

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

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| **Code assigned:** | **2021.035B** |  |
| **Short title:** Create one new subfamily (*Gracegardnervirinae*)for Che8-like siphoviruses (*Caudoviricetes*) | | |
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**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**

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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 2:** **NON-TAXONOMIC PROPOSAL**

**Text of proposal**

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

**Name of accompanying Excel module**

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| 2021.035B.R.Gracegardnervirinae |

**Abstract**

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| The Che8-like Mycobacterium siphoviruses are one of the biggest groups of phages in the Actinobacteriophage Database currently numbering 183 isolates (<https://phagesdb.org/clusters/F/>).  The *Cheoctovirus* genus was established by Taxonomy Proposal 2013.008b with 28 species. These are temperate bacteriophages infectious for Mycobacterium smegmatis with are classified to Cluster F by the Actinobacteriophage Database (<https://phagesdb.org/clusters/F/>). Most are grouped in Subcluster F1. Our reassessment based upon overall DNA sequence similarity analysis (VIRIDIC) indicates that this is an extremely diverse group of phages with no clear boundaries, unlike that which we observe with lytic phages. Therefore those siphoviruses which possess at least 54% DNA sequence similarity were included in this taxon.  The Actinobacteriophage Database now recognizes five Subclusters of phages for this group of viruses and this Taxonomy Proposal brings their classification closer to that proposed in the Actinobacteriophage Database with the creation of five new genera, and one new subfamily *Gracegardnervirinae*. |

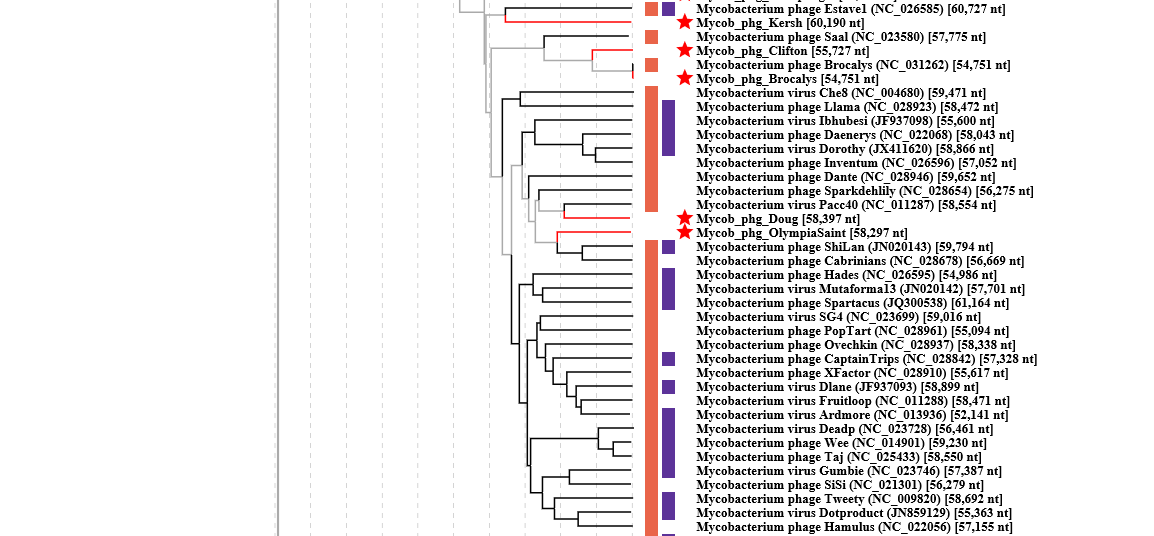
**Text of proposal**

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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.  **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 (in this case 54%) and that the genera form a clade in a marker tree phylogeny. [16] | |

