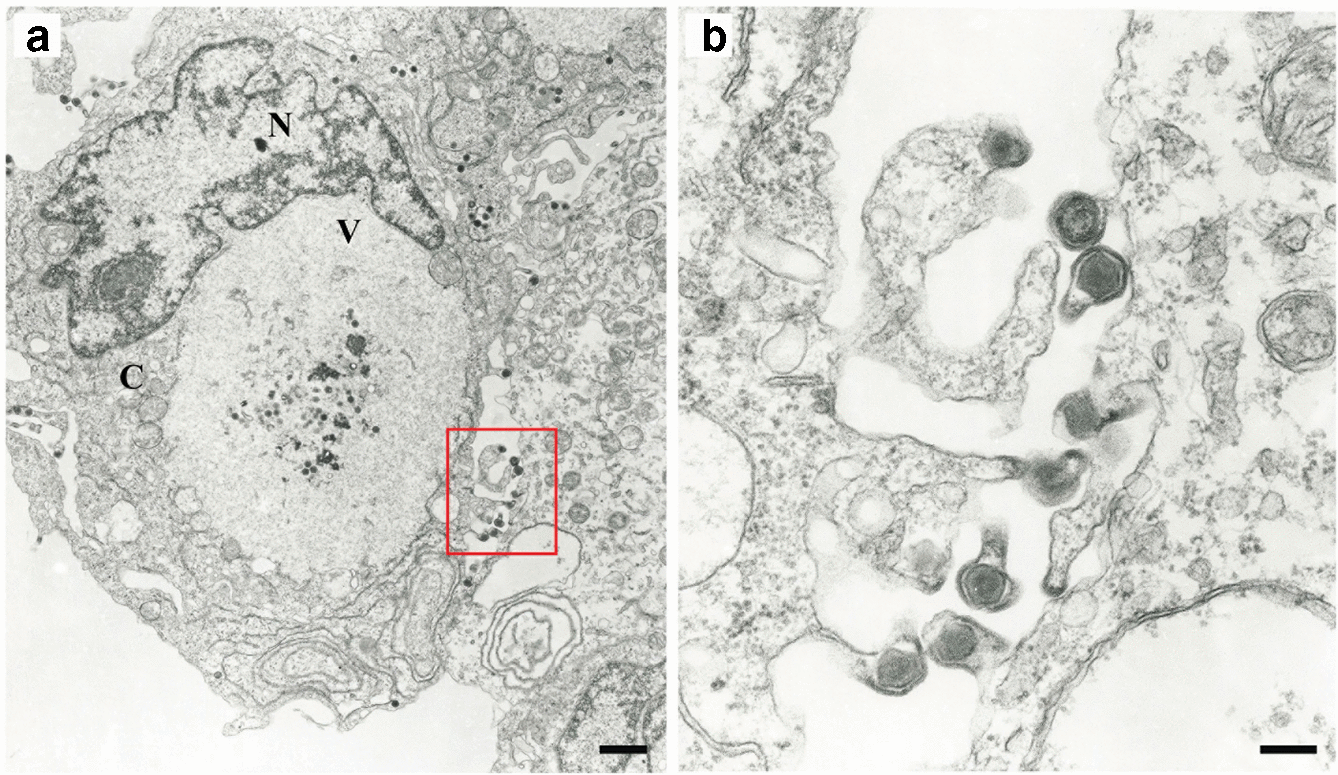


Fig. 1 TEM image of a BF-2 cell infected with ENARV isolate F24/15 [9]. Left panel: N, nucleus; C, cytoplasm; and V, viral assembly site; Bar = 1000 nm. Right panel: An enlargement of the insert shown in the right panel displaying viral particles acquiring an envelope at the plasma membrane. Bar = 200 nm. Reference: Stagg et al., 2020 [9].



Fig. 2: Visualization of locally collinear gene blocks (i.e., gene order) via Mauve [5].

