This form should be used for all taxonomic proposals. Please complete all those modules that are applicable (and then delete the unwanted sections). For guidance, see the notes written in blue and the separate document "Help with completing a taxonomic proposal"

Please try to keep related proposals within a single document; you can copy the modules to create more than one genus within a new family, for example.

MODULE 1: **TITLE, AUTHORS, etc**

Code assigned:	***2009.009a-pB***	(to be completed by ICTV officers)

Short title: Create the subfamily *Spounavirinae* in the family *Myoviridae*, order *Caudovirales*
(e.g. 6 new species in the genus *Zetavirus*)

Modules attached (modules 1 and 9 are required)	**1** ☒	**2** ☒	**3** ☒	**4** ☐	**5** ☐
	6 ☐	**7** ☒	**8** ☐	**9** ☒	

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Has this proposal has been seen and agreed by the relevant study group(s)? Please select answer in the box on the right	**Yes**

ICTV-EC or Study Group comments and response of the proposer:

Date first submitted to ICTV:
Date of this revision (if different to above): 2010-06-03

MODULE 2: **NEW SPECIES**

Part (a) to create and name one or more new species.
If more than one, they should be a group of related species belonging to the same genus (see Part b)

Code	***2009.009aB***	(assigned by ICTV officers)

To create 5 new species with the name(s):

Staphylococcus phage Twort
Staphylococcus phage K
Staphylococcus phage G1
Listeria phage P100
Listeria phage A511

Part (b) assigning new species to higher taxa
All new species must be assigned to a higher taxon. This is usually a genus although it is also permissible for species to be "unassigned" within a subfamily or family.

Code	***2009.009bB***	(assigned by ICTV officers)

To assign the species listed in section 2(a) as follows:

Genus:	***Twortlikevirus* (new)**
Subfamily:	***Spounavirinae (new)***
Family:	***Myoviridae***
Order:	***Caudovirales***

Fill in all that apply.
- If the higher taxon has yet to be created (in a later module, below) write "**(new)**" after its proposed name.
- If no genus is specified, enter "**unassigned**" in the genus box.

Reasons to justify the creation and assignment of the new species:
- Explain how the proposed species differ(s) from all existing species.
  - If species demarcation criteria (see module 3) have previously been defined for the genus, explain how the new species meet these criteria.
  - If criteria for demarcating species need to be defined (because there will now be more than one species in the genus), please state the proposed criteria.
- Provide GenBank accession numbers (not RefSeq accessions) for genomic sequences
- Further material in support of this proposal may be presented in the Appendix, Module 9

Genomic and proteomic comparison, the presence of unique genome regions (cfr Table 1 & local absence of BLASTN similarity) and different host specificity. Bacteriophages are morphologically identical (Figures 5, 6).

MODULE 2: **NEW SPECIES**

Part (a) to create and name one or more new species.
If more than one, they should be a group of related species belonging to the same genus (see Part b)

Code	***2009.009cB***	(assigned by ICTV officers)

To create 3 new species with the name(s):

Enterococcus phage phiEC24C
Lactobacillus phage LP65
Lactobacillus phage Lb338-1

Part (b) assigning new species to higher taxa
All new species must be assigned to a higher taxon. This is usually a genus although it is also permissible for species to be "unassigned" within a subfamily or family.

Code	***2009.009dB***	(assigned by ICTV officers)

To assign the species listed in section 2(a) as follows:

Genus:	**unassigned**
Subfamily:	***Spounavirinae (new)***
Family:	***Myoviridae***
Order:	***Caudovirales***

Fill in all that apply.
- If the higher taxon has yet to be created (in a later module, below) write "**(new)**" after its proposed name.
- If no genus is specified, enter "**unassigned**" in the genus box.

Reasons to justify the creation and assignment of the new species:

- Explain how the proposed species differ(s) from all existing species.
  - If species demarcation criteria (see module 3) have previously been defined for the genus, explain how the new species meet these criteria.
  - If criteria for demarcating species need to be defined (because there will now be more than one species in the genus), please state the proposed criteria.
- Provide Genbank accession numbers (not RefSeq accessions) for genomic sequences
- Further material in support of this proposal may be presented in the Appendix, Module 9

Morphologically similar to other *Spounavirinae*, but genome organization, proposed genome structure and proteomic comparison (Cfr Module 9) set them apart from members of the genera "SPO1-like viruses" (proposed name *Spo1likevirus*) and *Twortlikevirus*.

MODULE 3: **NEW GENUS**

creating and naming a new genus

Code	***2009.009eB***	(assigned by ICTV officers)
To create a new genus to contain the species listed below		

Code	***2009.009fB***	(assigned by ICTV officers)
To name the new genus: *Twortlikevirus*		

assigning a new genus to higher taxa

Code	***2009.009gB***	(assigned by ICTV officers)

To assign the new genus as follows: Ideally, a genus should be placed within a higher taxon, but if not, write "**unassigned**" in the box below.

Subfamily:	***Spounavirinae (new)***
Family:	***Myoviridae***
Order:	***Caudovirales***

If any of these taxa has yet to be created (in module 4, 5 or 6) please write "**(new)**" after its proposed name.

assigning type species and other species to a new genus

Code	***2009.009hB***	(assigned by ICTV officers)

To designate the following as the type species of the new genus

Staphylococcus phage Twort

Every genus must have a type species. This should be a well characterized species although not necessarily the first to be discovered

Code	***2009.009iB***	(assigned by ICTV officers)

To assign the following as additional species of the new genus:

Staphylococcus phage K
Staphylococcus phage G1
Listeria phage P100
Listeria phage A511

Reasons to justify the creation of a new genus:

Additional material in support of this proposal may be presented in the Appendix, Module 9

These viruses form a fairly homogeneous group of virulent phages infecting staphylococci (Twort, G1, K) (Kwan et al., 2005) and *Listeria* (A511, P100) (Carlton et al., 2005; Klumpp et al., 2008). The group is named after phage "Twort," which may be a descendant of the original bacteriophage described by F.W. Twort in 1915 (Twort, 1915). (Figure 5) Apparently, this phage was deposited at the Pasteur Institute of Paris in 1947 when Twort was invited there to retell the story of his discovery (personal communication by J.-F. Vieu, curator of the phage collection of the Pasteur Institute; 1983).

