

1 **A new bacteriophage subfamily - “Jerseyvirinae”**

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34 **Abstract**

35 Based upon morphology, comparative nucleotide and protein sequence analysis, a new subfamily of
36 the family *Siphoviridae* is proposed, named the “Jerseyvirinae” and consisting of three genera, the
37 “Jerseylikevirus”, the “Sp3unalikevirus” and the “K1glikevirus”. To date, this subfamily consists of 18
38 phages, for which the genomes have been sequenced. *Salmonella* phages Jersey, vB_SenS_AG11,
39 vB_SenS-Ent1, vB_SenS-Ent2, vB_SenS-Ent3, FSL SP-101, SETP3, SETP7, SETP13, SE2, SS3e and wksl3
40 form the “Jerseylikevirus”. The “K1glikevirus” consist of *Escherichia* phages K1G, K1H, K1ind1, K1ind2
41 and K1ind3. The Sp3unalikevirus contains one member so far. Jersey-like phages appear to be widely
42 distributed, as the above phages were isolated in the UK, Canada, USA and South Korea between
43 1970 and the present day. The distinguishing features of this subfamily include a distinct siphovirus
44 morphotype, terminally redundant, circularly permuted genomes of 40.7-43.6 kb (49.6-51.4 mol%
45 G+C), syntenic genome organisation and a high degree of nucleotide sequence identity and shared
46 proteins. All known members of the proposed subfamily are strictly lytic.

47

48 Introduction

49 The advent of affordable whole genome sequencing and the renewed interest in using
50 bacteriophages as alternatives to antibiotics or to decontaminate food substances has led to a
51 marked increase in submissions of complete phage genomes to public databases such as GenBank.
52 While this process has led to comparative genome analyses and resulted in the recognition of
53 relationships between newly submitted phage genomes and those already deposited, official
54 recognition of these new taxonomic units has lagged. This is particularly true for members of the
55 *Siphoviridae* family (double-stranded dsDNA genome, non-contractile tail), for which the present
56 International Committee on Virus Taxonomy (ICTV) only recognizes ten genera and thirty-one
57 species (www.ictvonline.org/virusTaxonomy.org). These species account for less than 10% of the
58 fully sequenced genomes from members of this family, a situation that must be addressed. A
59 significant number of these unclassified *Siphoviridae* infect *Salmonella* hosts.

60 Serovars of *Salmonella* are widespread aetiological agents of food and waterborne diseases in
61 humans and livestock. *Salmonella enterica* is classified into six subspecies by biochemical tests.
62 Based upon serology of the lipopolysaccharide (O) and flagella (H) antigens, over 2,600 serovars of
63 *Salmonella* have been described [23]. Not surprisingly, *Salmonellaphages* are also numerous and
64 varied. The morphology of 177 *Salmonella* phages was reviewed in 2007 [2] (updated in [56]) with
65 most representatives belonging to the *Caudovirales* order (dsDNA genome, tailed-phages) and its
66 three families, the *Siphoviridae*, *Myoviridae* and *Podoviridae*. A small number of phages representing
67 the *Inoviridae*, *Leviviridae*, *Microviridae* and *Tectiviridae* families have also been documented.

68 A number of *Salmonella* phages were originally isolated for the purposes of phage typing. Phage
69 typing represents a useful epidemiological tool whereby strains of a particular serovar may be
70 differentiated into phage types based upon susceptibility to a panel of bacteriophages. Phage Jersey
71 was originally isolated and used by Felix and Callow in the development of a typing scheme for
72 *Salmonella paratyphi* B [1].

73 Jersey presents a morphotype recognizable by electron microscopy with a tail terminal disk-like
74 structure with 6 club-shaped spikes [1, 2, 15]. To date, almost all phages examined which exhibit the
75 Jersey-type morphotype have been restricted to *Salmonella* and *Escherichia*, the sole exception to
76 this rule being *Serratia* phage η [16]. No phages with this morphotype have been observed to infect
77 other Gram-negative genera including *Aeromonas*, *Pseudomonas*, *Vibrio*, or *Rhizobium* (Table 1). The
78 genome of phage Jersey has recently been sequenced and discontinuous MEGABLAST revealed
79 similarity to a number of siphovirus genomes deposited in GenBank.

80

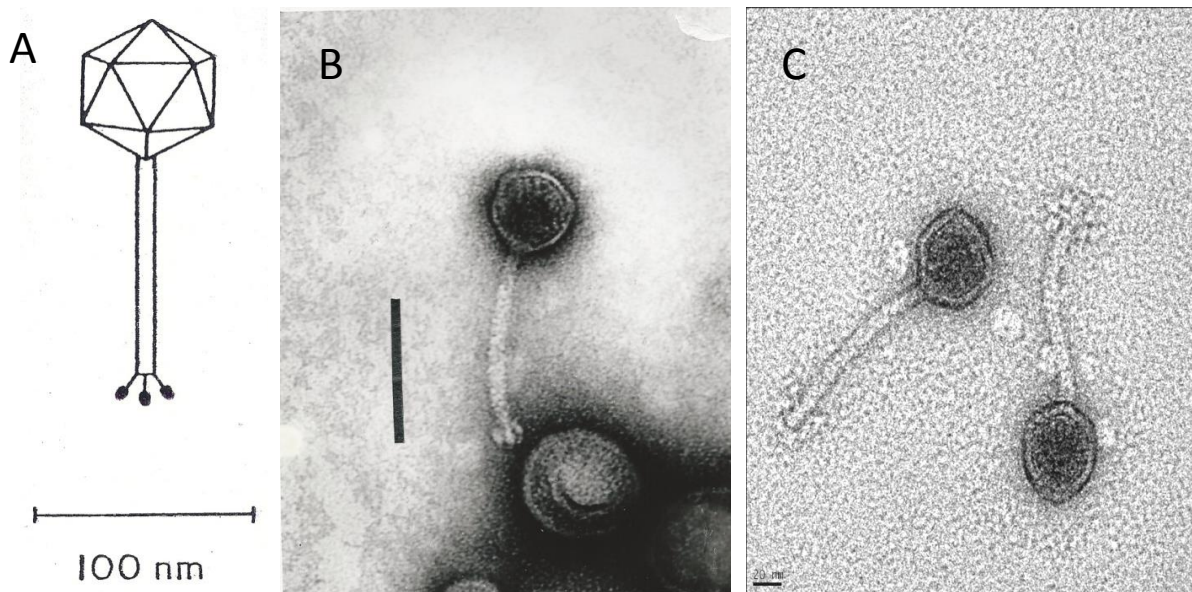
81 On the basis of an in-depth bioinformatics analysis, this work proposes the creation of three new
82 genera within a novel subfamily, the “Jerseyvirinae”.

