

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

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| **Code assigned:** | ***2023.018B*** |  |
| **Short title:** Create one new species in the genus *Kehishuvirus,* one species in the genus *Kolpuevirus,* and one new genus with one new species in the order *Crassvirales* |
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**Author(s) and email address(es)**

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| Papudeshi B, Vega AA, Souza C, Giles SK, Mallawaarachchi V, Roach MJ, An M, Jacobson N, McNair K, Mora MF, Pastrana K, Boling L, Leigh C, Harker C, Plewa WS, Grigson SR, Bouras G, Decewicz P, Luque A, Droit L, Handley SA, Wang D, Segall AM, Dinsdale EA, Edwards RA | nala0006@flinders.edu.au; alexvega619@gmail.com; colesouza017@gmail.com; sarah.giles@flinders.edu.au; mall0133@flinders.edu.au; michael.roach@flinders.edu.au; michellean92@gmail.com; njacobson@sdsu.edu; deprekate@gmail.com; moramariaf21@gmail.com;kpastrana0331@sdsu.edu; liquidgrey@gmail.com; chris.leigh@adelaide.edu.au; clarice.cram@flinders.edu.au; will.plewa@flinders.edu.au; p.decewicz@uw.edu.pl; susie.grigson@flinders.edu.au; george.bouras@adelaide.edu.au; aluque@sdsu.edu; ldroit@wustl.edu; shandley@wustl.edu; davewang@wustl.edu; asegall@sdsu.edu; elizabeth.dinsdale@flinders.edu.au; robert.edwards@flinders.edu.au  |

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**Corresponding author**

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| Robert A. Edwards, member of ICTV *Crassvirales* phages Study Group |

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

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| ICTV Bacterial Viruses Subcommittee, *Crassvirales* phages Study Group  |

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

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**ICTV Study Group votes on proposal**

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| **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 | 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**

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| 2023.018B.N.v1.Crassvirales\_1ng\_3ns.xlsx |

**Abstract**

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| The isolation of *Crassvirales* in vitro remains a challenge with only four successful pure isolates since the discovery of the first *Crassvirales* species in 2014. However, over 600 *Crassvirales* phages have been identified from metagenomes. In our study, we successfully isolated three novel *Crassvirales* phages from wastewater that infect the bacterial host Bacteroides cellulosilyticus WH2. Following the taxonomic demarcation and naming convention proposed by the ICTV for *Crassvirales,* we propose two novel species, Kehishuvirus tikkala (Bc01), Kolpuevirus frurule (Bc03), and a new genus, Rudgehvirus, with one new species Rudgehvirus jaberico (Bc11).  |

**Text of proposal**

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| The three isolates’ phages were visualized using transmission electron microscopy (TEM) and their genomes were sequenced. A nucleotide BLAST search against the nr database of the assembled genomes confirm their closely related genomes are other crass-like phages. Additionally, transmission electron micrographs revealed that the three phages share a podovirus morphology and have a genome length of 100kb which is consistent with the other crass-like phages. To determine their taxonomic assignment, we followed the *Crassvirales* order demarcation criteria. The genera within *Crassvirales* were defined based on the topology of the protein phylogenetic trees, which showed at least 80% shared orthologous groups. The species demarcation criteria included 95% nucleotide sequence identity over 85% of the complete genome length. Following the above taxonomic classification, we confirmed the three crAss-like phages isolated represent three novel species. Here are the details of each species:*Kehishuvirus tikkala*:This genome shares 80% orthologous genes within known genera, specifically *Kehishuvirus.* At the species level, the most closely related strain to this genome is *Kehishuvirus primarius,* with a sequence identity of 95.67% across 80.98% genome. *Kolpuevirus frurule:* This genome also shares 80% orthologous genes and is classified within the existing genus “*Kolpuevirus”*. However, it represents a novel species as it is most similar to the genome *Kolpuevirus hominis,* with an 82.58% sequence identity across 55.01% of the genome. We propose to call this new species *Kolpuevirus frurule.* *Rudgehvirus jaberico:* This genome was classified at the family level under *Intestiviridae,* with its closest related genome being *Jahgtovirus intestinalis* sharing 74.75% identity across 9.86%. Phylogenetic classification of the three conserved genes, portal protein, terminase large subunit and major capsid protein - suggests that these isolate forms a neighboring clade to its closely related genome. Following the ICTV *Crassvirales* genus naming convention, we propose to call this genus “*Rudgehvirus*” after Ridgeback dog breed.  |

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**Supporting evidence**

**History:** Since discovery of one of the most abundant bacteriophages in the human gut microbiome in 2014, crAssphage has been of interest [1]. Since there have been over 600 other crass-like phages that share some similarity with the crAssphage [4]. In 2021, these crass-like phages have been classified into a formal taxonomic system including a new order to represent all of them *Crassvirales* [3]. This order includes four new families, ten new subfamilies, 42 new genera and a total of 73 new species (Taxonomy Proposal 2021.021B.A.Crassvirales).

