

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

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| **Code assigned:** | **2021.060B** |  |
| **Short title:** Create one new family (*Orlajensenviridae*) including three new genera (*Caudoviricetes*) | | |
|  | | |

**Author(s) and email address(es)**

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**Corresponding author**

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| Andrew 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**

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

**Text of proposal**

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|  |

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

**Name of accompanying Excel module**

|  |
| --- |
| 2021.060B.R.Orlajensenviridae |

**Abstract**

|  |
| --- |
| This proposal formalizes the classification of the EE Cluster Microbacterium phages in the Actinobacteriophage Database. The ViPTree analyses reveal that this group of viruses have an extremely deep root, leading us to propose a new family. These three genera all fit within a single subfamily the members of which possess, on average, genomes of 17.43 kb (68.43 mol%G+C) and encode for 26 proteins and 0 tRNA. Using CoreGenes 5.0 (https://coregenes.ngrok.io/) revealed 20 homologs shared by this group of phages (76.9%). These homologs included: large subunit terminase, portal, major capsid and protease fusion protein, head-to-tail adaptor, tail terminator, major tail protein, two tail assembly chaperone, tape measure protein, two minor tail proteins and three DNA-binding proteins.  Based upon the ViPTree analysis one might consider adding Microbacterium phage Min1 but its genome size is almost three-times that of the phages described here. |

**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 (current isolates > 50%) and that the genera form a clade in a marker tree phylogeny.  **Family demarcation criteria: -** The family is represented by a cohesive and monophyletic group in the main predicted proteome-based clustering tools (VipTree, GRAViTy, vConTACT2). Members of the family share a significant number of orthologous genes (more than 10% of the genome).  (Taken from: Turner D et al. [9]) | |

**Supporting evidence**

**Proposals:**

**1.To create a new genus *Paopuvirus* with nine (9) species**

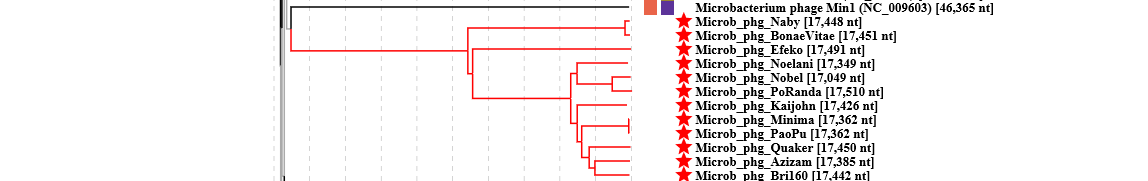
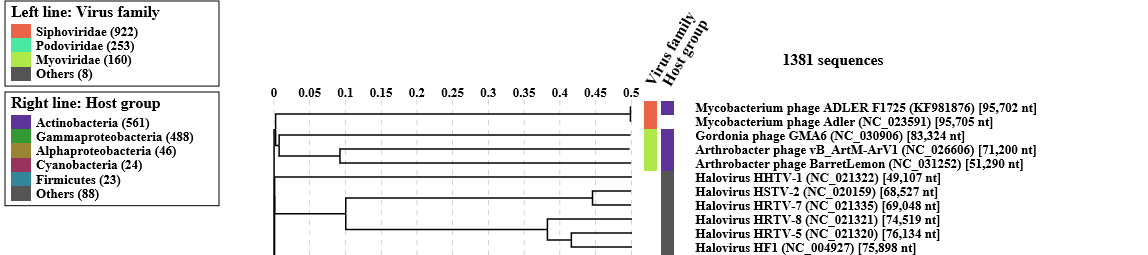
**2. To create a new genus *Bonaevitaevirus* with one (1) species**

**3. To create a new genus *Efekovirus* with one (1) species**

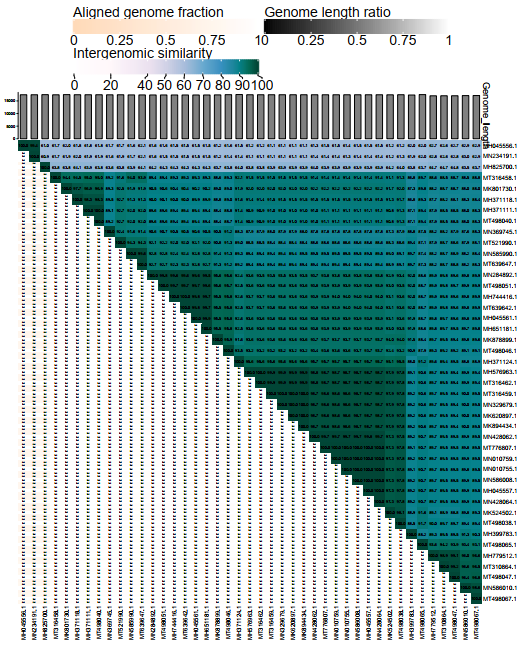
**4. To assign these three genera to a subfamily *Pelczarvirinae***

