

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

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| **Code assigned:** | **2021.028B** |  |
| **Short title:** Create two new genera of *Flavobacterium* phages (*Caudoviricetes*) | | |
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**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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| 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)** | 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 | 11 December 2020 |
| 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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| 2021.028B.R.Flavobacterium\_phages\_new\_genera |

**Abstract**

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| Based on whole genome sequencing and morphological analyses, we propose to group three newly characterized phages isolated from a Baltic Sea mesocosm experiment into one genus (*Immutovirus*) and one species within the family *Myoviridae.* The minimum genome similarity among the three phages was 99.2%. The maximum genome similarity identified to phage genomes available in the NCBI or IMG/ER database was 5.1%.  Based on whole genome sequencing and morphological analyses, we propose to group 62 newly characterized phages isolated from the same experiment into one genus (*Elemovirus*) and six species within Class *Caudoviricetes*. The minimum genome similarity among the 62 phages was 85.3% and the minimum genome similarity within the proposed species was 95.2%. The maximum genome similarity identified to phage genomes available in the NCBI or IMG/ER database was 2.2%. |

**Text of proposal**

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| |  | | --- | | Three phages were isolated in May/June 2016 from a Baltic Sea mesocosm experiment [1] on the host strain *Flavobacterium* sp. strain LMO8. The host was isolated in September 2015 from the Baltic Sea. We propose a new species within a new genus representing the three phage isolates.  Sixty-two phages were isolated from the same experiment [1] on the host strain *Flavobacterium* sp. strain BAL314. The host was isolated in May 2012 from the Baltic Sea. We propose six new species within a new genus representing these 62 isolates.  **Species demarcation criterion**  We used a threshold of 95% inter-genomic nucleotide similarity calculated using VIRIDIC (Virus Intergenomic Distance Calculator) [2] as species demarcation criterion. VIRIDIC implements the traditional algorithm used by the Bacterial Viruses Subcommitee of the ICTV to compute pairwise inter-genomic similarities based on BLASTN.  **Genus demarcation criterion**  We used a threshold of 70% inter-genomic nucleotide similarity calculated using VIRIDIC as genus demarcation criterion.  **Reference genomes**  To identify reference genomes for inter-genomic comparisons, the amino acid sequences of all protein coding genes of the largest genome within each proposed genus (vB\_FspM\_immuto\_2-6A and vB\_FspP\_elemo\_7-9A, respectively) were aligned to the NCBI non-redundant (nr) database [3] using Diamond v0.9.26 [4] in sensitive mode. Phage genomes yielding alignments with e-values <0.0001 were selected as references. Furthermore, the IMG/ER database [5] was screened for reference phage genomes that shared sequence similarity to vB\_FspM\_immuto\_2-6A or vB\_FspP\_elemo\_7-9A.  ***Immutovirus* (proposed genus)**  The reference genome with the highest inter-genomic similarity to vB\_FspM\_immuto\_2-6A was the viral single amplified genome (vSAG) 37-F16 from the Mediterranean Sea [6] showing only 5.1% nucleotide sequence similarity. We propose the name *Immutovirus* (from *immutare*, Latin verb for ‘change, alter, transform’) for the genus represented by vB\_FspM\_immuto\_2-6A, as its genome harbors a high proportion of genes putatively involved in cell surface modification and a gene set for biosynthesis of modified nucleosides.  We propose *Immutovirus Immuto* as the so far only known species affiliated to the genus.  ***Elemovirus* (proposed genus)**  The reference genome with the highest inter-genomic similarity to vB\_FspP\_elemo\_7-9A was the from Cellulophaga phage phi19:1 isolated from the Baltic Sea [7] showing only 2.2% nucleotide sequence similarity. We propose the name *Elemovirus*, referring to the Linnaeus Microbial Observatory (LMO) sampling station being the source of isolation of both the phage and its host and a site where the phages were detected in three consecutive years [1].  We propose six species affiliated to the genus (*Elemovirus ElemoA, Elemovirus ElemoB, Elemovirus ElemoC, Elemovirus ElemoD, Elemovirus ElemoE*, and *Elemovirus ElemoF*). | |

**Supporting evidence**

**Table 1. VIRIDIC results table** (provided in the separate file Table1\_VIRIDIC\_results.xlsx)

The table presents inter-genomic similarities among the newly described phages and selected reference genomes calculated using VIRIDIC [2]. The species clusters recommended by VIRIDIC based on the 95% similarity threshold are highlighted in different colors.