83 Results

84 Morphological characteristics

85 Members of this subfamily which have been examined by transmission electron microscopy are
86 typical siphoviruses with isometric heads of 60 nm in diameter between opposite apices and long,
87 non-contractile tails of 120 x 8 nm (Table 1). Capsids appear as hexagonal or pentagonal and are thus
88 icosahedral in shape. Tails have about 27 transverse striations with a thin, 17 nm wide baseplate
89 with 5-6 spikes of 10 x 3 nm. Capsid and tail dimensions differ slightly from those of
90 phosphotungstate-stained phage Jersey (head = 68 nm, tail length 116 nm), which were obtained in
91 1970 without the benefit of rigorous electron magnification calibration. A morphological diagram of
92 Jersey-like phages is presented in Figure 1. Phages exhibiting a Jersey-morphology have been
93 described many times in the literature (Table 1).

94



95

96 Fig 1: Scale drawing of Jersey-like phage (A). Representative negatively stained TEM images of phage
97 Jersey (B) and K1H (C). Scale bars are 100 nm and 20 nm, respectively

98 **Comparative genomics and proteomics of bacteriophages belonging to**
 99 **the subfamily “Jerseyvirinae”**

100 Phages considered in this manuscript are summarised in Table 2. Each was initially linked by similar
 101 morphology and discontinuous MEGABLAST analysis.

102 **Table1.** Jersey-like phages in the literature: (I) phages for which morphology and genome sequence
 103 are known, (II) phages with sequence only, and (III) phages with morphology only.

Group	Phages	Host	Origin	Capsid, nm	Tail, nm	References
I	Jersey , 1, 2, 3a, 3al, 1010	<i>S. Paratyphi B</i>	England	68	116	[1]
	AG11	<i>S. Enteritidis</i>	Canada	61	117 x 8	[5]
	Ent1	<i>S. Enteritidis</i>	England	64	116 x 9	[58]
	MB78	<i>S. Typhimurium</i>	India	60	90-100	[29]
	SE2	<i>S. Enteritidis</i>	South Korea	---	---	[57]
	SEPT3 , SEPT5, SEPT7, SEPT11, SEPT12, SEPT13	<i>S. Enteritidis</i>	England	63	120 x 7	[14]
	wksl3	<i>S. Enteritidis</i>	South Korea	63	121 x 8	[30]
	II	FSL SP-031	<i>S. Cerro</i>	USA		
FSL SP-038 [⌘]		<i>S. Cerro</i>	USA			[43]
FSL SP-049 [⌘]		<i>S. Cerro</i>	USA			[43]
FSL SP-101 [⌘]		<i>S. Dublin</i>	USA			[43]
K1G		<i>E. coli</i>	USA			[9]
K1H		<i>E. coli</i>	USA			[9]
K1ind1		<i>E. coli</i>	USA			[9]
K1ind2		<i>E. coli</i>	USA			[9]
K1ind3		<i>E. coli</i>	USA			[9]
SSe3		Broad range (*)	South Korea			[31]
L13		Broad range (**)	South Korea			[26]
ST4		Broad range(***)	South Korea			[26]
III		FGCSSa2	<i>S. Typhimurium</i>	New Zealand	66	122 x 9
	JS77.1, JS85.2	<i>E. coli</i> O127:K63	Bangladesh	---	---	[12]
	San3, San7, San8	<i>Salmonella</i> spp.	USA	57 x 60	113-119 x 7-11	[2], [3]
	ΦSH17, ΦSH18	<i>S. Typhimurium</i>	England	---	---	[27]
	2, 3	<i>S. Heidelberg</i>	Canada	58	115 x 8	[15]

104 Bold face: complete or partial ([⌘]) sequence. Dimensions in italics indicate size determination after
 105 calibration with catalase crystals or T4 phage tails.

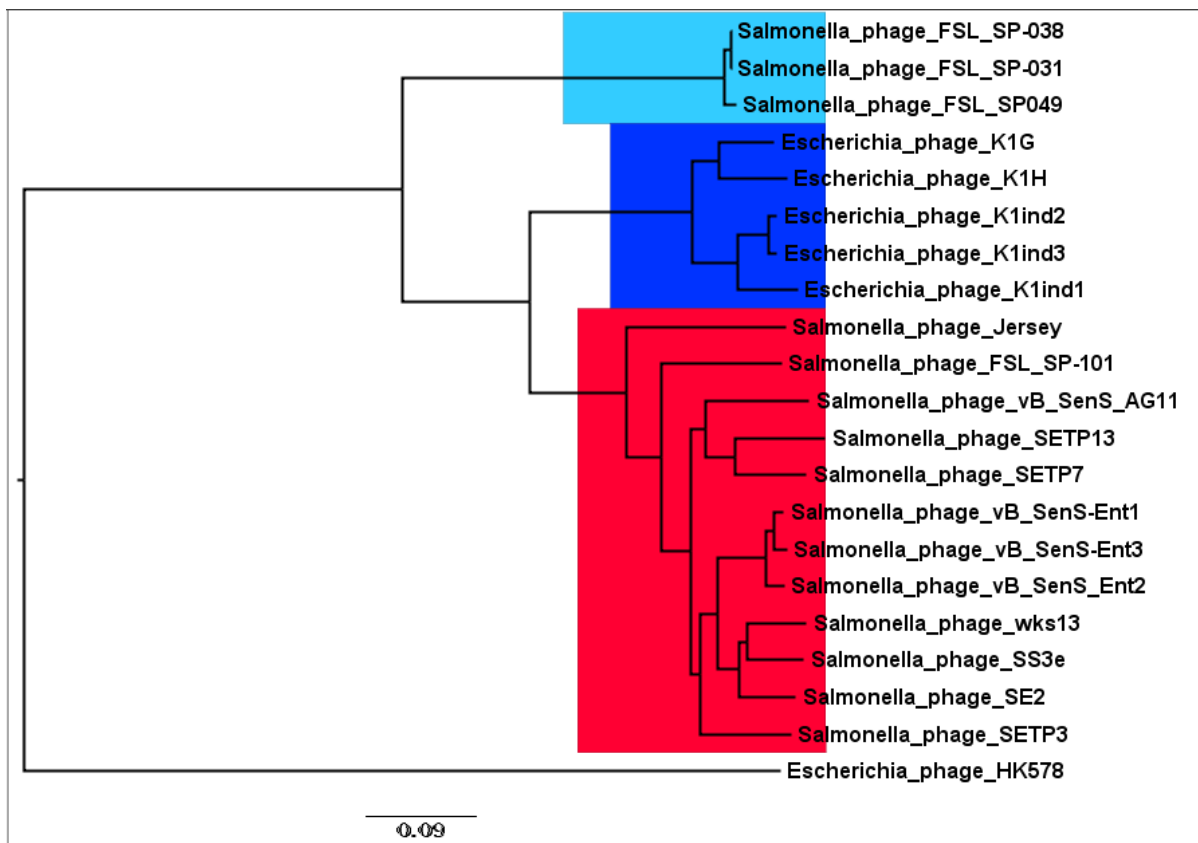
106 * Hosts: *E. coli*, *Shigella sonnei*, *Enterobacter cloacae*, *Serratia marcescens*.
 107 ** Hosts: *E. coli*, *S. Gallinarum*, *S. Pullorum*, *S. Enteritidis*, and *S. Typhimurium*.
 108 *** Hosts: *S. Gallinarum*, *S. Pullorum*, *S. Enteritidis*.
 109

110 **Table 2.** Bacteriophages with fully sequenced genomes belonging to the proposed subfamily
 111 “Jerseyvirinae”.

