

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

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| **Code assigned:** | **to be assigned by ICTV officers** |  |
| **Short title:** Create two new genera (*Deltatectivirus* and *Epsilontectivirus*) including three new species (*Kalamavirales*: *Tectiviridae*) | | |
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**List the ICTV Study Group(s) that have seen this proposal**

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| *Tectiviridae* Study Group, Bacterial and Archaeal Viruses Subcommittee |

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

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

|  |  |  |
| --- | --- | --- |
| **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 | July 2020 |
| Date of this revision (if different to above) |  |

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

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

**Name of accompanying Excel module**

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| 2020.042B.R.Deltatectivirus\_Epsilontectivirus |

**Abstract**

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| The 2019 Master Species List describes only seven species of tectiviruses divided in three genera. Three new tectiviruses infecting *Actinobacteria* have been recently identified and their genome fully sequenced. This proposal introduces two new genera: *Deltatectivirus,* including two species, and *Epsilontectivirus*, including one single species. |

**Text of proposal**

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| |  | | --- | | **Species demarcation criteria:** We have chosen 95% DNA sequence identity as the criterion for demarcation of species in these new genera. Each of the proposed species differs from the others with more than 5% at the DNA level as confirmed with the BLASTN algorithm. | |

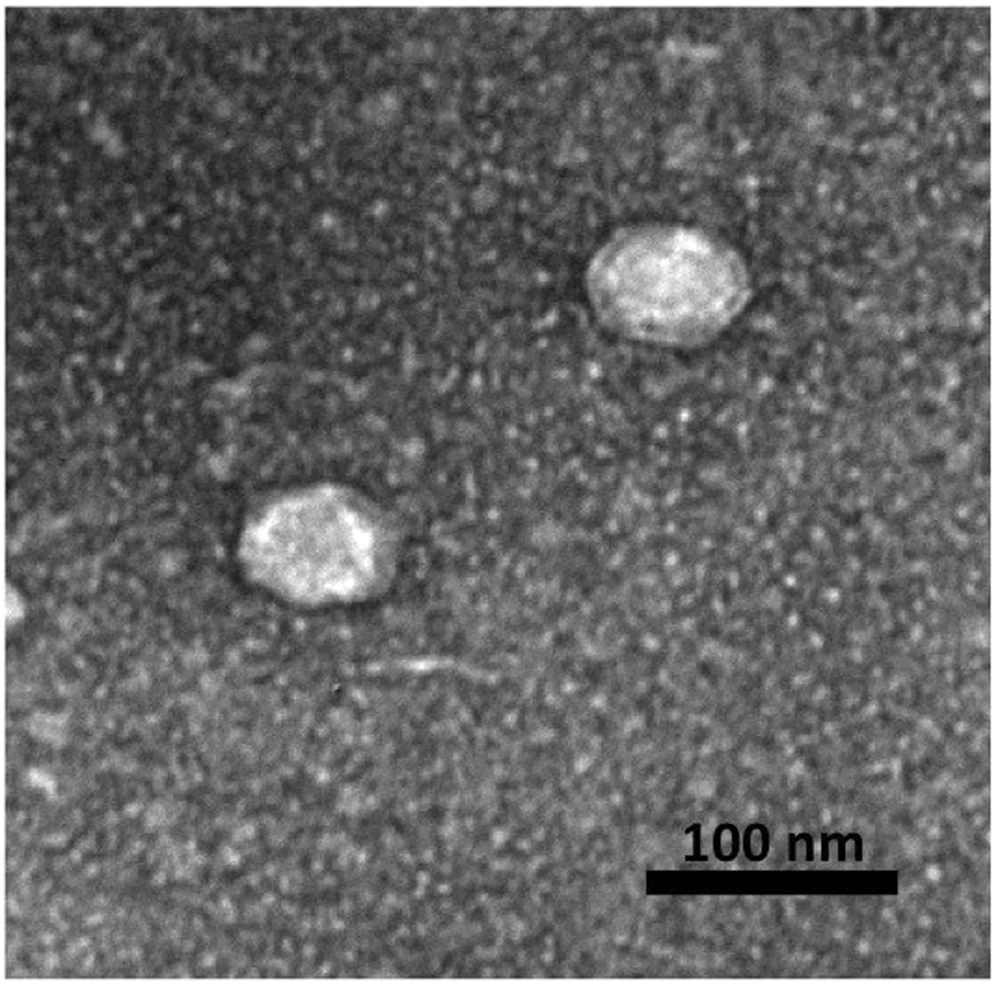
**Proposal 1:** **To create the new genus *Deltatectivirus*, including two (2) new species**

