

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

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| **Code assigned:** | **2021.032B** |  |
| **Short title:** Create one new subfamily (*Gclasvirinae*) containing five genera (*Caudoviricetes*) | | |
|  | | |

**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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| Actinobacteriophages 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**

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

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

**Submission dates**

|  |  |
| --- | --- |
| Date first submitted to SC Chair |  |
| 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**

|  |
| --- |
| 2021.032B.R.Gclasvirinae |

**Abstract**

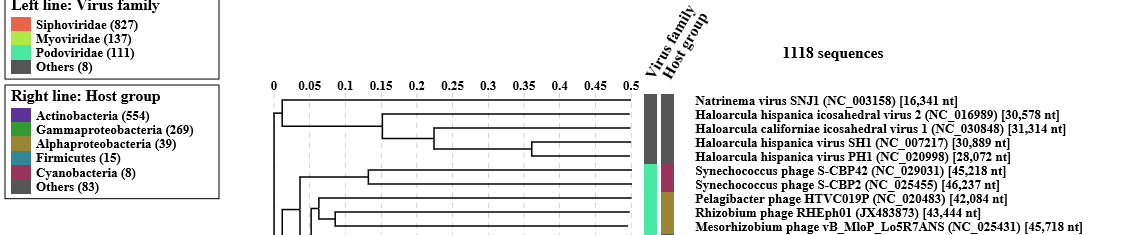
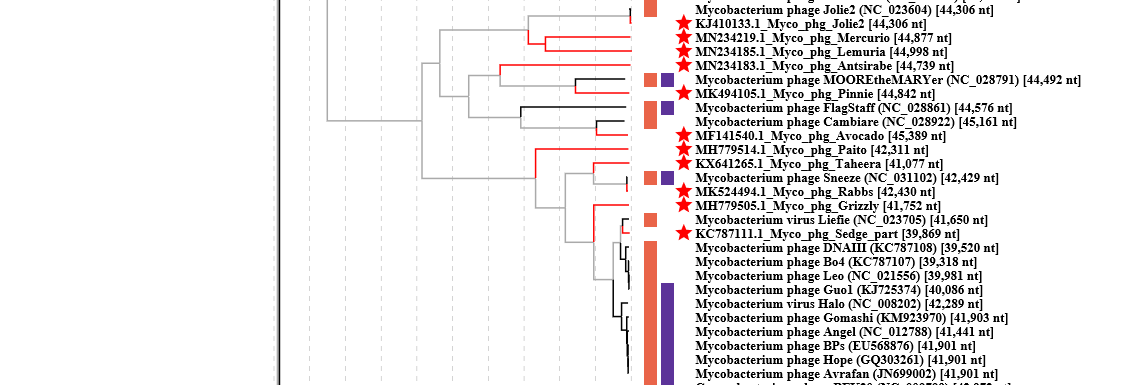
|  |
| --- |
| We have classified a number of new phages which The Actinobacteriophage Database group into Cluster G. These five genera all fit within a single subfamily the members of which possess, on average, genomes of 43.76 kb (67.9 mol%G+C) and encode for 65 proteins and 0 tRNA. Using CoreGenes 5.0 (https://coregenes.ngrok.io/) revealed 33 homologs shared by this group of phages (51%). These homologs included: small and large terminase subunits, portal, capsid maturation protease, scaffolding protein, major capsid protein, head-to-tail adaptor, head-to-tail stopper, tail terminator, major tail protein, tail tape measure protein, two tail assembly chaperone, five minor tail proteins, holin, RecE-like exonuclease, RecT-like ssDNA binding protein and RuvC-like resolvase. At the DNA level these phages share at least 39.9% DNA similarity. |