Phage name	Proposed genera	Genome size (bp)	Mol % G+C	No. of annotated CDSs	Reference	GenBank Accession Number
vB_SenS_AG11	Jerseylikevirus	41,546	49.91	66	-	JX297445
SETP3	Jerseylikevirus	42,572	49.85	63	[14]	EF177456
SETP7	Jerseylikevirus	42,749	49.90	68	[14]	KF562865
SETP13	Jerseylikevirus	42,665	49.79	68	[14]	KF562864
SS3e syn. KS7	Jerseylikevirus	40,793	50.08	58	[31]	AY730274
SE2	Jerseylikevirus	43,221	49.64	61	[57]	JQ007353
wksI3	Jerseylikevirus	42,633	49.80	64	[7]	JX202565
vB_SenS-Ent1	Jerseylikevirus	42,391	49.79	58	[58]	HE775250
vB_SenS-Ent2	Jerseylikevirus	42,093	49.92	56	-	HG934469
vB_SenS-Ent3	Jerseylikevirus	42,764	49.79	60	-	HG934470
FSLSP-101	Jerseylikevirus	41,873	50.27	57	[43]	KC139511
Jersey	Jerseylikevirus	43,447	49.97	69	-	KF148055
K1G syn. K1-dep(4)	K1glikevirus	43,587	51.07	52	[9]	GU196277
K1H syn. K1-dep(1)	K1glikevirus	41,632	51.17	50	[9]	GU196278
Kind1	K1glikevirus	42,292	51.27	51	[9]	GU196279
Kind2	K1glikevirus	42,765	51.35	48	[9]	GU196280
Kind3	K1glikevirus	43,461	51.15	49	[9]	GU196281
FSLSP-031	Sp3unalikevirus	42,215	51.07	59	[43]	KC139518

112
 113 In addition to phages with completely sequenced genomes, several phages with partial sequences in
 114 GenBank were also identified using discontinuous MEGABLAST; ST4 [JX233783], L13 [KC832325] and
 115 MB78 [AY040866, AF156970, AF349435, AJ277754, AJ249347, AJ245858, AJ245537, X87092,
 116 X86562, Y19202, Y19203, Y18133]. These phages are considered tentative members, subject to
 117 morphological examination and complete sequencing. Two *Salmonella* phages, FSL SP-038 and FSL
 118 SP-049, are also represented by partial genomic sequences in GenBank. Analyses suggest that they
 119 are part of the “Sp3unalikevirus” genus.

120
 121 Each of the phages listed in **Table 2** was colinearized and then examined for overall sequence
 122 similarity using progressiveMauve [13] (data not shown); and, DNA sequence identity using EMBOSS
 123 Stretcher [45] and CLUSTALW [34], which have been widely used for nucleotide sequence alignment
 124 of viruses [4, 42, 59]. Using the latter methodology, three clades were clearly defined (**Figure 2**).



125
 126 **Fig 2.** Clustal analysis reveals that the Jersey-like phages fall into three distinct groups, for which we
 127 proposed three genera “Jerseylikevirus” (red), “K1glikevirus” (dark blue), and “Sp3unalikevirus”
 128 (light blue) within a proposed subfamily, the “Jerseyvirinae.” The genomes were colinearized before
 129 analysis. *E. coli* phage HK578 was included as an outlier. The scale bar represents 0.09 substitutions
 130 per site.

131 In addition, the viral proteomes were subjected to pairwise comparisons using CoreGenes 3.0 which
 132 provided a measure of the total similarity at the protein level based upon pairs of proteins scoring
 133 above a pre-defined BLASTP bit score threshold [65]. The CoreGenes and Stretcher results are
 134 presented in **Table 3**. Lastly, using the Markov clustering algorithm OrthoMCL [38] with an e-value
 135 threshold of $1e^{-5}$ and an inflation value of 1.15, all the proteins encoded by members of the
 136 proposed subfamily were examined (data not shown). Functional annotations of proteins encoded
 137 by Jersey-like phages were obtained using BLAST tools and HHpred [51, 52]. Transmembrane
 138 domains were identified using TMHMM [32] and conserved domains using Pfam [17] and
 139 InterProScan [28]. In line with the 95% DNA sequence identity threshold used to delineate phage
 140 species specified by the Bacterial and Archaeal Viruses Subcommittee of the ICTV, the genus
 141 “Jerseylikevirus” is comprised of 10 species whose sequence identities range from 71.6% (FSL SP-
 142 101) to 67.6% (SETP13) and share between 79.7% and 68.1% proteins relative to Jersey (Table 3).

143 **Table 3.** Proteome and nucleotide sequence similarity of members of the “Jerseyvirinae”. Protein
 144 homology represents the percentage of shared proteins related to phage Jersey as determined using
 145 CoreGenes 3.0. Nucleotide sequence identity relative to phage Jersey was determined using
 146 EMBOSS Stretcher.

Genus	Phage name	GenBank Accession	Nucleotide sequence identity	Protein Homology
“Jerseylikevirus”	Jersey	KF148055	100%	100.0%
	AG11	JX297445	70.9%	79.7%
	wksI3	JX202565	71.2%	78.3%
	SE2	JQ007353	69.5%	71.0%
	Ent1	HE775250	69.5%	75.4%
	Ent2	HG934469	69.7%	73.9%
	Ent3	HG934470	69.4%	76.8%
	SS3e	AY730274.2	68.1%	68.1%
	SETP3	EF177456	71.1%	68.1%
	SETP7	KF562865	69.7%	79.7%
	SETP13	KF562864	67.6%	73.9%
	FSL SP-101*	KC139511	71.6%	72.5%
“K1glikevirus”	K1G	GU196277	59.5%	59.4%
	K1H	GU196278	59.0%	59.4%
	K1ind2	GU196280	58.0%	55.1%
	K1ind3	GU196281	59.5%	56.5%
	K1ind1	GU196279	59.7%	58.0%
“Sp3unalikevirus”	FSL SP-031	KC139518	53.4%	66.7%