**Proposal 2: To create the new genus *Epsilontectivirus*, including one (1) new species**

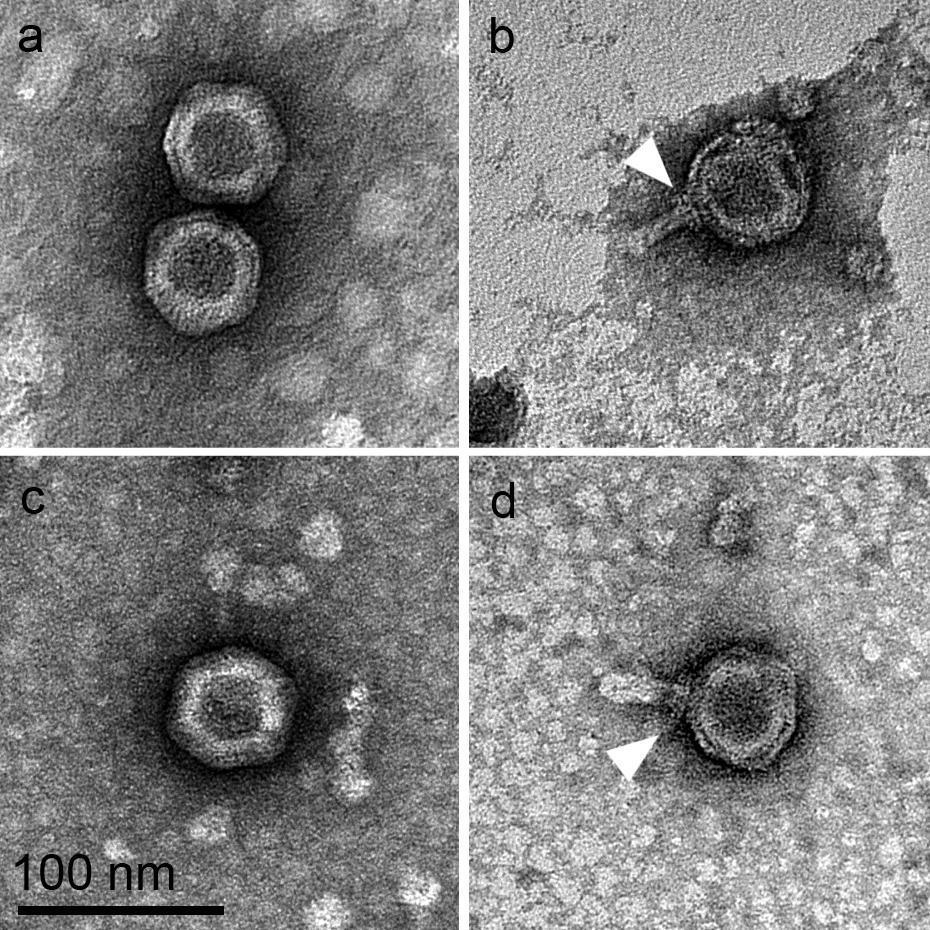
**Supporting evidence**

The lytic **Rhodococcus phage** **Toil** was isolated from a soil sample collected near an open-air-aerobic digestion basin of a wastewater treatment plant [1]. Toil infects *Rhodococcus opacus*, a mycolic acid-producing member of the order *Corynebacteriales*. Transmission electron microscopy (TEM) showed double-layered, tail-less icosahedral particles with a diameter of ~54 nm (Fig. 1). Based on its morphology, the presence of a lipid membrane, its genome architecture, the presence of terminal covalently-linked proteins and chloroform sensitivity, phage Toil is placed within the family *Tectiviridae*. Toil is the first reported tectivirus capable of infecting a member of the *Actinobacteria*. Phage toil genome is a non-permuted, linear dsDNA of 17,253 bp, containing 72 bp imperfect inverted terminal repeats with 75% identity, and encoding 35 predicted proteins. Only eight of these predicted proteins have any similarity to other proteins in the database as detectable by BLASTp at E < 0.001, and no Toil protein has directly detectable similarity (BLASTp, E < 10) with any proteins found in other tectivirus genomes. However, functional annotation of Toil genome identified several proteins that provide key tectiviral functions: DNA polymerase, DNA packaging ATPase, the major coat protein, the entry lysozyme, a receptor-binding protein, and the endolysins.

