



## Article

# BorMax, a Novel *Dhillonvirus* Infecting MDR *Escherichia coli*: Genomic Insights, Taxonomic Position, and Anti-CRISPR Repertoire

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## Abstract

*Escherichia coli* is a major pathogen responsible for urinary tract infections, septicemia, and other clinically relevant conditions, with increasing multidrug resistance (MDR) limiting available treatment options. In this context, bacteriophages represent a valuable resource for exploring novel antimicrobial and biotechnological tools. Here, we report the isolation and genomic characterization of BorMax, a novel lytic phage infecting multiple MDR *E. coli*. Transmission electron microscopy revealed a tailed morphology consistent with *Dhillonvirus*. Whole genome sequencing and de novo assembly showed a linear double-stranded DNA genome of 45,502 bp, encoding 70 predicted coding sequences (CDSs) and lacking tRNAs. Bioinformatic analyses confirmed the absence of lysogeny-associated genes, as well as virulence and antimicrobial resistance determinants. Comparative genomics using classified BorMax within the genus *Dhillonvirus* as a new species, sharing <77% intergenomic similarity with known members. Notably, predictions using PaCRISPR and AcRanker identified four CDSs with strong anti-CRISPR (Acr) potential, representing previously undescribed Acr candidates in this group. These genomic features highlight the novelty, safety, and potential biotechnological relevance of BorMax and contribute to the expanding genomic and functional diversity of *Dhillonvirus* and *E. coli*-infecting phages.

**Keywords:** bacteriophages; antimicrobial resistance; *E. coli*

## 1. Introduction

*Escherichia coli* is a common inhabitant of the intestinal microbiota of humans and animals, where it typically plays a commensal role in maintaining gut homeostasis. However, certain pathogenic strains have evolved virulence factors that enable them to cause a wide range of diseases, including Urinary Tract Infections (UTIs), neonatal meningitis, septicemia, and various gastrointestinal disorders [1]. Over the past decades, the emergence and dissemination of pathogenic *E. coli* strains have become a major public health concern worldwide [2–4]. These strains often carry Extended-spectrum  $\beta$ -lactamases (ESBLs) and carbapenemases, conferring resistance to critically important antibiotics and severely limiting therapeutic options [5]. The World Health Organization (WHO) has classified Enterobacterales resistant to third-generation cephalosporins and/or carbapenems as critical priority pathogens, emphasizing the urgency of developing new antimicrobial strategies [6]. The global crisis that represents AMR threatens to undermine the effectiveness of modern medicine, leading to increased morbidity, mortality, and healthcare costs [7]. The alarming



Academic Editor: Nicolai S. Panikov

Received: 20 November 2025

Revised: 16 January 2026

Accepted: 26 January 2026

Published: 2 February 2026

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spread of AMR genes through horizontal gene transfer further accelerates this problem, affecting both clinical and environmental settings. Therefore, there is an urgent need to explore innovative and sustainable therapeutic alternatives capable of controlling pathogenic bacteria and mitigating the escalating threat of AMR.

Bacteriophages, or bacterial viruses, represent a highly specific biological alternative for the control of pathogenic bacteria [8]. Phage therapy has gained renewed momentum due to the global rise of AMR, which limits the effectiveness of current treatments. Recent technological advances have improved phage isolation, characterization, and genomic analysis, while successful clinical trials continue to validate their therapeutic potential [9]. Moreover, international efforts are underway to standardize production, safety, and regulatory frameworks for medical application [10]. Phage therapy offers several advantages over conventional antibiotics, making it a promising alternative against antibiotic-resistant infections. Phages are highly specific, targeting only pathogenic bacteria without disturbing beneficial microbiota or human cells [11]. Their replication is self-limiting, ceasing once host bacteria are eliminated and they degrade naturally and do not persist in the environment like antibiotics [12]. Moreover, although bacterial resistance may emerge, phages can co-evolve to overcome these defenses [13].

Among the principal strategies that bacteria employ to defend themselves against invading Mobile Genetic Elements (MGEs)—including bacteriophages and plasmids—the CRISPR–Cas system stands out as a key adaptive immune mechanism [14,15]. In *E. coli*, multiple CRISPR–Cas types have been described, and several reports suggest that the absence or inactivation of these systems can correlate with increased antimicrobial resistance, indicating a possible trade-off between genome defense and the acquisition of resistance determinants [16,17]. At the same time, MGEs have evolved counter-defense mechanisms, such as Acr proteins, which enable them to bypass CRISPR–Cas-mediated immunity [18]. In *E. coli* populations, Acrs have been identified primarily in plasmids, although an increasing number of *E. coli* phages encoding Acr factors are beginning to be recognized [19].

