

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

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| **Code assigned:** | **2021.080B** |  |
| **Short title:** Create one new subfamily (*Stephanstirmvirinae*) including two genera and 14 new species (*Caudoviricetes*) | | |
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**Author(s) and email address(es)**

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| Andrew M. Kropinski |

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

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| Caudovirales 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)** | Y |

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| --- | --- | --- |
| **Taxon name** | **Person from whom the name is derived** | **Permission attached (Y/N)** |
| Stephanstirmvirinae | Stephan Stirm | Y |
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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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| 2021.080B.R.Stephanstirmvirinae |

**Abstract**

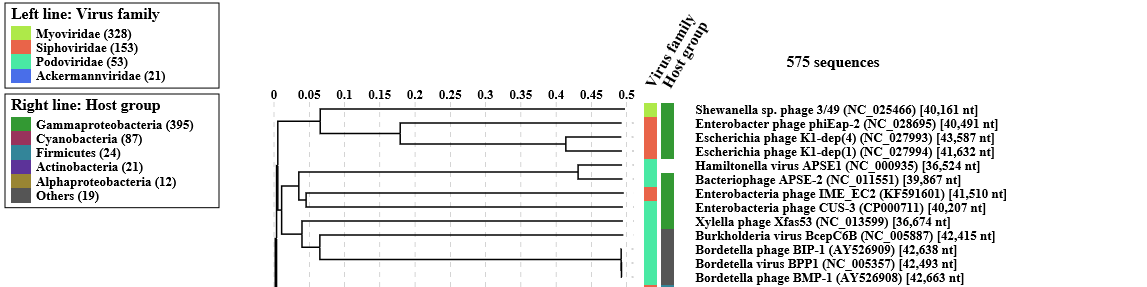
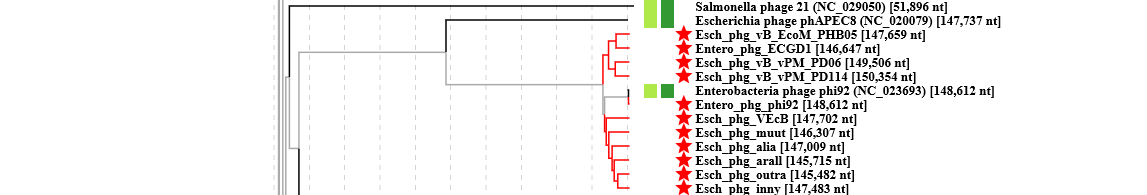
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| Here we create a new subfamily, *Stephanstirmvirinae*, comprising two genera, extracted as a monophyletic group from the order *Caudovirales* based on their genome content and phylogeny of the terminase large subunit. This subfamily is peripherally related to the *Vequintavirinae* suggesting that a new family or order should be proposed in the future. |

**Text of proposal**

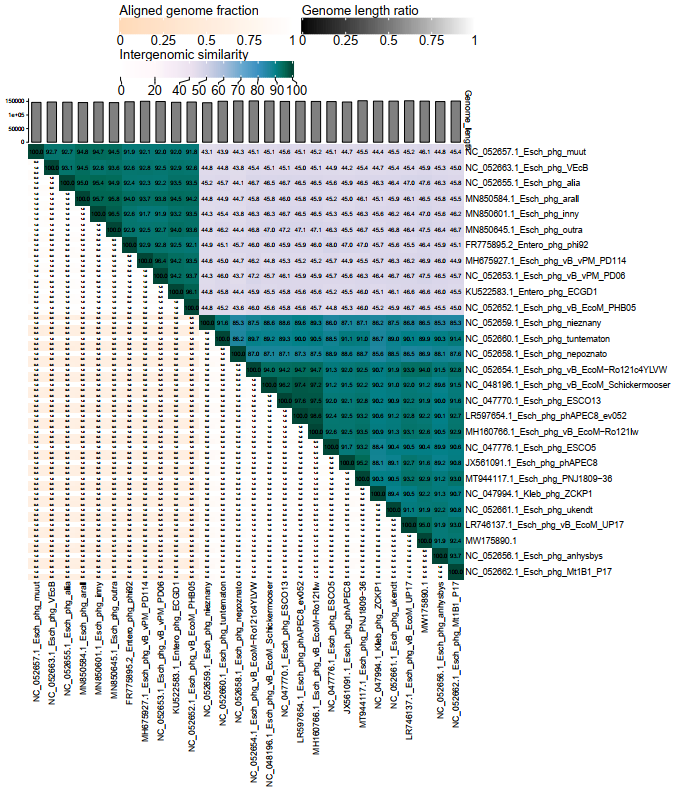
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| --- | --- |
| |  | | --- | | **Species demarcation criteria:** We have chosen 95% DNA sequence identity as the criterion for demarcation of species in this new genus. Each of the proposed species differs from the others with more than 5% at the DNA level as confirmed with the BLASTN algorithm  **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 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 and that the genera form a clade in a marker tree phylogeny.  (Taken from: Turner D et al. [9]) | |