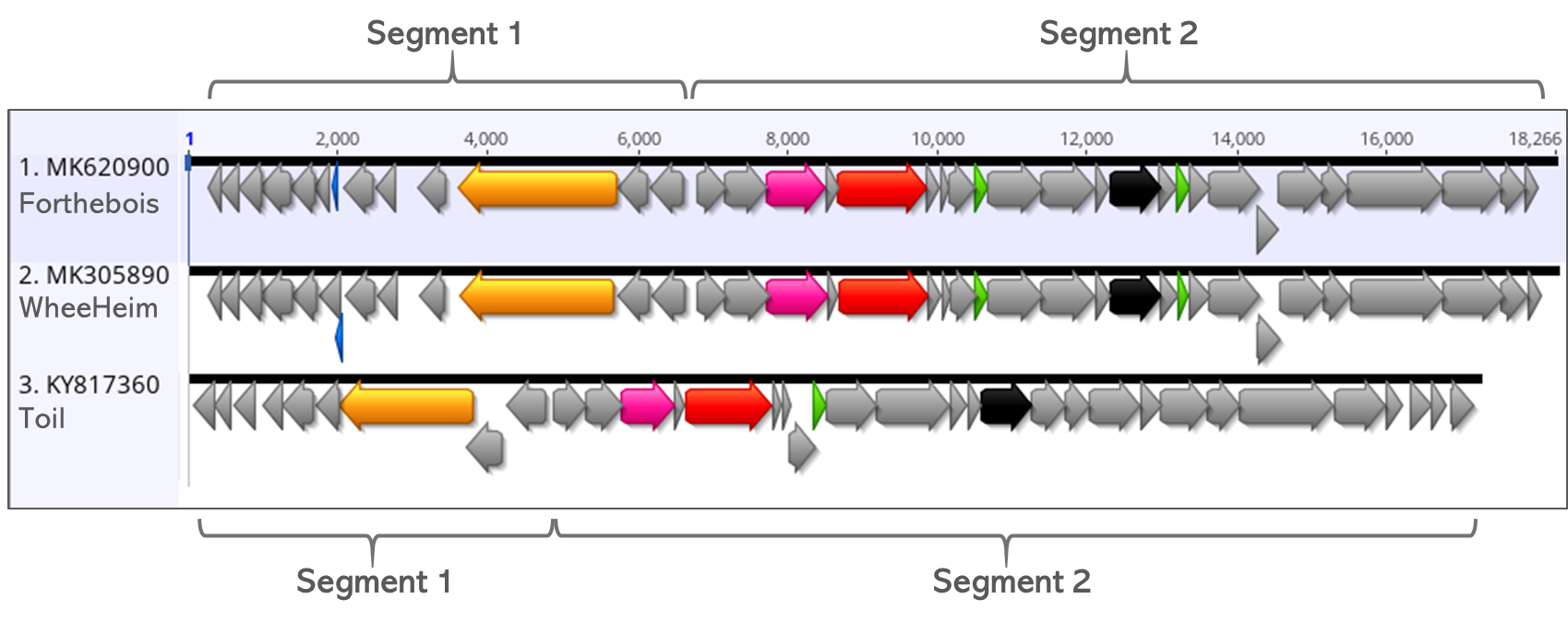
**Streptomyces phage WheeHeim** and **Streptomyces phage Forthebois** were isolated from soil samples. Both phages are temperate (lysogenic) and infect the plant pathogen *Streptomyces scabiei* [2]. TEM images of WheeHeim and Forthebois revealed tail-less viral particles with six-sided, icosahedral double-layered phage capsids (Fig. 2a, c) with a diameter of ~65.5 nm and ~64.4 nm, respectively. Several virion particles displayed protruding nanotubes (Fig. 2b, d) with a respective average length of 37.0 nm (WheeHeim) and 41.6 nm (Forthebois), consistent with the previous observation that the tectiviral lipid membrane becomes a tail-like structure used in genome delivery [3]. The genomes of both phages are linear dsDNA molecules of 18,266 bp (WheeHeim) and 18,251 bp (Forthebois), containing 24 bp inverted repeats at their ends, and encoding 36 predicted proteins. Additionally, both genomes have a tRNA-Asn gene. Both genomes also present the same overall two-segment arrangement, with a first segment of genes in the reverse strand and a second, larger one, in the forward strand. This arrangement is reminiscent of the one reported for phage Toil (Fig. 3) [1]. The first segment encompasses genes coding for a DNA polymerase, a single-stranded DNA binding protein and a homolog of MazG. The second, larger segment contains structural genes and genes involved in cell lysis, such as a homolog of the *Alphatectivirus* PRD1 major capsid protein, a LysM-like endolysin, membrane DNA delivery protein, a hydrolase and a glycosyltransferase. Based on their morphology, the presence of a lipid membrane, their genome architecture and the presence of terminal covalently-linked proteins, phages WheeHeim and Forthebois are placed within the family *Tectiviridae*.



