

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

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| **Code assigned:** | **2020.001B** |  |
| **Short title:**  Create two new species in the genus *Myohalovirus* (*Caudovirales*: *Myoviridae*) | | |
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**List the ICTV Study Group(s) that have seen this proposal**

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| Archaeal viruses Study Group (David Prangishvili) |

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

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

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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 | 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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| 2020.001B.R.Myohalovirus.xlsx |

**Abstract**

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| Two haloviruses, ChaoS9 and phiCh1, are proposed as novel species within the genus *Myohalovirus.* Like the type species of the genus, *Halobacterium virus phiH*, they infect members of the Class Halobacteria, are myoviruses with similar morphology, have linear dsDNA genomes of similar length (55-58 kb), similar G+C% (62-65%), and similar genome organization. In particular, they all carry an invertible tail-fiber gene module located near the middle of the genome. All genomes are terminally redundant and circularly permuted. Dot-plot comparisons (BLASTz) that included 15 other tailed haloviruses showed that phiH1, ChaoS9 and phiCh1 shared significant nucleotide sequence identity with each other but not with any other viruses. The average nucleotide similarity (ANI) between phiH1, ChaoS9 and phiCh1 varied between 72-79%. An inferred phylogenetic tree based on alignment of baseplate J (Bpj) proteins showed that ChaoS9 is part of a robust clade (100% bootstrap confidence) which includes phiCh1 and phiH1 but no other virus isolates. |

**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 this new genus. Each of the proposed species differs from the others with more than 5% at the DNA level as confirmed with the BLASTN algorithm.  New Species: Both of the proposed new species are considerably more than 5% different in DNA sequence from the type species *Halobacterium virus phiH*. Direct pairwise nucleotide alignments of the genomes of phiH1, ChaoS9 and phiCh1 show only patchy regions with high (≥ 90%) identity and it is not possible to align them along their full length using standard BLASTn (blastn suite-2sequences). Forced alignment using CLUSTAL gives similarity values of 50-60% but much of these alignments are not significant. Average Nucleotide Identity (ANI) values (<https://www.ezbiocloud.net/tools/ani>) varied between 72-79% but the proportion of aligned bases in each comparison was generally low; 9% (phiH1 vs ChaoS9), 11% (phiCh1 vs ChaoS9) and 31% (phiH1 vs phiCh1). *In silico* DDH estimates ([https://ggdc.dsmz.de/ggdc.php#](https://ggdc.dsmz.de/ggdc.php), formula 2) ranged from 21-58%. In summary, none of the three genomes meets the species demarcation criterion of an average of ≥95% nucleotide identity across their pairwise aligned genomes.  Currently, the genus *Myohalovirus* has a single member, the type species *Halobacterium virus phiH*, for which there are many supporting publications (see [2] and the references cited within). It has a fully sequenced genome [2]. *Natrialba* virus phiCh1 is a myovirus first described in 1997 [12], and infects the haloalkalophilic archaeon *Natrialba magadii*. A number of later publications have analysed the biology and genetics of this virus [1, 4-7, 9, 11]. ChaoS9 is a myovirus that infects *Halobacterium salinarum* strain S9, and was first described in 2019 [3]. In the latter study, ChaoS9 and phiCh1 were compared to each other and to the type species of the genus *Myohalovirus*, *Halobacterium virus phiH* [3].  