**Supporting evidence**

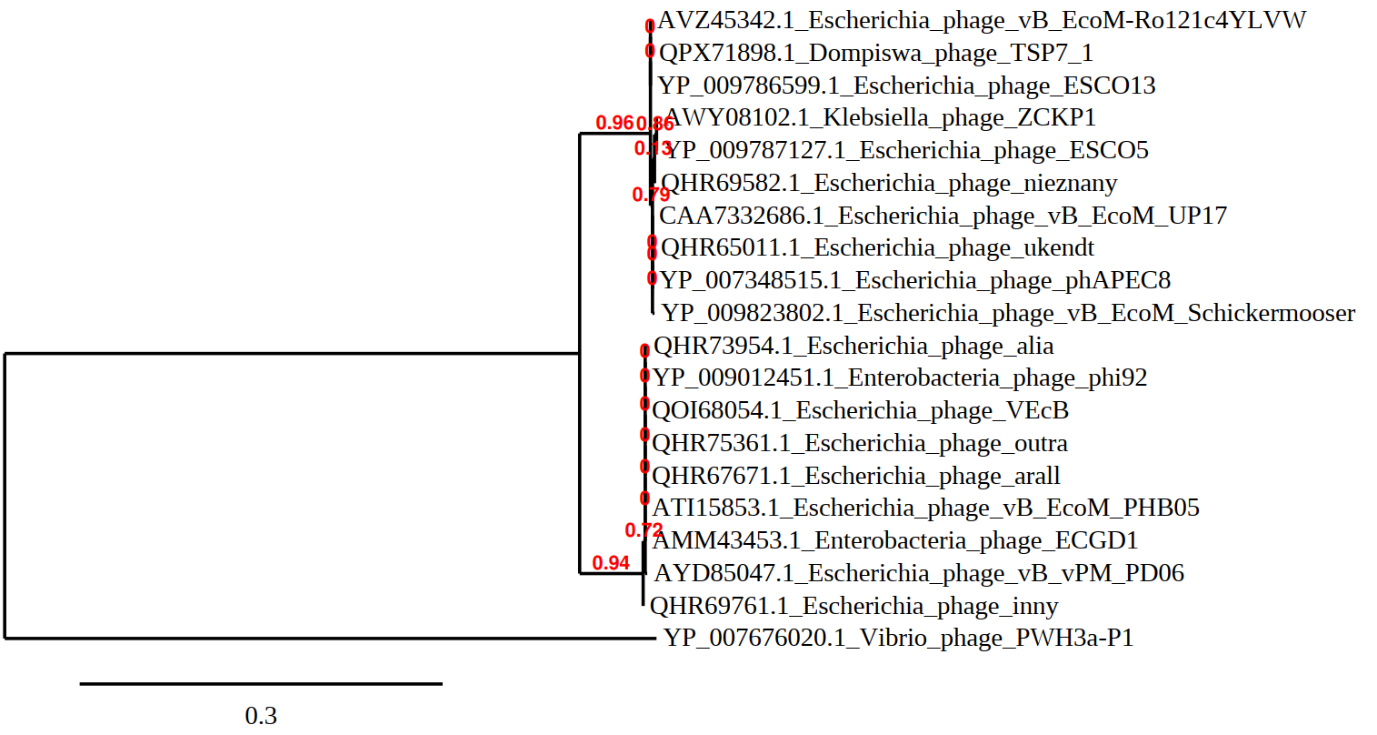
**ViPTree analysis:** ViPTree analysis ([https://www.genome.jp/viptree/](about:blank); [1]) is based upon Rohwer and Edwards (2002) famous Phage Proteomic Tree [2]. Some of the members of this subfamily are noted in **red**.