Origin of the new genus name:

After type species

Reasons to justify the choice of type species:

This is the first sequenced phage of this genus

Species demarcation criteria in the new genus:

- If there will be more than one species in the new genus, list the criteria being used for species demarcation and explain how the proposed members meet these criteria.
- Provide GenBank accession numbers (not RefSeq accessions) for genomic sequences of new species

Cfr. Modules 2

MODULE 4: **NEW SUBFAMILY**

creating and naming a new subfamily

Code	***2009.009jB***	(assigned by ICTV officers)

To create a new subfamily containing the genera listed below

Code	***2009.009kB***	(assigned by ICTV officers)

To name the new subfamily: *Spounavirinae*

assigning a new subfamily to a family

Code	***2009.009lB***	(assigned by ICTV officers)

To assign the new subfamily as follows:

Family:	***Myoviridae***
Order:	***Caudovirales***

If the family has yet to be created (in Module 5) please write "**(new)**" after the proposed name.
If there is no Order, write "**unassigned**" here.

genera and species assigned to the new subfamily

Code	***2009.009mB***	(assigned by ICTV officers)

genera assigned to the new subfamily

You may list several genera here. For each genus, please state whether it is new or existing.

- If the genus is new, it must be created in Module 3
- If the genus already exists, please state whether it is currently unassigned or is to be removed from another family. If the latter, complete Module 7 to 'REMOVE' it from that family

"SPO1-like viruses"

***Twortlikevirus* (new)**

Code	***2009.009nB***	(assigned by ICTV officers)

unassigned species in the new subfamily (i.e. within the subfamily but not assigned to any genus):

You may list several species here. For each species, please state whether it is new or existing. If the species is new, it must be created in Module 2

Enterococcus phage phiEC24C
Lactobacillus phage LP65

Lactobacillus phage Lb388-1

Reasons to justify the creation of the new subfamily:

Additional material in support of this proposal may be presented in the Appendix, Module 9

This proposed subfamily contains the ICTV-recognized genus "SPO1-like viruses" (proposed name *Spo1likevirus*) and, on the basis of our proteomic clustering (Figures 2, 3), a new genus (*Twortlikevirus*) and three peripherally related viruses: *Lactobacillus plantarum* phage LP65 (Chibani-Chennoufi et al., 2004), *Lactobacillus paracasei* phage Lb338-1 (Alemayehu et al., 2009) and *Enterococcus faecalis* phage phiEF24C (Uchiyama et al., 2008a & b). All of these are virulent, broad-host range phages which infect members of the *Firmicutes*. They possess

isometric heads of 84.5-94 nm in diameter and conspicuous capsomers, striated 140-219 nm long tails, a double base plate, and globular structures at the tail tip (Klumpp et al., 2010; Parker et al., 1983; Stewart et al., 2009). The latter have been resolved as base plate spikes and short kinked tail fibers with six-fold symmetry (Klumpp et al., 2008). Members of this group usually possess large (127–142 kb) nonpermuted genomes with additional 3.1–20 kb terminal redundancies (Allan et al., 1989; Perkus et al., 1985, Klumpp et al., 2008) (Table 2). The proposed name for this subfamily is derived from SPO plus *una* (latin for "one").

While the head diameter of *Bacillus* phage SPO1, of 84.5 (Parker et al., 1983) or 87 nm (Klumpp et al., 2010), is consistent with membership in the group, its tail is significantly shorter than that of most members (140-152 nm) (Figure 6), and, the DNA contains 5-hydroxymethyl uracil (HMU) rather than thymine (Table 2) (Stewart et al., 2009). No pronounced genome collinearity between SPO1 and the other *Spounavirinae* members is visible in whole-genome dot plots (Figure 4), but SPO1 clusters with all proposed candidate phages, when comparing large terminase subunit and major capsid protein sequences (Figure 3). A more closely related group is formed by *Listeria* phages A511 and P100 and *Staphylococcus* phages Twort and K. These phages are united by morphology (Figure 5, 6) genome collinearity (Figure 4) and protein similarities (Figure 2, 3). The outliers of this group comprise phages LP65, Lb338-1 and φEF24C. At 193 nm, the tail of phage LP65 is similar in length to that of other members of this group, however, it is more distantly related to the other phages on protein level (Figure 2, 3) and its genome is supposedly not terminally redundant (although no experimental evidence is presented by the authors). The genome size (142 kb), genome collinearity (Figure 4), proteome (Figure 2, 3) and morphology of *Enterococcus* phage φEF24C is clearly consistent with membership in this group (head diameter 93 nm; tail length 204-208 nm), but its genome is supposed to be circularly permuted (again, no experimental evidence is presented by the authors). A recent paper on these phages has commented on their close relationship (Klumpp et al., 2008). The third orphan, bacteriophage Lb338-1, exhibits the morphological characteristics of the *Spounavirinae* with a head diameter of 85 nm and a tail length of 200 nm (Alemayehu et al., 2009). However, no genome collinearity is observable (Figure 4) and Lb388-1 seem to be more distantly related to other phages of the proposed subfamily on protein level (Figure 3), although structural proteins display protein homologies to φEF24C, K, Twort, P100 and A511. Furthermore, no redundant genome ends could be found by the authors (Alemayehu et al., 2009).