**5. To create a new family *Orlajensenviridae* for these phages**

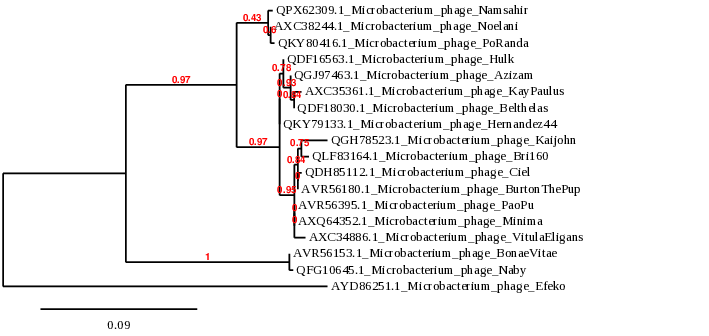
**ViPTree analysis:** ViPTree analysis ([https://www.genome.jp/viptree/](about:blank); [1]) is based upon Rohwer and Edwards (2002) famous Phage Proteomic Tree [2]. The phages are marked with **red stars**.



**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.

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**Phylogeny:** The phylogenetic tree was constructed using the terminase large subunit of these 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."



**1.To create a new genus *Paopuvirus* with nine (9) species**

**Source of the name of this taxon:** The name of this taxon is directly derived from that of one of the earliest phages of its type to be isolated, Microbacterium phage PaoPu.

**History:** Lytic siphovirus PaoPu was isolated in 2016 by Acacia Eleri from Germantown MD USA soil using Microbacterium foliorum NRRL B-24224 as the host bacterium. This was part of the Science Education Alliance-Phage Hunters Advancing Genomics and Evolutionary Science program. Its genome possesses 9 nt 3’-cohesive termini (CCCGCCCCA). It is classified into Cluster EE in the Actinobacteriophage Database <https://phagesdb.org/phages/PaoPu/>.

**Specific Reference:** Jacobs-Sera D, Abad LA, Alvey RM, Anders KR, Aull HG, Bhalla SS, Blumer LS, Bollivar DW, Bonilla JA, Butela KA, Coomans RJ, Cresawn SG, D'Elia T, Diaz A, Divens AM, Edgington NP, Frederick GD, Gainey MD, Garlena RA, Grant KW, Gurney SMR, Hendrickson HL, Hughes LE, Kenna MA, Klyczek KK, Kotturi H, Mavrich TN, McKinney AL, Merkhofer EC, Moberg Parker J, Molloy SD, Monti DL, Pape-Zambito DA, Pollenz RS, Pope WH, Reyna NS, Rinehart CA, Russell DA, Shaffer CD, Sivanathan V, Stoner TH, Stukey J, Sunnen CN, Tolsma SS, Tsourkas PK, Wallen JR, Ware VC, Warner MH, Washington JM, Westover KM, Whitefleet-Smith JL, Wiersma-Koch HI, Williams DC, Zack KM, Hatfull GF. Genomic diversity of bacteriophages infecting Microbacterium spp. PLoS One. 2020 Jun 18;15(6):e0234636. doi: 10.1371/journal.pone.0234636. PMID: 32555720; PMCID: PMC7302621.