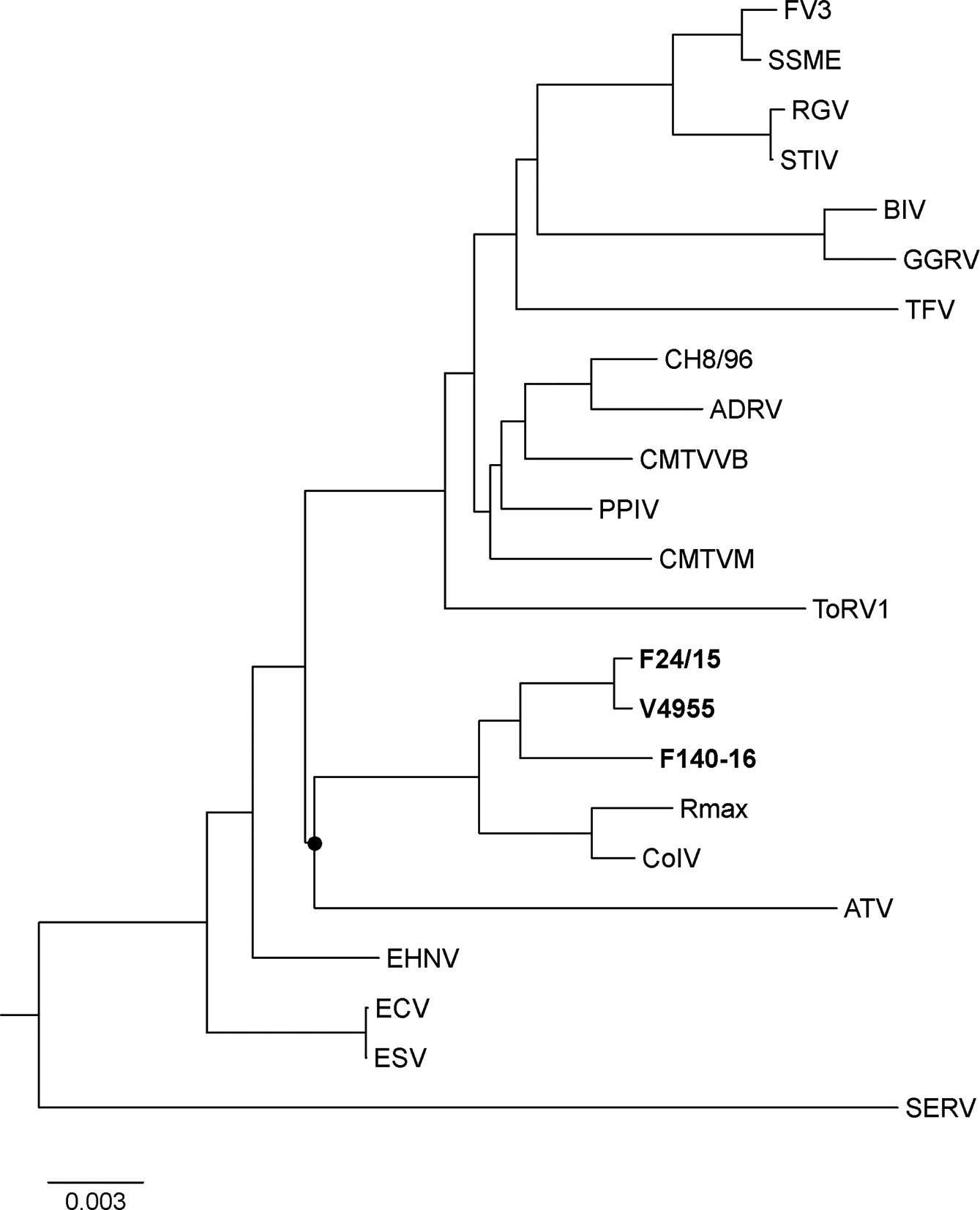


Fig. 3: Bayesian phylogram showing the relationship of three Icelandic ENARV isolates (F24/15, V4955, and F140-16) to representative members of the genus *Ranavirus* based on a concatenated set of 26 conserved genes (40467 nucleotide characters). All nodes are supported by posterior probability of 1.0 except one with a value of 0.8 (black circle). Abbreviations of viral names are as shown in Table 1 and in Table 2 from Stagg et al. 2020 [9]. Reference: Stagg et al., 2020 [9].

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