Using a BLASTP raw threshold score of 100 and CoreGenes 3.0 (http://binf.gmu.edu:8080/CoreGenes3.0/) to compare the proteomes of Twort, A511, LP65, and φEF24C against SPO1, we identified two clusters of genes which are conserved. These corresponded to morphogenesis genes from the large terminase subunit (SPO1 gp*2.11* to gp*16.2*), most likely reflecting the common morphology of these phages (Figure 5, 6); and the cluster of replication genes, including helicase, exonuclease, primase, and resolvase (SPO1 gp*19.5* – gp*24.1*). The DNA polymerases (SPO1 gp*31* and homologs) of these phages are related more closely to bacterial-type I DNA polymerases than other phage deoxynucleotide polymerizing enzymes.

Origin of the new subfamily name:

Named after the best-studied of these phages, *Bacillus* phage SPO1 .

MODULE 7: **REMOVE and MOVE**

Use this module whenever an existing taxon needs to be removed:

- *Either* to abolish a taxon entirely (when only part (a) needs to be completed)
- *Or* to move a taxon and re-assign it e.g. when a species is moved from one genus to another (when BOTH parts (a) and (b) should be completed)

Part (a) taxon/taxa to be removed or moved

Code	***2009.009oB***	(assigned by ICTV officers)

To remove the following taxon (or taxa) from their present position:

"SPO1-like viruses"

The present taxonomic position of these taxon/taxa:

Genus:	
Subfamily:	
Family:	***Myoviridae***
Order:	***Caudovirales***

Fill in all that apply.

If the taxon/taxa are to be abolished (i.e. not reassigned to another taxon) write "yes" in the box on the right	

Reasons to justify the removal:
Explain why the taxon (or taxa) should be removed

The taxon is now incorporated as a new genus in the new subfamily *Spounavirinae.* See part B below.

Part (b) re-assign to a higher taxon

Code	***2009.009pB***	(assigned by ICTV officers)

To re-assign the taxon (or taxa) listed in Part (a) as follows:

Genus:	**"SPO1-like viruses"** (proposed new name *Spo1likevirus*)
Subfamily:	***Spounavirinae (new)***
Family:	***Myoviridae***
Order:	***Caudovirales***

Fill in all that apply.

- If the higher taxon has yet to be created write "**(new)**" after its proposed name and complete relevant module to create it.

If no genus is specified, enter "**unassigned**" in the genus box.

Reasons to justify the re-assignment:

- If it is proposed to re-assign species to an existing genus, please explain how the proposed species differ(s) from all existing species.
  - If species demarcation criteria (see module 3) have previously been defined for the genus, explain how the new species meet these criteria.
  - If criteria for demarcating species need to be defined (because there will now be more than one species in the genus), please state the proposed criteria.
- Provide Genbank accession numbers (not RefSeq accessions) for genomic sequences
- Further material in support of this proposal may be presented in the Appendix, Module 9

Cfr modules 4 and 9

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

References:

Alemayehu D, Ross PR, O'Sullivan O, Coffey A, Stanton C, Fitzgerald GF, McAuliffe O: Genome of a virulent bacteriophage Lb338-1 that lyses the probiotic Lactobacillus paracasei cheese strain. Gene 2009, 448: 29-39.

Allan BJ, Davies P, Carstens EB, Kropinski AM: Characterization of the genome of Pseudomonas aeruginosa bacteriophage phi PLS27 with particular reference to the ends of the DNA. Journal of Virology 1989, 63: 1587-1594.

Carlton RM, Noordman WH, Biswas B, de Meester ED, Loessner MJ: Bacteriophage P100 for control of Listeria monocytogenes in foods: genome sequence, bioinformatic analyses, oral toxicity study, and application. Regulatory Toxicology and Pharmacology 2005, 43: 301-312.

Chibani-Chennoufi S, Dillmann ML, Marvin-Guy L, Rami-Shojaei S, Brüssow H: Lactobacillus plantarum bacteriophage LP65: a new member of the SPO1-like genus of the family Myoviridae. Journal of Bacteriology 2004, 186: 7069-7083.

Klumpp J, Dorscht J, Lurz R, Bielmann R, Wieland M, Zimmer M et al.: The terminally redundant, nonpermuted genome of Listeria bacteriophage A511: a model for the SPO1-like myoviruses of gram-positive bacteria. Journal of Bacteriology 2008, 190: 5753-5765.

Klumpp J, Lavigne, R, Loessner, M J, Ackermann, H-W: The SPO1-related Bacteriophages. Archives of Virology 2010, submitted.

Kwan T, Liu J, DuBow M, Gros P, Pelletier J: The complete genomes and proteomes of 27 Staphylococcus aureus bacteriophages. Proceedings of the National Academy of Sciences of the United States of America 2005, 102: 5174-5179.

Lavigne R, Darius P, Summer EJ, Seto D, Mahadevan P, Nilsson AS, Ackermann H-W, Kropinsky AM: Classification of Myoviridae bacteriophages using protein sequence similarity. BMC Microbiology 2009, 9:224

Parker ML, Ralston EJ, Eiserling FA: Bacteriophage SPO1 structure and morphogenesis. II. Head structure and DNA size. Journal of Virology 1983, 46: 250-259.

Perkus ME, Shub DA: Mapping the genes in the terminal redundancy of bacteriophage SPO1 with restriction endonucleases. Journal of Virology 1985, 56:

References:

40-48.