**GenBank Summary:**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Phage name | INSDC | Size (Kb) | GC% | Protein | Overall DNA sequence identity (\*) | % common proteins (\*\*) |
| Microbacterium phage PaoPu | [MH045561.1](https://www.ncbi.nlm.nih.gov/nuccore/MH045561.1) | 17.36 | 68.5 | [25](https://www.ncbi.nlm.nih.gov/genome/browse/#!/proteins/69017/373001%7CMicrobacterium%20phage%20PaoPu/viral%20segment/) | 100 | 100 |
| Microbacterium phage Azizam | [MN585990.1](https://www.ncbi.nlm.nih.gov/nuccore/MN585990.1) | 17.39 | 68.7 | [25](https://www.ncbi.nlm.nih.gov/genome/browse/#!/proteins/85765/744425%7CMicrobacterium%20phage%20Azizam/viral%20segment/) | 92.8 | 100 |
| Microbacterium phage Bri160 | [MT521990.1](https://www.ncbi.nlm.nih.gov/nuccore/MT521990.1) | 17.44 | 68.6 | [25](https://www.ncbi.nlm.nih.gov/genome/browse/#!/proteins/93300/927345%7CMicrobacterium%20phage%20Bri160/viral%20segment/) | 92.1 | 100 |
| Microbacterium phage Kaijohn | [MN369745.1](https://www.ncbi.nlm.nih.gov/nuccore/MN369745.1) | 17.43 | 68.8 | [26](https://www.ncbi.nlm.nih.gov/genome/browse/#!/proteins/85562/743721%7CMicrobacterium%20phage%20Kaijohn/viral%20segment/) | 90.6 | 100 |
| Microbacterium phage Nobel | [MT310864.1](https://www.ncbi.nlm.nih.gov/nuccore/MT310864.1) | 17.05 | 69.0 | [25](https://www.ncbi.nlm.nih.gov/genome/browse/#!/proteins/89534/893396%7CMicrobacterium%20phage%20Nobel/viral%20segment/) | 89.7 | 100 |
| Microbacterium phage Noelani | [MH399783.1](https://www.ncbi.nlm.nih.gov/nuccore/MH399783.1) | 17.35 | 68.2 | [25](https://www.ncbi.nlm.nih.gov/genome/browse/#!/proteins/71221/390997%7CMicrobacterium%20phage%20Noelani/viral%20segment/) | 91.9 | 100 |
| Microbacterium phage PoRanda | [MT498065.1](https://www.ncbi.nlm.nih.gov/nuccore/MT498065.1) | 17.51 | 69.1 | [25](https://www.ncbi.nlm.nih.gov/genome/browse/#!/proteins/92666/916855%7CMicrobacterium%20phage%20PoRanda/viral%20segment/) | 88.7 | 100 |
| Microbacterium phage Quaker | [MH371111.1](https://www.ncbi.nlm.nih.gov/nuccore/MH371111.1) | 17.45 | 68.6 | [25](https://www.ncbi.nlm.nih.gov/genome/browse/#!/proteins/71203/390979%7CMicrobacterium%20phage%20Quaker/viral%20segment/) | 89.4 | 100 |
| Microbacterium phage Scamander | [MH576963.1](https://www.ncbi.nlm.nih.gov/nuccore/MH576963.1) | 17.45 | 68.7 | [25](https://www.ncbi.nlm.nih.gov/genome/browse/#!/proteins/71957/399829%7CMicrobacterium%20phage%20Scamander/viral%20segment/) | 93.6 | 100 |