147 * Partially sequenced

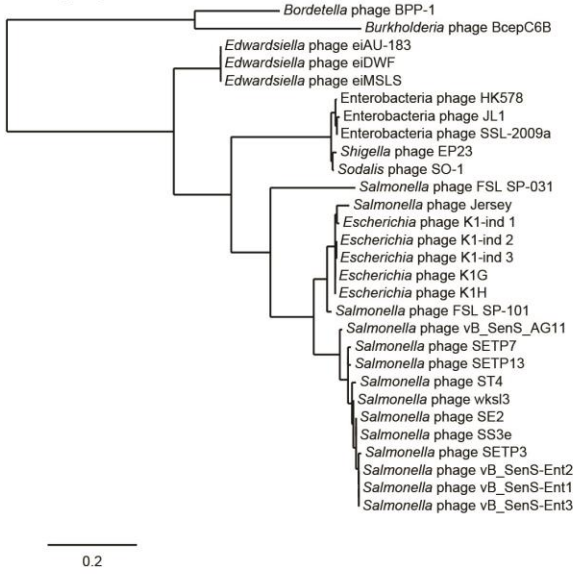
148 The “K1glikevirus” genus consists of four species - K1H, K1G, K1ind1 and K1ind2. They share
 149 between 79% and 97.1 % DNA sequence identity and a minimum of 84% homologous proteins.
 150 Relative to phage Jersey, the “K1glikevirus” exhibit at minimum 58% nucleotide sequence identity.
 151 Despite exhibiting significant protein homology (66.7%), the DNA sequence of FSL SP-031 shows only
 152 53.4% identity to Jersey, a difference sufficient to indicate that this phage is distinct from other
 153 members and warrant the creation of a separate genus, the “Sp3unalikevirus” within the subfamily.

154 The results of DNA sequence alignment, homologous proteins, morphology, and genome
 155 organisation indicate that the Jersey-like phages form three distinct groups, united by 53% DNA
 156 sequence identity, which are proposed to represent three genera; the “Jerseylikevirus”,
 157 “Sp3unalikevirus” and “K1glikevirus” (Fig. 2). In the following sections the common properties of
 158 viruses, which belong to the “Jerseyvirinae”, are discussed alongside specifics of the three genera.

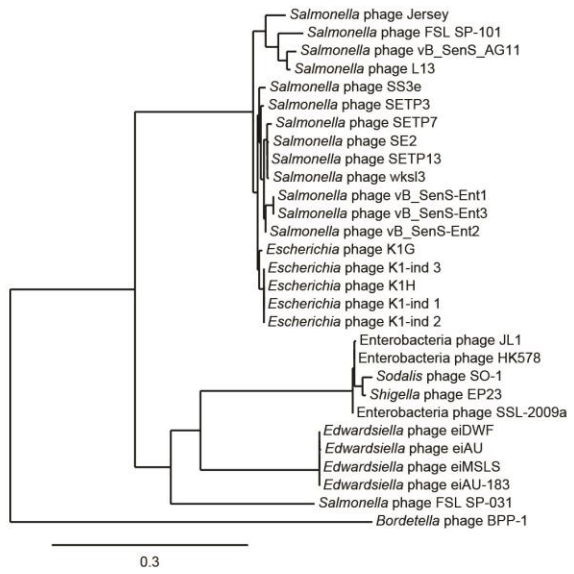
159 **Protein phylogeny**

160 Phylogenetic trees were constructed to investigate common proteomic features of the
161 “Jerseyvirinae” for the large terminase subunit (TerL), portal protein, DNA polymerase (Dpol),
162 helicase, major capsid, and major tail (MTP) proteins (Fig. 3). Analysis of the helicase proteins
163 indicates that this group of phages are phylogenetically related and distinct from the
164 “Hk574likevirus”. The trees constructed using the TerL, portal and Dpol sequences clearly indicate
165 that this group of viruses can be subdivided into three clades. Analysis of the major capsid and
166 MTPs reveals that the K1G clade is significantly different from the “Jerseylikevirus” group.
167 Interestingly, the K1H major capsid protein is distinct from other members of the subfamily, a
168 feature corroborated by the OrthoMCL groupings.

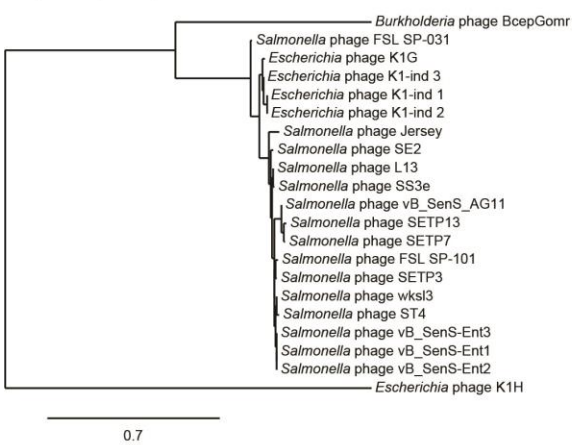
DNA polymerase



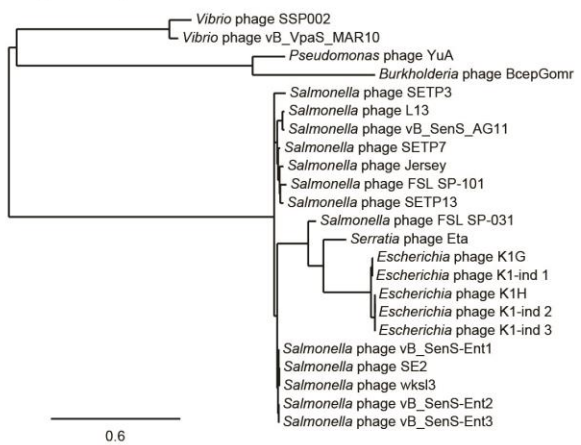
Helicase



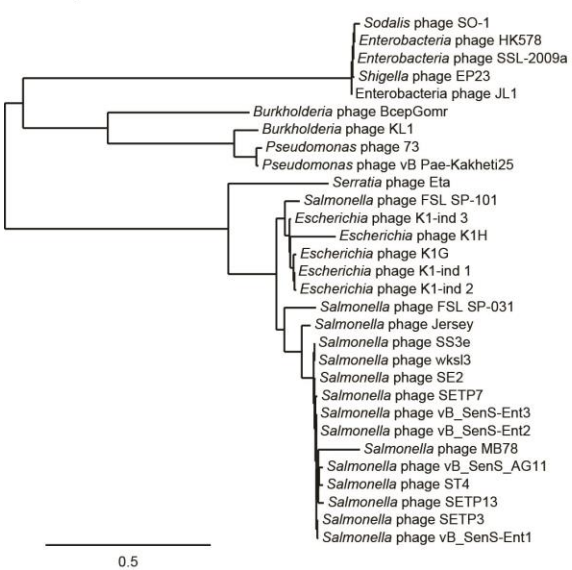
Major capsid protein



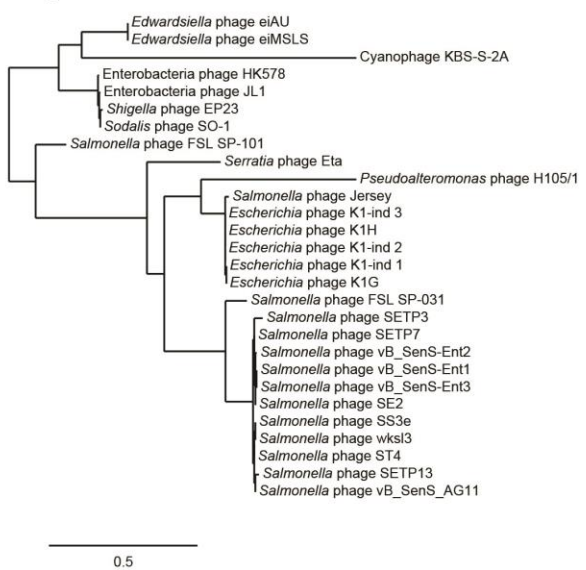
Major tail protein



Portal protein



Large terminase subunit



170 **Fig 3.** Phylogenetic analysis of the “Jerseyvirinae” common proteins produced using
171 www.phylogeny.fr. Branch length is proportional to the number of substitutions per site.

