

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

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| **Code assigned:** | **2021.064B** |  |
| **Short title:** Create two new genera (*Pharaohvirus, Refugevirus*) including four new species (*Caudoviricetes*) | | |
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**Author(s) and email address(es)**

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| Turner D, Tolstoy I, Kropinski AM | dann2.turner@uwe.ac.uk;  tolstoy@ncbi.nlm.nih.gov;  Phage.Canada@gmail.com |

**Author(s) institutional address(es) (optional)**

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| University of the West of England at Bristol, UK [DT]  NCBI, USA [IT]  University of Guelph, Canada [AMK] |

**Corresponding author**

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| Andrew M. Kropinski |

**List the ICTV Study Group(s) that have seen this proposal**

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| Bacterial Viruses Subcommittee |

**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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| --- | --- |
| **Is any taxon name used here derived from that of a living person (Y/N)** | N |

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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 | May 2021 |
| Date of this revision (if different to above) |  |

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

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| “Based on the overall sequence identity (64,9 and 65,7%) the classification of Mycobacterium phage DarthPhader and Mycobacterium phage Refuge to the genus Pharaoviris is not convincing; according to Viptree analysis and this low level of identity these two phages may be classified into a separate genus; additionally, names of bacterial genera and species should be italicized”  Proposers reply: A new genus, *Refugevirus*, has been proposed. |

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

**Name of accompanying Excel module**

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| 2021.064B.A.v1.Pharaohvirus\_Refugevirus.xlsx |

**Abstract**

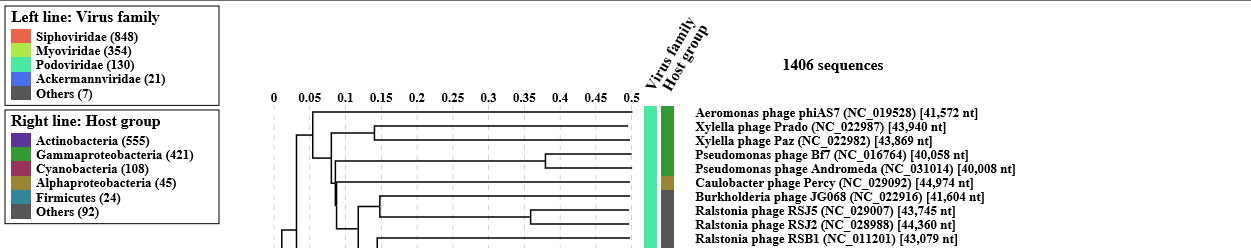
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| A detailed molecular and phylogenetic reexamination of the temperate *Mycobacterium* phages which the Actinobacteriophage Database placed in Cluster A, and ICTV classified as members of the genus *Fromanvirus* has revealed great diversity. This proposal will create two new genera, *Pharaohvirus* and *Refugevirus,* for similar phages which belong to Subcluster A12. |

**Text of proposal**

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| |  | | --- | | **Species demarcation criteria:** Two phages are assigned to the same species if their genomes are more than 95% identical over their genome length for isolates.  These values can be calculated by a number of tools, such as BLASTn – usually calculated using intergenomic distance calculator VIRIDIC [10].  **Genus demarcation criteria:** In search for criteria that create cohesive and distinct genera that are reproducible and monophyletic, the Bacterial Viruses Subcommittee has established 70% nucleotide identity of the genome length as the cut-off for genera. Genus-level groupings should always be monophyletic in the signature genes, as tested with a phylogenetic tree. [4] | |

**Supporting evidence**

**ViPTree analysis:** ViPTree analysis ([https://www.genome.jp/viptree/](about:blank); [11]) is based upon Rohwer and Edwards (2002) famous Phage Proteomic Tree [12]. The phages of interest are indicated with **red arrow (*Pharaohvirus*) and blue arrow (*Refugevirus*)**.

  
**A picture containing schematic

Description automatically generated**  
**VIRIDIC heat map:** VIRIDIC (Virus Intergenomic Distance Calculator; [10]; [http://rhea.icbm.uni-oldenburg.de/VIRIDIC/](about:blank)) computes pairwise intergenomic distances/similarities amongst phage genomes. 

Chart

Description automatically generated

**Phylogeny:** The phylogenetic tree was constructed using the major capsid proteins of these phages with phylogeny.fr in “one click” mode [8]. "The "One Click mode" targets users that do not wish to deal with program and parameter selection. By default, the pipeline is already set up to run and connect programs recognized for their accuracy and speed (MUSCLE for multiple alignment and PhyML for phylogeny) to reconstruct a robust phylogenetic tree from a set of sequences." It also includes the use of Gblocks to eliminate poorly aligned positions and divergent regions. "The usual bootstrapping procedure is replaced by a new confidence index that is much faster to compute. See: Anisimova M., Gascuel O. Approximate likelihood ratio test for branches: A fast, accurate and powerful alternative [9] for details." The new genera are indicated with arrows or boxes.



**Proposal A. To create a new genus, *Pharaohvirus* with two species**

**Origin of the name of this taxon:** This genus is named after the first virus of its type, Mycobacterium phage Pharaoh

**Historical aspects:** Temperate phage Pharaoh was isolated in 2015 by Allison Lindquist (Hope College, Louisville, CO USA) from soil using Mycobacterium smegmatis mc²155 as the host bacterium. This was part of the Science Education Alliance-Phage Hunters Advancing Genomics and Evolutionary Science program. Its genome contains 10 nt 3’-cohesive termini (CGGGACGTAA). The Actinobacteriophage Database classifies this phage to Cluster A/Subcluster A12.