Table 1 summarizes their main features of all three viruses. They share similar morphologies (head diameters and tail lengths), all have linear dsDNA genome of similar size (55-58 kbp), and have similar types of genome ends that are terminally redundant (TR) and circularly permuted (CP). The genbank accessions are: phiH1 (MK002701), phiCh1 (MK450543) and ChaoS9 (MK310226).  The nucleotide similarity of their linear, dsDNA genomes was compared each to other and to 15 other caudoviruses isolated from various haloarchaeal hosts (Class Halobacteria), and the results are summarized in Figure 1. ChaoS9, phiCh1 and phiH1 share significant sequence similarity to each other by dot-plot alignment (Fig. 1a) and by ANI (Fig. 1b), but do not show any significant similarity to other sequenced caudoviruses.  A BLASTx dot-plot comparison of just the three viruses is presented in Figure 2, showing that phiH1 and phiCh1 are more similar to each other (63% nucleotide identity) than either is to ChaoS9, and also highlights the invertible tail-fiber module (a cross in the dotplot) that is near central in all the panels.  A gene map comparison of the three genomes is shown in Figure 3, with BLASTx (inferred protein) similarities between them indicated by pink shading. They share many similar protein coding genes, and all carry a similar invertible tail fibre module that is near-central in each genome (labeled as ‘invertible region’ below phiH1). The function of this region in switching tail fiber expression has been examined in most detail in phiCh1 [7, 9]. Overall, the three share a similar gene organization across their genomes, with virus assembly and DNA packaging modules occupying the left half of the genome while genes relating to lysogeny, plasmid replication and DNA modification are located in the right half of the genome. While virus assembly proteins are generally well conserved (left half), particularly in tail genes and the invertible region, there is more variation between the three viruses within the right half where the lysogeny/replication/modification genes are found.  In many taxonomic studies of prokaryotic caudoviruses, the large subunit terminase (TerL) and major capsid proteins (Mcp) are used to help study their evolutionary relationships [8]. While phiH1 and phiCh1 share similar Mcp and TerL sequences (89% and 83% aa identity, respectively), the ChaoS9 Mcp differs completely from the other two, showing instead a relatively low similarity (36% aa identity) to the Mcp of halovirus HHTV-1 (AGM11277.1). There is no other similar protein between ChaoS9 and HHTV-1. It appears that there has been a recombination event that has swapped out just the head/packaging module of ChaoS9. Because of this, other conserved proteins were examined and the base-plate J like protein found to be the most useful, as it was strongly conserved in sequence in all three viruses and also displayed sequence similarity to a range of other Bpj-like proteins of other viruses. A phylogenetic tree reconstruction using Bpj protein sequences is shown in Figure 4. The three viruses (phiH1, phiCh1 and ChaoS9) branch together to the exclusion of other known viruses, and form a clade with 100% bootstrap confidence. They branch with chromosomal genes from proviruses or provirus remnants in the genomes of four haloarchaea, indicating that members of this virus genus are widespread in nature.  In summary, the myoviruses phiCh1 and ChaoS9 are specifically related to the *Halobacterium virus phiH* but their genomes show far less than the threshold nucleotide similarity of 95% to be members of the type species. They also show far less than 95% nucleotide similarity to each other . We propose that phiCh1 and ChaoS9 be included in the genus *Myohalovirus* as two new species, with the names *Natrialba virus phiCh1* and *Halobacterium virus ChaoS9*. | |