Stewart C, Casjens S, Cresawn SG, Houtz JM, Smith AL, Ford ME, Peebles CL, Hatfull GF, Hendrix RW, Huang WM, Pedulla ML: The Genome of Bacillus subtilis Bacteriophage SPO1. Journal of Molecular Biology 2009, 388:48-70.

Twort FW: An investigation on the nature of the ultramicroscopic viruses. Lancet 1915, 189: 1241-1243.

Uchiyama J, Rashel M, Maeda Y, Takemura I, Sugihara S, Akechi K et al.: Isolation and characterization of a novel Enterococcus faecalis bacteriophage fEF24C as a therapeutic candidate. FEMS Microbiology Letters 2008, 278: 200-206.

Uchiyama J, Rashel M, Takemura I, Wakiguchi H, Matsuzaki S: In silico and in vivo evaluation of bacteriophage fEF24C, a candidate for treatment of Enterococcus faecalis infections. Applied and Environmental Microbiology 2008, 74: 4149-4163.

Annex:

Include as much information as necessary to support the proposal, including diagrams comparing the old and new taxonomic orders.
The use of Figures and Tables is strongly recommended.

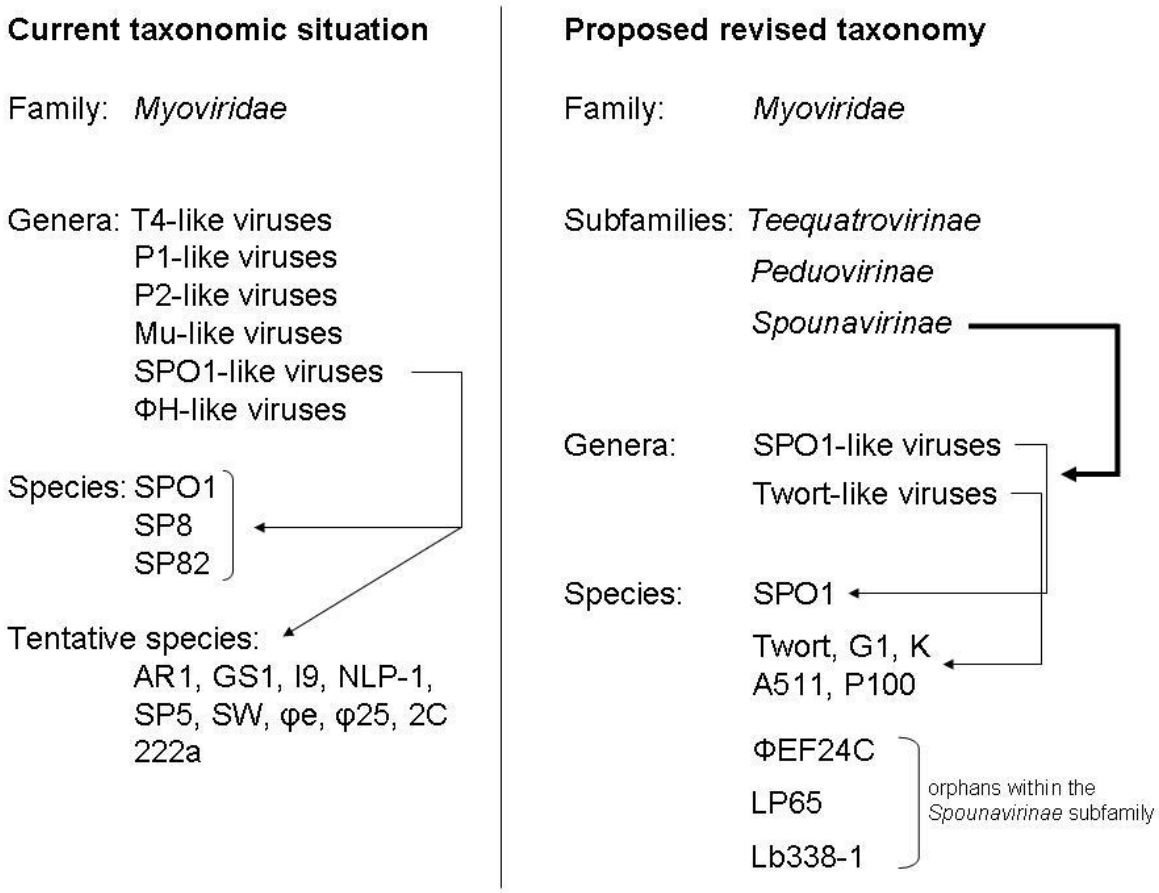

Figure 1: Comparison of the current taxonomic situation of the *Myoviridae* family and the proposed revised taxonomic scheme (Lavigne et al., 2009; Klumpp et al., 2010).