**Specific References:** None

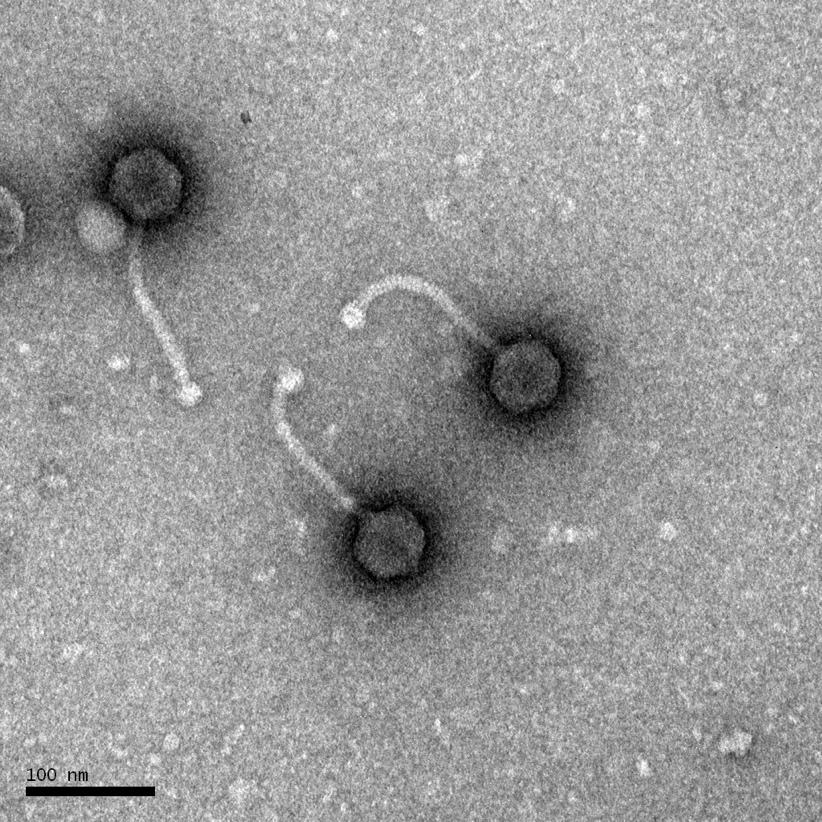
**Genome summary:**

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| --- | --- | --- | --- | --- | --- | --- | --- |
| Phage name | INSDC | Size (Kb) | GC% | Protein | tRNA | Overall % DNA sequence identity (\*) | Overall % homologous proteins (\*\*) |
| Mycobacterium phage Pharaoh | [MK524530.1](about:blank) | 50.35 | 63.7 | [80](about:blank) | 1 | 100 | 100 |
| Mycobacterium phage Steamy | [MH513984.1](about:blank) | 53.42 | 63.4 | [90](about:blank) | 2 | 71.5 | 83.7 |

**(\*) Determined using VIRIDIC [10]**

**(\*\*) Determined using CoreGenes 3.5 at** [**http://binf.gmu.edu:8080/CoreGenes3.5/**](about:blank) **[6]**

**Electron micrograph:** Electron micrographs of negatively stained Mycobacterium phage Pharaoh ([https://phagesdb.org/phages/Pharaoh/](about:blank)). Limited permission was granted by The Actinobacteriophages Database ([https://phagesdb.org/](about:blank)), funded by the Howard Hughes Medical Institute, to use this electron micrograph for this taxonomy proposal; it cannot be reused without permission of The Actinobacteriophages Database.

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**Proposal B. To create a new genus, *Refugevirus* with two species**

**Origin of the name of this taxon:** This genus is named after the first virus of its type, Mycobacterium phage Refuge

**Historical aspects:** Temperate phage Refuge was isolated in 2016 by Brittnie Hodsdon, Hateya Levesque, Billy & Jon (University of Maine, Fort Kent, ME, USA) from sediment near the Jalbert crossing along the St. John river using Mycobacterium smegmatis mc²155 as the host bacterium. This was part of the Science Education Alliance-Phage Hunters Advancing Genomics and Evolutionary Science program. Its genome contains 10 nt 3’-cohesive termini (CGGGACGTAA). The Actinobacteriophage Database classifies this phage to Cluster A/Subcluster A12.

**Specific References:** None

**Genome summary:**

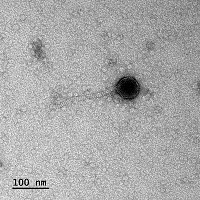
|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Phage name | INSDC | Size (Kb) | GC% | Protein | tRNA | Overall % DNA sequence identity (\*) | Overall % homologous proteins (\*\*) |
| Mycobacterium phage Refuge | [MK494113.1](about:blank) | 53.59 | 63.5 | [91](about:blank) | 1 | 100 | 100 |
| Mycobacterium phage DarthPhader | [KX657793.1](about:blank) | 53.43 | 63.2 | [91](about:blank) | 1 | 77.1 | 82.4 |

**(\*) Determined using VIRIDIC [10]**

**(\*\*) Determined using CoreGenes 3.5 at** [**http://binf.gmu.edu:8080/CoreGenes3.5/**](about:blank) **[6]**

**N.B. There is a close relationship between *Refugevirus* and *Pharaohvirus*, but, at this time, we choose not to propose a new subfamily.**

**Electron micrograph:** Electron micrographs of negatively stained Mycobacterium phage Refuge (<https://phagesdb.org/phages/Refuge/>). Limited permission was granted by The Actinobacteriophages Database ([https://phagesdb.org/](about:blank)), funded by the Howard Hughes Medical Institute, to use this electron micrograph for this taxonomy proposal; it cannot be reused without permission of The Actinobacteriophages Database.

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**References**

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