

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

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| **Code assigned:** | **2020.002B** |  |
| **Short title:** Create one new genus (*Haloferacalesvirus*) including five new species (*Caudovirales*: *Myoviridae*) | | |
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**List the ICTV Study Group(s) that have seen this proposal**

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| Archaeal viruses Study Group (David Prangishvili) |

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

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**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 2020 |
| 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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| 2020.002B.R.Haloferacalesvirus.xlsx |

**Abstract**

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| We propose a new genus to accommodate the archaeal myoviruses HF1 and HF2, which infect species within the Order *Haloferacales*. Both viruses have linear, dsDNA genomes of 76-78 kb with long terminal direct repeats, and they share 94.4-95.1% sequence identity (depending on the alignment algorithm), and have distinct host ranges. Although on the borderline of species demarcation (95%; see below) we propose that they be placed in separate species within the same genus. This is because of their long history of publication as separate viruses, and classifying them into one species now is likely to cause confusion. The suggested genus name is *Haloferacalesvirus* in order to encompass the different host genera (*Haloferax* and *Halorubrum*) of HF1 and HF2. HF1 is the proposed type species.  We also propose to include the more recently described haloviruses HRTV-5, HRTV-8 and HRTV-7 within this genus as separate species. From the published data, they share a similar particle morphology, genome type (linear, dsDNA), gene organization, and sequence similarity to HF1. Like HF2, they infect members of the genus *Halorubrum*. |