Although members of the genus *Dhillonvirus* have been increasingly identified in environmental samples, most are reported only at the genomic level, with limited insights into their structural diversity, anti-defense mechanisms, or molecular traits associated with host interaction. In particular, the presence and biological relevance of anti-CRISPR systems in *Dhillonvirus* remain largely unexplored, and the genomic diversity within the group is still underestimated. Here, we describe BorMax, a novel lytic phage infecting multiple MDR *Escherichia coli*, isolated from wastewater and representing a new species within *Dhillonvirus*. We combined genome sequencing, taxonomic assignment, comparative genomics, and prediction of anti-CRISPR elements to characterize its genetic architecture and evaluate its safety-related genomic features, including the absence of lysogeny-associated, virulence, and antimicrobial resistance genes. Notably, BorMax harbors multiple putative anti-CRISPR proteins not previously reported in this genus, offering new insights into phage–host coevolution and expanding the current understanding of the molecular diversity within *Dhillonvirus*. These findings contribute to closing important knowledge gaps on the taxonomy, genome organization, and anti-defense repertoire of phages infecting MDR *E. coli*, providing a valuable genomic resource for future functional and biotechnological studies.

## 2. Materials and Methods

### 2.1. Bacterial Strains

The novel phage was isolated using *Escherichia coli* ATCC 25922 as the host strain. To evaluate its host range, an additional panel of 50 clinical *E. coli* isolates was included. These

strains were identified using standard biochemical methods. All bacterial isolates were preserved at  $-80^{\circ}\text{C}$  in LB broth containing 20% glycerol and routinely cultured in LB broth at  $37^{\circ}\text{C}$ .

## 2.2. Sample Collection and Processing

Samples of domestic wastewater from Concepción, Chile, were collected in sterile 50 mL tubes (50 mL per sample) and stored at  $4^{\circ}\text{C}$  until processing within 48 h. The samples were centrifuged at  $8000 \times g$  for 45 min at  $4^{\circ}\text{C}$ , after which the supernatant was recovered to eliminate large particulates and most bacterial cells. To further remove residual bacterial debris while preserving the viral fraction, the clarified supernatant was filtered through a sterile 25 mm Whatman glass fiber membrane with a pore size of  $0.22\text{ }\mu\text{m}$ .

## 2.3. Isolation, Purification and Propagation of the Novel Phage

The conventional Double Layer Agar (DLA) method was performed [20] using 3 mL of soft agar (0.5%) supplemented with  $\text{CaCl}_2$  (5 mM) heated at  $55^{\circ}\text{C}$ , 100  $\mu\text{L}$  of wastewater samples previously filtered and 100  $\mu\text{L}$  of the overnight culture of *E. coli* ATCC 25922. After mixing, the solutions were poured onto LB plates (1.5% agar) and incubated overnight at  $37^{\circ}\text{C}$ . Successive single-plaque purifications were performed by picking a plaque, incubating it with a fresh bacterial overnight culture, and replating it in LB top agar. Pure suspensions were stored in SM Buffer ( $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  8 mM, Tris-HCl 50 mM, NaCl 100 mM) at  $4^{\circ}\text{C}$ . The titer of the phage suspension was measured by DLA using serial dilutions in SM buffer. Subsequently, lysis plaques were counted, and the titer was expressed as Plaque-Forming Units per Milliliter (PFU/mL).

For phage propagation, an overnight culture of *Escherichia coli* ATCC 25922 was mixed with the phage suspension at a multiplicity of infection (MOI) of 0.1. Briefly, 1 mL of phage suspension ( $10^7$  PFU/mL) and 1 mL of an overnight bacterial culture were added to 100 mL of LB broth supplemented with  $\text{CaCl}_2$  (5 mM). The mixture was incubated at  $37^{\circ}\text{C}$  for 16 h without shaking to allow phage replication. Following incubation, the culture was centrifuged at 4000 rpm for 15 min to remove bacterial debris. The supernatant was collected, filtered through a  $0.22\text{ }\mu\text{m}$  pore-size membrane, and supplemented with polyethylene glycol 8000 (PEG 8000; final concentration 10% [wt/vol]) and NaCl (final concentration 0.5 M). The mixture was incubated overnight at  $4^{\circ}\text{C}$  to precipitate phage particles. Phages were pelleted by centrifugation at 8000 rpm for 2 h at  $4^{\circ}\text{C}$ , after which the supernatant was discarded and the pellet was resuspended in 750  $\mu\text{L}$  of SM buffer. Phage titers were determined by DLA using serial dilutions in SM buffer, as described above.