**Text of proposal**

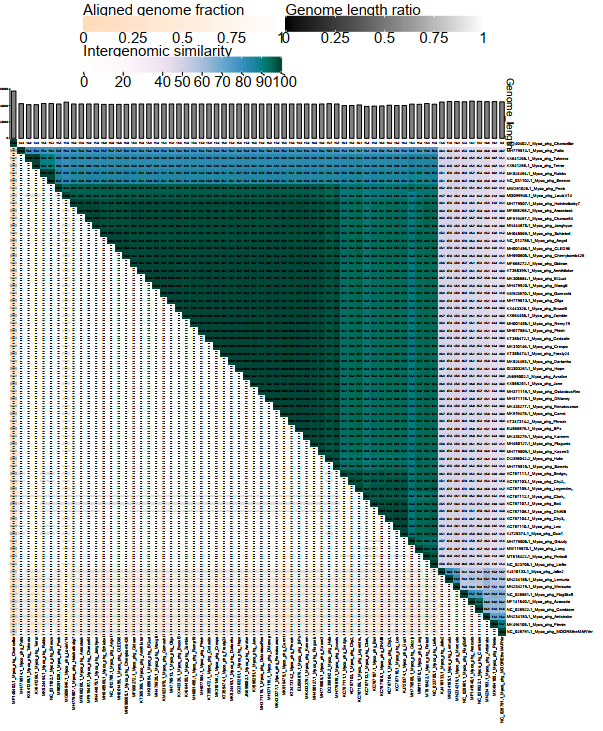
|  |  |
| --- | --- |
| |  | | --- | | **Species demarcation criteria:** Two phages are assigned to the same species if their genomes are more than 95% identical over their genome length for isolates.  These values can be calculated by a number of tools, such as BLASTn – usually calculated using intergenomic 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.  **Subfamily demarcation criteria:** Subfamilies are to be created when two or more genera are related below the family level. In practical terms, this usually means that they share a low degree of sequence similarity (here minimum 39%) and that the genera form a clade in a marker tree phylogeny. [9] | |

**Supporting evidence**

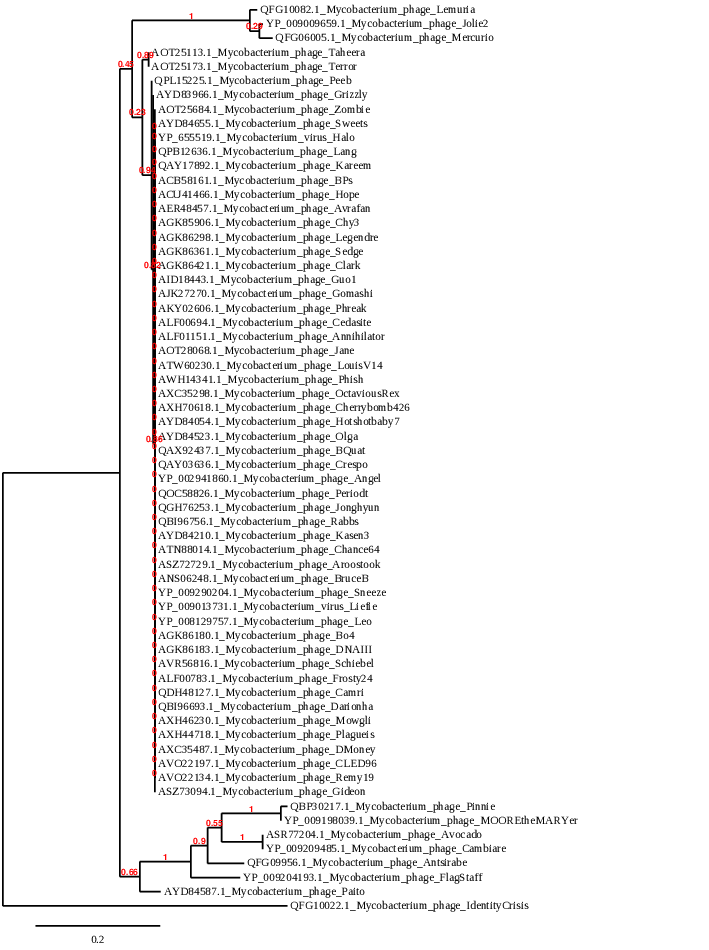
**ViPTree analysis:** ViPTree analysis ([https://www.genome.jp/viptree/](about:blank); [1]) is based upon Rohwer and Edwards (2002) famous Phage Proteomic Tree [2]. The phages of interest are indicated with **red lines**.