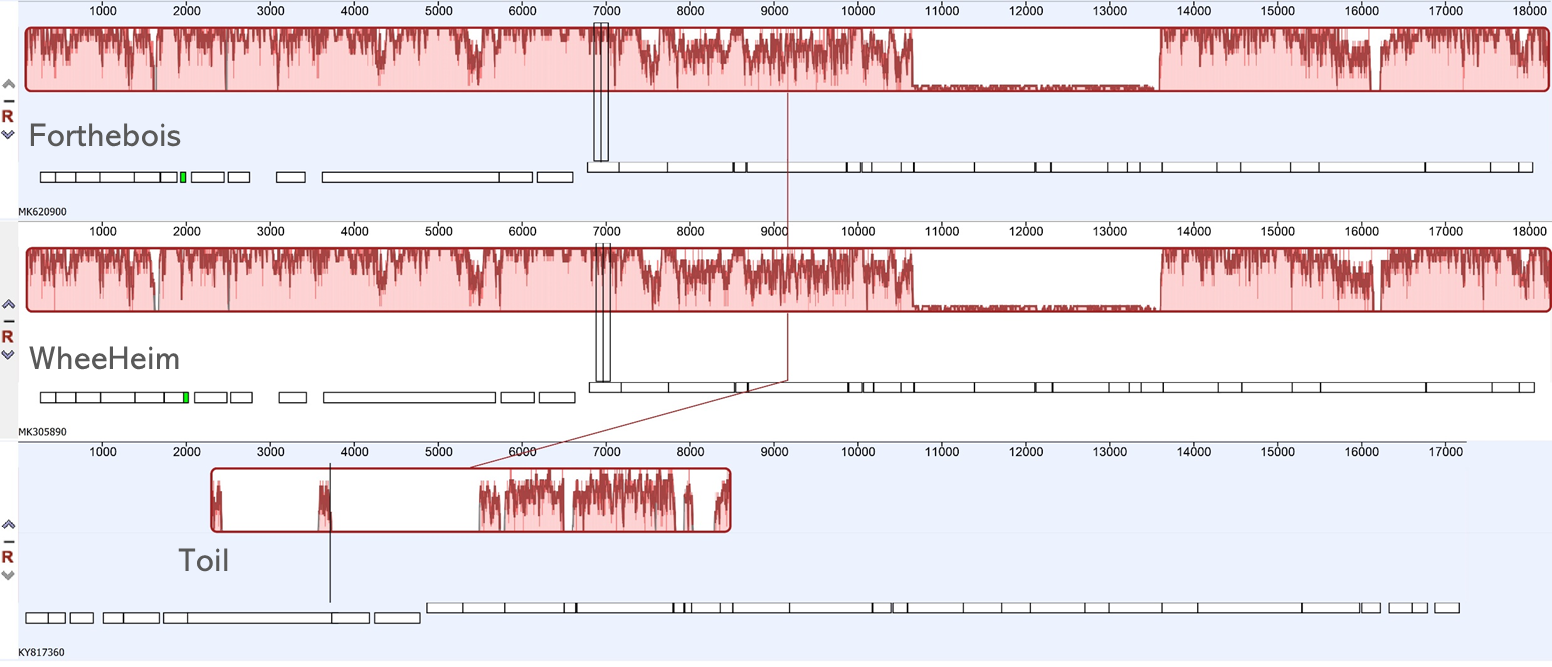
**Figure 1.** Transmission electron micrograph of Toil virions. Scale bar, 100 nm. The figure is reproduced from Ref [1] (*Scientific Reports*, 2018, CC BY 4.0).

**Figure 2.** Transmission electron micrographs of WheeHeim **(a, b)** and Forthebois **(c, d)** virions. Micrographs on the right show the nanotube protruding from the capsids. White arrowheads indicate collar-like structure. Scale bar, 100 nm for all panels. The figure is reproduced from Ref [2] (*Viruses*, 2019, CC BY 4.0).