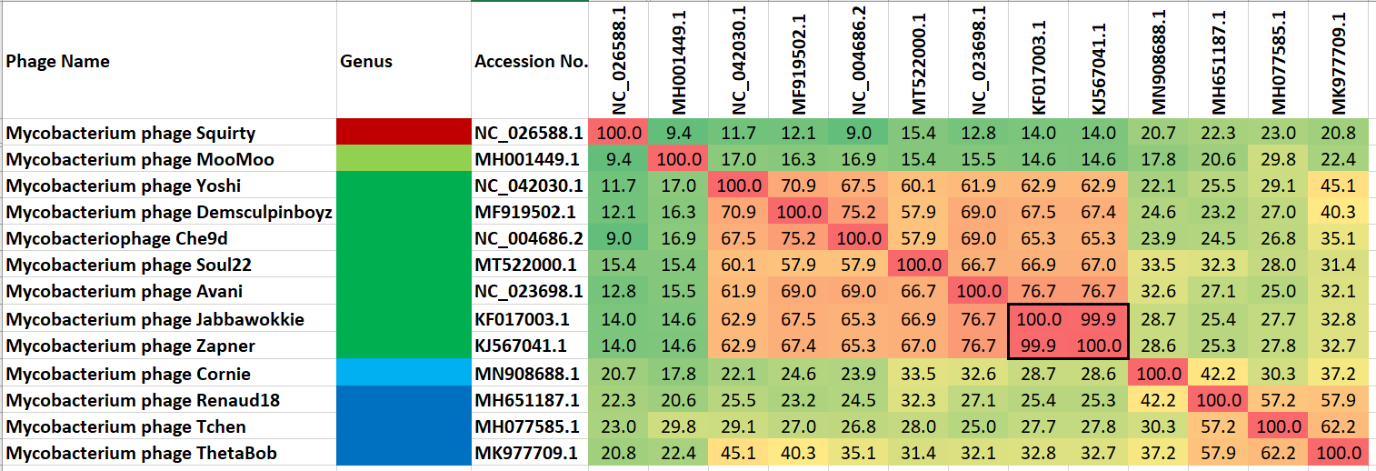
**Supporting evidence**

**Molecular analysis:**

**ViPTree analysis:** ViPTree analysis ([https://www.genome.jp/viptree/](about:blank); [1]) is based upon Rohwer and Edwards (2002) famous Phage Proteomic Tree [2]. Phages under consideration are marked with a **blue bar**.

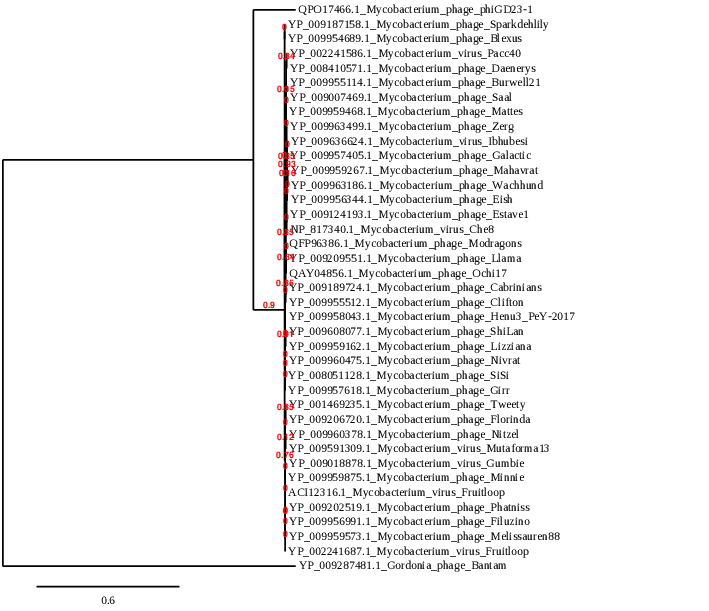
  
  


**VIRIDIC heat map:** VIRIDIC (Virus Intergenomic Distance Calculator; [3]; [http://rhea.icbm.uni-oldenburg.de/VIRIDIC/](about:blank)) computes pairwise intergenomic distances/similarities amongst phage genomes. The black box delineates strains. Because of the huge number of isolates the *Cheoctovirus* heat map is attached as a separate file. Two files are also attached one for *Cheoctovirus* and other for the other genera which make up this subfamily.