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**VIRIDIC heat map:** VIRIDIC (Virus Intergenomic Distance Calculator; [3]; [http://rhea.icbm.uni-oldenburg.de/VIRIDIC/](about:blank)) computes pairwise intergenomic distances/similarities amongst phage genomes. (High resolution version attached)



**Phylogeny:** The phylogenetic tree was constructed using the major capsid proteins of *Gclasvirinae* phages with phylogeny.fr in “one click” mode [5]. "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 [6] for details."

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**Proposals:**

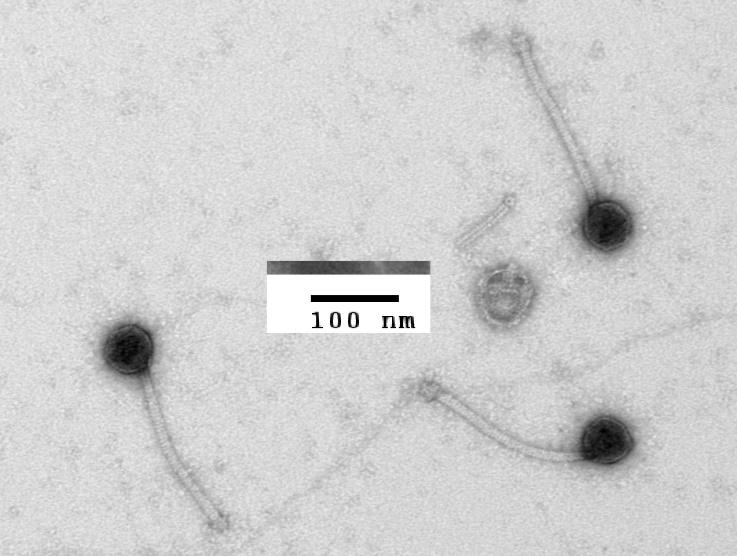
1. **To add four (4) new species to the genus *Liefievirus***
2. **To create a new genus, *Avocadovirus* with three (3) species**
3. **To create a new genus, *Antsirabevirus* with a single species**
4. **To create a new genus, *Jolieduovirus* with three (3) species**
5. **To create a new genus, *Pinnievirus* with two (2) species**
6. **To create a new subfamily *Gclasvirinae***

**To add four (4) new species to the genus *Liefievirus***

**Source of the name of this taxon:** This taxon was named directly from the first virus of its type, Mycobacterium phage Liefie.

**History:** The *Liefievirus* genus, with two temperate species, was created from the *Halolikevirus* through Taxonomy Proposal 2015.006aB. The Actinobacteriophage Database places this phage in Cluster G, Subcluster G1.

**Electron micrograph:** Electron micrographs of negatively stained Microbacterium phage Liefie (https://phagesdb.org/phages/Liefie/). 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.



**Specific Reference:** Pope WH, Bowman CA, Russell DA, Jacobs-Sera D, Asai DJ, Cresawn SG, Jacobs WR, Hendrix RW, Lawrence JG, Hatfull GF; Science Education Alliance Phage Hunters Advancing Genomics and Evolutionary Science; Phage Hunters Integrating Research and Education; Mycobacterial Genetics Course. Whole genome comparison of a large collection of mycobacteriophages reveals a continuum of phage genetic diversity. Elife. 2015 Apr 28;4:e06416. doi: 10.7554/eLife.06416. PMID: 25919952; PMCID: PMC4408529.