172 **Common features of the “Jerseyvirinae”**

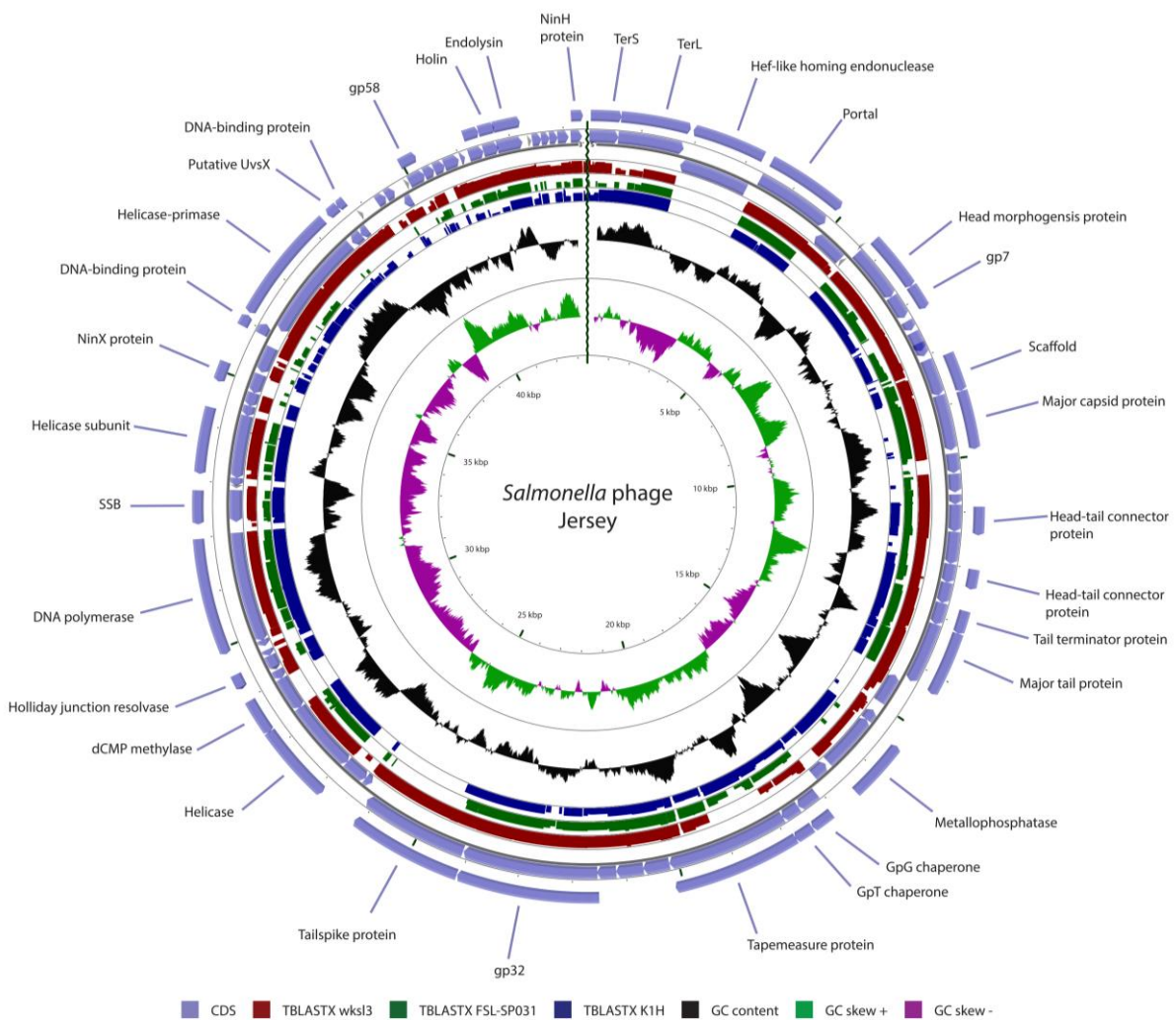
173 The genome of the proposed “Jerseyvirinae” phages is ranging in size from 40.7 to 43.6 kb with a
174 G+C content from 49.6 to 51 %. They also encode between 48 to 69 proteins but no tRNAs. These
175 phages appear to be strictly lytic/virulent as none have been shown capable of lysogeny or harbour
176 homologues of known integrases, recombinases or excisionases. As with most phages, their
177 genomes exhibit a modular structure and the organisation of genes shows a high degree of synteny
178 across all members (Fig. 4). The genome may be divided into four modules on the basis of the
179 predicted function of component genes; (i) virion structure and assembly, (ii) regulation/immunity,
180 (iii) genome replication and (iv) host lysis. Despite the presence of a number of deduced proteins of
181 unknown function, the roles of some gene products can be predicted on the basis of BLAST and
182 HHpred searches or by the presence of conserved domains.

183 Using OrthoMCL, the 1,057 proteins encoded by the 18 members of the three proposed genera
184 formed 90 clusters of orthologous proteins and 51 singletons. Of these, 25 clusters were present in
185 all members of the three proposed genera representing conserved proteins in the structural,
186 replicative and lysis gene modules. In addition to these ‘core’ genes/proteins, a further 1, 5 and 12
187 clusters were present in all members of the “Jerseylikevirus”, “K1glikevirus”, and “Sp3unalikevirus”,
188 respectively. The structural gene module represents the most highly conserved region with 17
189 ortholog clusters. Almost half of the genes encoded by the “Jerseyvirinae” phages are devoted to
190 genome packaging, assembly and structure of mature virions. The structural module follows a
191 strongly conserved gene order, encoding genes involved in DNA packaging followed by the virion
192 capsid, tail and adsorption apparatus in an arrangement reminiscent of that observed in many
193 phages.