**Text of proposal**

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| |  | | --- | | Two myoviruses, HF1 and HF2, were first described in 1993 [5], and were isolated from the crystallizer pond of an Australian saltern. Both infect haloarchaeal host species (Class Halobacteria, Order Haloferacales). HF1, was isolated on *Haloferax lucentense* (originally called *Haloferax* Aa2.2) but could also infect several other species, including *Haloferax volcanii*, *Halobacterium salinarum*, *Haloarcula trapanicum* and *Haloarcula marismortui*. HF2 was isolated on *Halorubrum coriense* (originally called Ch2) but can also infect *Hrr. saccharovorum.* The general characteristics of these viruses are shown in Table 1.  The particle morphologies of HF1 and HF2 appear identical by negative-stain electron-microscopy [5, 9]. An example of HF2 is given in Figure 1 below. Contracted tails can be seen in the upper left corner and the upper right edge, confirming that they are both myoviruses. Virion dimensions were reported in [9] as 90 nm tail length and 67.8 nm head diameter.  HF2 has been listed on the ICTV website for many years as “Halorubrum phage HF2“ in the section “*List of other related viruses which may be members of the family Myoviridae but have not been approved as species*”. HF1 appears not to have been listed.  link: https://talk.ictvonline.org/ictv-reports/ictv\_9th\_report/dsdna-viruses-2011/w/dsdna\_viruses/68/myoviridae  The HF2 genome was sequenced in 2002, and the HF1 genome sequence reported in 2004 [8, 9] (accessions, Table 1). The two genomes were compared to each other in the 2004 study [9]. Both genomes have terminal direct repeats of 306 bp, and their sequences share very high identity (94.4%). The presence of terminal repeats suggested replication via concatemeric intermediates, which was confirmed experimentally with HF2 by examining of the state of the phage genome in infected cells [6].  Pairwise comparison of HF1 and HF2 revealed that their similarity is divided between the first 48 kb, which is near identical between the two (just one base difference), and the remaining ~28 kb which has a much lower (87%) nucleotide similarity. The clear separation of sequence similarity between the two viruses suggests that one of the two viruses is the result of a recombination event with another, closely related virus.  A dotplot comparison of the HF1 and HF2 genomes (Figure 2) shows the high nucleotide sequence similarity between them that extends throughout the length of each genome.  A gene map comparison focusing on the region beyond 40 kb is shown in Figure 3, and indicates the regions of difference (indels and replacements) that characterize the right ends of their genomes, starting from about 48kb. This right end region carries genes for virus assembly and packaging, and is the likely reason for the very different host ranges of HF1 and HF2. Both carry tRNA genes, one of which is a partial tRNA-Thr(GGT) that is found nearby a site-specific integrase. It most likely represents the virus *attP*.  A transcription map for HF2 was described in 2002 [8] and showed that most of the genome was transcribed and that transcription occurred in a highly organized and reproducible pattern over a 5 h infection cycle.  In 2012, a number of novel caudoviruses infecting haloarchaea were described by Atanasova and colleagues [1], and in 2015 the genome sequences of these viruses were reported [7]. In particular, *Halorubrum* head-tail (HRTV) viruses 5, 7 and 8 are myoviruses that show significant similarity to HF1 and HF2. Table 2 summarizes the characteristics of these three novel caudoviruses. A dotplot comparison of these caudoviruses is shown below in Figure 4a, and average nucleotide identities (ANI) are summarized in Figure 4b.  The data shown in Table 2 and Figure 4 confirm that the genomes of HF1 and HF2 share significant similarity with *Halorubrum* head-tail viruses 5, 7 and 8 [4]. In addition, the group that includes these 5 viruses does not show significant similarity to the genomes of other known tailed viruses.  Table 3 highlights just the group of HF1-related viruses, and shows their pairwise ANI values and their percentage DNA similarity (from Mauve alignments). From these data, it is clear that HF1, HF2, HRTV-5 and HRTV-8 are a closely related group (ANI ≥83%) while HRTV-7 is the least similar genome (ANI = 62-63%) to all the others.  The OPTSIL phylogenetic method of virus clustering and taxonomy [3] was used to indicate species and genus level taxa of these five viruses (Tables 4 and 5). Using nucleotide sequences or protein sequences, all five viruses were grouped into the same genus. Using nucleotide sequences (Table 4), the five viruses were separated into distinct species, while the protein analysis (Table 5) clustered HF1 and HF2 into the same species and separated the other three.  The protein sequences of the five viruses were used to infer phylogenetic trees using the VICTOR webservice at the DSMZ (<https://victor.dsmz.de>). This implements the Genome-BLAST Distance Phylogeny method (GBDP) as described in [3], and is designed to assist delineating virus taxa, particularly at the genus and species levels. A representative tree is shown in Figure 5. Four of the five viruses form a tightly clustered and strongly supported clade, while HRTV-7 branches before this clade but is still specifically related to them.  In summary, the evidence presented supports the proposal that these five myoviruses that infect haloarchaea should be classified into the same genus, with each belonging to a separate species. We propose that the genus name be *Haloferacalesvirus*, a name selected so as to include main host genera of the five viruses (*Haloferax* and *Halorubrum* are genera within distinct families within the same Order, *Haloferacales*). The type species is HF1.  Species demarcation criteria: We have chosen 95% DNA sequence identity as the criterion for demarcation of species in this new genus. However, we propose that HF1 and HF2 be an exception, and be classified as separate species to avoid confusion in the literature. The other proposed species differ from each other by more than 5% at the DNA level as confirmed with the BLASTN algorithm.    New Genus with type species and one additional strain: The demarcation of a novel genus is based on the OPTSIL criteria applied to full viral genome sequences, as described by [3] and implemented online at the DSMZ webserver (<http://ggdc.dsmz.de/victor.php>), using the formula d0 (nucleotide sequences). | |

**Supporting evidence**

Table 1. General characteristics of the myoviruses HF1 and HF21

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Virus** | **Can Infect** | **Morphotype** | **Plaque morphology** | **Unit Genome  length**1 **(nt)** | **%G+C** | **Genome**  **Ends 2** | **GenBank Accession** |
| HF1 | *Hfx. lucentense*  *Hfx. volcanii*  *Hbt. salinarum*  *Har. trapanicum*  *Har. marismortui* | myovirus | clear | 75,898 | 55.8 | dsDNA, linear, TDR (306 bp) | AY190604 |
| HF2 | *Hrr. coriense*  *Hbt. saccharovorum* | myovirus | clear | 77,672 | 55.8 | dsDNA, linear, TDR (306 bp) | AF222060 |
|  |  |  |  |  |  |  |  |

1Data from [5, 9]. 2TDR, terminal direct repeats.