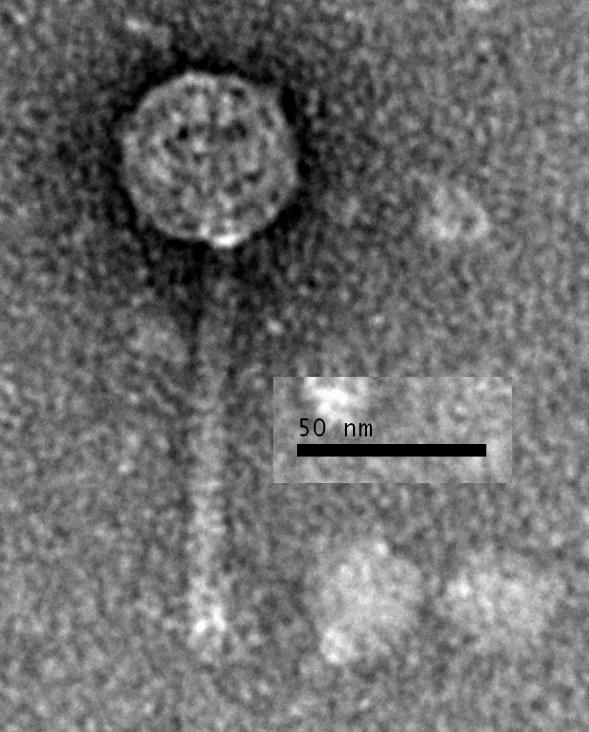
**(\*) Determined using VIRIDIC [3]**

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

**Strains:**

|  |  |  |
| --- | --- | --- |
| **Phage name** | **Accession No.** | **Strain of:** |
| Microbacterium phage Belthelas | MK801730.1 | *Paopuvirus Quaker* |
| Microbacterium phage Bradley2 | MN284892.1 | *Paopuvirus* *PaoPu* |
| Microbacterium phage Bullzi2019 | MT498067.1 | *Paopuvirus* *Nobel* |
| Microbacterium phage BurtonThePup | MH045557.1 | *Paopuvirus* *Scamander* |
| Microbacterium phage Ciel | MN010759.1 | *Paopuvirus* *Scamander* |
| Microbacterium phage Danno | MT316462.1 | *Paopuvirus* *Scamander* |
| Microbacterium phage Dongwon | MH744416.1 | *Paopuvirus* *PaoPu* |
| Microbacterium phage HarperAnne | MN586010.1 | *Paopuvirus* *Nobel* |
| Microbacterium phage Hernandez44 | MT498046.1 | *Paopuvirus* *PaoPu* |
| Microbacterium phage Hulk | MK878899.1 | *Paopuvirus* *PaoPu* |
| Microbacterium phage Jahseh | MT498051.1 | *Paopuvirus* *PaoPu* |
| Microbacterium phage JRok | MT639647.1 | *Paopuvirus* *Azizam* |
| Microbacterium phage KayPaulus | MH371118.1 | *Paopuvirus* *Quaker* |
| Microbacterium phage LaviMo | MK894434.1 | *Paopuvirus* *Scamander* |
| Microbacterium phage Livingwater | MT498040.1 | *Paopuvirus Quaker* |
| Microbacterium phage Luxx | MT498038.1 | *Paopuvirus* *Scamander* |
| Microbacterium phage Majesty | MT776807.1 | *Paopuvirus* *Scamander* |
| Microbacterium phage McShie | MT316458.1 | *Paopuvirus* *Quaker* |
| Microbacterium phage Miaurora | MH779512.1 | *Paopuvirus* *Nobel* |
| Microbacterium phage Minima | MH65118.1 | *Paopuvirus* *PaoPu* |
| Microbacterium phage Otwor | MT316459.1 | *Paopuvirus* *Scamander* |
| Microbacterium phage Owens | MN428064.1 | *Paopuvirus* *Scamander* |
| Microbacterium phage Rhysand | MK620897.1 | *Paopuvirus* *Scamander* |
| Microbacterium phage Scrunchy | MN586008.1 | *Paopuvirus* *Scamander* |
| Microbacterium phage Slentz | MN010755.1 | *Paopuvirus* *Scamander* |
| Microbacterium phage TeddyBoy | MN428062.1 | *Paopuvirus* *Scamander* |
| Microbacterium phage TimoTea | MK524502.1 | *Paopuvirus* *Scamander* |
| Microbacterium phage TinyTruffula | MT498047.1 | *Paopuvirus* *Nobel* |
| Microbacterium phage Vanisius | MN329679.1 | *Paopuvirus* *Scamander* |
| Microbacterium phage VitulaEligans | MH371124.1 | *Paopuvirus* *Scamander* |
| Microbacterium phage YertPhresh | MT639642.1 | *Paopuvirus* *PaoPu* |

**Electron micrograph:** None available for PaoPu.Electron micrographs of negatively stained Microbacterium phage Minima (https://phagesdb.org/phages/Minima/). 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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**2. To create a new genus *Bonaevitaevirus* with one (1) species**

**Source of the name of this taxon:** The name of this taxon is directly derived from that of one of the earliest phages of its type to be isolated, Microbacterium phage BonaeVitae.

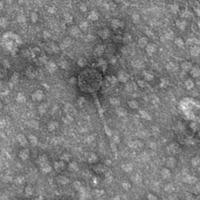
**History:** Lytic siphovirus BonaeVitae was isolated in 2015 by Dan Novinski (Nebraska Wesleyan University) from Grand Island, NE USA creek mud using Microbacterium paraoxydans NWU1 as the host bacterium. This was part of the Science Education Alliance-Phage Hunters Advancing Genomics and Evolutionary Science program. Its genome possesses 9 nt 3’-cohesive termini (CCCGCCCCA). It is classified into Cluster EE in the Actinobacteriophage Database <https://phagesdb.org/phages/BonaeVitae/>. Our proteomic, genomic and phylogenetic analyses indicate that it is sufficiently different to be part of a new genus.