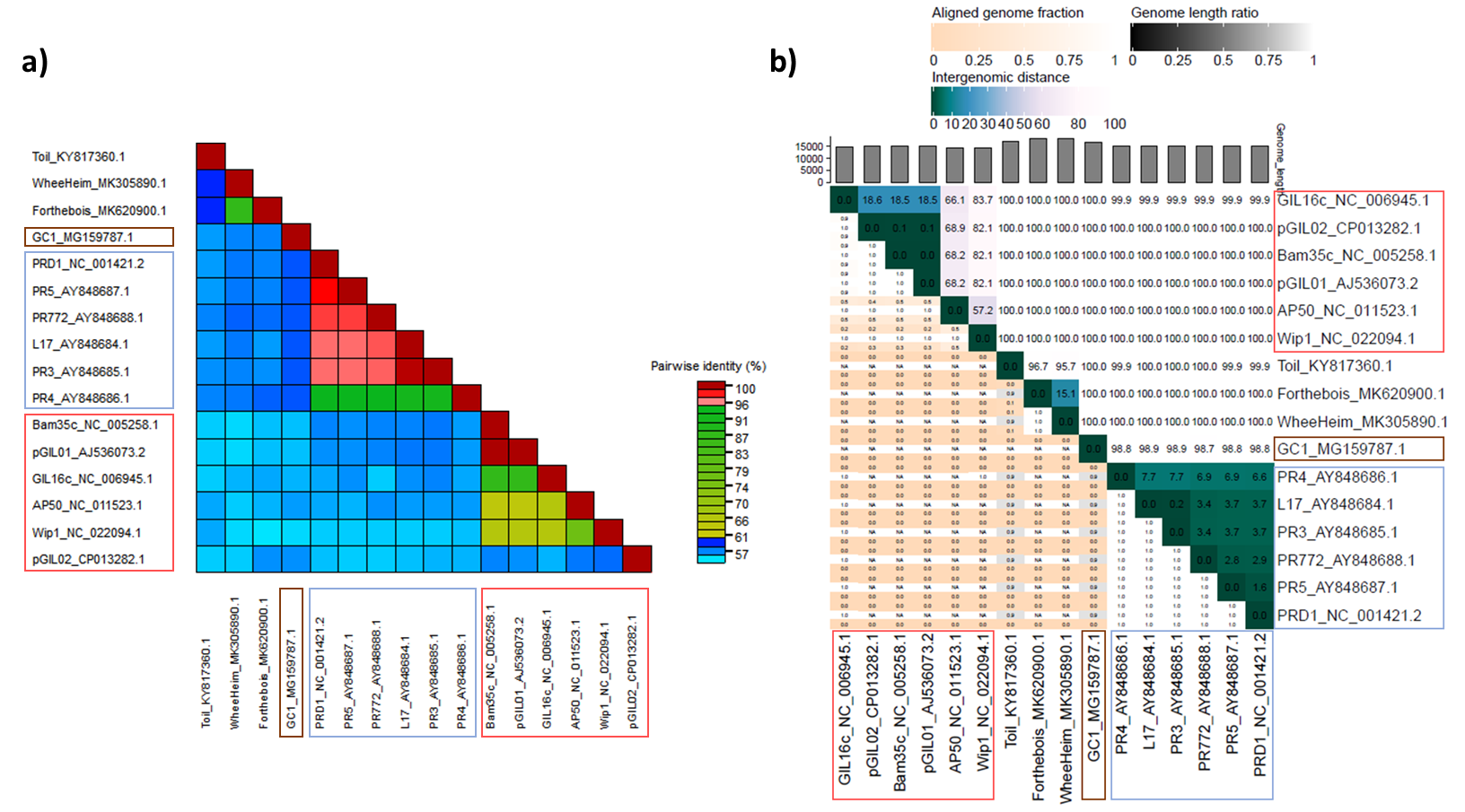
The taxonomical placement of Rhodococcus phage Toil and Streptomyces phages WheeHeim and Forthebois was investigated through genome organization comparisons (Fig. 3), genome alignments (Fig. 4), DNA sequence identity comparisons (Fig. 5, Tables 1 and 2), phylogenetic analyses (Fig. 6 and 7). Genome-wide comparisons placed these three phages separated from other tectiviruses belonging to the genera *Alphatectivirus, Betatectivirus* and *Gammatectivirus* (Fig. 6 and 7). Also, phages WheeHeim and Forthebois clustered together and phage Toil formed a well-supported sister group of WheeHeim and Forthebois. Phylogenetic analysis and pairwise intergenomic distances between all completely sequenced tectiviruses (Fig. 5-7, Tables 1 and 2), confirmed that tectiviruses WheeHeim, Forthebois and Toil are highly divergent members of the family *Tectiviridae*, and cannot be placed into the three existing genera based on the ICTV recommended demarcation criteria. Thus, we propose that Streptomyces phage WheeHeim be classified as a type species of a new genus, ***Deltatectivirus***, within the family *Tectiviridae*; Streptomyces phage Forthebois be classified as a new species of genus *Deltatectivirus*; and Rhodococcus phage Toil be classified as a type species of a new genus, ***Epsilontectivirus***, within the family *Tectiviridae*. All performed analysis indicate that the proposed genera, *Deltatectivirus* and *Epsilontectivirus*, are cohesive and distinct from each other.