**Supporting evidence**

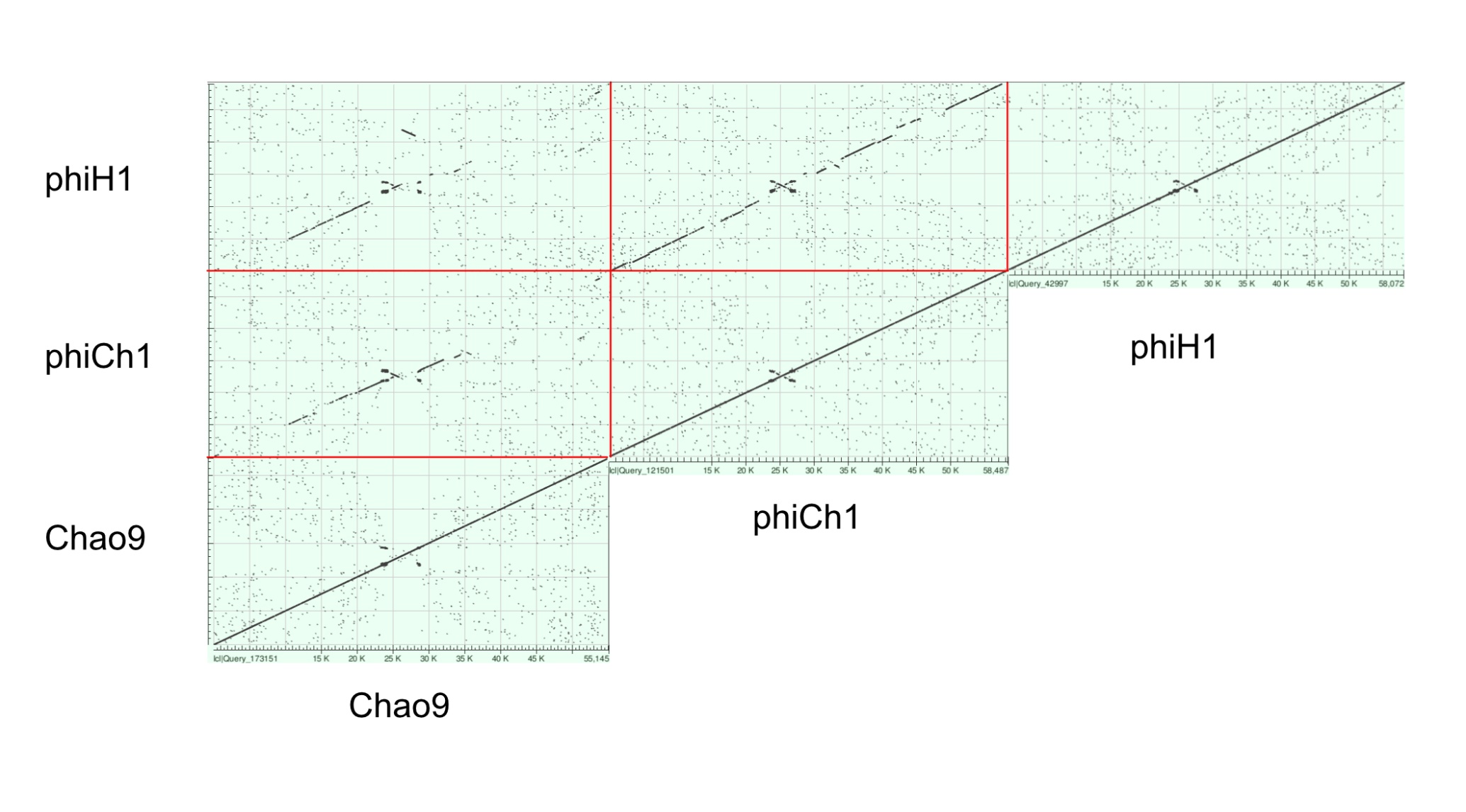
**Table1**. Major characteristics of haloviruses PhiH1, PhiCh1 and ChaoS9

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| **Virus1** | **Head diameter (nm)** | **Tail length  × width (nm)** | **Morphotype** | **Plaque morphology** | **Unit Genome  length**1 **(nt)** | **%G+C** | **Genome**  **Ends in Virus 2** | **Temperate (genome form)** |
| ChaoS9 | 61 | 128 × 17 | myovirus | turbid | 55,145 | 65.3 | ds, linear, TR,CP, >1 unit length | ? |
| phiH1 | 64 | 170 × 18 | myovirus | turbid | 58,072 | 63.7 | ds, linear, TR,CP, >1 unit length | Yes, provirus  is a plasmid (circular, ds, 1 unit length) |
| phiCh1 | 70 | 130 × 20 | myovirus | turbid | 58,487 | 61.9 | ds, linear, TR,CP, >1 unit length | Yes, provirus  is a plasmid  (circular, ds, 1 unit length) |