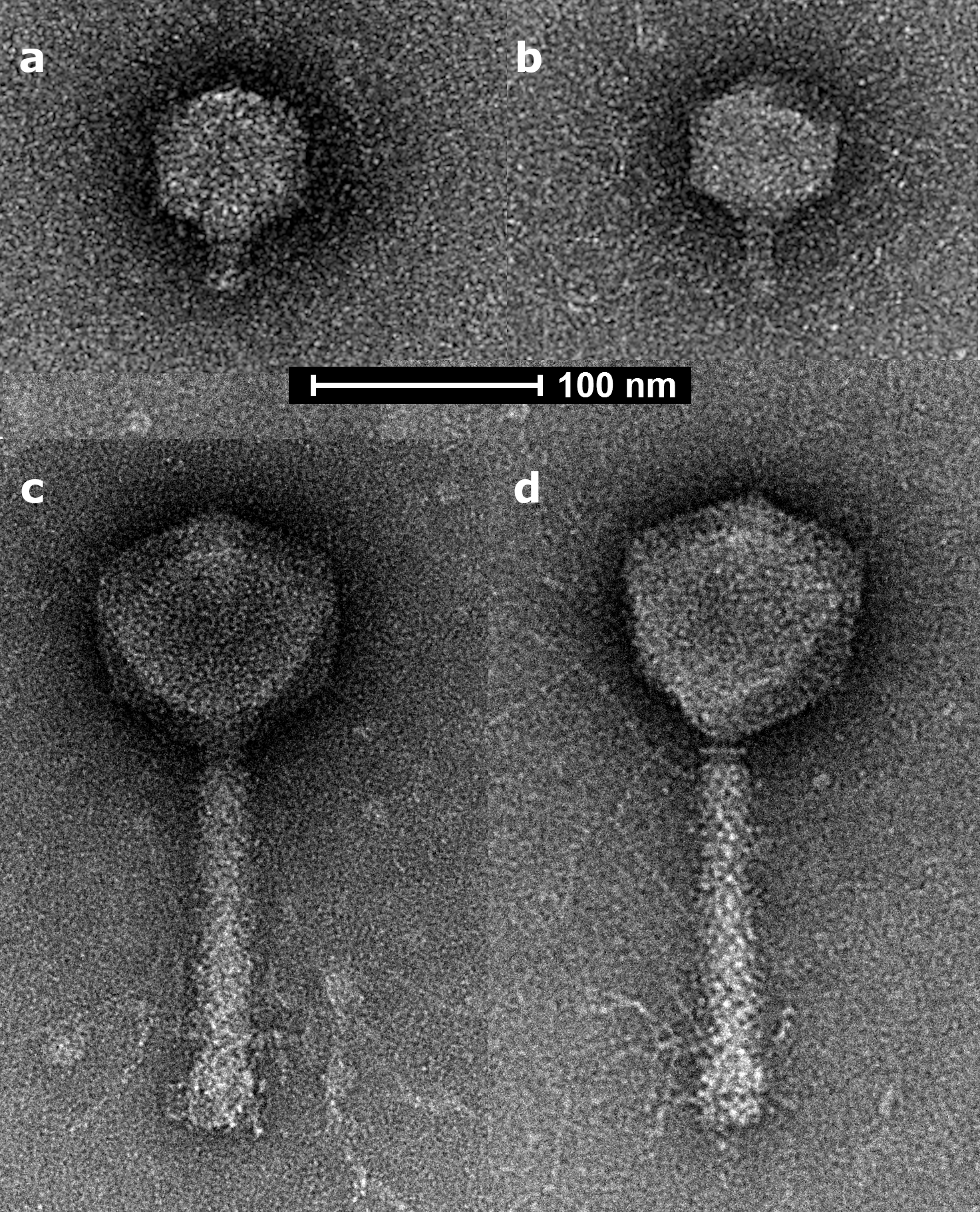
**Table 2. Isolated phages affiliated to the proposed taxa** (next page)

Genome characteristics and GenBank accession numbers of three isolates affiliated to the proposed genus *Immutovirus* and 62 isolates affiliated to six proposed species within the genus *Elemovirus*.