**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.

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**Phylogeny:** The phylogenetic tree was constructed using the terminase large subunit of related 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 create a new genus, *Justusliebigvirus* with six (6) species**

**2. To add eight (8) new species to the genus *Phapecoctavirus***

**3. To create a new subfamily, *Stephanstirmvirinae*, with these genera**

**Source of the name of this taxon:** This genus is named after the university in Germany (Justus-Liebig-Universität Giessen) where in the Institut für Medizinische Virologie the first phage of its type, Enterobacteria phage phi92, was isolated.

**History:** Bacteriophage phi92 is a large, lytic myovirus isolated in 1983 from pathogenic Escherichia coli strains that carry a polysialic acid capsule. The GenBank record for this phage indicates 343 bp direct terminal repeats. It has been pointed out [Schwarzer et al. 2012] that phage phi92 is related to Escherichia phage rV5 (DQ832317.1, *Vequintavirinae*) at the protein level. Indeed CoreGenes 3.5 analysis reveals that these two phages share 25.6% homologous proteins.

**Specific Reference:** Kwiatkowski B, Boschek B, Thiele H, Stirm S. Substrate specificity of two bacteriophage-associated endo-N-acetylneuraminidases. J Virol. 1983 Jan;45(1):367-74. doi: 10.1128/JVI.45.1.367-374.1983. PMID: 6401818; PMCID: PMC256418.

Schwarzer D, Buettner FF, Browning C, Nazarov S, Rabsch W, Bethe A, Oberbeck A, Bowman VD, Stummeyer K, Mühlenhoff M, Leiman PG, Gerardy-Schahn R. A multivalent adsorption apparatus explains the broad host range of phage phi92: a comprehensive genomic and structural analysis. J Virol. 2012 Oct;86(19):10384-98. doi: 10.1128/JVI.00801-12. Epub 2012 Jul 11. PMID: 22787233; PMCID: PMC3457257.

Olsen NS, Forero-Junco L, Kot W, Hansen LH. Exploring the Remarkable Diversity of Culturable Escherichia coli Phages in the Danish Wastewater Environment. Viruses. 2020 Sep 4;12(9):986. doi: 10.3390/v12090986. PMID: 32899836; PMCID: PMC7552041. **[muut, alia]**

**GenBank Summary:**

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| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Phage name | RefSeq No. | INSDC | Size (Kb) | GC% | Protein | t  RNA | Overall DNA sequence identity (\*) | % common proteins (\*\*) |
| Escherichia phage phi92 | [NC\_023693.1](about:blank) | [FR775895.2](about:blank) | 148.61 | 37.4 | [250](about:blank) | 18 | 100 | 100 |
| Escherichia phage muut |  | [MN850573.1](about:blank) | 146.31 | 37.4 | [239](about:blank) | 15 | 91.9 | 89.2 |
| Escherichia phage VEcB |  | [MT932211.1](about:blank) | 147.7 | 37.6 | [249](about:blank) | 13 | 92.6 | 92.8 |
| Escherichia phage alia |  | [MN850632.1](about:blank) | 147.01 | 37.5 | [240](about:blank) | 15 | 92.4 | 91.6 |
| Escherichia phage vB\_vPM\_  PD06 |  | [MH816848.1](about:blank) | 149.51 | 37.7 | [238](about:blank) | 17  (\*\*\*) | 92.8 | 89.6 |
| Escherichia phage vB\_EcoM\_  PHB05 |  | [MF805809.1](about:blank) | 147.66 | 37.5 | [231](about:blank) | 17  (\*\*\*) | 92.1 | 88.4 |
|  |  |  |  |  |  |  |  |  |