**Table 2:** General characteristics of *Halorubrum* head-tail viruses 5, 7 and 8

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| --- | --- | --- | --- | --- | --- | --- |
| **Virus1** | **Isolation**  **Host** | **Morphotype** | **Genome  length2****(bp)** | **%G+C** | **Genome**  **Ends 2** | **GenBank Accession** |
| HRTV-5 | *Halorubrum* sp  s5a-3 | myovirus | 76,134 | 56.4 | dsDNA, linear, TDR (271 bp) | KC292022 |
| HRTV-8 | *Halorubrum* sp  B2-2 | myovirus | 74,519 | 57.1 | dsDNA, linear, TDR (346 bp) | KC292020 |
| HRTV-7 | *Halorubrum* sp  B2-2 | myovirus | 69,048 | 59.6 | dsDNA, linear, TDR (340 bp) | KC292021 |

1Data from [1] and [7]

**Table 3**. ANI and % similarity values for members of the *Haloferacalesvirus* genus

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Virus** | **Average Nucleotide Identity (ANI)**1**/Pairwise DNA % similarity** | | | | |
|  | HF1 | HF2 | HRTV-5 | HRTV-8 | HRTV-7 |
| HF1 | 100 | 92.4 | 82.5 | 71.7 | 17.7 |
| HF2 | 99 | 100 | 78.5 | 68.1 | 16.6 |
| HRTV-5 | 89 | 88 | 100 | 72.8 | 17.5 |
| HRTV-8 | 85 | 83 | 86 | 100 | 18.1 |
| HRTV-7 | 62 | 63 | 62 | 62 | 100 |

1 ANI values (below the diagonal) derived using EZbiocloud webserver (<https://www.ezbiocloud.net/tools/ani>).  
2 %DNA similarity values output from Mauve alignment tool within the Geneious package.

**Table 4.** D0-OPTSIL clustering using virus genomes1

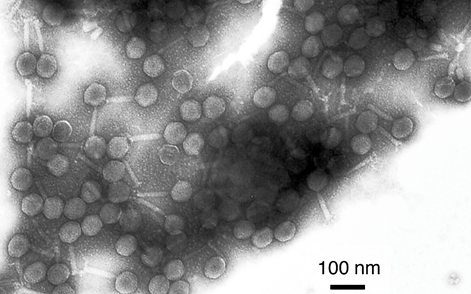
|  |  |  |  |
| --- | --- | --- | --- |
| **Genomes** | **species** | **genus** | **family** |
| HF1 | 1 | 1 | 1 |
| HF2 | 2 | 1 | 1 |
| HRTV-5 | 3 | 1 | 1 |
| HRTV-8 | 4 | 1 | 1 |
| HRTV-7 | 5 | 1 | 1 |

