

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

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| **Code assigned:** | **2021.053B** |  |
| **Short title:** Create one new genus (*Microwolfvirus*)including five species (*Caudoviricetes*) | | |
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**Author(s) and email address(es)**

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**Corresponding author**

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| Andrew M. Kropinski |

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

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| Actinobacteriophage Study Group, 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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| Acceptance of proposal 2021.001B.abolish\_Caudovirales by EC53 results in removal of the order *Caudovirales* and families *Myoviridae*, *Podoviridae* and *Siphoviridae*. All underlying taxa are to be assigned directly to the class *Caudoviricetes*. The Excel module of this proposal has been altered to reflect the future changes; however, the Word module has been unaltered while awaiting the ratification vote. |

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

**Name of accompanying Excel module**

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| 2021.053B.R.Microwolfvirus |

**Abstract**

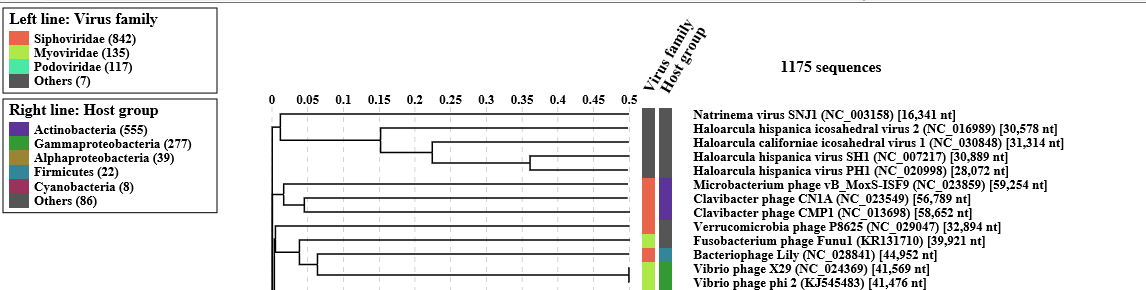
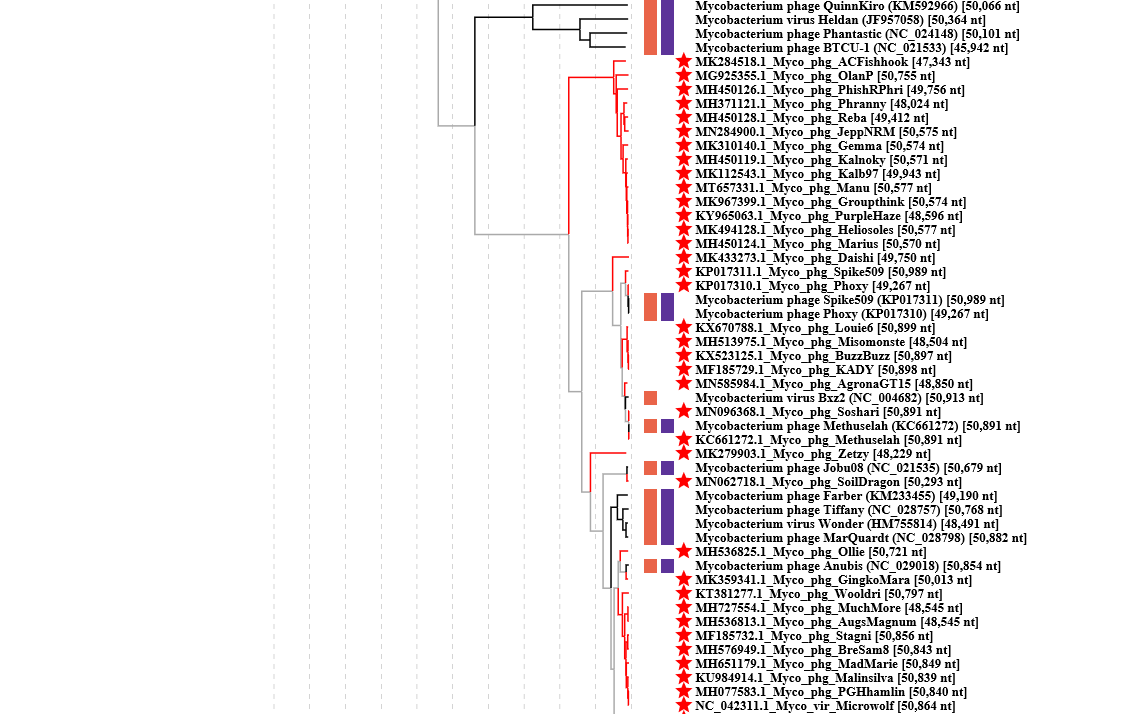
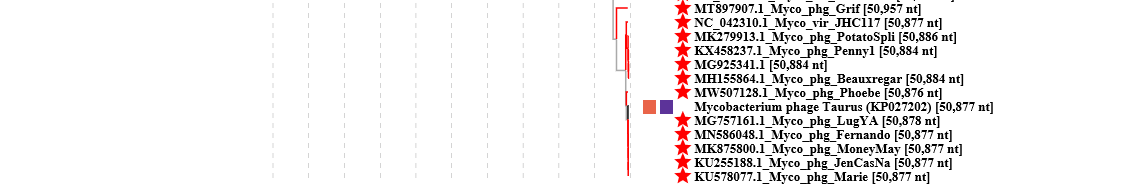
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| Mycobacterium temperate siphovirus Microwolf is currently classified in the Actinobacteriophage Database to Cluster A/Subcluster A3, and to the *Fromanvirus* genus by ICTV. An in-depth reanalysis of this genus reveals that it is not monophyletic and we have decided to split-out the Microwolf-like viruses. |

