

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

|  |  |  |
| --- | --- | --- |
| **Code assigned:** | **2020.055B** |  |
| Create one new genus (*Enterogokushovirus*) in the subfamily *Gokushovirinae* (*Microviridae*) | | |
|  | | |

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

|  |  |
| --- | --- |
|  |  |
| Kirchberger PC, Ochman H | pkirchberger@utexas.edu; howard.ochman@austin.utexas.edu |

**Author(s) institutional address(es) (optional)**

|  |
| --- |
| University of Texas at Austin (PCK, HO) |

**Corresponding author**

|  |
| --- |
| Kirchberger, PC |

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

|  |
| --- |
| **Bacterial and Archaeal Viruses Subcommittee; *Microviridae* Study Group** |

**ICTV study group comments and response of proposer**

|  |
| --- |
|  |

**Authority to use the name of a living person**

|  |  |  |
| --- | --- | --- |
| **Taxon name** | **Person from whom the name is derived** | **Permission attached (Y/N)** |
|  |  |  |
|  |  |  |
|  |  |  |

**Submission dates**

|  |  |
| --- | --- |
| Date first submitted to SC Chair |  |
| Date of this revision (if different to above) |  |

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

|  |
| --- |
|  |

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

**Name of accompanying Excel module**

|  |
| --- |
| 2020.055B.R.Enterogokushovirus.xlsx |

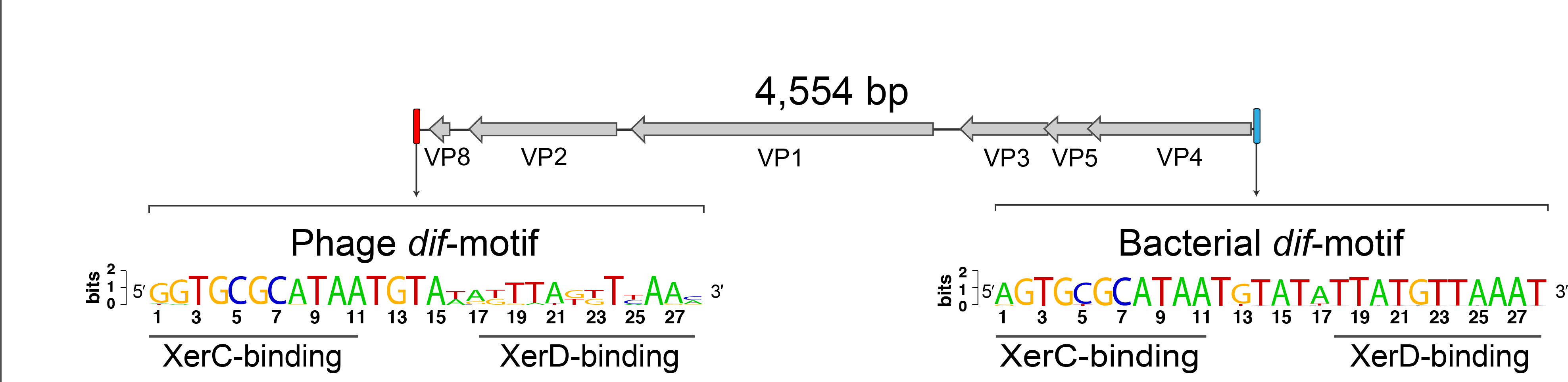
**Abstract**

|  |
| --- |
| A phage of the subfamily *Gokushovirinae (*Family *Microviridae)* was isolated from a prophage of *Escherichia marmotae*. These phages represent the first known lysogenic members of the *Gokushovirinae*. The type strain, EC6098, averages approximately 50% nucleotide sequence identity with other prophage sequences found in the genera *Escherichia*, *Citrobacter*, *Kosakonia*, *Enterobacter* and *Salmonella*. The genomes of these (pro)phages are 4.5­–4.6 kilobases in length and encode six protein coding genes, including the hallmark gene Rep and a major capsid protein characteristic of gokushoviruses. The phage genomes encode a single 28-bp *dif-*motif required for integration into the bacterial genome*.* The genomic cohesion of this group, phylogenetic differentiation from other gokushoviruses, and the ability to lysogenize enterobacteria necessitates the creation of a new genus *Enterogokushovirus* |