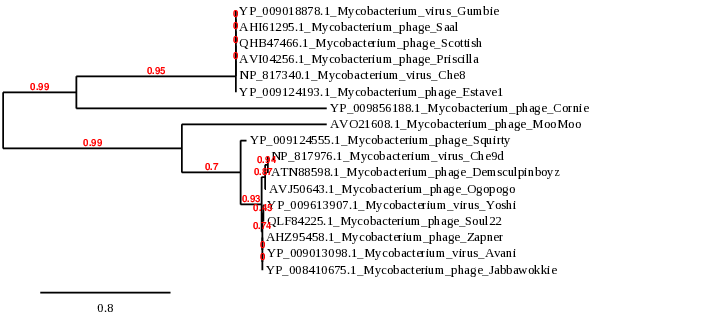
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**Phylogeny:** The phylogenetic tree was constructed using the terminase large subunit of some of these phages with phylogeny.fr in “one click” mode with Gordonia phage Bantam as outlier (A) [5]. "The "One Click mode" targets users that do not wish 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."

1. ***Cheoctovirus* group**

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1. **Remainder of the subfamily**



**Proposals:**

**Proposal A: To create a new genus *Moomoovirus***

**Proposal B: To create three (3) new species in the genus *Avanivirus***

**Proposal C: To create 79 new species within the genus *Cheoctovirus***

**Proposal D: To create a new subfamily *Gracegardnervirinae* containing these genera**

**Proposal A: To create a new genus *Moomoovirus***

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

**History:** Temperate Mycobacterium phage MooMoo was isolated from Sandy Hook, KY USA creek by Cole Blair (Western Kentucky University) as part of the Science Education Alliance-Phage Hunters Advancing Genomics and Evolutionary Science program in 2011. The host bacterium was Mycobacterium smegmatis mc²155. Its genome possess 9 nt 3'-cohesive termini (CCCGCCTGA). The Actinobacteriophage Database places this phage in Singleton (<https://phagesdb.org/phages/MooMoo/>).

**Specific Reference:** None

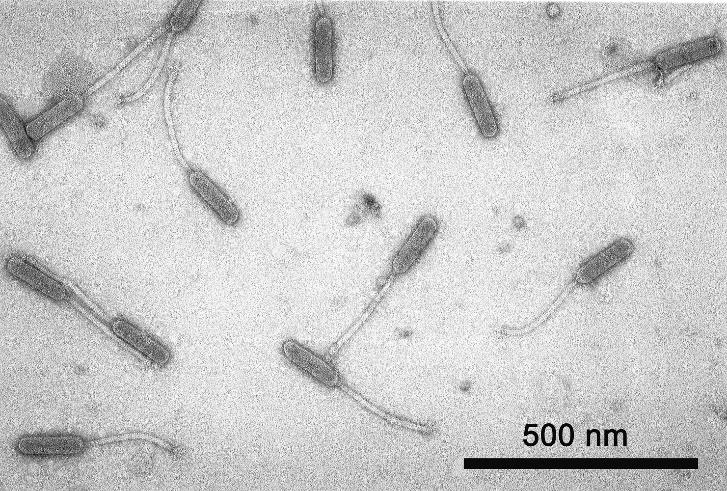
**GenBank Summary:**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Phage name | RefSeq No. | INSDC | Size (Kb) | GC% | Protein | Overall DNA sequence identity (\*) | % common proteins (\*\*) |
| Mycobacterium phage MooMoo |  | [MH001449.1](https://www.ncbi.nlm.nih.gov/nuccore/MH001449.1) | 55.18 | 62.0 | [97](https://www.ncbi.nlm.nih.gov/genome/browse/#!/proteins/68622/369771%7CMycobacterium%20phage%20MooMoo/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]**

**Electron micrograph:** Electron micrographs of negatively stained Mycobacterium phage MooMoo (https://phagesdb.org/phages/MooMoo/). 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. The authors of this proposal are concerned that the accompanying electron micrography does not represent phage MooMoo.

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**Proposal B: To create three new species in the genus *Avanivirus***

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

**History:** Temperate Mycobacterium phage Avani was isolated by Stacey Heaver (Gettysburg College, Gettysburg, PA USA) from soil as part of the Science Education Alliance-Phage Hunters Advancing Genomics and Evolutionary Science program in 2011. The host bacterium was Mycobacterium smegmatis mc²155. Its genome possess 10 nt 3'-cohesive termini (CTCAGGGCAT). The Actinobacteriophage Database places this phage in Cluster/Subcluster F/F2 (<https://phagesdb.org/phages/Yoshi/>). Phages Avani, Yoshi and Che9d were assigned to the *Che8likevirus* genus by Taxonomy Proposal 2013.008a-dB. New evidence suggest that they are sufficiently different to be part of a separate genus