Here we are presenting the supporting evidence on how we are classifying the three novel crass-like phages isolated [2] into the *Crassvirales* order.

**Podovirus morphology**

All three phages isolated in Papudeshi et al., 2023 [2] were confirmed to have podovirus morphology when visualized as an electron micrograph (Figure 1).

 A) *Kehishuvirus tikkala* B) *Kolpuevirus frurule* C) *Rudgehvirus jaberico*

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Figure 1: TEM image of negatively stained crass-like virions, under 49,000x magnification. Image taken from Papudeshi et al., 2023 [2].

**Phylogenomics**

Taxonomic classification of the three phages were assigned using three signals, 1) phylogeny 2) orthologous genes shared 3) nucleotide identity using a CrassUS program (<https://github.com/dcarrillox/CrassUS>).

Open reading frame (ORFs) were predicted Prodigal. In this study, a revised version of prodigal [4] was employed by CrassUS, to specifically detect codon reassignment within the three isolated phages. The genomes were annotated to predict the ORFs using both the standard codon table, and the codon table with TAG and TGA reassigned, as observed in some of the *Crassvirales* phages[4]. Upon analyzing coding potential, the highest values were observed when using the standard codon table. These identified ORFs were subsequently utilized for taxonomic classification.

1. **Orthologous genes**

Orthologous genes were analyzed for the three phages, after predicting the open reading frames (ORFs) using revised Prodigal bioinformatic tool. Amino acid sequences of the predicted ORFs were aligned against known *Crassvirales* genome protein clusters from Yutin et al [4], using mmseqs2 v13.45. The clustered proteins were then used to build presence/absence matrix. If the genome shared 80% of its proteins with a known genus, the genome is assigned a taxon (Table 1).

**Table 1:** Taxonomic classification based on shared orthologous groups when compared against known *Crassvirales* genomes.

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| **Genome** | **Reference shared proteins** | **Most similar family** | **Most similar subfamily** | **Most similar genus** |
| Bc01 | 84.0 | *Steigviridae* | *Asinivirinae* | *Kehishuvirus* |
| Bc03 | 82.4 | *Steigviridae* | *Asinivirinae* | *Kolpuevirus* |
| Bc11 | 50.0 | *Intestiviridae* | -  | -  |

1. **Nucleotide identity**

Average nucleotide identity was calculated, comparing each isolate to the known *Crassvirales* genomes using BLAST alignment. From the BLAST results, the average nucleotide identity and query coverage was calculated using scripts in CrassUS (<https://github.com/dcarrillox/CrassUS>). If the genome shared 95% DNA sequence identity over 85% query coverage to a complete reference genome, to assign taxonomy to species level classification (Table 2).

**Table 2:** Taxonomic classification based on shared nucleotide identity when compared against known *Crassvirales* genomes.

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| **Genome** | **Most similar reference species** | **Percent identity (pid)** | **Query coverage** |
| Bc01 | *Kehishuvirus primarius* | 95.51 | 79.08 |
| Bc03 | *Kolpuevirus hominis* | 82.79 | 53.73 |
| Bc11 | *Jahgtovirus intestinalis* | 74.72 | 9.86 |

1. **Phylogeny**

Three conserved proteins, large terminase subunit, portal, and major capsid proteins from the genome annotations were used for phylogenetic reconstruction. These genes are then aligned using MAFFT v7.49, the poorly aligned regions are then trimmed using trimal v1.4.1. The resulting alignment was used to infer phylogenetic relationship FastTree Version 2.1.10 that generated maximum likelihood tree using the Jones-Taylor-Thornton (JTT) model and Continuous Rate Ancestral State Reconstruction (CAT) approximation with 20 rate categories to account for heterogeneity in substitution rates across the alignment. The resulting trees were visualized using iTol (Figure 2), and the outgroup is set to *Cellulophaga phage phi13:2.*



Figure 2: Maximum likelihood phylogenetic tree of known *Crassvirales* phages with *Cellulophaga phage phi13:2* set as the outgroup. The phylogenetic trees were plotted with three conserved genes A) portal, B) major capsid protein (MCP), and C) terminase large subunit (*terL*). The tips of the tree are color coded based on family level classification, with the three isolates highlighted in bold, along with the proposed names for the three phages. This figure was taken from Papudeshi et al., 2023 [2]

**References**

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