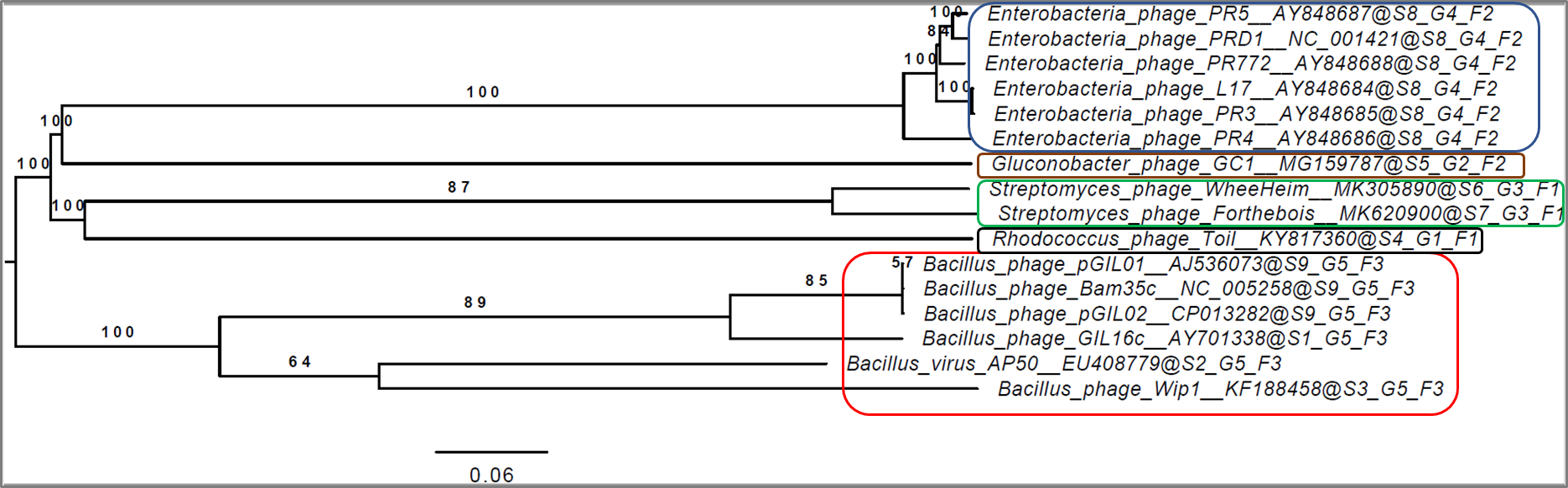
**Figure 3.** Functional maps of phages Forthebois, WheeHeim and Toil. Predicted CDSs and their direction of transcription are represented as block arrows. Predicted function of major tectiviral genes are color-coded: yellow, DNA polymerase; fuchsia, packaging ATPase; red, major capsid protein; green, phage membrane protein; black, lytic transglycosylase; blue, tRNA-Asn. *Segment 1* indicates genes transcribed from the reverse strand and *Segment 2* highlights genes transcribed from the forward strand. GenBank accession numbers for each phage are indicated on the left. The ruler represents base pairs. Map was generated using Geneious Prime® 2020.1.2 (http://www.geneious.com) [4].