**GenBank Summary:**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Phage name | INSDC | Size (Kb) | GC% | Protein |
| Microbacterium phage BonaeVitae | [MH045556.1](https://www.ncbi.nlm.nih.gov/nuccore/MH045556.1) | 17.45 | 68.2 | [26](https://www.ncbi.nlm.nih.gov/genome/browse/#!/proteins/69012/372996%7CMicrobacterium%20phage%20BonaeVitae/viral%20segment/) |
|  |  |  |  |  |

**Strain:** Microbacterium phage Naby (MN234191) strain of *Bonaevitaevirus BonaeVitae*

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



**3. To create a new genus *Efekovirus* with one (1) species**

**Source of the name of this taxon:** The name of this taxon is directly derived from that of one of the earliest phages of its type to be isolated, Microbacterium phage Efeko.

**History:** Lytic siphovirus Efeko was isolated in 2017 by Brenna Franke (University of Wisconsin-River Falls) from Hudson, WI USA compost using Microbacterium paraoxydans NRRL B-14843 as the host bacterium. This was part of the Science Education Alliance-Phage Hunters Advancing Genomics and Evolutionary Science program. Its genome possesses 9 nt 3’-cohesive termini (CCCGCCCCA). It is classified into Cluster EE in the Actinobacteriophage Database <https://phagesdb.org/phages/Efeko/>. Our proteomic, genomic and phylogenetic analyses indicate that it is sufficiently different to be part of a new genus.

**GenBank Summary:**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Phage name | INSDC | Size (Kb) | GC% | Protein |
| Microbacterium phage Efeko | [MH825700.1](https://www.ncbi.nlm.nih.gov/nuccore/MH825700.1) | 17.49 | 68.6 | [28](https://www.ncbi.nlm.nih.gov/genome/browse/#!/proteins/72776/409397%7CMicrobacterium%20phage%20Efeko/viral%20segment/) |
|  |  |  |  |  |

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



**4. To assign these three genera to a subfamily *Pelczarvirinae***

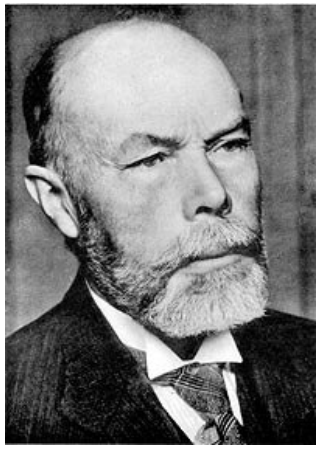
**Source of the name of this taxon:** This taxon is named in honour of Michael Joseph Pelczar, Jr. (b. 1916, Baltimore (MD) d. 2009 Kent Island (MD)) American microbiologist, educator, and university administrator. Ph.D., University of Iowa, Iowa City, 1941. University of Maryland; president, 1978-84. American Society of Microbiology, chairman - Governor's Scientific Advisory Board, 1970-93. Author/co-author, *Microbiology*, 5 editions. He worked on the nutritional requirements of Microbacterium in the 1940s.



**Rationale:** The genomic comparison of these three genera reveal that while they are distinguishable they are closely related.

**5. To create a new family *Orlajensenviridae* for these phages**

**Source of the name of this taxon:** This taxon is named in honour of Danish chemist and dairy bacteriologist Professor, Dr.phi. et scient., Sigurd Orla-Jensen (1870-1949). “In 1904 his doctoral thesis was published, on the types and origin of volatile fatty acids in cheese. This paper so much increased the interest in Orla-Jensen's work that in 1906 he was called to occupy a special chair at the Technical University of Denmark, and in 1908 he was appointed professor of fermentation physiology and agricultural chemistry, later termed bio-technical chemistry.” He held this position for 40 years. His seminal work was *The Lactic Acid Bacteria* (1919). (OLSEN E. S. Orla-Jensen (1870-1949). J Gen Microbiol. 1950 May;4(2):107-9. doi: 10.1099/00221287-4-2-107. PMID: 15415563.). He is responsible for the isolation and taxonomy of the genus Microbacterium.



**Rationale:** The ViPTree analyses reveal that this group of viruses have an extremely deep root, leading us to propose a new family. These three genera all fit within a single subfamily the members of which possess, on average, genomes of 17.43 kb (68.43 mol%G+C) and encode for 26 proteins and 0 tRNA. Using CoreGenes 5.0 (<https://coregenes.ngrok.io/>) revealed 20 homologs shared by this group of phages (76.9%). These homologs included: large subunit terminase, portal, major capsid and protease fusion protein, head-to-tail adaptor, tail terminator, major tail protein, two tail assembly chaperone, tape measure protein, two minor tail proteins and three DNA-binding proteins.

Based upon the ViPTree analysis one might consider adding Microbacterium phage Min1 but its genome size is almost three-times that of the phages described here.

**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.

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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