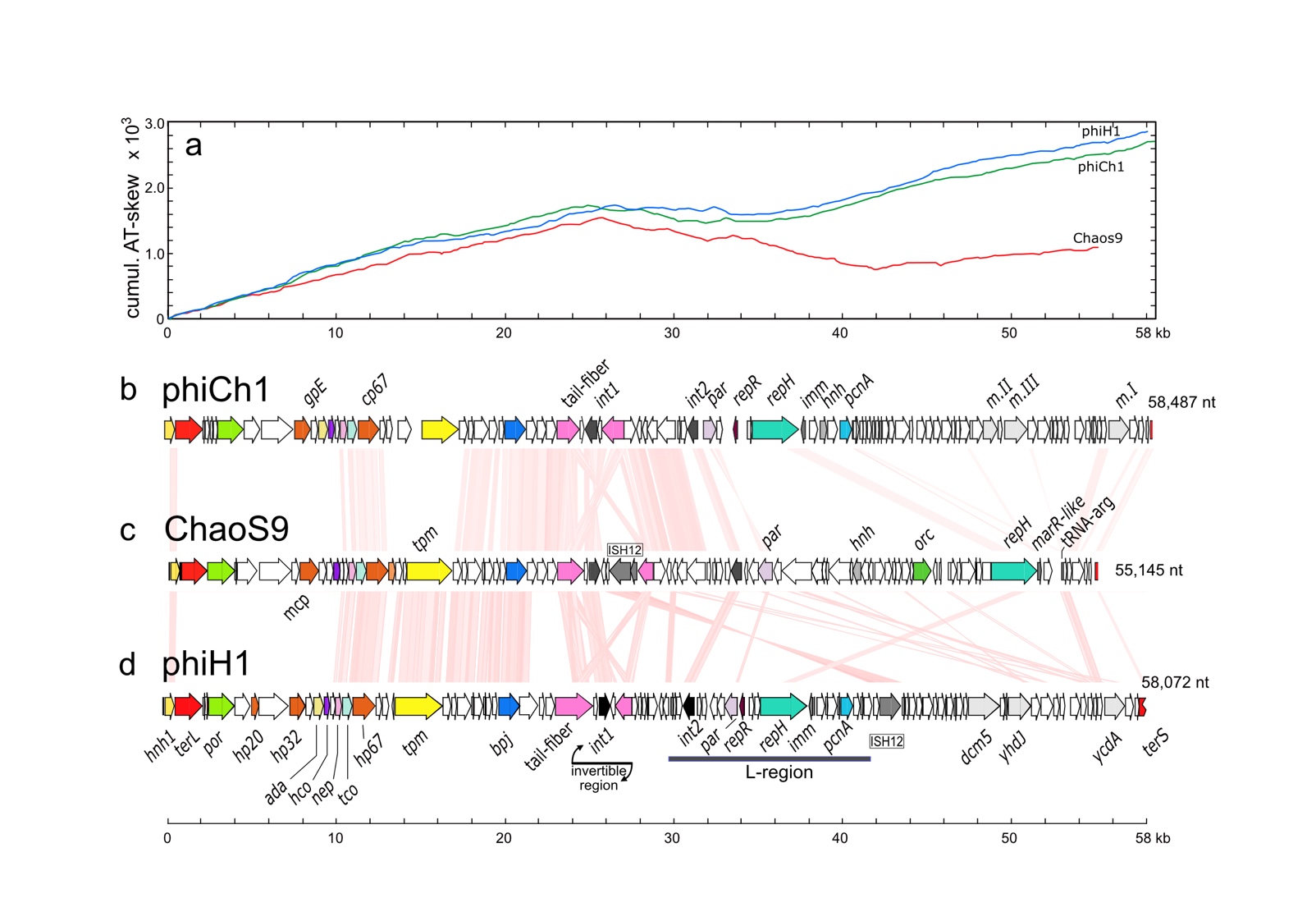
1Data for ChaoS9 was from [3] (open access), for phiH1 was from [10] and [2], and for phiCh1 was from [12] and [6]. 2TR, CP; terminally redundant, circularly permuted.