**GenBank Summary:**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Phage name | INSDC | Size (Kb) | GC% | Protein | tRNA | Overall DNA sequence identity (\*) | % common proteins (\*\*) |
| Mycobacterium phage Liefie | [JN412593.1](https://www.ncbi.nlm.nih.gov/nuccore/JN412593.1) | 41.65 | 66.8 | [61](https://www.ncbi.nlm.nih.gov/genome/browse/#!/proteins/24516/460516%7CMycobacterium%20virus%20Liefie/viral%20segment%20Unknown/) | 0 | 100 | 100 |
| Mycobacterium phage Paito | [MH779514.1](https://www.ncbi.nlm.nih.gov/nuccore/MH779514.1) | 42.31 | 66.0 | [68](https://www.ncbi.nlm.nih.gov/genome/browse/#!/proteins/72816/409437%7CMycobacterium%20phage%20Paito/viral%20segment/) | 0 | 76.4 | 85.2 |
| Mycobacterium phage Taheera | [KX641265.1](https://www.ncbi.nlm.nih.gov/nuccore/KX641265.1) | 41.08 | 66.4 | [60](https://www.ncbi.nlm.nih.gov/genome/browse/#!/proteins/63065/465688%7CMycobacterium%20phage%20Taheera/viral%20segment/) | 0 | 86.1 | 91.8 |
| Mycobacterium phage Rabbs | [MK524494.1](https://www.ncbi.nlm.nih.gov/nuccore/MK524494.1) | 42.43 | 66.4 | [65](https://www.ncbi.nlm.nih.gov/genome/browse/#!/proteins/76386/468441%7CMycobacterium%20phage%20Rabbs/viral%20segment/) | 0 | 87.5 | 93.4 |
| Mycobacterium phage Grizzly | [MH779505.1](https://www.ncbi.nlm.nih.gov/nuccore/MH779505.1) | 41.75 | 66.8 | [62](https://www.ncbi.nlm.nih.gov/genome/browse/#!/proteins/72808/409429%7CMycobacterium%20phage%20Grizzly/viral%20segment/) | 0 | 93.5 | 95.1 |

**(\*) Determined using VIRIDIC [3]**

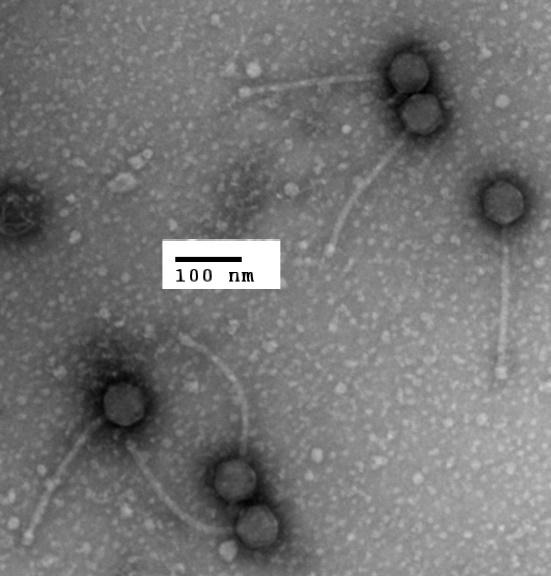
**(\*\*) Determined using CoreGenes 3.5 at** [**http://binf.gmu.edu:8080/CoreGenes3.5/**](http://binf.gmu.edu:8080/CoreGenes3.5/) **[4]**

**To create a new genus, *Avocadovirus* with three (3) species**

**Source of the name of this taxon:** This taxon is named directly from the first virus of its type, Mycobacterium phage Avocado

**History:** Temperate Mycobacterium phage Avocado was isolated in 2015 by L. Hudson (Seton Hill University, Greensburg, PA 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. The genome possesses 10 nt 3’-cohesive termini (CCCATGGCAT). The Actinobacteriophage Database places this phage in Cluster G, Subcluster G2.

**Electron micrograph:** Electron micrographs of negatively stained Mycobacterium phage Flagstaff (https://phagesdb.org/phages/Flagstaff/). 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.



**Specific Reference:** Pope WH, Augustine DA, Carroll DC, Duncan JC, Harwi KM, Howry R, Jagessar B, Lum BA, Meinert JW, Migliozzi JS, Milliken KA, Mitchell CJ, Nalatwad AS, Orlandini KC, Rhein MJ, Saravanan V, Seese BA, Schiebel JG, Thomas KB, Adkins NL, Cohen KL, Iyengar VB, Kim H, Kramer ZJ, Montgomery MT, Schafer CE, Wilkes KE, Grubb SR, Warner MH, Bowman CA, Russell DA, Hatfull GF. Genome Sequences of Cluster G Mycobacteriophages Cambiare, FlagStaff, and MOOREtheMARYer. Genome Announc. 2015 Jun 18;3(3):e00595-15. doi: 10.1128/genomeA.00595-15. PMID: 26089410; PMCID: PMC4472887.

