

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

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| **Code assigned:** | ***2022.017B*** |  |
| **Short title:** Create a new genus (*Carnodivirus*) containing two new species (*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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**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 |

|  |  |  |
| --- | --- | --- |
| **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 | April 2022 |
| 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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| --- |
| 2022.017B.N.v1.Carnodivirus\_ng.xlsx |

**Abstract**

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| Carnobacterium phages cd2, cd3 and cd4 are lytic phages that infect various strains of *Carnobacterium divergens*. We have analyzed the genomes of these phages and propose that these phages constitute a new genus called *Carnodivirus*, within the class *Caudoviricetes*, where phages cd2 and cd3 belong to the same species and phage cd4 is a different species. |

**Text of proposal**

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| |  | | --- | | Recently, we isolated and sequenced three lytic phages (*Carnobacterium* phages cd2, cd3 and cd4) that infect various strains of *Carnobacterium divergens* (1). Phages cd2, cd3 and cd4 have genome sizes of 56.7-57.2 kb, with G+C content of 38.7-39.0%, and are predicted to encode for 109-111 proteins (Table 1). A BlastN search of the genomes did not find closely related bacteriophages. Electron microscopy shows that phage cd2 has an elongated head (length: 111 nm; width: 45 nm) and a long, noncontractile tail (length: 157 nm). This morphology reveals that phage cd2 is a siphophage and displays the B3 morphotype (Fig 1). Based on the morphology and sequencing data, we propose the following taxonomic changes:   * Creating a new genus, called *Carnodivirus*, within the class Caudoviricetes * Creating two new species, *Carnodivirus cd2* and *Carnodivirus cd4,* within this new genus.   **Rationale for change #1: Creating a new genus, *Carnodivirus***  We have chosen 70% sequence identity as the demarcation for a new genus (2). As described below, analyses by BlastN, CoreGenes, VipTree and Viridic all indicate that the proposed genus, *Carnodivirus*, is cohesive and distinct from other genera of viruses.   * BlastN reveals that phages cd2, cd3 and cd4 are related to each other (93-99% identity), but are unrelated to other reported phages as the top 10 matched nucleotide sequences displayed limited coverage (less than 5%) and identity (less than 75%). * ViPTree shows that phages cd2, cd3 and cd4 have limited homology to a group of *Enterococcus* and *Streptococcus* phages (genus *Saphexaviru*s) and *Listeria* phages (genus *Homburgvirus*) (Fig 2). A genomic alignment of phages cd2, cd3 and cd4 with these other phages illustrates that phages cd2, cd3 and cd4 are highly similar to each other, but distinct from phages in genera *Saphexaviru*s and *Homburgvirus* (Fig 3). * Using Viridic (2), a heatmap displaying the intergenomic similarity/dissimilarity between phages cd2, cd3 and cd4 with the phages in genera *Saphexaviru*s and *Homburgvirus* was generated (Fig 4). The results show that phages cd2, cd3 and cd4 display < 70% similarity to the other phages and therefore, comprise a new genus of phage. * Phylogenetic trees for the major capsid protein and primase enzymes show that phages cd2, cd3 and cd4 form a cohesive group, distinct from other known phages (Fig 5).   **Rationale for change #2: Create two new species within the genus *Carnodivirus***  We have chosen 95% sequence identity as the demarcation for a new species (2). As illustrated in the Viridic heatmap (Fig 4), phages cd2 and cd3 belong to the same species (98.9% nucleotide similarity to each other) whereas phage cd4 is a different species (less than 95% nucleotide similarity to phages cd2 and cd3). | |  | |

**Supporting evidence**

**Table 1.** Phage genomes belonging to the proposed genus *Carnodivirus*

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| Phage name | GenBank accession number | Genome size (bp) | GC content (%) | No. of ORFs | % nucleotide identity a | % shared proteinsb |
| Phage cd2 | MZ398135 | 57,220 | 39.0 | 111 | 100 | 100 |
| Phage cd3 | MZ398136 | 57,171 | 38.9 | 110 | 98.9 | 99 |
| Phage cd4 | MZ399596 | 56,713 | 38.7 | 109 | 91.1 | 85 |