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|  |  |
| **a** | **b** |

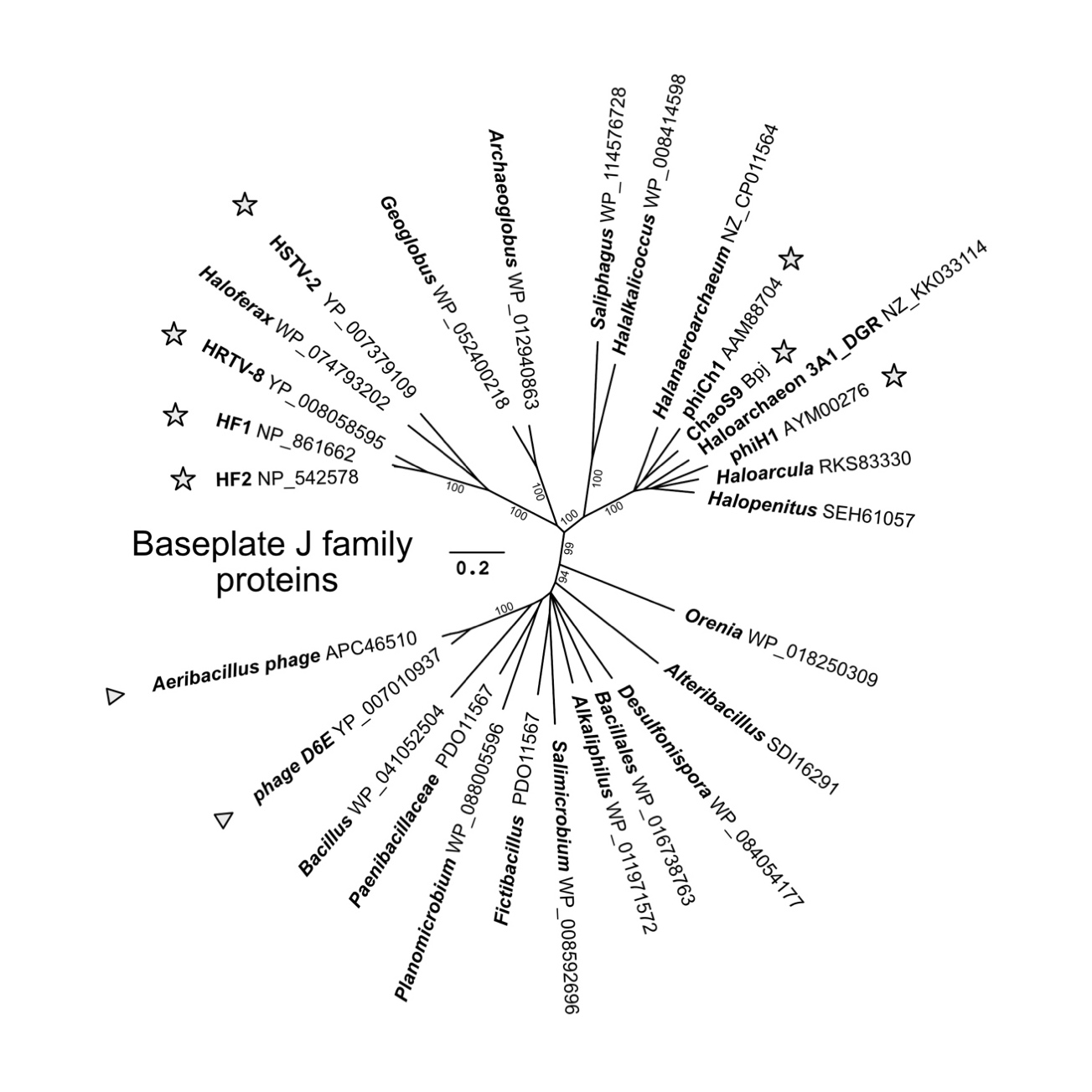
**Figure 1.** Dot-plot comparison (panel a) and average nucleotide identities (ANI; panel b) of haloviruses ChaoS9, phiCh1, phiH1 and 15 other tailed haloviruses. Data from [3](open access). The dot plot was performed using zPicture (https://zpicture.dcode.org/) and the ANI calculations were performed on the EZbiocloud webserver (<https://www.ezbiocloud.net/tools/ani>).



**Figure 2.** Dot-plot comparison (BLASTx) of haloviruses ChaoS9, phiCh1, phiH1. Pairwise comparisons were performed using BLASTx option at (<https://blast.ncbi.nlm.nih.gov/>).



**Figure 3**. Genome map comparison of ChaoS9, phiCh1 and phiH1. Scale bar at bottom shows length, in kb. Pink shading between genome maps indicates similarity of encoded proteins (tBLASTx) at ≥ 30% amino acid identity level. Colour coding of genes indicates those encoding proteins with predicted (or experimentally determined) functions. Some of these genes are labeled along the gene maps. Data from [3](open access).

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**Figure 4.** Phylogenetic tree reconstruction using baseplate J family (Bpj) proteins. Halovirus proteins are indicated by stars and bacteriophage proteins are marked by triangles. The consensus tree was produced using the Neighbor-Joining algorithm and 100 bootstrap replications. Bootstrap values are shown near branch points. Scale bars represent the estimated number of amino acid replacements per position. Data from [3](open access)

**Acknowledgement:** Most figures and the table were from reference [3], an open access publication where copyright is held by the authors, and the article is distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/). All four authors of this proposal are authors of that publication.

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