194 Several features are of interest within the morphogenesis module. Using phage Jersey as reference,
195 the major capsid protein (gp12) shows structural similarity to *Bacillus* phage SPP1 and coliphage
196 HK97 (Protein Data Bank/PDB accession numbers: 4an5 and 3p8q, respectively) suggesting that the
197 capsid protein shares the evolutionary conserved HK97-like fold [18]. Three gene products each
198 return structural similarity to Ig-set domain proteins with HHpred. Two of these proteins, Jersey gp7
199 (AGP24895) and SETP13 gp12 (AGX84616), return matches to the Hoc protein of Enterobacteria
200 phage RB49 (PDB 3shs) while a third, Jersey gp13 (AGP24902), returned a match to the head fibre of
201 *Bacillus* phage Φ 29 (PDB 3qc7). Ig-like domains are found widely within the order *Caudovirales* and

202 are predominantly associated with structural proteins suggesting that these proteins play a role
 203 within capsid assembly or completion and may also be involved in non-specific binding to host cells
 204 [19]. The presence of a weak match to an Ig-like I-set domain (pfam: PF07679) in gp07 and the
 205 proximity of its gene to the gene coding for the major capsid protein might suggest that this gene
 206 encodes a capsid decoration protein [53]. Head decoration proteins have been described in a
 207 number of phages such as L, λ and ES18 and are thought to aid stabilisation of the capsid structure
 208 [21]. However, to date none of the structural proteins present in mature Jersey-like virions have
 209 been identified using mass spectrometry.

210



211

212 Fig 4. Genetic and physical map of *Salmonella* phage Jersey prepared using CGView [22]. For
 213 sequence similarity comparison, TBLASTX was used versus wks13 (red), FSL-SP031 (dark green) and
 214 K1H (dark blue). GC content is depicted in black while positive and negative GC skew is denoted by
 215 green and purple, respectively.

216

217 Two coding sequences positioned immediately upstream of the gene coding for the putative tape
218 measure form a single cluster using OrthoMCL, which is present in all members of the three
219 proposed genera. The positioning of these genes is similar to the ones of λ tail assembly chaperones
220 gpG and gpGT. In λ , gpG and the translational frameshift product gpGT interact with the tape
221 measure and major tail proteins and are required for correct tail formation [61-63]. Analysis of the
222 putative gpG and gpGT genes in phage Jersey (AGP24914 and AGP24915) with MFOLD and
223 HPKNOTTER provided evidence for the presence of a stem-loop structure and a pseudoknot,
224 respectively, suggesting that the Jersey-like phages produce a gpGT-like fusion product.

225 The putative tail fibre (Jersey gp32, AGP24920) shows distant similarity to the central tail fibre gpJ of
226 phage λ and p33 of phage T1 using PSI-BLAST and is conserved among all members of the
227 “Jerseyvirinae”. However, a central tail tip fibre has not been observed in electron micrographs so
228 the precise role and location of this protein remains unclear. This protein may in fact form the virion
229 baseplate in conjunction with a gene (Jersey gp31, AGP24919) encoded immediately upstream
230 which exhibits similarity to the endolysin of *Pseudomonas* phage ϕ KZ (PBD: 3bkh), suggesting a role
231 in cell wall degradation.

232 In all “Jerseyvirinae”, the morphogenesis gene module is interrupted after the gene coding for the
233 major tail protein by a cassette of between 1 to 5 genes encoded in the opposite orientation where
234 no single protein is present across the subfamily. With the exception of phages SS3e and SP-031, all
235 members of the “Jerseyvirinae” encode a putative serine/threonine protease in this region (pfam:
236 PF12850, metallophos_2), which is linked by HHpred to the Ninl protein phosphatase in λ (PDB
237 1g5b).

238 Four critical units involved in genome replication, a replicative family A DNA polymerase (pfam:
239 PF00476), DEAD-box helicase-primase (pfam: PF13481) and helicase (pfam: PF00176) are also
240 conserved across all members of the proposed subfamily. Analysis of the replication gene cluster
241 using HHpred provides additional evidence for the presence of a Holliday junction resolvase (PDB
242 1hh1), helicase subunit (PDB 3h4r) and a helix-destabilising ssDNA-binding protein similar to gp2.5 of
243 phage T7 (PDB 1je5).

244 Finally, the lysis gene cluster codes for proteins facilitating lysis of the infected host cell, allowing
245 egress of newly formed virions into the surrounding environment. The lysis or late gene module
246 represents an area of significant divergence among the three genera and between individual phages.
247 The module is replete with ORFs of unknown function and only three gene products, the endolysin,

248 holin and a protein of unknown function are conserved across the subfamily. While the holin formed
249 a single cluster using OrthoMCL, the number of predicted transmembrane domains differed
250 between phage. On the basis of predicted transmembrane domains, phages AG11, Jersey, SE2,
251 SETP3, wksl3, Ent2 and SP-031 are presumed to encode class I holins while class II holins are
252 encoded by Ent1, Ent3, SP-101, SETP7, SETP13 and SS3e [64]. The endolysin belongs to the glycoside
253 hydrolase 24 family (muraminidase, pfam:PF00959). Many of the lysis gene products are shared
254 between only some members of each genus, while others are found between limited
255 representatives of one or more genera, suggesting that this region has been the site of frequent
256 genetic exchange.

257 **Description of individual genera**

258 **“Jerseylikevirus”**

259 This proposed genus is named after the first characterized phage of this morphotype, *Salmonella*
260 phage Jersey [1]. The genus is distinguished by a distinct morphology, a similar genome size among
261 its members, conserved gene organisation and the use of a P22-like tailspike to facilitate host
262 recognition and adsorption. The latter distinguished them from the “K1glikevirus” and
263 “Sp3unalikevirus” as well as by an additional 25 accessory genes shared between two or more
264 species. To date, 12 members, isolated from four different countries have been fully sequenced and
265 annotated (**Table 2**). Members of the “Jerseylikevirus” have an average G+C content of 49.79 %,
266 slightly lower than the average of 52 % reported for serovars of *Salmonella enterica* [41].

267 Host specificity is conferred by six tailspikes, observed as short clubs attached to the tail terminus.
268 Like the tailspikes of podophages P22, Sf6 and HK620, they exhibit a modular design consisting of a
269 conserved N-terminal binding domain and a P22-like C-terminal catalytic domain (data not shown).
270 In phage P22, the tailspike facilitates adsorption to *Salmonella* O-antigen 12 of the cell surface LPS,
271 expressed by members of White-Kauffmann-Le Minor serogroups A, B and D1 [23]. The high degree
272 of conservation of the P22-like domain and catalytic residues combined with host range data
273 available [15, 30, 57] for SETP3, SETP7, SETP13, vB_SenS-Ent1 and wksl3 suggests that
274 “Jerseylikevirus” isolated to date are limited to these *Salmonella* serogroups. However, SS3e appears
275 to be an exception, being reported as capable of lysing enterobacterial genera other than
276 *Salmonella*, including *E. coli*, *Enterobacter cloacae*, *Shigella sonnei* and *Serratia marcescens* [57].