**Text of proposal**

|  |  |
| --- | --- |
| |  | | --- | | **Genus demarcation criteria:**  The isolate of the proposed genus as well as all detected prophage sequences are roughly 4,500-4,600 bases in length, encode six genes in conserved order, and process a characteristic 28-base *dif*-motif enabling integration downstream of the homologous *dif*-motif of bacterial genomes(Figure 1) [1]. Known hosts include the members of the bacterial genera *Escherichia*, *Citrobacter*, *Salmonella*, *Enterobacter* and *Kosakonia* (Fam. *Enterobacteriaceae*). (Pro)phages of this genus share at least 50% average nucleotide identity (Table 1). All members form a phylogenetically distinct lineage based on their conserved Rep (VP4) and major capsid protein (VP1) sequences (Figure 2). The name *Enterogokushovirus* is derived from their host family, the *Enterobacteriaceae*, and the type species name *Enterogokushovirus* *EC6098* is based on the first isolate Escherichia phage EC6098, initially found lysogenizing *Escherichia marmotae* MOD1-EC6098.  **Species demarcation criteria:**  The representative Escherichia phage EC6098 should be placed into the proposed species *Enterogokushovirus EC6098.* As only one isolate of this genus is available, we do not suggest species demarcation criteria at this point. | |

**Supporting evidence**

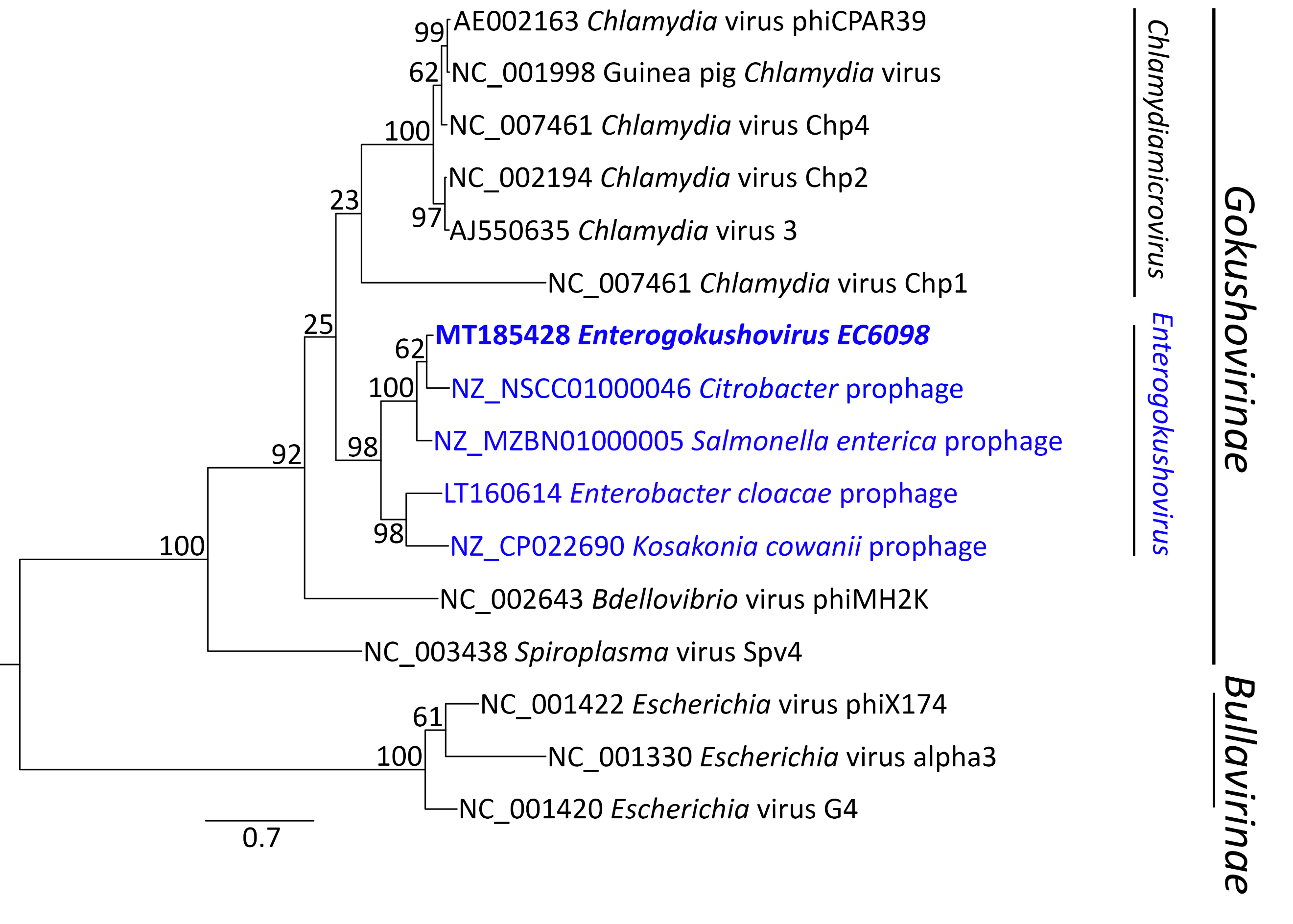


**Figure 1: Gene order of *Enterogokushovirus* and sequence logo of characteristic integration sites into enterobacterial genomes.** Arrows indicate conserved open reading frames, red bar indicates the 28-base *dif*-motif encoded on phage genome, blue bar the homologous *dif*-motif of bacteria directly upstream of conserved phage integration site (absent in the closed circular phage genome). Sequence logos for both *dif*-motifs are shown.Figure adapted from [1]

**Table 1:** **Average nucleotide identity of *Enterogokushovirus* strains found in different bacterial hosts.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | A | B | C | D | E |
| A *Enterogokushovirus* *EC6098* |  | 78 | 73 | 53 | 52 |
| B *Citrobacter* prophage | 78 |  | 70 | 54 | 52 |