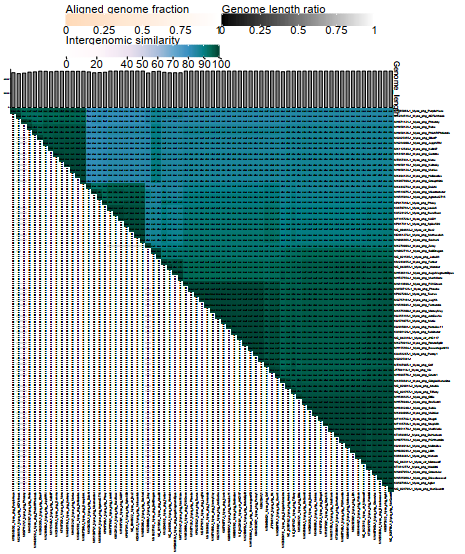
**Text of proposal**

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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 [3].  **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] | |

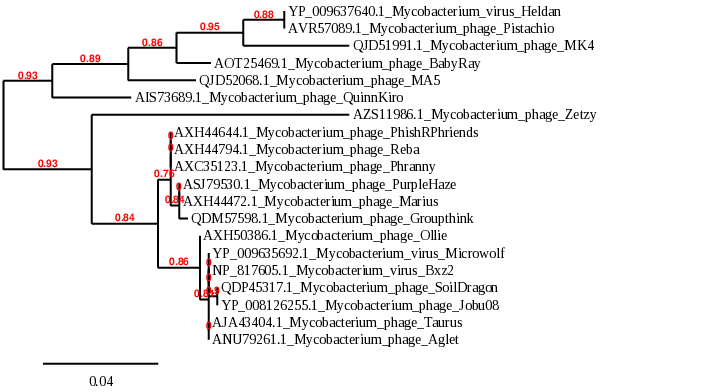
**Supporting evidence**

**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 lines and stars**.

  
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**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.



**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.

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**Origin of the name of this taxon:** The name is directly derived from Mycobacterium phage Microwolf.

**Historical aspects:** Phage Microwolf, along with Mycobacterium phages Bxz2, JHC117 and Wonder is currently classified as a member of the genus *Fromanvirus* but our reanalysis of this cluster reveals that it is not cohesive. The Actinobacteriophage Database places this virus in Cluster A, subcluster A3. Microwolf was isolated in 2009 by Hope Hendricks (North Carolina State University) from Rayleigh, NC 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. This temperate phage has 10 nt 3’-cohesive termini (CGGGTGGTAA).