277 Several members of the “Jerseylikevirus” (Jersey, Ent1, Ent2, Ent3, SE2, wksl3 and SP-101) encode a
278 putative DNA binding protein containing one or two Pfam family domains; ANT (PF03374) and pRha
279 (PF09669). In phage P22, *ant* encodes an anti-repressor which inhibits binding of the c2 repressor to

280 the PL and PR operators enabling the expression of genes necessary for the lytic development [10].
281 The pRha domain represents a family of proteins whose expression is detrimental for lytic growth in
282 the absence of integration host factor function [55].

283 A gene product with similarity to inner membrane immunity (Imm) proteins, which protect against
284 superinfecting phages [33], is also found in all Jersey-like phages, except Jersey and AG11. This gene
285 product contains an Imm_superinfect motif (pfam: PF14373), is predicted to localize at the
286 cytoplasmic membrane (PSORTb) and contains two transmembrane domains (TMHMM). Only the
287 putative immunity protein [AAZ41745] is annotated in SS3e, although this appears to be due to an
288 incomplete genome sequence rather than an absence of further proteins in this region.

289 An interesting feature of the “Jerseylikevirus” is that intein insertion is evident in the DNA helicase of
290 phages vB_SenS-Ent1, Ent2, Ent3, SETP3 and SETP7 and also within the DNA polymerase of phages
291 FSL SP-101, Ent1, Ent2, Ent3, SE2 and SETP3 (data not shown). Inteins are defined as protein
292 sequences embedded within a precursor sequence which, upon translation, catalyzes self-excision
293 from the host polypeptide and ligation of the flanking sequences to yield two stable products; the
294 mature protein (extein) and the intein [48].

295 In SE2 and SS3e the DNA polymerase appears to be encoded by more than one gene. For SE2, two
296 gene products, gp05 (AEX56144) and gp06 (AEX56145) have predicted DNA polymerase activity. The
297 large subunit, gp06, is predicted to possess an intein similar to that found in SETP3 and vB_SenS-
298 Ent1. Like SE2, the DNA polymerase of SS3e also appears to be split into two coding sequences (gp41
299 and gp43) although in this case, the subunits are interrupted by an additional gene (gp42;
300 AAW51247) with a predicted C-terminal HNH endonuclease domain. Notably all three gene products
301 show short matches to either the N- or C-terminal ends of the SETP3 intein.

302 A total of 15 accessory genes are encoded within the lysis/late gene cassette of which only 3 have
303 homologues with predicted functions; a putative protease (9 members), a HNH homing
304 endonuclease (7 members) and a putative RNA-binding protein (2 members).

305 “K1glikevirus”

306 The *Escherichia* phages K1G, K1H, K1ind1, K1ind2 and K1ind3, comprising the “K1glikevirus” are
307 described as K1-dependant or -independent, denoting the requirement for the K1 capsule for
308 productive infection [9]. The GC content of their genome is slightly higher than the “Jerseylikevirus”
309 ranging between 51.1 to 51.5%, a value closer to that of their host *E. coli* (50.8 %). Moreover, they
310 encode fewer proteins than the “Jerseylikevirus”.

311 Members of the “K1glikevirus” possess tailspikes, which are of similar size to the “Jerseylikevirus”.
312 These proteins exhibit similarity to the conserved N-terminal domain identified within the
313 “Jerseylikevirus” but possess divergent C-terminal domains, indicative of their different host
314 specificity. Tailspikes from the K1-independent phages K1ind1, K1ind2 and K1ind3 exhibit high
315 similarity to the HK620 tailspike [PDB 2x6w] and appear to belong to the pectate lysase 3 family
316 (pfam: PF12708). The HK620 tailspike possess endo-*N*-acetylglucosaminidase activity that degrades
317 the O-antigen of *E. coli* serotype O18A1 [7]. In contrast, the tailspike encoded by the K1-dependant
318 phages K1G and K1H exhibit endo-*N*-acyl-neuraminidase (endosialidase) activity [9] and are nearly
319 identical to the tailspike of the T7-like phage K1F (PDB 3ju4). Endosialidases bind to and degrade the
320 K1 capsular polysaccharide, a homopolymer made up of α 2,8-linked sialic acid residues [6]. Each of
321 the K1-dependant tailspikes are predicted to contain a C-terminal Peptidase_S74 domain (PF13884)
322 which shows homology to the protease domains of K1F, K1E, K1-5 tailspikes as well as the long tail
323 fibre of T5. In these phages this domain has been shown to function as an intramolecular chaperone,
324 whose presence and subsequent auto-cleavage is essential for folding and assembly of mature
325 proteins [50]. These data indicate that the K1-dependant tailspikes undergo a distinct maturation
326 process to their counterparts in the “Jerseylikevirus” and K1-independent members of the
327 “K1glikevirus”.

328 With one or two exceptions, members of the “K1glikevirus” share the complete structural module
329 with phages of the “Jerseylikevirus” genus. Phage K1ind3 has a HNH homing endonuclease gene
330 immediately downstream of a gene coding for a gp7 family morphogenesis protein (gp04) related to
331 *Bacillus* phage SPO1 (PBD 1u3e). K1G encodes another homing endonuclease (gp12) with 31 %
332 identity to MobE of coliphage T6. Notably, the K1H major capsid sequence differs substantially to
333 other “Jerseyvirinae” phages, but is predicted to have a similar structure to SPP1 and HK97 using
334 HHpred. A further gene product of unknown function, K1H gp09, not found in other members of the
335 proposed subfamily is encoded immediately downstream of the gene coding for the major capsid
336 protein (K1H gp08, ADA82303).

337 Three gene products are conserved across all K1G-like phages in the immunity gene module, a
338 serine/threonine protein phosphatase (pfam: PF12850), an acid-phosphatase B domain protein
339 (pfam: PF03767) and a protein with inferred ATPase activity (pfam: P13207). With the exception of
340 K1H, all members of the “K1glikevirus” encode a super-infection immunity protein. Neither Jersey,
341 AG11 nor K1H appear to harbour an immunity protein, instead these phages encode a hypothetical
342 protein forming a single OrthoMCL cluster.

343 In addition to the core replication proteins of the “Jerseyvirinae”, each of the K1G-like phages encode
344 a C-5 cytosine specific methylase (pfam: PF00145) which bears little sequence similarity to the
345 methylase of phage Jersey or FSL SP-101 and falls under a separate cluster using OrthoMCL. All K1G-
346 like phages except K1ind1 encode a putative UvsX-like protein. In coliphage T4, UvsX functions as a
347 RecA-like recombinase interacting with the UvsY helicase to promote strand-exchange during
348 genome replication [20].