**GenBank Summary:**

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| --- | --- | --- | --- | --- | --- | --- | --- |
| Phage name | INSDC | Size (Kb) | GC% | Protein | tRNA | Overall DNA sequence identity (\*) | % common proteins (\*\*) |
| Mycobacterium phage Avocado | [MF141540.1](https://www.ncbi.nlm.nih.gov/nuccore/MF141540.1) | 45.39 | 68.7 | [68](https://www.ncbi.nlm.nih.gov/genome/browse/#!/proteins/63824/466445%7CMycobacterium%20phage%20Avocado/viral%20segment/) | 0 | 100 | 100 |
| Mycobacterium phage FlagStaff | [KR080197.1](https://www.ncbi.nlm.nih.gov/nuccore/KR080197.1) | 44.58 | 68.5 | [65](https://www.ncbi.nlm.nih.gov/genome/browse/#!/proteins/42354/461937%7CMycobacterium%20phage%20FlagStaff/viral%20segment%20Unknown/) | 0 | 78.7 | 86.8 |
| Mycobacterium phage Cambiare | [KR080198.1](https://www.ncbi.nlm.nih.gov/nuccore/KR080198.1) | 45.16 | 68.8 | [67](https://www.ncbi.nlm.nih.gov/genome/browse/#!/proteins/42353/461936%7CMycobacterium%20phage%20Cambiare/viral%20segment%20Unknown/) | 0 | 93.9 | 91.2 |

**(\*) Determined using VIRIDIC [3]**

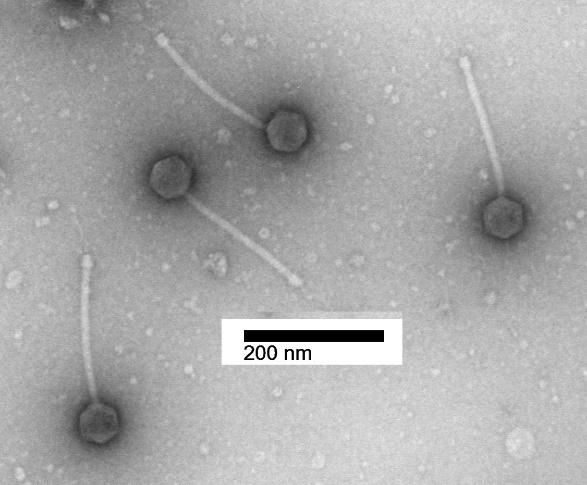
**(\*\*) Determined using CoreGenes 3.5 at** [**http://binf.gmu.edu:8080/CoreGenes3.5/**](http://binf.gmu.edu:8080/CoreGenes3.5/) **[4]**

**To create a new genus, *Antsirabevirus*,with a single species**

**Source of the name of this taxon:** This taxon is named directly from the first virus of its type, Mycobacterium phage Antsirabe

**History:** Temperate Mycobacterium phage Antsirabe was isolated in 2018 by Stephanie and Deborah Jacobs-Sera from Antisrabe, Madagascar soil using Mycobacterium smegmatis mc²155 as the host bacterium. This was part of the Phage Hunters Integrating Research and Education program. The genome possesses 10 nt 3’-cohesive termini (CCCGTGGCAT). The Actinobacteriophage Database places this phage in Cluster G, Subcluster G5.

**Electron micrograph:** Electron micrographs of negatively stained Mycobacterium phage Antsirabe (https://phagesdb.org/phages/Antsirabe/). 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.

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**Specific Reference:** None

**GenBank Summary:**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Phage name | INSDC | Size (Kb) | GC% | Protein | Overall DNA sequence identity (\*) | % common proteins (\*\*) |
| Mycobacterium phage Antsirabe | [MN234183.1](https://www.ncbi.nlm.nih.gov/nuccore/MN234183.1) | 44.74 | 69.0 | [66](https://www.ncbi.nlm.nih.gov/genome/browse/#!/proteins/84815/708372%7CMycobacterium%20phage%20Antsirabe/viral%20segment/) | 100 | 100 |