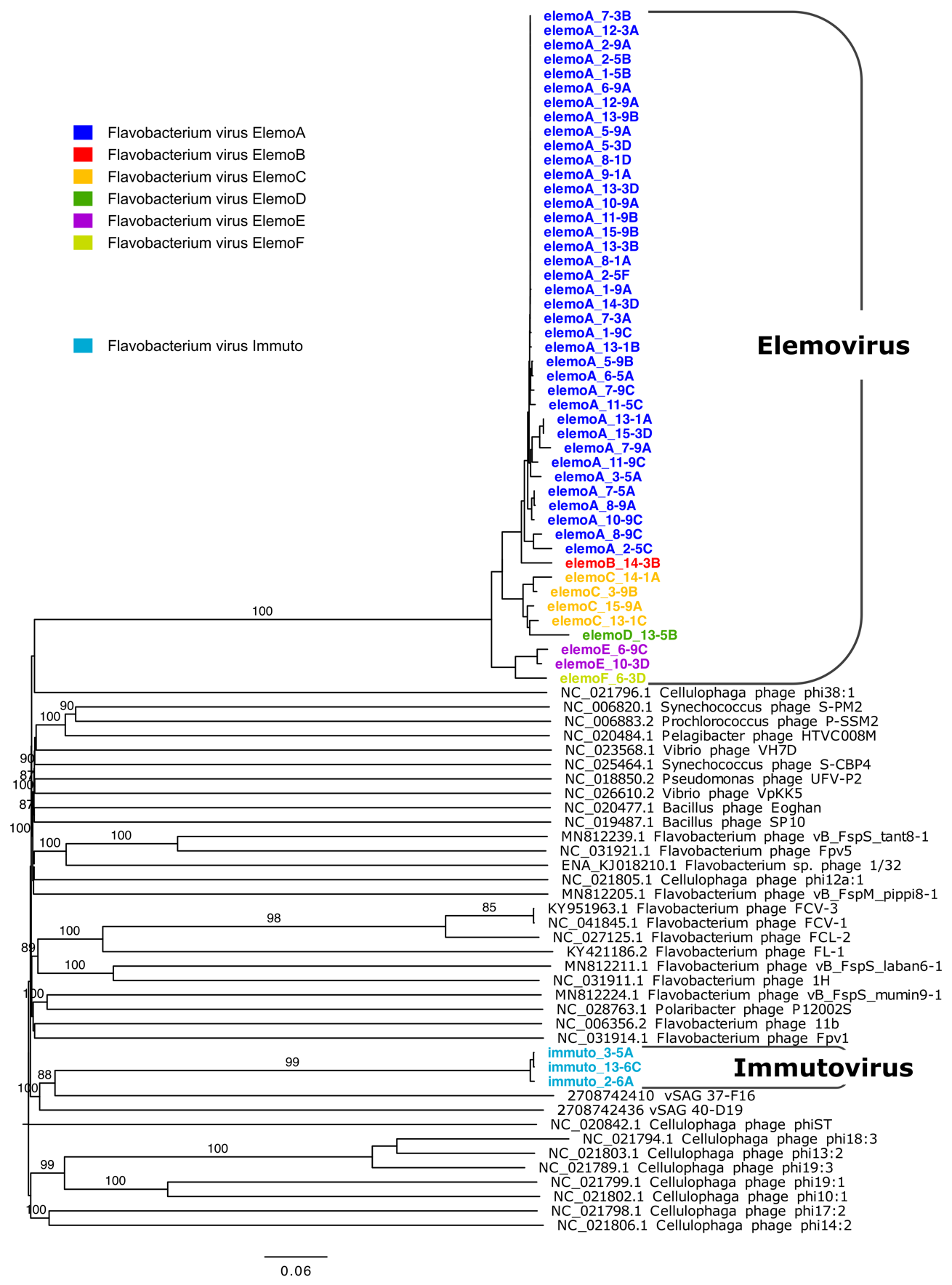
**Figure 1. Morphology**

Transmission electron micrographs of elemoA\_7-9A (a) and elemoD\_13-5B (b) showing a podovirus morphology. Measurements of multiple virions revealed similar sizes for both strains (capsid diameter ~75 nm, tail length ~18 nm). Micrographs of immuto\_2-6A (c, d) revealing a myovirus morphology (capsid diameter ~115 nm, tail length ~168 nm, sheath length ~133 nm, tail width ~25 nm). The scale is the same in all four micrographs.



**Figure 2. VICTOR tree**

Phylogenomic tree comprising the newly described phages and reference genomes calculated using the VICTOR web server [8]. The tree is based on the Genome-BLAST Distance Phylogeny (GBDP) method using the formula D0 [8]. The resulting inter-genomic distances were used to infer a balanced minimum evolution tree with branch support via FASTME including SPR post-processing [9]. Branch support was inferred from 100 pseudo-bootstrap replicates. Only bootstrap values >80% are shown. The different proposed species are highlighted by different colors.



**References**

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