**Specific References:** Hatfull GF; Science Education Alliance Phage Hunters Advancing Genomics and Evolutionary Science Program; KwaZulu-Natal Research Institute for Tuberculosis and HIV Mycobacterial Genetics Course Students; Phage Hunters Integrating Research and Education Program. Complete genome sequences of 138 mycobacteriophages. J Virol. 2012 Feb;86(4):2382-4. doi: 10.1128/JVI.06870-11. PMID: 22282335; PMCID: PMC3302410. **[Microwolf, HC117]**

Pedulla ML, Ford ME, Houtz JM, Karthikeyan T, Wadsworth C, Lewis JA, Jacobs-Sera D, Falbo J, Gross J, Pannunzio NR, Brucker W, Kumar V, Kandasamy J, Keenan L, Bardarov S, Kriakov J, Lawrence JG, Jacobs WR Jr, Hendrix RW, Hatfull GF. Origins of highly mosaic mycobacteriophage genomes. Cell. 2003 Apr 18;113(2):171-82. doi: 10.1016/s0092-8674(03)00233-2. PMID: 12705866. **[Bxz2]**

**Genome summary:**

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| Phage name | RefSeq No. | INSDC | Size (Kb) | GC% | Protein | tRNA | Overall % DNA sequence identity (\*) | Overall % homologous proteins (\*\*) |
| Mycobacterium phage Microwolf | [NC\_042311.1](https://www.ncbi.nlm.nih.gov/nuccore/NC_042311.1) | [JF704101.1](https://www.ncbi.nlm.nih.gov/nuccore/JF704101.1) | 50.86 | 64.0 | [85](https://www.ncbi.nlm.nih.gov/genome/browse/#!/proteins/66516/467175%7CMycobacterium%20virus%20Microwolf/viral%20segment/) | 3 | 100 | 100 |
| Mycobacterium phage Bxz2 | NC\_004682.1 | AY129332.1 | 51.00 | 64.2 | 83 | 3 | 90.7 | 94.1 |
| Mycobacterium phage JHC117 | [NC\_042310.1](https://www.ncbi.nlm.nih.gov/nuccore/NC_042310.1) | [JF704098.1](https://www.ncbi.nlm.nih.gov/nuccore/JF704098.1) | 50.88 | 64.0 | [85](https://www.ncbi.nlm.nih.gov/genome/browse/#!/proteins/64073/466553%7CMycobacterium%20virus%20JHC117/viral%20segment/) | 3 | 97.0 | 97.6 |
| Mycobacterium phage Wonder | [NC\_042058.1](https://www.ncbi.nlm.nih.gov/nuccore/NC_042058.1) | [HM755814.1](https://www.ncbi.nlm.nih.gov/nuccore/HM755814.1) | 48.49 | 64.0 | [33](https://www.ncbi.nlm.nih.gov/genome/browse/#!/proteins/65302/884356%7CMycobacterium%20virus%20Wonder/viral%20segment/)(\*\*\*) | 3 (Δ) | 97.1 | ND |
| Mycobacterium phage Purple Haze |  | [KY965063.1](https://www.ncbi.nlm.nih.gov/nuccore/KY965063.1) | 48.60 | 64.0 | [83](https://www.ncbi.nlm.nih.gov/genome/browse/#!/proteins/63575/466197%7CMycobacterium%20phage%20PurpleHaze/viral%20segment/) | 3 | 86.3 | 91.8 |
| Mycobacterium phage SoilDragon |  | [MN062718.1](https://www.ncbi.nlm.nih.gov/nuccore/MN062718.1) | 50.29 | 64.0 | [90](https://www.ncbi.nlm.nih.gov/genome/browse/#!/proteins/83115/639506%7CMycobacterium%20phage%20SoilDragon/viral%20segment/) | 3 | 95.2 | 98.8 |
| Mycobacterium phage Zetzy |  | [MK279903.1](https://www.ncbi.nlm.nih.gov/nuccore/MK279903.1) | 48.23 | 64.0 | [81](https://www.ncbi.nlm.nih.gov/genome/browse/#!/proteins/75094/435071%7CMycobacterium%20phage%20Zetzy/viral%20segment/) | 3 | 91.9 | 92.9 |