349 Finally, a total of seven proteins are conserved across all K1G-like phages in the lysis or late gene
350 module, three of which are conserved across the subfamily; the holin, lysin and a hypothetical
351 protein. Unlike the “Jerseylikevirus”, each of the K1G-like phages are predicted to encode a separate
352 class I and class II holin, with three and two transmembrane domains, respectively. Each K1G-like
353 phage has a NinH-like domain protein (pfam: PF06322) in addition to two gene products of
354 unknown function. Lastly, a gene product found only in the K1-independent phages, returns a
355 DUF3850 family motif (pfam: PF12961), suggested to be involved in RNA recognition.

356 “Sp3unalikevirus”

357 This genus was recently proposed by Moreno Switt et al. (2014). To date, three members (FSL SP-
358 031, FSL SP-038, and FSL SP-049) have been reported, though only the genome of phage FLS SP-031
359 is fully sequenced, hence the proposed genus “Sp3unalikevirus” [43]. Phages in the
360 “Sp03unalikevirus” genus were isolated from dairy farms in the state of New York, with history of
361 *Salmonella* isolation [44]. Sp031-like viruses have a GC content of 51.1%, closer to the *Salmonella*
362 G+C of approximately 52% [47].

363 Unique characteristics of “Sp3unalikevirus” are non-essential “cargo” genes and genes associated
364 with host-specificity. The genes coding for a number of unique hypothetical proteins with unknown
365 function are located in the replication module of SP-031-likeviruses. Two annotated proteins that are
366 absent in the “Jerseylikevirus” and “K1glikevirus” are homing endonucleases. These homing
367 endonucleases are inserted in the replication module of FLS SP-031, one endonuclease shows 41%
368 identity over the 93% of the protein with an endonuclease in *Synechococcus* phage S-SSM7
369 (GenBank YP_004324330.1), and the other endonuclease, with the homing endonuclease domain
370 PF13392, showed 42% identity over the 89% with an endonuclease in the genome of *Salmonella*
371 phage FLS SP-126 (GenBank AGF87903.1). Another difference of Sp03unalikeviruses is the presence
372 of a gene encoding a putative phosphoadenosine phosphosulfate (PAPS) reductase (pfam: PF01507)
373 in the virion assembly module. PAPS reductases are involved in the reduction of sulfate to hydrogen
374 sulfide, which is generated by *Salmonella* in the gut of humans and animals [60]. While PAPS

375 reductases have previously been reported in temperate phages (e.g., BcepB1A [54]), there is no
376 current evidence to indicate whether this enzyme confers a fitness advantage to the host.

377 Sp031-like phages have a very narrow host range. When 23 different *Salmonella* serovars were
378 tested, only *Salmonella* Cerro was lysed [44]. This host specificity is likely related with the tail spikes
379 in the “Sp3unlikevirus”. BLAST of the tail spike amino acid sequence of FSL SP-031 showed as the
380 best hit (78% over 80 % of the protein) a tail spike of a P22-like prophage found in the genome of *S.*
381 Cerro str. 818 (GenBank ESH26034), with the divergence at N-terminal. This prophage shows no
382 further identity to any other CDSs of Sp031-like phages. . To further investigate the potential genetic
383 mechanism involved in this host range, an alignment of the tail spike amino acid sequences of
384 representative Jersey-likeviruses (i.e., Jersey, L3, SETP7, SETP3, SE2, Ent2, SETP12, and FSL SP-101)
385 and Sp031-like viruses (FSL SP-031) showed a conserved N-terminal (approx. 87 % aa identity) and a
386 divergent C-terminal (approx. 15% aa identity). This finding corresponds with the host specificity
387 reported; while other *Salmonella* phages in the “Jerseyvirinae” subfamily infect serogroups A, B, and
388 D; Sp031-likeviruses only infect serogroup K.

389 Discussion

390 The availability of an increasing number of genome sequences and improvements in gene prediction
391 methods has resulted in a sizeable shift towards the inclusion of genomic and proteomic data for the
392 taxonomic classification of bacteriophages [37]. However, a number of different, and sometimes
393 conflicting, approaches have been reported in the literature for the purposes of delineating
394 evolutionary relationships between phages including the proteomic tree [49], numbers of shared
395 homologous/orthologous proteins [35, 36] and reticulate classification based on gene content [39,
396 40]

397 Our analysis reveals that the classification criteria introduced for members of the *Podoviridae* and
398 *Myoviridae* [35, 36], based upon the existence of protein homologs, tend to “lump” taxa rather than
399 represent the true taxonomic relationship within a genus. Using an alternative approach employing
400 NCBI BLASTN and TBLASTX, genera can be defined a possessing $\geq 65\%$ DNA sequence identity while
401 subfamilies show $\geq 40\%$ protein homologs (Kropinski, Edwards and Mahadevan, unpublished results).
402 These values are those derived by Niu et al. in their assessment of the T1-likeviruses [46].

403 Phylogenetic analysis also demonstrates that the *Salmonella* and *Escherichia* phages described here
404 fall into three clusters, substantiating the establishment of three genera. Analysis using EMBOSS
405 Stretcher demonstrated that while phages within each genera were closely related, a significant

406 relationship based upon shared homologous proteins identified using CoreGenes and OrthoMCL
407 existed between genera. Based upon these data we propose the establishment of a subfamily of
408 *Siphoviridae*, the “Jerseyvirinae” comprised of three genera, the “Jerseylikevirus”, “K1Glikevirus” and
409 “Sp3unalikevirus”. The three genera of closely related phages were isolated from geographically
410 disparate locations. This suggests that phages of this subfamily are widely distributed and have
411 persisted and evolved in various environments. Considering the length of time between the isolation
412 of Jersey and the other members, horizontal gene transfer does not always mask the identification
413 of taxomic relationships between phages. Grose and Casjens have recently clustered 337 phages
414 infecting various members of family *Enterobacteriaceae* [24] based upon average nucleotide
415 identity, conserved gene product content in addition to whole genome nucleotide and amino acid
416 dotplots. They report a “SETP3 supercluster”, formed of 5 clusters of lytic phages; SETP3-like, SO-1-
417 like, ECO1230-10-like, Gj1-like and PY100-like phages. Our independent findings are in broad
418 agreement with those reported by Grose and Casjens but provide a more detailed analysis of the
419 phages comprising the SETP3-like subcluster.

420 All Jersey-like phages isolated to date exhibit a strictly lytic lifestyle and encode no proteins with
421 homology to known toxins or allergens. As such members of this subfamily appear suited to
422 biocontrol applications [8, 25], particularly the “Jerseylikevirus” which exhibit a broad host range
423 encompassing important serogroups of *Salmonella*.

424 Undoubtedly more Jersey-like bacteriophages will be isolated in the future. The authors hope the
425 data provided here will act as a starting point for the annotation of these future isolates.

426 **Author’s contributions**

427 DT, AIMS, HA, AK, SM and HWA wrote the manuscript. DT, AIMS, HA, AMK, JEN, HWA, NDL and SM
428 contributed towards the analysis of data. All authors read and approved the final manuscript.

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433 **Conflict of interest**

434 The authors declare that they have no competing interests.

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