

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

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| **Code assigned:** | ***2023.074B*** |  |
| **Short title:** Create new genus (*Vespunovirus*) in the class *Caudoviricetes* |
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**Author(s) and email address(es)**

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| --- |
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**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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| --- |
| No comments |

**ICTV Study Group votes on proposal**

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| --- | --- |
| **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 |

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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 |  |
| 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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| 2023.074B.N.v1.Vespunovirus\_ng.xlsx |

**Abstract**

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| Hereby we propose the genus of temperate bacteriophages infecting *Enterococcus* spp. The group is strongly supported by molecular phylogeny and network analyses, but the representatives have very low degree of intergenomic similarity. |

**Text of proposal**

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| Isolation of the bacteriophage VEsP-1 and the description of the taxonomic group was published in the journal Viruses [1]. The phage originated from water of Vietnamese river and was isolated using *Enterococcus faecalis* as a host. The phage had siphovirus morphology (Figure 1). Upon whole genome sequencing, no significant relationship to existing phage families or genera was found. In vContact2 cluster analysis, the genome sequence of the phage formed statistically significant genus group with eight other genomes retrieved from GenBank (Table 1). From this number, seven sequences were obtained in metagenomic studies [2]. The genus confidence score for the respective subcluster was calculated at 0.8889. The same group of nine genomes belonged to their own OPTSIL genus hypothesis in VICTOR analysis (Figure 2). Also, the group was monophyletic as determined by phylogenetic analysis with major capsid protein sequences (Figure 3). In CoreGenes analysis, the nine genomes shared 16 protein coding genes, including integrase-coding gene and 15 genes from structural module. The conserved structural genes coded for portal protein, minor capsid protein, major capsid protein, adaptor protein, neck protein, tail completion protein, tail tube protein, tail assembly chaperone protein, tail length tape-measure protein T, tail protein, upper baseplate protein and four hypothetical proteins. Though nucleotide sequence similarity values were as low as 17-77 percent (Figure 4), the exclusion of the least similarto VEsP-1 metagenomic phages ctCNj1 (BK016516) and ctyrd11 (BK030209) was not supported by MCP phylogeny (Figure 3). Nor we could find a possibility to refine the group on the basis of XtmB terminase large subunit sequences (Figure 5). Therefore, we believe the provided evidence is sufficient to describe a genus “Vespunovirus” with 9 members, as listed in the original reference. The name of the taxon is derived from the name of bacteriophage VEsP-1. Due to the abandonment of *Siphoviridae* [3], we propose to create the genus directly in the class *Caudoviricetes.* |

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**Supporting evidence**

**Specific reference: [1]**

**Electron micrographs:**

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Figure 1. Scanning electron microscopy images of Enterococcus phage VEsP-1 particles [1]. Scale bar, 100 nm.

**BLASTN homologs:**

The closest match to the obtained whole genome sequence of VEsP-1 was metagenomic sequence of bacteriophage cts681 (BK058556.1) with 53% similarity to VEsP-1 genome as determined by multiplication of percent identity by percent coverage. The closest genome with known taxonomic position had 14% similarity and belonged to Enterococcus phage phiFL3B, a member of the genus *Phifelvirus* (GQ478087.1).

**GenBank Summary:**

Table 1. The genomes of “Vespunovirus” representatives.

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Phage name** | **Sample location** | **Sample type** | **GenBank accession** | **Length, bp** | **ORFs** | **tRNAs** | **GC content, %** | **Terminase large subunit** | **Top bacterial BLAST hit; query cover / identity, %** |
| VEsP-1 | Vietnam | River water | MZ333456 | 39221 | 63 | 1 | 35.1 | XtmB | *Enterococcus faecalis*; 51 / 94 |
| cts681 | ND | Human feces | BK058556 | 40006 | 63 | 2 | 35.4 | XtmB | *E. faecalis*; 71 / 99 |
| ct63w4 | Estonia | — " — | BK017522 | 38654 | 59 | 2 | 35.4 | XtmB | *E. faecalis*; 72 / 96 |
| ctinV5 | Denmark | — " — | BK025106 | 41201 | 66 | 3 | 35.0 | XtmB | *E. faecalis*; 69 / 95 |
| SEsuP-1 | Russia | — " — | MZ333458 | 39319 | 51 | 2 | 33.5 | XtmB | *E. faecalis*; 96 / 100 |
| cthD61 | USA | — " — | BK033083 | 39498 | 54 | 1 | 34.1 | XtmB | *E. faecalis*; 99 / 100 |
| ct40X5 | Russia | — " — | BK020573 | 35370 | 52 | 2 | 33.9 | COG5362 | *E. faecalis*; 100 / 100 |
| ctCNj1 | USA | — " — | BK016516 | 42320 | 66 | 1 | 37.9 | COG5362 | *E. avium*; 23 / 100 |
| ctyrd11 | Ethiopia | — " — | BK030209 | 38401 | 52 | 0 | 33.8 | DEXDc | *E. hirae*; 83 / 98 |

(The Table was modified from ref. [1])

**Phylogeny:**

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Figure 2. VICTOR phylogenomic tree [1].

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Figure 3. Maximum Likelihood phylogenetic tree inferred from major capsid protein sequences [1]. The amino acid sequence sample was obtained by querying NCBI Protein database with VEsP-1 major capsid protein QYI86490.1 in a blastp search. The tree was calculated with the use of PhyML 3.1 program as implemented in SeaView 5.0.4 package. The substitution model was LG, assuming 12 across site rate variation categories. The number of invariable sites was algorithm-optimized. The stability of branches was assessed with 100 bootstrap replicates calculated by the Transfer Bootstrap Expectation method. Branch support values above 75% are shown. The VEsP-1 sequence is highlighted in bold. The topology of the tree supports the “Vespunovirus” hypothesis.

**Intergenomic similarity:**

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Figure 4. VIRIDIC intergenomic similarity analysis of proposed “Vespunovirus” member genomes.

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Figure 5. Maximum Likelihood phylogenetic tree inferred from XtmB terminase large subunit sequences. The amino acid sequence sample was obtained by querying NCBI Protein database with VEsP-1 terminase large subunit QYI86497.1 in a blastp search. The tree was calculated with the use of PhyML 3.1 program as implemented in SeaView 5.0.4 package. The substitution model was LG, assuming 12 across site rate variation categories. The number of invariable sites was algorithm-optimized. The stability of branches was assessed with 100 bootstrap replicates. Branch support values above 75% are shown. The “Vespunovirus” sequences are shown in blue.

**References**

1. Pchelin IM, Tkachev PV, Azarov DV, Gorshkov AN, Drachko DO, Zlatogursky VV, Dmitriev AV, Goncharov AE (2023) A genome of temperate *Enterococcus* bacteriophage placed in a space of pooled viral dark matter sequences. Viruses 15(1):216. https://doi.org/10.3390/v15010216. PMID: 36680256.

2. Tisza MJ, Buck CB (2021) A catalog of tens of thousands of viruses from human metagenomes reveals hidden associations with chronic diseases. Proc Natl Acad Sci U S A 118(23):e2023202118. doi: 10.1073/pnas.2023202118. PMID: 34083435

3. Adriaenssens EM, Kropinski AM, Turner D, Krupovic M, Millard A, Dutilh BE, Oksanen HM, Lood C, Reyes A, Moraru C, et al. Abolish the order *Caudovirales* and the families *Myoviridae*, *Siphoviridae* and *Podoviridae* (*Caudoviricetes*). Taxonomic proposal 2021.001B. (https://ictv.global/filebrowser/download/6040, accessed o