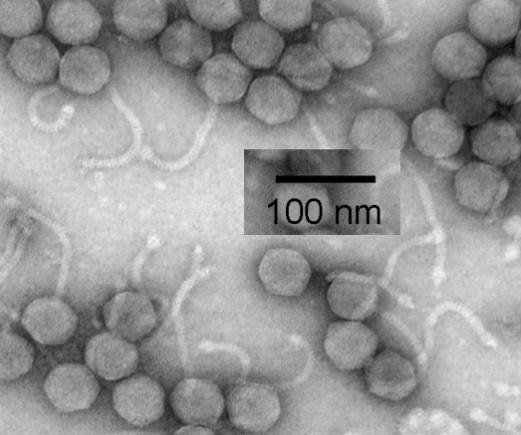
**(\*) Determined using VIRIDIC [10]**

**(\*\*) Determined using CoreGenes 3.5 at** [**http://binf.gmu.edu:8080/CoreGenes3.5/**](http://binf.gmu.edu:8080/CoreGenes3.5/) **[4]**

**(\*\*\*) underannotated**

**(Δ) The GenBank record has 43 ambiguous bases and no indication of tRNAs. These were identified using tRNAscan-SE at** [**http://lowelab.ucsc.edu/tRNAscan-SE/**](http://lowelab.ucsc.edu/tRNAscan-SE/) **[7]**

**Electron micrograph:** Electron micrographs of negatively stained Mycobacterium phage Microwolf (<https://phagesdb.org/phages/Microwolf/>). Limited permission was granted by The Actinobacteriophages Database (<https://phagesdb.org/>), 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.



**References**

1: Sayers EW, Agarwala R, Bolton EE, Brister JR, Canese K, Clark K, et al. Database resources of the National Center for Biotechnology Information. Nucleic Acids Res. 2019;47(D1):D23-D28. doi: 10.1093/nar/gkz899. PMID: 31602479.

2: Tolstoy I, Kropinski AM, Brister JR. Bacteriophage Taxonomy: An Evolving Discipline. Methods Mol Biol. 2018;1693:57-71. doi: 10.1007/978-1-4939-7395-8\_6. PMID: 29119432.

3: O'Leary NA, Wright MW, Brister JR, Ciufo S, Haddad D, McVeigh R, et al. Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. Nucleic Acids Res. 2016;44(D1):D733-45. doi: 10.1093/nar/gkv1189. PMID: 26553804.

4: Turner D, Kropinski AM, Adriaenssens EM. A Roadmap for Genome-Based Phage Taxonomy. Viruses. 2021 Mar 18;13(3):506. doi: 10.3390/v13030506. PMID: 33803862; PMCID: PMC8003253.

5: Chan PP, Lowe TM. tRNAscan-SE: Searching for tRNA Genes in Genomic Sequences. Methods Mol Biol. 2019;1962:1-14. doi: 10.1007/978-1-4939-9173-0\_1. PMID: 31020551.

6: Turner D, Reynolds D, Seto D, Mahadevan P. CoreGenes3.5: a webserver for the determination of core genes from sets of viral and small bacterial genomes. BMC Res Notes. 2013;6:140. doi: 10.1186/1756-0500-6-140. PMID: 23566564.

7: Darling AE, Mau B, Perna NT. progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. PLoS One. 2010;5(6):e11147. doi: 10.1371/journal.pone.0011147. PMID: 20593022.

8: Dereeper A, Guignon V, Blanc G, Audic S, Buffet S, Chevenet F, Dufayard JF, Guindon S, Lefort V, Lescot M, Claverie JM, Gascuel O. Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Res. 2008;36(Web Server issue):W465-9. doi: 10.1093/nar/gkn180. Epub 2008 Apr 19. PMID: 18424797.

9: Anisimova M, Gascuel O. Approximate likelihood-ratio test for branches: A fast, accurate, and powerful alternative. Syst Biol. 2006;55(4):539-52. PMID: 16785212. DOI: 10.1080/10635150600755453.

10: Moraru C, Varsani A, Kropinski AM. VIRIDIC-A Novel Tool to Calculate the Intergenomic Similarities of Prokaryote-Infecting Viruses. Viruses. 2020 Nov 6;12(11):1268. doi: 10.3390/v12111268. PMID: 33172115; PMCID: PMC7694805.

11: Nishimura Y, Yoshida T, Kuronishi M, Uehara H, Ogata H, Goto S. ViPTree: the viral proteomic tree server. Bioinformatics. 2017; 33(15):2379-2380. doi:10.1093/bioinformatics/btx157. PubMed PMID: 28379287.

12: Rohwer F, Edwards R. The Phage Proteomic Tree: a genome-based taxonomy for phage. J Bacteriol. 2002 Aug;184(16):4529-35. PubMed PMID: 12142423