**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.

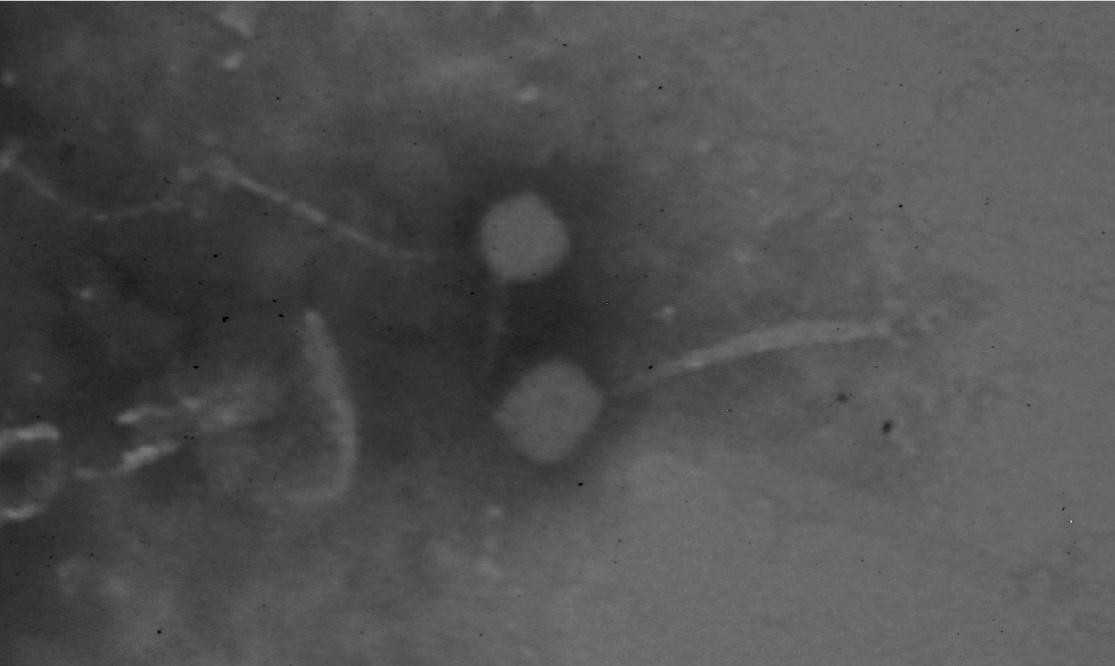
**GenBank Summary:**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Phage name | RefSeq No. | INSDC | Size (Kb) | GC% | Protein | Overall DNA sequence identity (\*) | % common proteins (\*\*) |
| Mycobacterium phage Avani | [NC\_023698.1](https://www.ncbi.nlm.nih.gov/nuccore/NC_023698.1) | [JQ809702.1](https://www.ncbi.nlm.nih.gov/nuccore/JQ809702.1) | 54.47 | 61.0 | [107](https://www.ncbi.nlm.nih.gov/genome/browse/#!/proteins/24530/460529%7CMycobacterium%20virus%20Avani/viral%20segment%20Unknown/) | 100 | 100 |
| Mycobacterium phage Demsculpinboyz |  | [MF919502.1](https://www.ncbi.nlm.nih.gov/nuccore/MF919502.1) | 57.44 | 60.9 | [117](https://www.ncbi.nlm.nih.gov/genome/browse/#!/proteins/64204/466611%7CMycobacterium%20phage%20Demsculpinboyz/viral%20segment/) | 69.0 | 74.9 |
| Mycobacterium phage Soul22 |  | [MT522000.1](https://www.ncbi.nlm.nih.gov/nuccore/MT522000.1) | 54.81 | 61.5 | [102](https://www.ncbi.nlm.nih.gov/genome/browse/#!/proteins/93306/927351%7CMycobacterium%20phage%20Soul22/viral%20segment/) | 66.7 | 67.3 |
| Mycobacterium phage Zapner |  | [KJ567041.1](https://www.ncbi.nlm.nih.gov/nuccore/KJ567041.1) | 55.31 | 61.1 | [108](https://www.ncbi.nlm.nih.gov/genome/browse/#!/proteins/69824/467368%7CMycobacterium%20phage%20Zapner/viral%20segment/) | 76.7 | 78.5 |