| C *Salmonella* prophage | 73 | 70 |  | 54 | 53 |
| D *Enterobacter* prophage | 53 | 54 | 54 |  | 63 |
| E *Kosakonia* prophage | 52 | 52 | 53 | 63 |  |

Average nucleotide identity was calculated in Geneious Prime (Biomatters Ltd.) from whole genome alignments of phage and prophages (minus bacterial *dif*-motif) constructed using Clustal Omega (standard settings) [2]. Accession numbers for genomes are indicated in Figure 2.

****

**Figure 2: Phylogeny of *Microviridae*.** Members of the proposed genus *Enterogokushovirus* are labeled blue, with the type strain indicated in bold. Maximum likelihood phylogeny was constructed using the PROTGAMMAWAG algorithm implemented in RAxML 8 [3], based on a 1270 aa concatenated alignment of the conserved major capsid and Rep proteins created in Clustal Omega (standard settings) [2]. Bootstrap support was assessed using 100 fast bootstrap replicates. Scalebar indicates amino acid substitutions/site.

**References**

Please list references in *Archives of Virology* style and include PMID/PMCID and doi.

[1] Kirchberger PC and Ochman H (2020) Resurrection of a global, metagenomically defined gokushovirus. eLife 9: e51599. PMID: 32101162,

doi: 10.7554/eLife.51599

[2] Sievers F and Higgins DG (2018) Clustal Omega for making accurate alignments of many protein sequences. Protein Sci 27(1): 135-145. PMID: 28884485, doi: 10.1002/pro.3290

[3] Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis

of large phylogenies. Bioinformatics 30(9):1312-1313. PMID: 24451623, doi: 10.1093/bioinformatics/btu033

[4] Sayers EW, Agarwala R, Bolton EE, Brister JR, Canese K, Clark K, et al. Database resources of the National Center for Biotechnology Information. Nucleic Acids Res. 2019;47(D1):D23-D28. doi: 10.1093/nar/gkz899. PMID: 31602479.

[5] Tolstoy I, Kropinski AM, Brister JR. Bacteriophage Taxonomy: An Evolving Discipline. Methods Mol Biol. 2018;1693:57-71. doi: 10.1007/978-1-4939-7395-8\_6. PMID: 29119432

[6] O'Leary NA, Wright MW, Brister JR, Ciufo S, Haddad D, McVeigh R, et al. Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. Nucleic Acids Res. 2016;44(D1):D733-45. doi: 10.1093/nar/gkv1189. PMID: 26553804