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Unveiling the Genetic Ties of Singleton RomansRevenge

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Bacteriophages are categorized through a dynamic system of clusters based on genetic similarities. Despite the clustering system’s flexibility, many bacteriophages, known as singletons, do not fit within the existing categories. By exploring novel singleton RomansRevenge’s genome through bioinformatic tools including DNA extraction and sequencing, annotation, SplitsTree, gene content similarity (GCS) heatmaps, dotplot analysis, and Phamerator, RomansRevenge was compared with other bacteriophage clusters to investigate its identity. When comparing RomansRevenge to the AP, EG, B, and DL clusters, the AP cluster was shown to be the most similar to RomansRevenge. AP bacteriophages generated the highest GCS with RomansRevenge in comparison to other clusters, with GCS values of 25.8%. Amino acid sequence similarity was discovered in the left-end region of the genome between RomansRevenge and bacteriophages from the AP subclusters. Synteny and pham similarity in the between RomansRevenge and AP bacteriophages was also present. Aligning with function annotations towards the right-end region of RomansRevenge’s genome, where most genes were classified as no known function, a divergence in functional evolution from AP bacteriophages is suggested. RomansRevenge demonstrated close relationships with AP bacteriophages within shared phams for minor tail proteins, indicating similarity to AP in tail assembly and infection cycle. Despite the genomic similarities, lack of comprehensive congruence across the entire genome underscored the rationale for maintaining RomansRevenge’s classification as a singleton. The study of singletons like RomansRevenge is critical for mapping the uncharted territories of bacteriophage genomics and uncovering gaps where new clusters may emerge, enriching known bacteriophage biodiversity.