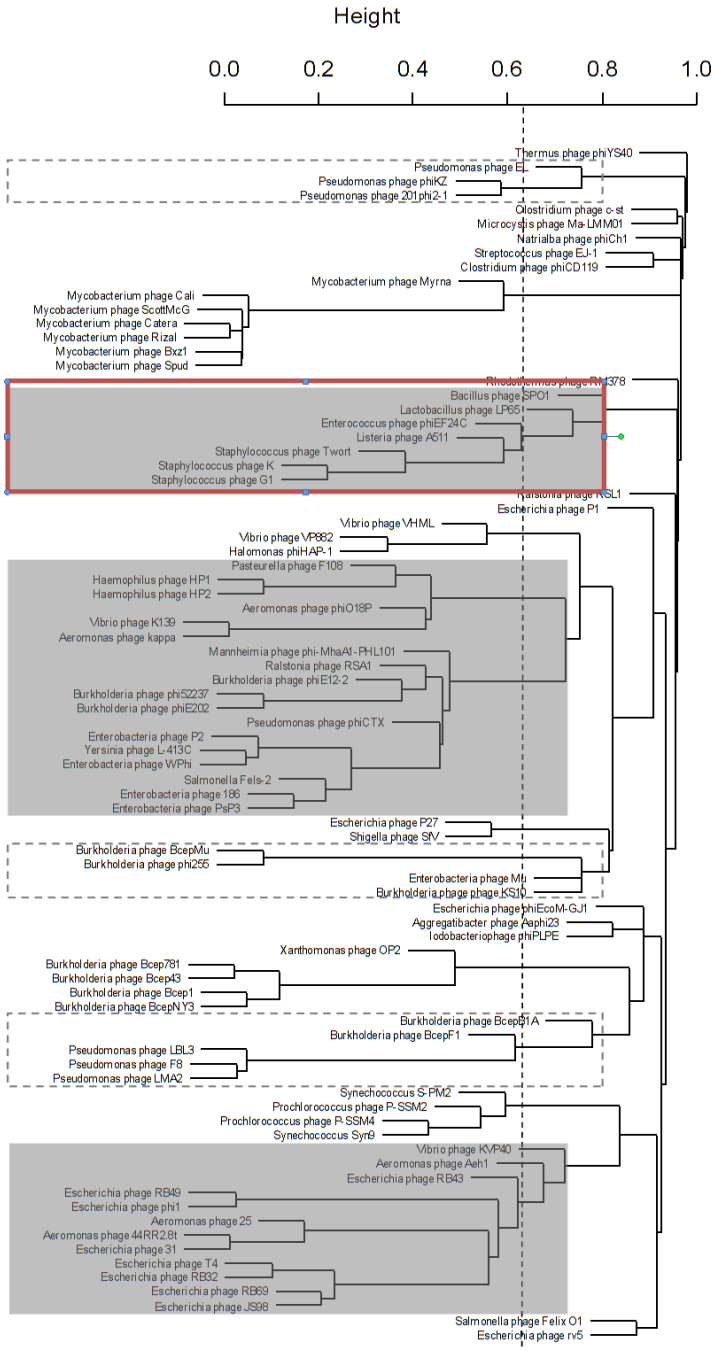

Figure 2: Hierarchical cluster dendrogram of the *Myoviridae*. The relative dissimilarity between the phage proteomes (between 0.0 and 1.0) forms the basis for the proposed groupings. The dotted lines reflect the cut-off value used for the establishment of genera, used consistently for all *Myoviridae* and the previously defined *Podoviridae* (Lavigne et al., 2008; Lavigne et al., 2009). Subfamily and tentative subfamily groupings are indicated in the grey and dotted boxes, respectively.