**(\*) 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]**

**Electron micrograph:** Electron micrographs of negatively stained Mycobacterium phage Avani (https://phagesdb.org/phages/Avani/). 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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**Proposal C: To create 79 new species in the genus *Cheoctovirus***

**Source of the name of this taxon:** This genus is named after the first virus of its type, Mycobacterium phage Che8

**History:** This taxon was established through Taxonomy Proposal 2013.008a-dB.

**Specific Reference:** Hatfull GF. The secret lives of mycobacteriophages. Adv Virus Res. 2012;82:179-288. doi: 10.1016/B978-0-12-394621-8.00015-7. PMID: 22420855.

Pham TT, Jacobs-Sera D, Pedulla ML, Hendrix RW, Hatfull GF. Comparative genomic analysis of mycobacteriophage Tweety: evolutionary insights and construction of compatible site-specific integration vectors for mycobacteria. Microbiology (Reading). 2007 Aug;153(Pt 8):2711-2723. doi: 10.1099/mic.0.2007/008904-0. PMID: 17660435; PMCID: PMC2884959.

**GenBank Summary:**

|  |  |  |
| --- | --- | --- |
| **Phage Name** | **Accession No.** | **% DNA sequence identity (\*)** |
| Mycobacterium phage Che8 | AY129330.1 | 100 |
| Mycobacterium phage ArcusAngelus | MH744415.1 | 62.1 |
| Mycobacterium phage Batiatus | MH020235.1 | 54.6 |
| Mycobacterium phage Blexus | MN586012.1 | 62.6 |
| Mycobacterium phage BobaPhett | MH155865.1 | 53.7 |
| Mycobacterium phage BodEinwohner17 | MN945900.1 | 63.3 |
| Mycobacterium phage Bubbles123 | KY348865.1 | 63.8 |
| Mycobacterium phage Burwell21 | MH651169.1 | 56.6 |
| Mycobacterium phage BuzzLyseyear | KM347889.1 | 55.4 |
| Mycobacterium phage Byougenkin | MH155866.1 | 60.7 |
| Mycobacterium phage CaptainTrips | KM652553.1 | 59.2 |
| Mycobacterium phage Chuckly | MN585995.1 | 58.3 |
| Mycobacterium phage Dante | KT309034.1 | 59.4 |
| Mycobacterium phage DillTech15 | MH077578.1 | 58.1 |
| Mycobacterium phage Donkeykong | MN586001.1 | 58.5 |
| Mycobacterium phage Doug | MN310544.1 | 67.1 |
| Mycobacterium phage DRBy19 | MT310893.1 | 58.5 |
| Mycobacterium phage Eish | MN585975.1 | 64.0 |
| Mycobacterium phage EleanorGeorge | MH669001.1 | 58.3 |
| Mycobacterium phage Emma | MF668270.1 | 62.5 |
| Mycobacterium phage Empress | KY012363.1 | 61.1 |
| Mycobacterium phage Estave1 | KM279937.1 | 54.5 |
| Mycobacterium phage Fancypants | MK524503.1 | 69.2 |
| Mycobacterium phage Filuzino | MK016494.1 | 54.2 |
| Mycobacterium phage Firehouse51 | MT889374.1 | 59.8 |
| Mycobacterium phage Florinda | KR997930.1 | 58.3 |
| Mycobacterium phage Galactic | MH727548.1 | 61.8 |
| Mycobacterium phage Gandalph | MT684586.1 | 63.9 |
| Mycobacterium phage Geralt | MF668271.1 | 62.0 |
| Mycobacterium phage Girr | MH669003.1 | 61.8 |
| Mycobacterium phage Gorge | MH590602.1 | 56.9 |
| Mycobacterium phage Hades | KM101122.1 | 62.1 |
| Mycobacterium phage Hamulus | KF024723.1 | 57.1 |
| Mycobacterium phage Harley | MH632119.1 | 66.3 |
| Mycobacterium phage Hlubikazi | MT684601.1 | 58.5 |
| Mycobacterium phage Inventum | KM066034.1 | 61.9 |
| Mycobacterium phage JoeyJr | MH669005.1 | 60.5 |