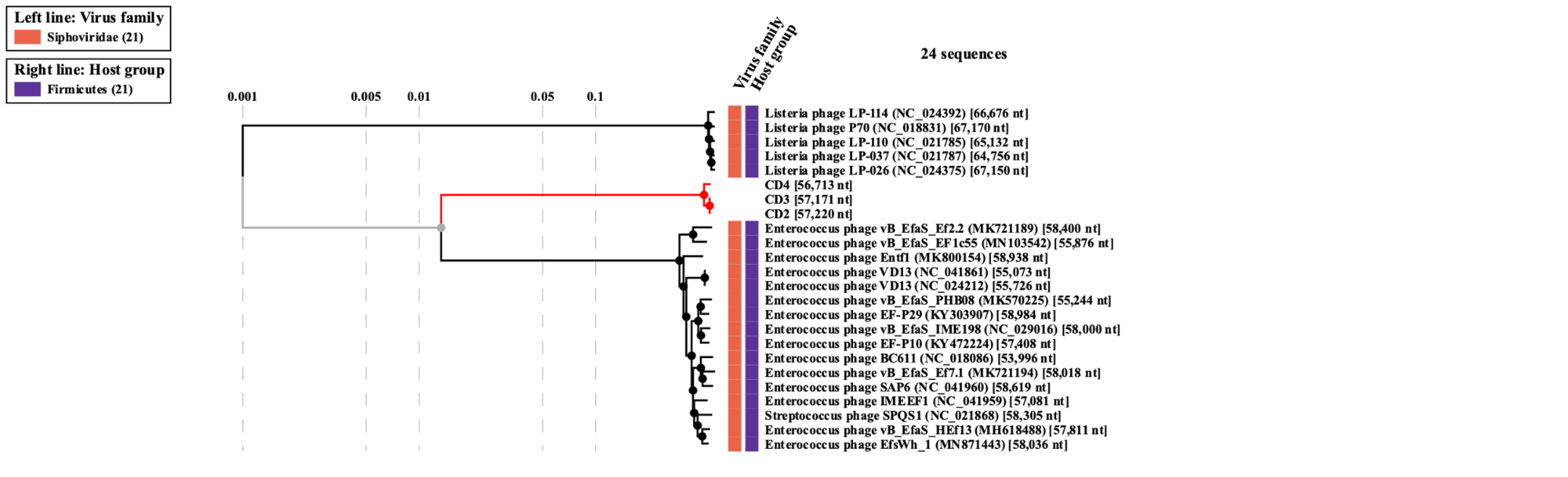
a Calculated by multiplying the query coverage by the percent identity values, as reported by BlastN.

b Relative to phage cd2, calculated using CoreGenes 3.5 (<http://binf.gmu.edu:8080/CoreGenes3.5/>) (3–5)