**(\*) Determined using VIRIDIC [3]**

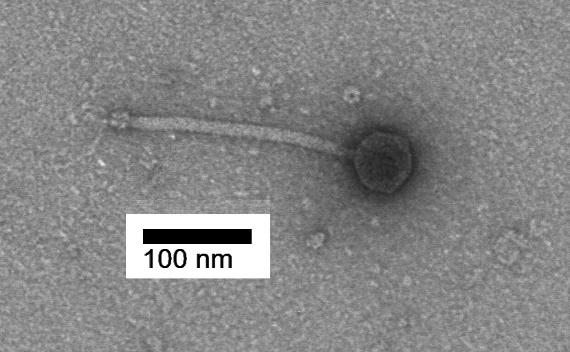
**(\*\*) Determined using CoreGenes 3.5 at** [**http://binf.gmu.edu:8080/CoreGenes3.5/**](http://binf.gmu.edu:8080/CoreGenes3.5/) **[4]**

**To create a new genus, *Jolieduovirus* with three (3) species**

**Source of the name of this taxon:** This taxon is named directly from the first virus of its type, Mycobacterium phage Jolie2

**History:** Temperate Mycobacterium phage Jolie2 was isolated in 2014 by Franceschelli,J.J., Suarez,C.A., Teran,L., Raya,R. (Universidad Nacional de Rosario, Argentina) from soil using Mycobacterium smegmatis mc²155 as the host bacterium. The Actinobacteriophage Database places this phage in Cluster G, Subcluster C4.

**Electron micrograph:** Electron micrographs of negatively stained Mycobacterium phage Mercurio (https://phagesdb.org/phages/Mercurio/). 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.



**Specific Reference:** Franceschelli JJ, Suarez CA, Terán L, Raya RR, Morbidoni HR. Complete genome sequences of nine mycobacteriophages. Genome Announc. 2014 May 29;2(3):e00181-14. doi: 10.1128/genomeA.00181-14. PMID: 24874666; PMCID: PMC4038871.

**GenBank Summary:**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Phage name | INSDC | Size (Kb) | GC% | Protein | tRNA | Overall DNA sequence identity (\*) | % common proteins (\*\*) |
| Mycobacterium phage Jolie2 | [KJ410133.1](https://www.ncbi.nlm.nih.gov/nuccore/KJ410133.1) | 44.31 | 68.1 | [62](https://www.ncbi.nlm.nih.gov/genome/browse/#!/proteins/24343/460447%7CMycobacterium%20phage%20Jolie2/viral%20segment%20Unknown/) | 0 | 100 | 100 |
| Mycobacterium phage Mercurio | [MN234219.1](https://www.ncbi.nlm.nih.gov/nuccore/MN234219.1) | 44.88 | 69.0 | [64](https://www.ncbi.nlm.nih.gov/genome/browse/#!/proteins/84890/708447%7CMycobacterium%20phage%20Mercurio/viral%20segment/) | 0 | 74.4 | 95.2 |
| Mycobacterium phage Lemuria | [MN234185.1](https://www.ncbi.nlm.nih.gov/nuccore/MN234185.1) | 45 | 68.8 | [65](https://www.ncbi.nlm.nih.gov/genome/browse/#!/proteins/84817/708374%7CMycobacterium%20phage%20Lemuria/viral%20segment/) | 0 | 80.3 | 93.5 |

**(\*) Determined using VIRIDIC [3]**

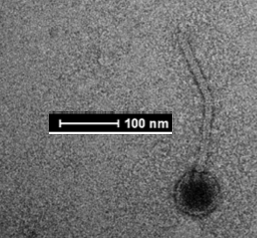
**(\*\*) Determined using CoreGenes 3.5 at** [**http://binf.gmu.edu:8080/CoreGenes3.5/**](http://binf.gmu.edu:8080/CoreGenes3.5/) **[4]**

**To create a new genus, *Pinnievirus* with two (2) species**

**Source of the name of this taxon:** This taxon is named directly from the second virus of its type, Mycobacterium phage Pinnie

**History:** Temperate Mycobacterium phage Pinnie was isolated in 2014 by Rachael Snodgrass and Emily Smith (Gonzaga University, Spokane, WA 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. The genome possesses 10 nt 3’-cohesive termini (CTCGTGGCAT). The Actinobacteriophage Database places this phage in Cluster G, Subcluster G3.