| Mycobacterium phage Kersh | KX610764.1 | 58.9 |
| Mycobacterium phage Kimberlium | KR935214.1 | 54.7 |
| Mycobacterium phage Krakatau | MH590598.1 | 60.5 |
| Mycobacterium phage KristaRAM | MH651178.1 | 56.7 |
| Mycobacterium phage LilMoolah | MN586011.1 | 62.6 |
| Mycobacterium phage Lizziana | MH834617.1 | 59.3 |
| Mycobacterium phage Mahavrat | MK967397.1 | 68.1 |
| Mycobacterium phage Mantra | MH590596.1 | 66.3 |
| Mycobacterium phage Mattes | MH155871.1 | 57.7 |
| Mycobacterium phage Melissauren88 | MH077580.1 | 57.4 |
| Mycobacterium phage MilleniumForce | MH825707.1 | 62.3 |
| Mycobacterium phage MinionDave | MN369749.1 | 60.8 |
| Mycobacterium phage Minnie | MN369755.1 | 56.9 |
| Mycobacterium phage Moonbeam | MT684593.1 | 62.7 |
| Mycobacterium phage Nitzel | MN310545.1 | 65.7 |
| Mycobacterium phage Nivrat | MH651183.1 | 62.7 |
| Mycobacterium phage Ochi17 | MK359307.1 | 67.0 |
| Mycobacterium phage OldBen | MG770213.1 | 61.5 |
| Mycobacterium phage OlympiaSaint | MH371120.1 | 63.7 |
| Mycobacterium phage Ovechkin | KR824843.1 | 58.8 |
| Mycobacterium phage OwlsT2W | MH051257.1 | 54.1 |
| Mycobacterium phage Phasih | MF919526.1 | 67.4 |
| Mycobacterium phage Phatniss | KT279576.1 | 63.1 |
| Mycobacterium phage Plumbus | MW055915.1 | 56.8 |
| Mycobacterium phage Poenanya | MK305886.1 | 60.0 |
| Mycobacterium phage Polka14 | MK875792.1 | 61.0 |
| Mycobacterium phage PopTart | KT281792.1 | 59.5 |
| Mycobacterium phage Priscilla | MG872841.1 | 59.6 |
| Mycobacterium phage QuickMath | MH669012.1 | 64.9 |
| Mycobacterium phage RitaG | MF668281.1 | 60.1 |
| Mycobacterium phage Royals2015 | MT897904.1 | 66.3 |
| Mycobacterium phage Saal | KJ025956.1 | 58.5 |
| Mycobacterium phage Sandalphon | MH371123.1 | 59.5 |
| Mycobacterium phage SiSi | KC661278.1 | 59.7 |
| Mycobacterium phage Sparkdehlily | KT895280.1 | 66.1 |
| Mycobacterium phage Spikelee | MH669014.1 | 55.5 |
| Mycobacterium phage Spoonbill | MF190168.1 | 60.3 |
| Mycobacterium phage SuperGrey | KX808131.1 | 57.4 |
| Mycobacterium phage SwagPigglett | MK494110.1 | 54.0 |
| Mycobacterium phage Veteran | MT114164.1 | 59.9 |
| Mycobacterium phage Wachhund | MF668287.1 | 65.2 |
| Mycobacterium phage Whouxphf | MK016504.1 | 62.6 |
| Mycobacterium phage WillSterrel | KX576644.1 | 61.7 |
| Mycobacterium phage XFactor | KT281795.1 | 55.4 |
| Mycobacterium phage Zerg | MK112556.1 | 60.3 |

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

**Electron micrograph:** N/A

**Proposal D: To create a new subfamily *Gracegardnervirinae* containing these genera, and *Squirtyvirus, Cornievirus* and *Thetabobvirus*.**

**Source of the name of this taxon:** This genus is in honour of Grace M. Gardner (1924-2007) who together with her mentor Russell S. Weiser (1906-2000) isolated the first phages against Mycobacterium smegmatis in 1947 in the Department of Microbiology, School of Medicine, University of Washington, Seattle (USA).

**Evidence:** Phylogenetic, genomic (VIRIDIC) and proteomic (ViPTree) analysis reveals that all these phages belong to a single group.

**References**

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