

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

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| **Code assigned:** | ***2023.033B*** |  |
| **Short title:** To create a new subfamily, *Heleneionescovirinae* with two genera – *Zhangjivirus* and *Kenyatavirus* [*Caudoviricetes*] | | |
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| Andrew M. Kropinski |

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

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| Caudoviricetes Study Group |

**ICTV Study Group comments and response of proposer**

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**ICTV Study Group votes on proposal**

|  |  |  |  |
| --- | --- | --- | --- |
| **Study Group** | **Number of members** | | |
| **Votes support** | **Votes against** | **No vote** |
|  |  |  |  |
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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 | June 2023 |
| Date of this revision (if different to above) |  |

**ICTV-EC comments and response of the proposer**

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**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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| 2023.033B.N.v1.Heleneionescovirinae\_nsf.xlsx |

**Abstract**

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| We have created a new subfamily, *Heleneionescovirinae*, consisting of two existing genera of Bacillus siphophages, *Zhangjivirus* and *Kenyatavirus*. |

**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 [1,2] – 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 [10].  **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 (usually about 40-50%) and that the genera form a clade in a marker tree phylogeny [10]. | |

**Supporting evidence**

**Proposal Data:**

1. **To create a new species in the genus *Zhangjivirus***
2. **To create a new subfamily, *Heleneionescovirinae***
3. **To transfer *Kenyatavirus* and *Zhangjivirus* to this subfamily**

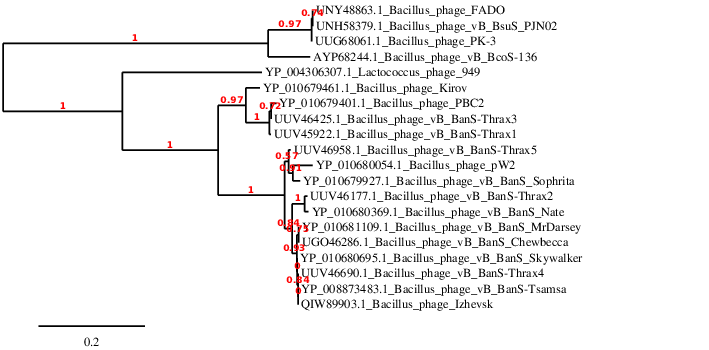
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**Figure 1. VIRIDIC heat map:** VIRIDIC (Virus Intergenomic Distance Calculator; VIRIDIC (Virus Intergenomic Distance Calculator; [3]; http://rhea.icbm.uni-oldenburg.de/VIRIDIC/) computes pairwise intergenomic distances/similarities amongst phage genomes. Data values which are bordered in black correspond to strains. Abbreviations: phg = phage; Baci = Bacillus.

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**Figure 2. ViPTree analysis:** ViPTree analysis ([https://www.genome.jp/viptree/](about:blank); [4]) is based upon Rohwer and Edwards (2002) famous Phage Proteomic Tree [5]. The phages of interest are indicated with **red lines and stars**.

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**Figure 3. Phylogeny:** The phylogenetic tree was constructed using the large subunit terminase proteins from these and related 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.”

**Taxonomic Proposals:**

1. **To create a new species in the genus *Zhangjivirus***

**Historical aspects:** Temperate siphophages FADO, PJN02 and PK-3 were isolated in Portugal, China and Pakistan, respectively. The first two were cultivated on Bacillus subtilis. Because it is underannotated and significantly smaller than the other two phages, we will not be including phage PK-3 in this genus.

**Genome summary:**

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| --- | --- | --- | --- | --- | --- | --- |
| Phage name | INSDC | Size (Kb) | GC% | Protein | Overall % DNA sequence identity (\*) | Overall % homologous proteins (\*\*) |
| Bacillus phage FADO | [OM236516.1](https://www.ncbi.nlm.nih.gov/nuccore/OM236516.1) | 164.76 | 33.6 | [222](https://www.ncbi.nlm.nih.gov/genome/browse/#!/proteins/112401/1820422|Bacillus phage FADO/viral segment/) | 89.2 | ND |
| Bacillus phage vB\_BsuS\_PJN02 | [OM634653.1](https://www.ncbi.nlm.nih.gov/nuccore/OM634653.1) | 165.87 | 33.6 | [230](https://www.ncbi.nlm.nih.gov/genome/browse/#!/proteins/111732/1813520|Bacillus phage vB_BsuS_PJN02/viral segment/) |  |  |
| Bacillus phage PK-3 | [ON881243.1](https://www.ncbi.nlm.nih.gov/nuccore/ON881243.1) | 142.57 | 34.0 | [111](https://www.ncbi.nlm.nih.gov/genome/browse/#!/proteins/117025/1910184|Bacillus phage PK-3/viral segment/)(\*\*\*) | ND | ND |

**Conclusion:** On the basis of DNA (Fig. 1) and protein (Fig. 2) similarity; and phylogenetic analysis of the TerL proteins (Fig. 3) this is a cohesive genus.

1. **To create a new subfamily, *Heleneionescovirinae***

**Origin of the name of this taxon:** This taxon was named in honour of Hélène Ionesco (b. 1914 - d. 1985, Paris France), who was part of André Lwoff's Service de Physiologie microbienne at the Pasteur Institute. In 1954 "She had found that when a certain strain of Bacillus megaterium was lysogenized with a certain bacteriophage, rough colonies formed instead of the normal smooth ones." van Helvoort, A. A. F. J. (1993). Research styles in virus studies in the twentieth century: controversies and the formation of consensus. [Doctoral Thesis, Maastricht University]. Rijksuniversiteit Limburg. DOI: 10.26481/dis.19930402ah]



(Photograph derived from: <https://phototheque.pasteur.fr/fr/asset/fullTextSearch/search/H%C3%A9l%C3%A8ne%20Ionesco/page/1>)

**Rationale:** Bacillus phage vB\_BsuS\_PJN02 (*Zhangjivirus*) and vB\_BcoS-136 (*Kenyatavirus*; 2022.005B.A.Andregratiavirinae\_Joanripponvirinae\_nsf) contain temperate siphoviruses. They share a similar size (162.6 kb), GC content (32.9%). At the DNA level they are 30.7% similar while at the protein level they share 99 homologs (45% similarity). These values are well within the parameter set for the creation of a subfamily [10].

1. **To transfer *Kenyatavirus* and *Zhangjivirus* to this subfamily**

**Rationale:** Bacillus phage vB\_BsuS\_PJN02 (*Zhangjivirus*) and vB\_BcoS-136 (*Kenyatavirus*; 2022.005B.A.Andregratiavirinae\_Joanripponvirinae\_nsf) contain temperate siphoviruses. They share a similar size (162.6 kb), GC content (32.9%). At the DNA level they are 30.7% similar while at the protein level they share 99 homologs (45% similarity). These values are well within the parameter set for the creation of a subfamily [10].

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