**Electron micrograph:** Electron micrographs of negatively stained Mycobacterium phage Pinnie (https://phagesdb.org/phages/Pinnie/). 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.

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**Specific Reference:** Pope WH, Augustine DA, Carroll DC, Duncan JC, Harwi KM, Howry R, Jagessar B, Lum BA, Meinert JW, Migliozzi JS, Milliken KA, Mitchell CJ, Nalatwad AS, Orlandini KC, Rhein MJ, Saravanan V, Seese BA, Schiebel JG, Thomas KB, Adkins NL, Cohen KL, Iyengar VB, Kim H, Kramer ZJ, Montgomery MT, Schafer CE, Wilkes KE, Grubb SR, Warner MH, Bowman CA, Russell DA, Hatfull GF. Genome Sequences of Cluster G Mycobacteriophages Cambiare, FlagStaff, and MOOREtheMARYer. Genome Announc. 2015 Jun 18;3(3):e00595-15. doi: 10.1128/genomeA.00595-15. PMID: 26089410; PMCID: PMC4472887.

**GenBank Summary:**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Phage name | INSDC | Size (Kb) | GC% | Protein | tRNA | Overall DNA sequence identity (\*) | % common proteins (\*\*) |
| Mycobacterium phage Pinnie | [MK494105.1](https://www.ncbi.nlm.nih.gov/nuccore/MK494105.1) | 44.84 | 68.8 | [66](https://www.ncbi.nlm.nih.gov/genome/browse/#!/proteins/78106/478559%7CMycobacterium%20phage%20Pinnie/viral%20segment/) | 0 | 100 | 100 |
| Mycobacterium phage MOOREtheMARYer | [KR080202.1](https://www.ncbi.nlm.nih.gov/nuccore/KR080202.1) | 44.49 | 68.6 | [67](https://www.ncbi.nlm.nih.gov/genome/browse/#!/proteins/42351/461934%7CMycobacterium%20phage%20MOOREtheMARYer/viral%20segment%20Unknown/) | 0 | 89.7 | 92.4 |

**(\*) Determined using VIRIDIC [3]**

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

**To create a new subfamily *Gclasvirinae***

**Source of the name of this taxon:** The names of most of the subfamilies of Actinobacteriophages are derived from the Cluster names. These phages all belong to Cluster G in the Actinobacteriophage Database (G CLuster Actinophage Siphovirus).

**Rationale:**

These five genera all fit within a single subfamily the members of which possess, on average, genomes of 43.76 kb (67.9 mol%G+C) and encode for 65 proteins and 0 tRNA. Using CoreGenes 5.0 (<https://coregenes.ngrok.io/>) revealed 33 homologs shared by this group of phages (51%). These homologs included: small and large terminase subunits, portal, capsid maturation protease, scaffolding protein, major capsid protein, head-to-tail adaptor, head-to-tail stopper, tail terminator, major tail protein, tail tape measure protein, two tail assembly chaperone, five minor tail proteins, holin, RecE-like exonuclease, RecT-like ssDNA binding protein and RuvC-like resolvase. At the DNA level these phages share at least 39.9% DNA similarity.

**References:**

1: 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.

2: 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

3: 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. <http://rhea.icbm.uni-oldenburg.de/VIRIDIC/>

4: 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.

5: 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.

6: 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.

7: Lowe, T.M. and Chan, P.P. (2016) tRNAscan-SE On-line: Search and Contextual Analysis of Transfer RNA Genes. Nucl. Acids Res. 44: W54-57.

8: Zimmermann L, Stephens A, Nam SZ, et al. A Completely Reimplemented MPI Bioinformatics Toolkit with a New HHpred Server at its Core. J Mol Biol. 2018;430(15):2237-2243. doi:10.1016/j.jmb.2017.12.007

9: Turner D, Kropinski AM, Adriaenssens EM. 2021. A Roadmap for Genome-Based Phage Taxonomy. Viruses 2021, 13, 506. https://doi.org/10.3390/v13030506