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

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

**(\*\*\*) None listed in GenBank record; these discovered using tRNAscan-SE [7]**

**Electron micrograph:** None available

**2. To add eight (8) new species to the genus *Phapecoctavirus***

**History:** This genus was established through Taxonomy Proposal 2019.075B

**Reference:** Khalifeh A, Kraberger S, Dziewulska D, Stenzel T, Varsani A. Complete Genome Sequence of a Phapecoctavirus Isolated from a Pigeon Cloacal Swab Sample. Microbiol Resour Announc. 2021 Feb 4;10(5):e01471-20. doi: 10.1128/MRA.01471-20. PMID: 33541888. **[TSP7\_1]**

Olsen NS, Forero-Junco L, Kot W, Hansen LH. Exploring the Remarkable Diversity of Culturable Escherichia coli Phages in the Danish Wastewater Environment. Viruses. 2020 Sep 4;12(9):986. doi: 10.3390/v12090986. PMID: 32899836; PMCID: PMC7552041. **[anhysbys, nepoznato, nieznany, tuntematon, ukendt]**

**GenBank Summary:**

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| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Phage name | RefSeq No. | INSDC | Size (Kb) | GC% | Protein | t  RNA | Overall DNA sequence identity (\*) | % common proteins (\*\*) |
| Escherichia phage phAPEC8 | [NC\_020079.1](about:blank) | [JX561091.1](about:blank) | 147.74 | 39.1 | [269](about:blank) | 11 | 100 | 100 |
| Dompiswa phage TSP7\_1 |  | MW175890 | 150.89 |  | 269 | 11 | 91.6 | 92.9 |
| Escherichia phage anhysbys |  | [MN850648.1](about:blank) | 149.34 | 39.1 | [272](about:blank) | 10 | 98.2 | 92.9 |
| Escherichia phage Mt1B1\_P17 |  | [MT496970.1](about:blank) | 151.2 | 38.9 | [284](about:blank) | 11 | 90.8 | 95.5 |
| Escherichia phage nepoznato |  | [MN850571.1](about:blank) | 151.51 | 38.9 | [266](about:blank) | 9 | 88.6 | 89.2 |
| Escherichia phage nieznany |  | [MN850598.1](about:blank) | 145 | 39.1 | [256](about:blank) | 10 | 87.1 | 85.1 |
| Escherichia phage tuntematon |  | [MN850618.1](about:blank) | 150.47 | 39.1 | [280](about:blank) | 10 | 91.1 | 93.7 |
| Escherichia phage ukendt |  | [MN850565.1](about:blank) | 150.95 | 39.0 | [269](about:blank) | 10 | 89.1 | 92.6 |
| Escherichia phage vB\_EcoM-Ro121c4YLVW |  | [MH051333.1](about:blank) | 151.67 | 39.1 | [266](about:blank) | 10 | 92.0 | 92.2 |

**3. To create a new subfamily, *Stephanstirmvirinae*, with these genera**

**Source of the name of this taxon:** This genus is named in honour of Professor Dr. Stephan Stirm formerly at the Max Planck-Institut für Immunbiologie and Institut für Biologie II der Universität, Freiburg, Germany was the Direktor of the Biochemisches Institut, University of Giessen. He was a noted carbohydrate chemist who worked with Bartłomiej Kwiatkowski on the digestion of capsular polysaccharides by bacteriophages.

**Rationale:** The two genera *Phapecoctavirus* and *Justusliebigvirus* share approximate 45% DNA sequence relatedness. In addition, Enterobacteria phage phi92 and Escherichia phage phAPEC8 share 179 protein homologs (66.5% common proteins).

**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/](about:blank)

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