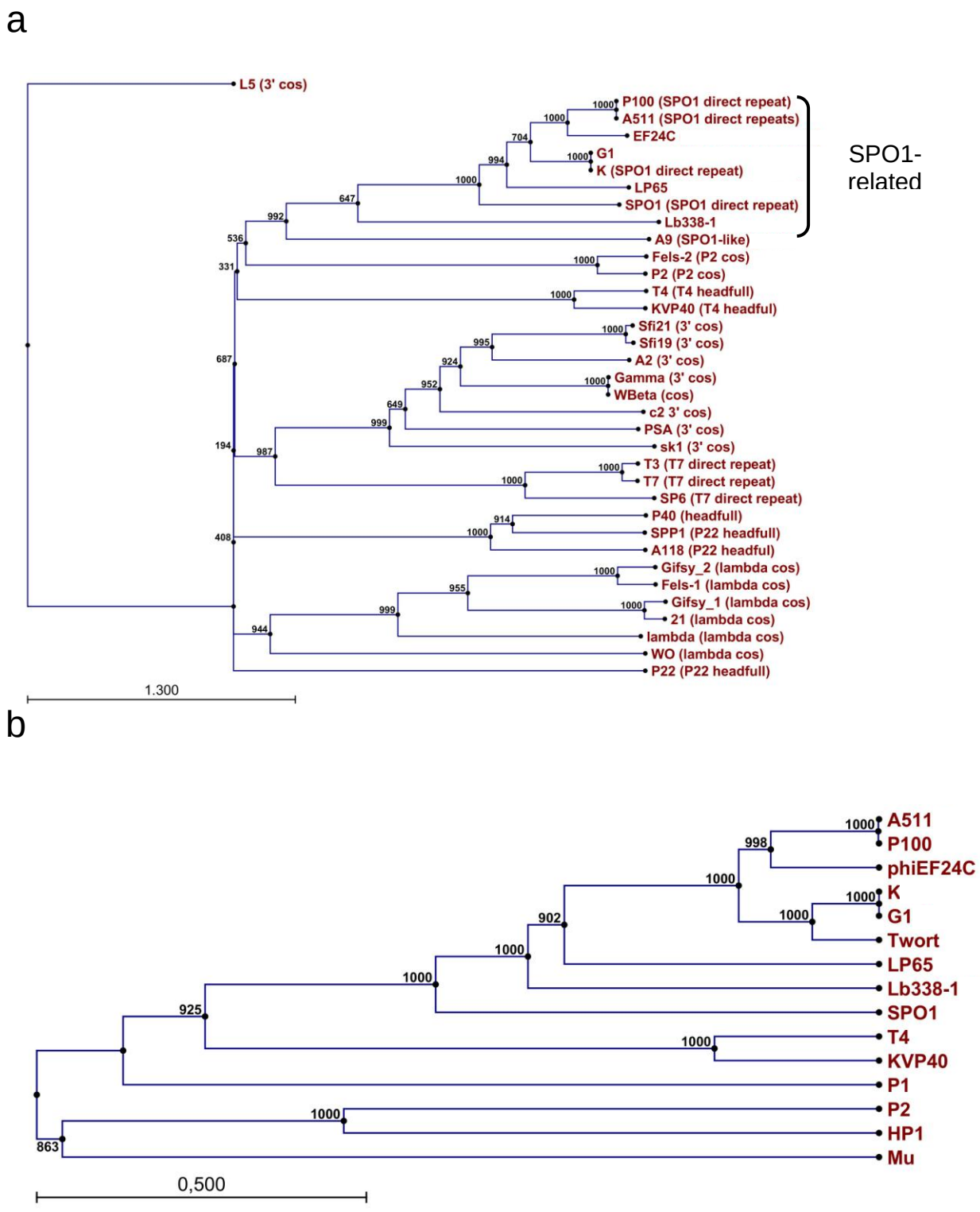

Figure 3: a. Phylogenetic tree calculated from ClustalW alignments of large terminase subunit sequences from SPO1-like phages and a selection of phages with known packaging mechanism using the Neighbor Joining method and 1000 bootstrap replicates. Numbers of successful bootstrap replicates are indicated. **b.** Phylogenetic tree calculated from ClustalW-alignments of the major capsid protein of SPO1-like phages and unrelated phages. The tree was constructed using UPGMA method and 1000 bootstrap replicates. (Klumpp et al., 2010).

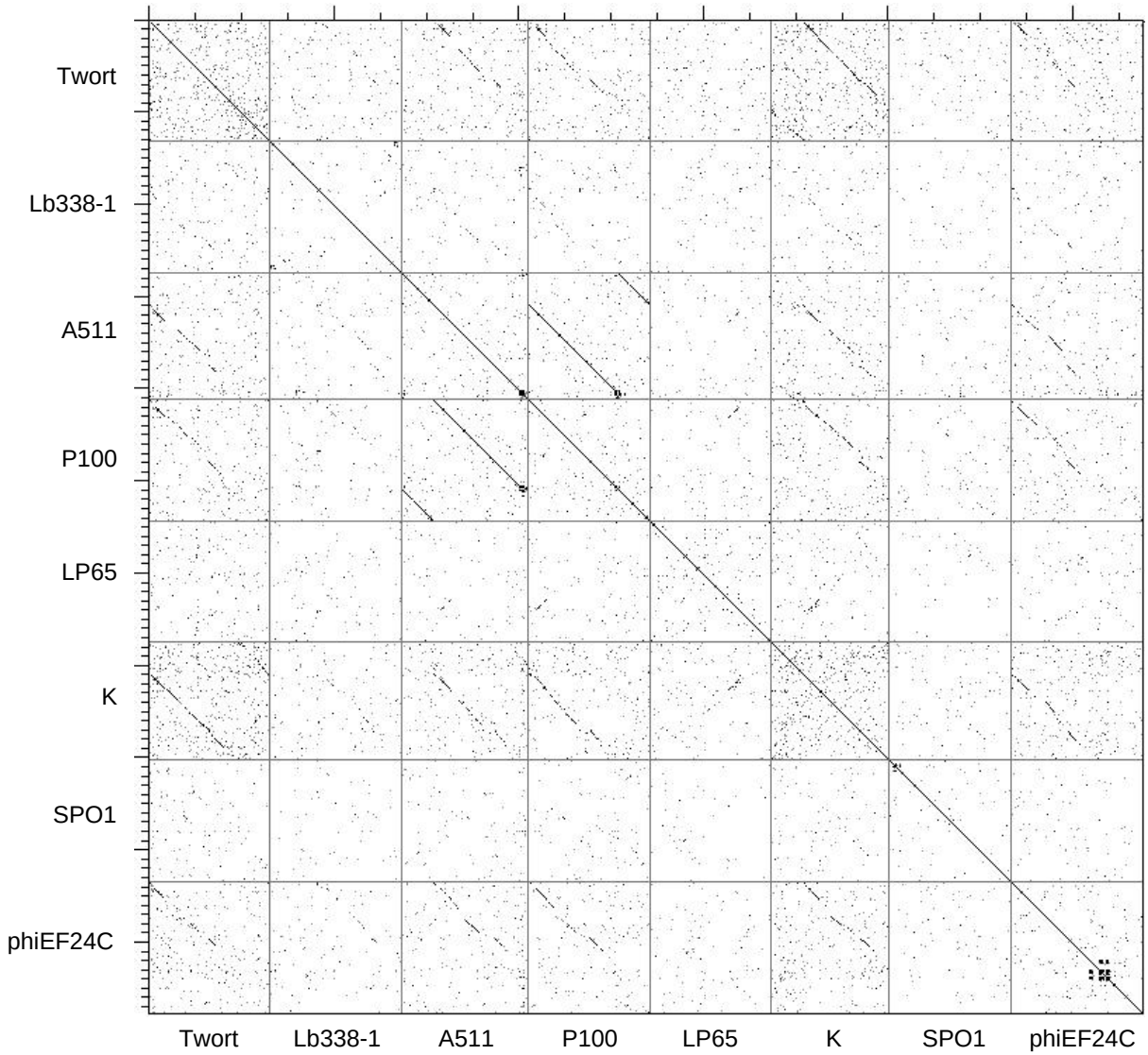

Figure 4: Dot plot of nucleotide sequences from published SPO1-like phages. The plot was generated using Dotter Linux version 2002 with a sliding window size of 25. The position of phage genomes is indicated on the X and Y axes. Scale of Y-axis is 1000 bp; scale of Y-axis is 50 kb. Genbank accession numbers of used sequences are: Twort (AY954970), Lb338-1 (FJ822135), A511 (DQ003638), P100 (DQ004855), LP65 (AY682195), K (NC_005880), SPO1 (FJ230960), φEF24C (NC_009904). (Klumpp et al., 2010).