1data from the online webserver at <http://ggdc.dsmz.de/victor.php>

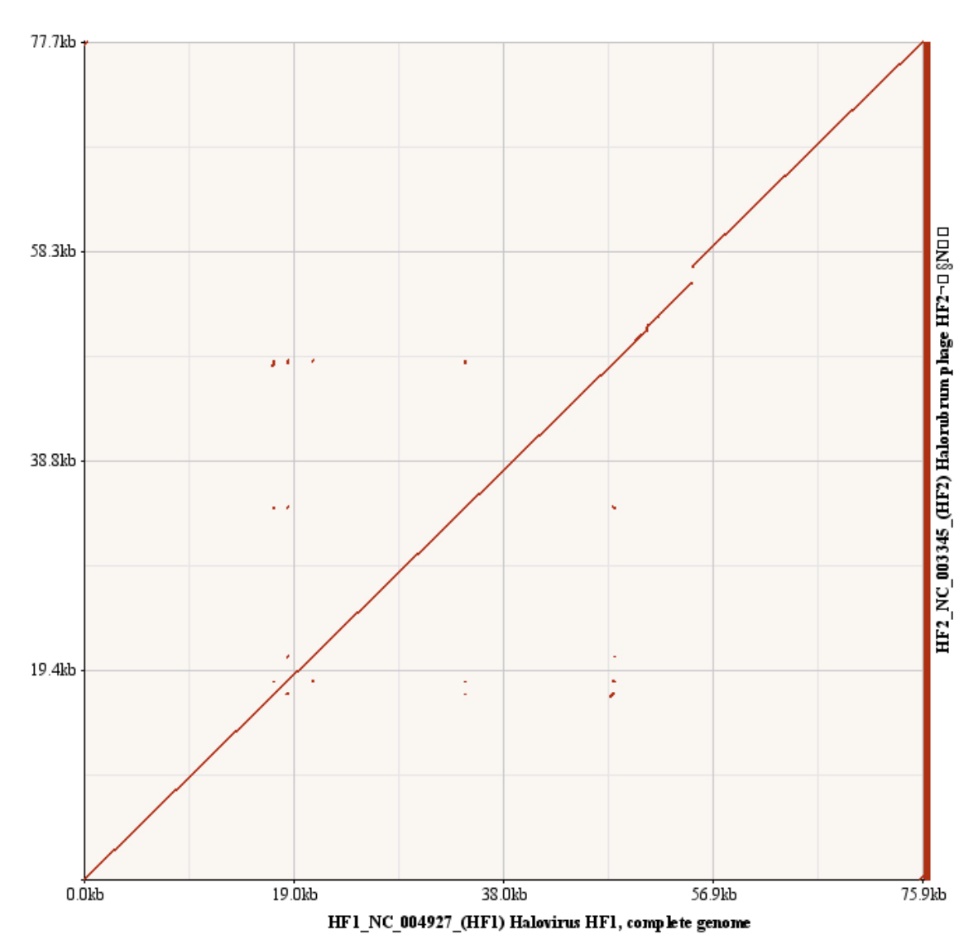
**Table 5.** D6-OPTSIL clustering using virus proteins1

|  |  |  |  |
| --- | --- | --- | --- |
| **Virus** | **species** | **genus** | **family** |
| HF1 | 1 | 1 | 1 |
| HF2 | 1 | 1 | 1 |
| HRTV-5 | 2 | 1 | 1 |
| HRTV-8 | 3 | 1 | 1 |
| HRTV-7 | 4 | 1 | 1 |

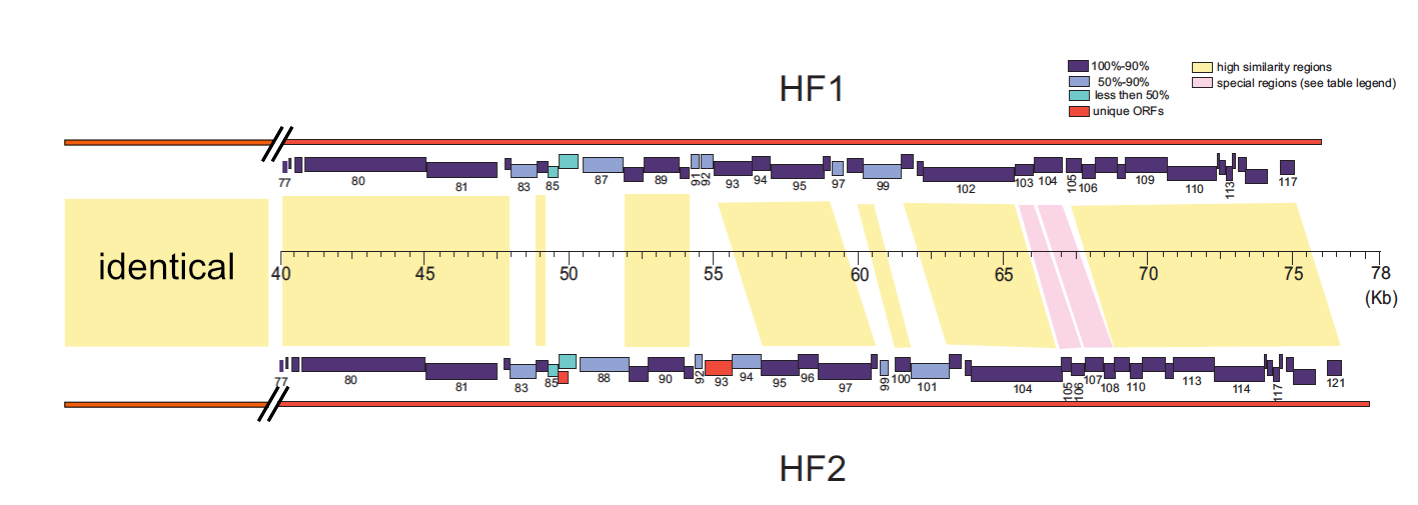
1data from the online webserver at <http://ggdc.dsmz.de/victor.php>