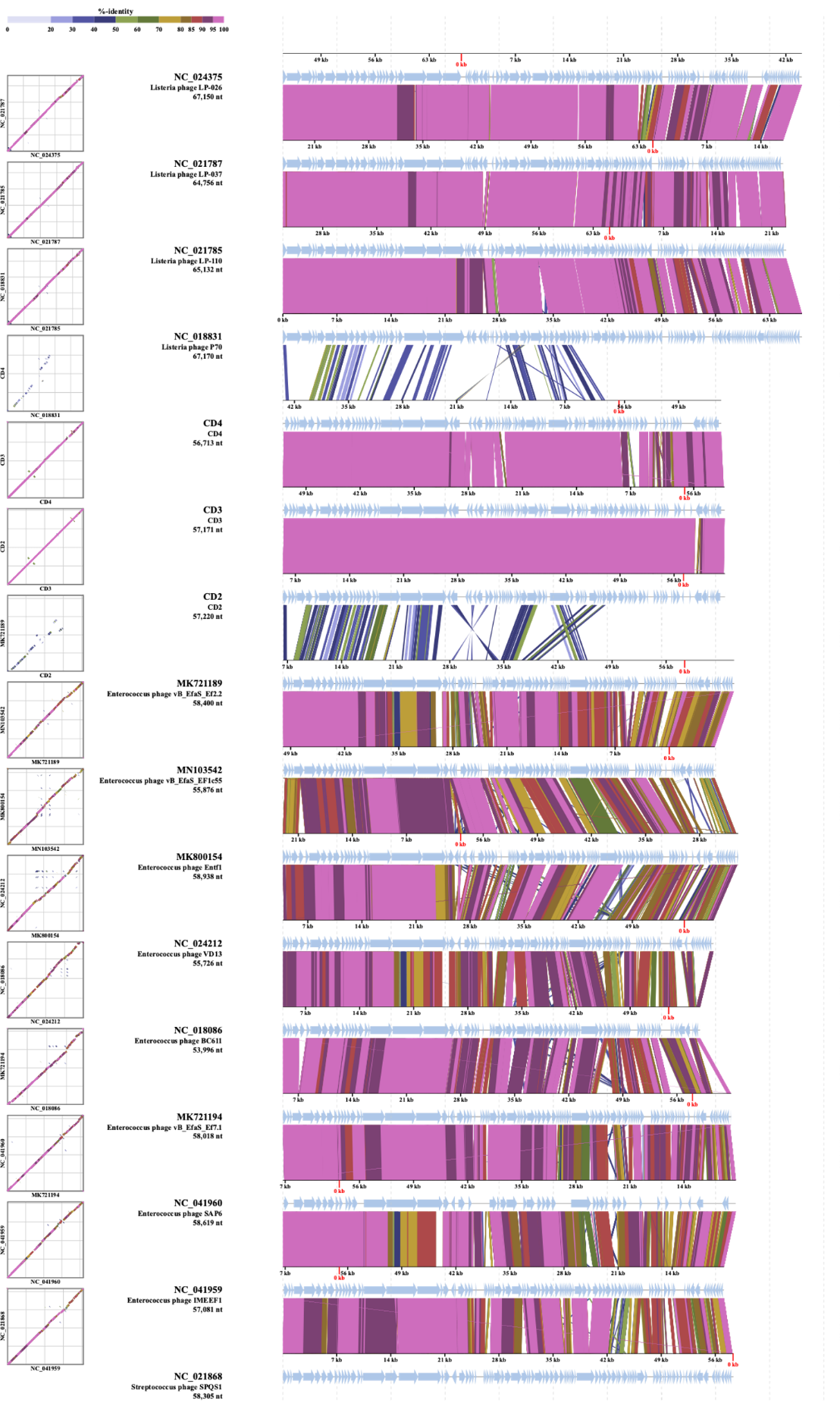
**A picture containing graphical user interface

Description automatically generated**

**Figure 1.** Electron micrographs of phage cd2 using cryo-EM (image 1) and TEM (image 2, staining with uranyl acetate), illustrating that it is a siphophage.