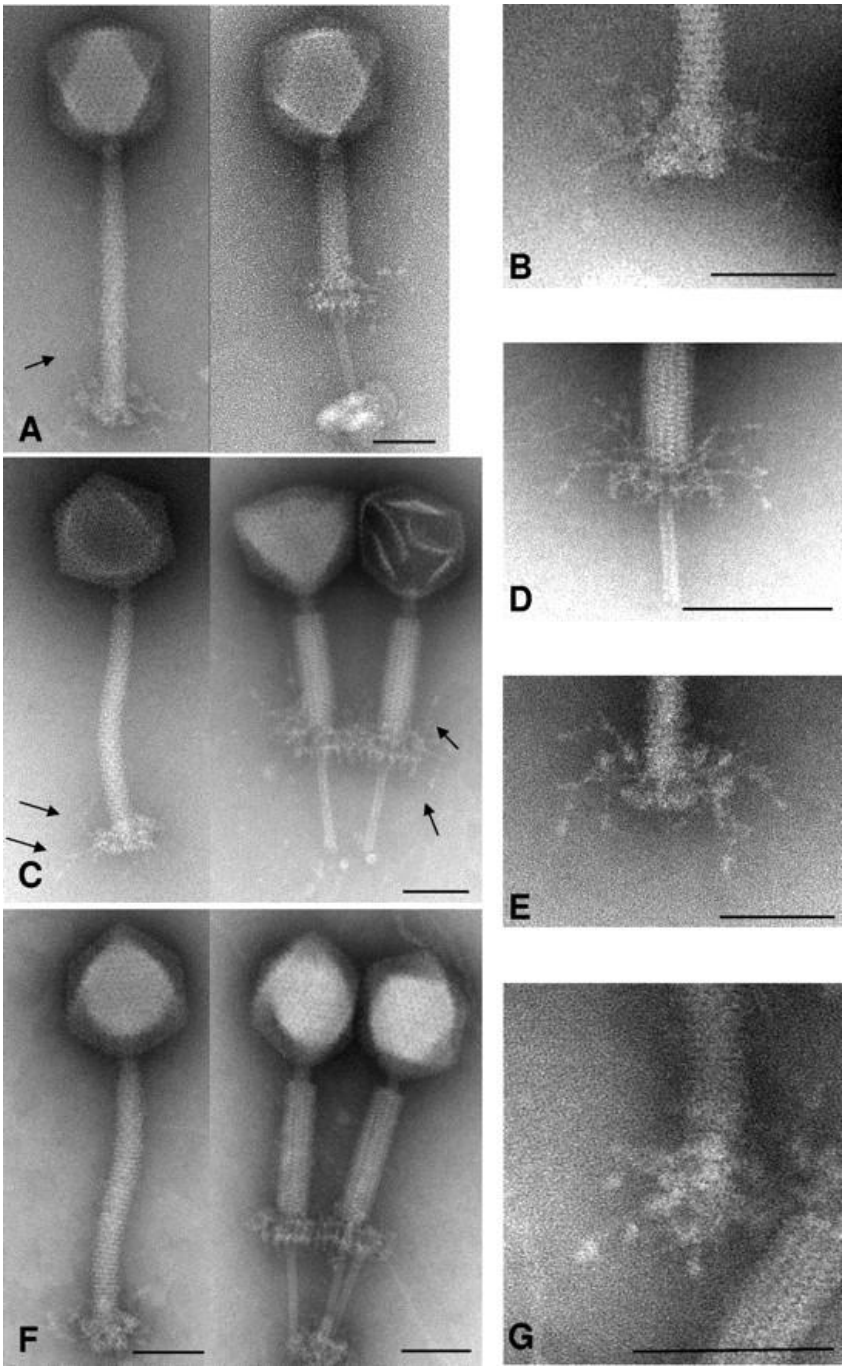

Figure 5. Electron micrographs of A511, P100, and K (Klumpp et al., 2008)
(A) Phage A511 negatively stained with uranyl acetate with its tail in a native and contracted states. Arrows indicate the thin, long tail fibers (whiskers) originating at the base plate. The inner tube extends approximately 70 nm beyond the contracted outer tail shaft and base plate. (B) Close-up view of A511 base plate stained with ammonium molybdate. (C) Phage P100, in noncontracted (left panel [ammonium molybdate]) and contracted status (right panel [stained with phosphotungstic acid]). Arrows indicate tail fibers. In the right panel, the lighter phage capsid (left phage) is packed with DNA, whereas the darker capsid (right phage) is empty as a result of DNA ejection. (D and E) Close-up views of P100 tail in contracted and noncontracted states (stained with phosphotungstic acid) including the long, flexible tail fibers. (F) Phage K with uncontracted (left) and contracted tail (right), stained with ammonium molybdate. (G) Close-up view of the K adsorption apparatus (stained with uranyl acetate) from diagonal below; the symmetrical arrangement of tail fibers is clearly visible. Bars represent 50 nm.