**Figure 1**. Electron micrograph of purified HF2 virus, negatively stained with uranyl acetate (2% wt/vol). Contracted tails can be seen upper left and at right upper edge. Scale bar is 100 nm. Picture: MDS, unpublished data.



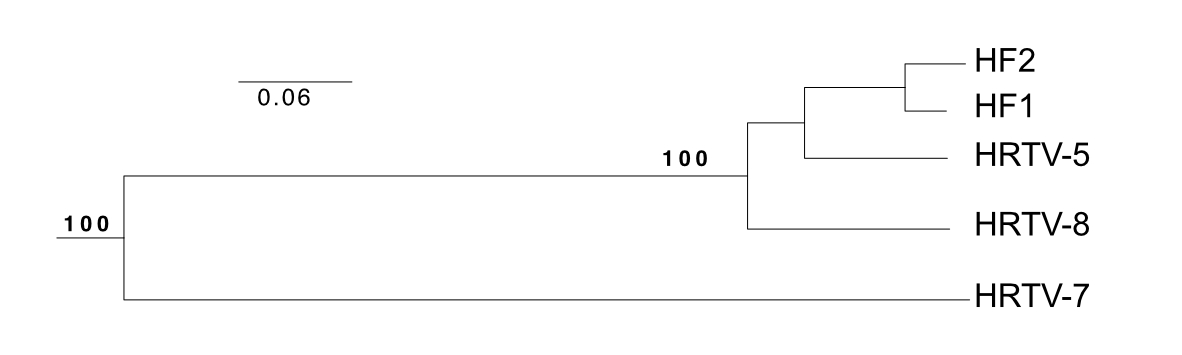
**Figure 2**. Dotplot comparison of the HF1 (horizontal axis) and HF2 (vertical axis) genomes. The dotplot was performed using zPicture: <https://zpicture.dcode.org/>. The overall nucleotide sequence identity between the two viruses is 94.4%.

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**Figure 3**. Comparative gene maps of HF1 and HF2 genomes, focusing on the region after 40 kb. The light green boxes indicate the similarity of amino acid sequences of corresponding ORFs was lower than 50%, the light-blue boxes between 50% to 90% and the indigo boxes more than 90%. Light yellow regions between the two genomes indicate a similarity value above 90%. Data modified from [9].

|  |  |
| --- | --- |
|  |  |
| **a** | **b** |

**Figure 4.** Dot-plot comparison (panel a) and average nucleotide identities (ANI; panel b) of tailed haloviruses. Data from [2](open access). The dot plot was performed using zPicture (https://zpicture.dcode.org/) and the ANI calculations were performed on the EZbiocloud webserver (<https://www.ezbiocloud.net/tools/ani>).

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**Figure 5.** Phylogenetic tree reconstruction of viruses inferred from protein sequences using the Genome-BLAST Distance Phylogeny method (GBDP) under optimal settings (formula VICTOR *d6*), as implemented at the DSMZ webserver <https://victor.dsmz.de>. Branches showing 100 percent support are labeled. The branch lengths are scaled in terms of the GBDP distance formula *d6* [3]. Tree scale (0.06) is indicated by the bar.

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