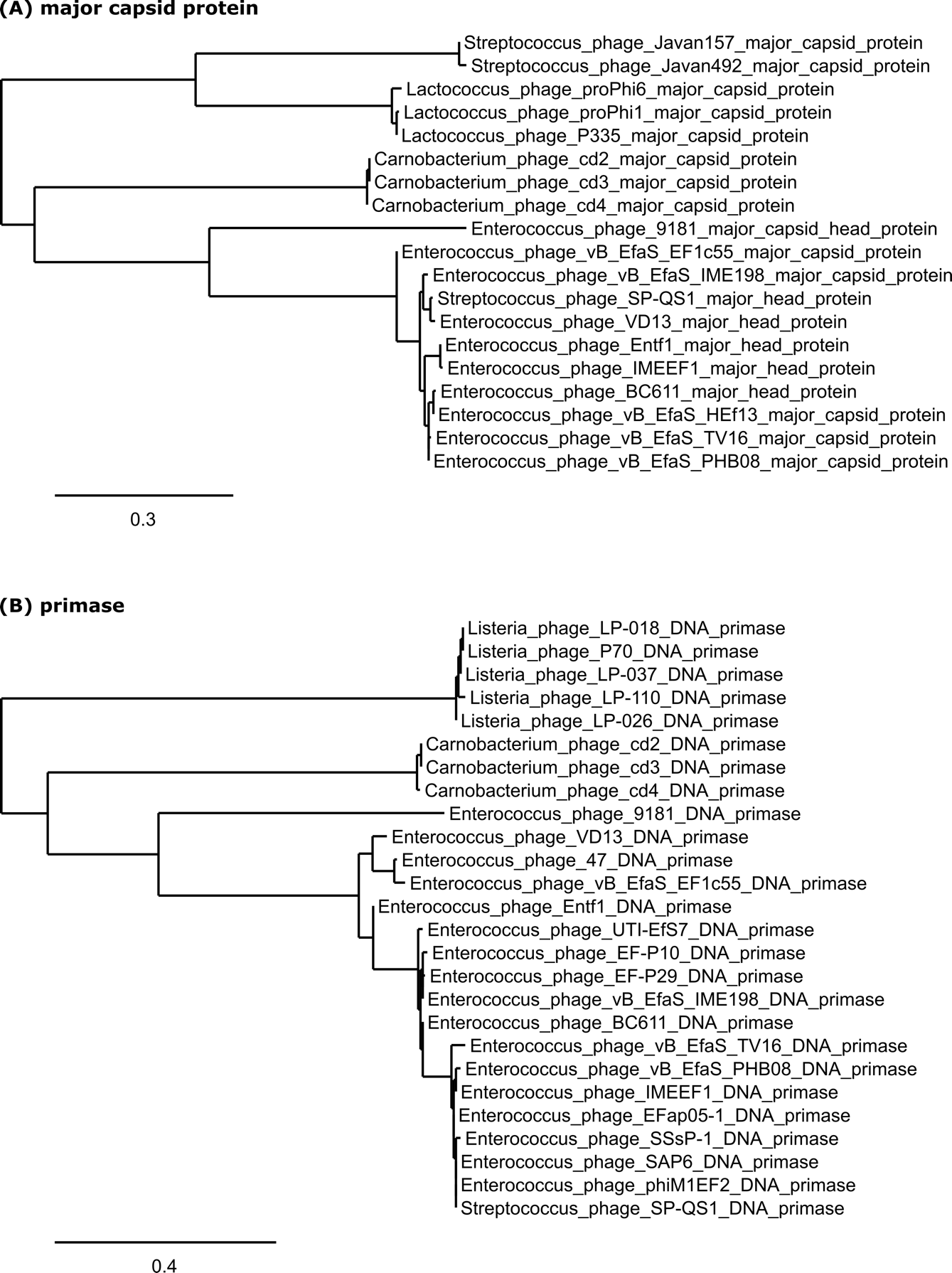
**Figure 2.** Proteomic tree for phages cd2, cd3 and cd4. Only viruses displaying a genomic similarity score greater than 0.05 (SG > 0.05) are included. Figure generated using ViPTree (6).



**Figure 3.** Genomic alignment of phages cd2, cd3 and cd4 with viruses belonging to genus *Homburgvirus* (Listeria viruses LP-026, LP-037, LP-110, P70) and viruses belonging to genus *Saphexovirus* (Enterococcus viruses vB\_EfaS\_Ef2.2, vB\_EfaS\_EF1c55, Entf1, VD13, BC611, vB\_EfaS\_Ef7.1, SAP6, IMEEF1 and Streptococcus phage SPQS1). The coloured vertical blocks indicate the level of nucleotide similarity between sequences. Sequences were adjusted and reordered to begin at the terminase small subunit. Figure generated using ViPTree (6).

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**Figure 4.** Intergenomic similarities heatmap generated with Viridic, using default settings (2). The results suggest that phages cd2, cd3 and cd4 comprise a new genus since they display < 70% similarity to the Listeria phages (LP-037, LP-110, LP-114, P70), Enterococcus phages (vB\_EfaS\_EF1c55, IME-EF1, vB\_EfaS\_HEf13, Entf1, vB\_EfaS\_PHB08, EF-P29, vB\_EfaS\_IME198, VD13, BC611, SAP6, vB\_EfaS\_Ef7.1) and Streptococcus phage SPQS1. Within this new genus, phages cd2 and cd3 are the same species (>95% similarity), while phage cd4 is a different species (<95% similarity compared to phages cd2 and cd3).



**Figure 5:** Phylogenetic trees for the major capsid proteins (A) and DNA primases (B) for phages cd2, cd3 and cd4 and other related phages, as identified by BLASTP. Figure generated using “one click” mode by Phylogeny.fr (<http://www.phylogeny.fr/>) (7). The positions of phages cd2, cd3 and cd4 are indicated with a yellow box.

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