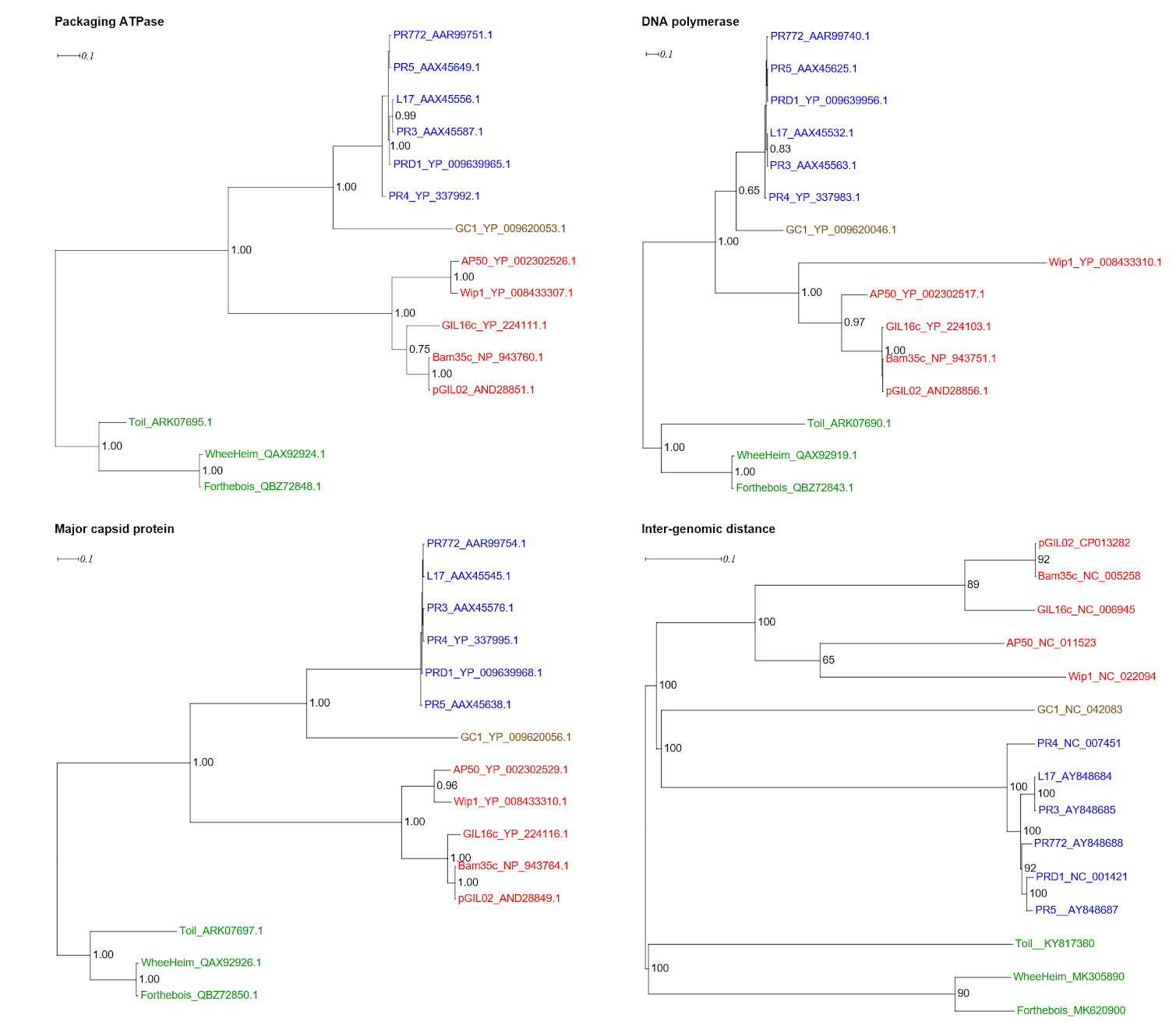
**Figure 4.** Progressive MAUVE [5] alignment of the phages Forthebois, WheeHeim and Toil. Progressive MAUVE uses a sum-of-pairs breakpoint score, which facilitates accurate detection of rearrangement breakpoints when genomes have unequal gene content [5]. Each genome is displayed horizontally with annotated coding regions shown as white boxes. GenBank accession numbers for each phage are indicated.

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**Figure 5.** Graphical representation of percent pairwise nucleotide sequence identity of complete genomes of phages belonging to the family *Tectiviridae*. **a)** Percent pairwise nucleotide sequence identity calculated using the Sequence Demarcation Tool (SDTv1.2) (www.cbio.uct.ac.za/SDT) [6]. SDT aligns every unique pair of sequences, calculates pairwise similarity scores, and displays a color-coded matrix of these scores [6]. The color scale bar indicates the percentage of nucleotide sequence identity among 16 tectiviruses. **b)** Virus Intergenomic Distance Calculator (VIRIDIC, viridic.icbm.de) [7] generated heatmap incorporating intergenomic similarity values (right half) and alignment indicators (left half and top annotation). VIRIDIC computes pairwise intergenomic distances/similarities amongst phage genomes. In the right half, the color coding allows a rapid visualization of the clustering of the phage genomes based on intergenomic similarity: the closer related the genomes, the darker the color [7]. GenBank accession numbers for each phage are indicated. Color boxes highlight phages belonging to *Alphatectivirus* (blue), *Betatectivirus* (red), and *Gammatectivirus* (brown).



