

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

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| **Code assigned:** | ***2023.048B*** |  |
| **Short title:** Create one new subfamily (*Munstervirinae*) including four new genera within the class *Caudoviricetes* |
|  |

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

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

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**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** |
|  |  |  |  |
|  |  |  |  |

**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 | May 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**

|  |
| --- |
| 2023.048B.N.v1.Munstervirinae\_nsf.xlsx |

**Abstract**

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| Here, we propose the creation of a new subfamily “Munstervirnae” comprising four new genera, the new genera “Ahaonvirus” and “Adovirus” both comprising two new species and the new genera “Atrivirus” and “Aceathervirus”, each containing one new species. These proposals are based on genomic comparisons. |

**Text of proposal**

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**Supporting evidence**

**Source of the names used for taxa:**

***Munstervirnae*:** The name is the province of Ireland where phages were first isolated [1].

**Genus names:** Derived from the names of numbers in the Irish language.

**Species demarcation criteria:**

In this proposal, we have chosen 95% DNA sequence idenitity/similarity as the criterion for the demarcation of a species. Each proposed species genome differs by more than 5% in nucleotide identity/similarity as determined by the BLASTN algorithm and VIRIDIC (Table 1-2, Figure 2) [2].

**Subfamily and genus demarcation criteria:**

*Ruminococcus* phages phiRgPS\_6, phiRgIBDN1, phiRg519T2, phiRg507T2\_2, phiRg507T2\_3 and phiRgRM10 are all temperate phages infecting *Ruminococcus gnavus*. Genomes of these phages are homologous sharing >50% nucleotide similarity and possessing gene synteny allowing their placement within a novel subfamily (Figure 2 & 3). Nucleotide identity/similarity can be found between phages phiRg507T2\_2 and phiRg507T2\_3 on one hand, and phages phiRg519T2 and phiRgIBDN1 on the other, of >70% allowing placement within two separate novel genera (Figure 2, Table 1 & 2). Similarly, phages phiRgRM10 and phiRgPS\_6 can be placed within their own separate novel genera (Figure 2). Nucleotide similarity of >70% can be found between phage phiRgIBDN1 and phages phiRg507T2\_2. The convention of placing phage into different genera based on the demarcation of sharing nucleotide identity/similarity of ≥70 can be difficult to apply to temperate phages due their more recombinogenic nature, as typified by phage phiRgIBDN. However, gene products related to DNA functions are more conserved between phage phiRgIBDN1 and phiRg519T2, indicating these phages are better placed within the same genus (Figure 4). Designation of proposed subfamily and genera are also supported by phylogenetic analysis using the proteome of these phages (Figure 5).

All phages with the proposed subfamily possess genomes that range between 36,510 and 37,780 bp with a GC content of 41-42%. The number of open reading frames (ORFs) found on the genomes of these phages ranged from 54 to 59, with each possessing either one or two tRNA genes (Tables 1 to 4). All phages possess an integrase gene and have been demonstrated to be able to form prophage on the genome of their host bacterium [1]. Transmission electron microscopy of phiRgPS6, phiRg507T2\_2,phiRg507T2\_3 and phiRg519T2 revealed virions with a classic siphovirus morphology with icosahedral heads (55-61 nm in diameter), with long flexible non-contractile tails (195-204 nm) possessing very discreet baseplates (Figure 1) [1].

**History:**

Isolation source of the phages forming the proposed subfamily of the *Munstervirnae*:

* Phage phiRgPS6 was isolated from a pooled human faecal sample in Co. Cork, Ireland [1].
* Phages phiRgIBDN1, phiRg519T2, phiRg507T2\_2 and phiRg507T2/3 were isolated from the faeces of individuals suffering from inflammatory bowel disease in Co. Cork, Ireland [1].
* Phage phiRgRM10 was isolated from a sample obtained from a farm in Co. Tipperary, Ireland [1].

**Electron micrograph:**



**Figure 1.** Transmission electron micrographs. The micrographs of *Ruminococcus gnavus* phages (A) phiRg507T2\_2, (B) phiRg507T2\_3, and (C) phiRgPS\_6. Phages where negatively stained with 0.5% w/v uranyl acetate. The scale represents 100 nm [1].

**GenBank Summary:**

**Table 1.** Properties of the two phages belonging to the genus *Ahaonvirus*

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| --- | --- | --- | --- | --- | --- | --- | --- |
| **Phage** | **INSDC accession number** | **Genome size (bp)** | **GC content (%)** | **Number of ORFs** | **Number of tRNA genes** | **Nucleotide homology (%)\*** | **Homologous Proteins (%)\*\*** |
| phiRg507T2\_2 | MT980836 | 37,304 | 42 | 54 | 2 | 100 | 100 |
| phiRg507T2\_3 | MT980837 | 36,510 | 42 | 54 | 2 | 86 | 89 |

**Table 2.** Properties of the two phages belonging to the genus *Adovirus*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Phage** | **INSDC accession number** | **Genome size (bp)** | **GC content (%)** | **Number of ORFs** | **Number of tRNA genes** | **Nucleotide homology (%)\*** | **Homologous Proteins (%)\*\*** |
| phiRgIBDN1 | MT980840 | 37,233 | 41 | 58 | 2 | 100 | 100 |
| phiRg519T2 | MT980838 | 36,865 | 42 | 58 | 2 | 73 | 81 |

**Table 3.** Properties of the phage forming the genus *Atrivirus*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Phage** | **INSDC accession number** | **Genome size (bp)** | **GC content (%)** | **Number of ORFs** | **Number of tRNA genes** | **Nucleotide homology (%)\*** | **Homologous Proteins (%)\*\*** |
| phiRgPS6 | MT980839 | 37,780 | 41 | 59 | 2 | - | - |

**Table 4.** Properties of the phage forming the genus *Aceathervirus*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Phage** | **INSDC accession number** | **Genome size (bp)** | **GC content (%)** | **Number of ORFs** | **Number of tRNA genes** | **Nucleotide homology (%)\*** | **Homologous Proteins (%)\*\*** |
| phiRM10 | MT980841 | 36,920 | 42 | 58 | 1 | - | - |

\* Determined using BLASTN; \*\* Determined using CoreGenes 5 [3]

**Phylogeny:**



**Figure 2**. Heatmap showing nucleotide similarity of *Ruminococcus* phages as calculated with VIRIDIC [2].



**Figure 3.** Comparison of the genomes of *Ruminococcus* phages isolated in this study employing BLASTN and visualisation with Easyfig [4]. The genome maps display arrows indicating the locations and orientation of ORFs among different phage genomes. Arrows have been colour-coded describing their predicted roles (see key), and shading between the genome maps indicates the level of identity.



**Figure 4.** Heatmap illustrates shared genes as calculated by Proteinortho [5] (Identity = 30%, coverage = 70%) among *Ruminococcus* phages where function of their gene product could be determined. Key describes color coding for the presence absence of genes among phages and the category gene product function is allocated for the gene product in question.



**Figure 5**. Amino acid VICTOR-generated phylogenomic Genome-BLAST Distance Phylogeny (GBDP) tree inferred using the formula D4 and yielding average support of 67% [6]. The phylogram includes this study’s *Ruminococcus* phages and those that share an evolutionary connection. The genus (if allocated) of phages in the analysis is illustrated. Branch support was inferred from 100 pseudo-bootstrap replicates.

**References**

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