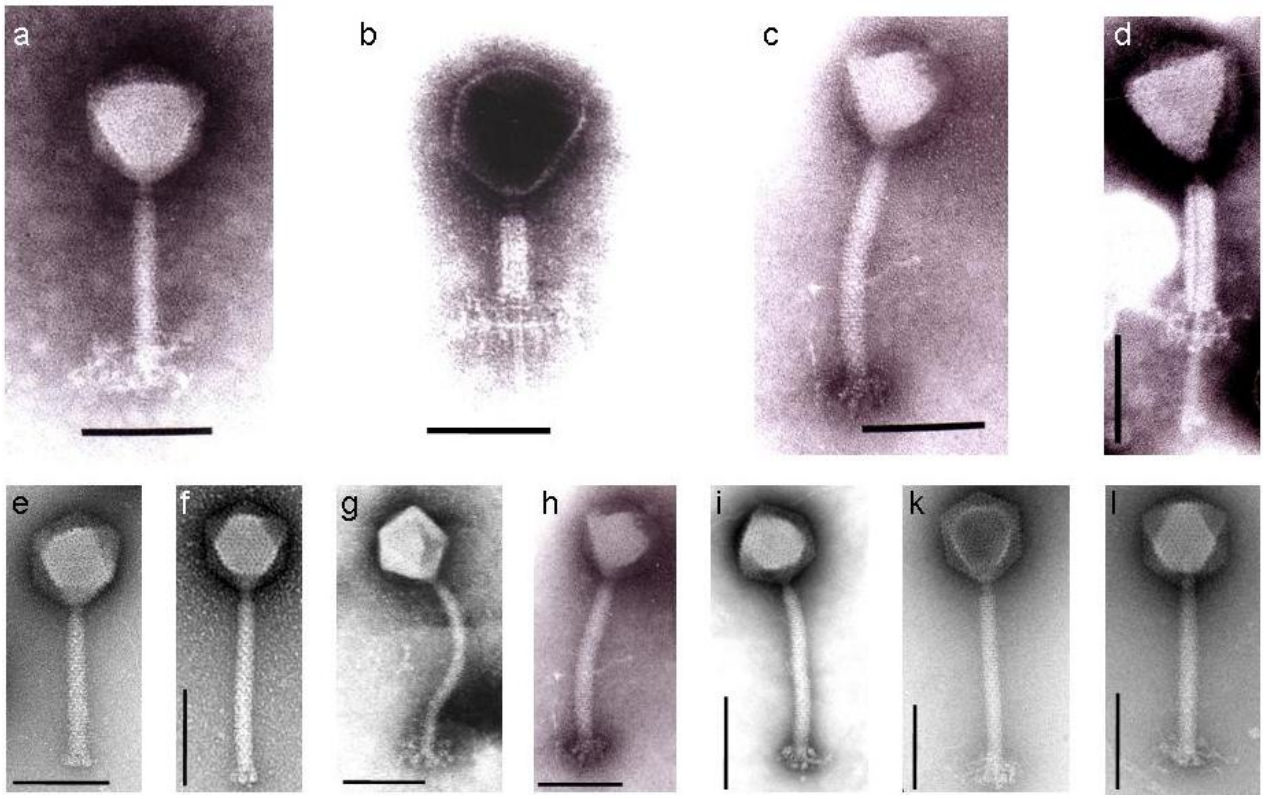

Figure 6: Transmission electron microscopic images of SPO1 and SPO1-related bacteriophages. a and **b**: *B. subtilis* phage SPO1; **c** and **d:** *S. hyicus* phage Twort; **e:** *B. cereus* phage CP-51; **f:** *B. thuringiensis* phage Bastille; **g**, **h and i**: *S. aureus* phages G1, G4 and K; **k** and **l**: *L. monocytogenes* phages P100 and A511.

Table 1: List of the proposed Spounavirinae and their Accession numbers:

Bacillus phage SPO1	NC_011421
Staphylococcus phage Twort	NC_007021
Staphylococcus phage K	NC_005880
Staphylococcus phage G1	NC_007066
Listeria phage P100	NC_007610
Listeria phage A511	NC_009811
Enterococcus phage φEC24C	NC_009904
Lactobacillus phage LP65	NC_006565
Lactobacillus phage Lb338-1	NC_012530

Table 2: Characteristics of completely sequenced SPO1-related phages and *Spounavirinae* candidates

Host	Designation	Genome size, kb	Predicted ORFs	tRNAs	Genome structure	HMU
Bacillus subtilis	SPO1	145.747	204	5	Tr, 13.2 kb fixed repeats	Present
B. cereus	CP-51	~138	~200	2	Fixed ends, N.D.	Present
Brochothrix thermosphacta	A9	127 ± 1[b]	199	6	Tr, 11 kb fixed repeats	Absent
Enterococcus faecalis	φEF24C	142.072	221	5	Circularly permuted[a]	
Lactobacillus paracasei	Lb338-1	141.832	199	2[c]	Nonredundant[a]	
L. plantarum	LP65	131.573	165	14	Nonredundant[a]	
Listeria monocytogenes	A511	137.619	199	16	Tr, 3.125 kb repeats	Absent
L. monocytogenes	P100	131.384	174	18	Tr, ca. 3 kb repeats	Absent
Staphylococcus aureus	G1	138.715	214	3[c]	N.D.	
S. aureus	K	127.395	118	3[c]	Tr, ca. 20 kb repeats	
S. aureus	Twort	130.706	195	1[c]	N.D.	Absent

Genome sizes include size of the terminal redundancy (if known); HMU, 5-hydroxymethyl uracil; kb, kilobase; N.D., not determined; Tr: terminally redundant, non-permuted; [a] no data presented by authors; [b] The A9 genome size has been determined with 1 kb uncertainty due to the presence of a highly repetitive AT-sequence, which could not be fully sequenced; [c] tRNA numbers are either taken from published genomes or predicted using tRNA-Scan SE [65].

(Klumpp et al., 2010)