**Figure 6.** Genome-wide nucleotide phylogeny of tectiviruses. The analysis was conducted with VICTOR Virus Classification and Tree Building Online Resource (https://victor.dsmz.de) [8] with settings recommended for prokaryotic viruses. The analysis yielded five genera (G1–G5). The members of the different genera are highlighted by color boxes: *Alphatectivirus* (blue), *Betatectivirus* (red) *Gammatectivirus* (brown), *Deltatectivirus* (green), and *Epsilontectivirus* (black).



**Figure 7.** Phylogenetic trees resulting from Bayesian inference on multiple sequence alignments of the packaging ATPase, the DNA polymerase and the major capsid protein sequences, and from minimal evolution inference on BLAST-derived inter-genomic distances. Only support values above 0.5 probability or 50% bootstrap support are shown. Trees were rooted arbitrarily for display purposes. Colors denote phages belonging to *Alphatectivirus* (blue), *Betatectivirus* (red), *Gammatectivirus* (brown) and phages Toil, WheeHeim and Forthebois (green). The figure is reproduced from Ref [2] (*Viruses*, 2019, CC BY 4.0).

**Proposal 1: To create the new genus *Deltatectivirus*, including two (2) new species**

**Genus demarcation:** BLASTN, genomic comparisons (Fig.4), pairwise nucleotide sequence identity (Fig. 5), and phylogenetic analyses (Fig. 6 and 7), all indicate that the proposed genus, *Deltatectivirus*, is cohesive and distinct from other genera.

**Type species:** *Streptomyces virus WheeHeim*. WheeHeim was the first described member of this genus.

**Genus name:** *Deltatectivirus*; from *delta*, the fourth letter of the Greek alphabet, indicating fourth genus of this family and from Latin *tectus*, meaning “covered”.

**Specific reference:** Caruso SM, deCarvalho TN, Huynh A, Morcos G, Kuo N, Parsa S, Erill I. A novel genus of actinobacterial *Tectiviridae*. Viruses 2019; 12: 1134.

**Species demarcation criteria:** we have chosen 95% DNA sequence identity as the criterion for demarcation of species in this new genus. The members of the proposed species differ from each other with more than 5% at the DNA level, as confirmed with the BLASTN algorithm (Table 2).

Table 2. Properties of the phages in genus *Deltatectivirus*.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Phage Name** | **INSDC** | **Genome length (bp)** | **% G+C** | **No. of CDSs** | **No. tRNA** | **% DNA**  **sequence**  **identity\*** |
| Streptomyces phage WheeHeim † | MK305890 | 18266 | 54.6 | 36 | 1 | 100 |
| Streptomyces phage Forthebois # | MK620900 | 18251 | 53.6 | 36 | 1 | 90.5 |

\* Determined by NCBI BLASTN, using the MK305890 sequence as reference.

† Represents a new type species.

# Represents a new species.

**Electron micrograph:** see figure 2.

**Proposal 2: To create the new genus *Epsilontectivirus*, including one (1) new species**

**Genus demarcation:** BLASTN, genomic comparisons (Fig.4), pairwise nucleotide sequence identity (Fig. 5), and phylogenetic analyses (Fig. 6 and 7), all indicate that the proposed genus, *Epsilontectivirus*, is cohesive and distinct from other genera.

**Type species:** *Rhodococcus virus Toil*. Toil is the only described member of this genus.

**Genus name:** *Epsilontectivirus*; from *epsilon*, the fifth letter of the Greek alphabet, indicating fifth genus of this family and from Latin *tectus*, meaning “covered”.

**Specific reference:** Gill JJ, Wang B, Sestak E, Young R, Chu, K-H. Characterization of a novel tectivirus phage Toil and its potential as an agent for biolipid extraction. Sci. Rep. 2018; 8: 1062.

**Species demarcation criteria:** Not applicable. There is only one species in the genus (Table 1).

Table 1. Properties of the phage in genus *Epsilontectivirus*.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Phage Name** | **INSDC** | **Genome length (bp)** | **% G+C** | **No. of CDSs** | **No. tRNA** |
| Rhodococcus phage Toil † | KY817360 | 17253 | 54.5 | 35 | 0 |

† Represents a new type species.

**Electron micrograph:** see figure 1.

**References**

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