## 2.4. Host Range and Efficiency of Plating (EOP)

The host range was determined against *E. coli* as well as *Salmonella enterica* subsp. *enterica* ATCC 25928 and *Pseudomonas aeruginosa* ATCC 27853. For this, mixtures of overnight bacterial cultures and phage suspension were incubated at MOI 1 during 15 min and then 20  $\mu\text{L}$  were spotted onto LB agar plates by triplicate.

The efficiency of plating (EOP) of phage BorMax was determined for all BorMax-susceptible clinical *Escherichia coli* isolates. Briefly, overnight cultures of each strain were prepared in LB broth and adjusted to exponential phase. Serial tenfold dilutions of the phage suspension were prepared in SM buffer and plated by DLA using each test strain as the host. In parallel, the same phage dilutions were plated on the reference host *E. coli* ATCC 25922. After incubation at  $37^{\circ}\text{C}$  for 16–18 h, plaques were enumerated and phage titers were calculated as plaque-forming units per milliliter (PFU/mL). The EOP for each isolate was calculated as the ratio of the PFU/mL obtained on the test strain to the PFU/mL obtained on *E. coli* ATCC 25922. All assays were performed in triplicate.

The antibiotic susceptibility of the BorMax-sensitive strains was assessed using a standard disk diffusion assay on Mueller–Hinton agar, following the Clinical & Laboratory Standards Institute (CLSI) recommendations. After incubation, the diameters of the inhibition halos generated by each antimicrobial disk were recorded and interpreted according to CLSI breakpoints. The antimicrobial panel tested included imipenem, meropenem, ertapenem, ceftriaxone, cefotaxime, ceftazidime, ceftazidime/avibactam, aztreonam, gentamicin, amikacin, trimethoprim/sulfamethoxazole and ciprofloxacin.

### 2.5. DNA Extraction

Highly concentrated phage stocks ( $10^{11}$  PFU/mL) were treated with DNase and RNase according to Parra et al. [21]. Then, DNA was extracted using PureLink® Viral RNA/DNA Mini Kit (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA). The purified DNA was then visualized by 0.8% agarose gel electrophoresis and stored at  $-20^{\circ}\text{C}$ . The concentration was quantified using the Qubit 1× dsDNA High Sensitivity Assay Kit (Thermo Fisher Scientific) and a Nanodrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

### 2.6. Sequencing and Genome Assembly

Sequencing was performed at SeqCenter Inc. (Pittsburg, PA, USA). Sequencing was performed on an Illumina NovaSeq X Plus system, generating  $2 \times 151$  bp paired end reads. Demultiplexing and adapter trimming were performed using BCL-convert v4.2.4. The quality of reads was measured using FastQC v0.12.1 [22]. Genome assembly was performed de novo using SPAdes v3.14 [23]. Assembly quality metrics, including coverage and completeness, were evaluated during genome reconstruction. A 2% random subsampling of reads was applied to reduce redundancy and computational bias while maintaining sufficient coverage for accurate genome assembly. Reads were randomly subsampled using seqtk v1.4 [24].

### 2.7. Genome Analysis and Annotation

A Megablast analysis was performed. Similar phages were checked on the International Committee on Taxonomy of Viruses (ICTV) webpage, and their sequences were downloaded from GenBank. Pairwise comparisons of sequences were performed using VIRIDIC [25]. For phage classification, the online services Virus Classification and Tree Building Online Resource (VICTOR) [26] and TaxMyPhage [27]. Open reading frames (ORFs) were predicted with Phanotate [28] in pharokka v1.3.2 software [29], using the PHROGs database [30], and subsequently validated manually by searches with BLASTn 2.17.0. The tRNAs were predicted with ARAGORN v2.36 [31]. Genomic maps phage was generated with Proksee [32].

### 2.8. Detection of ARGs, Virulence Genes and Acrs

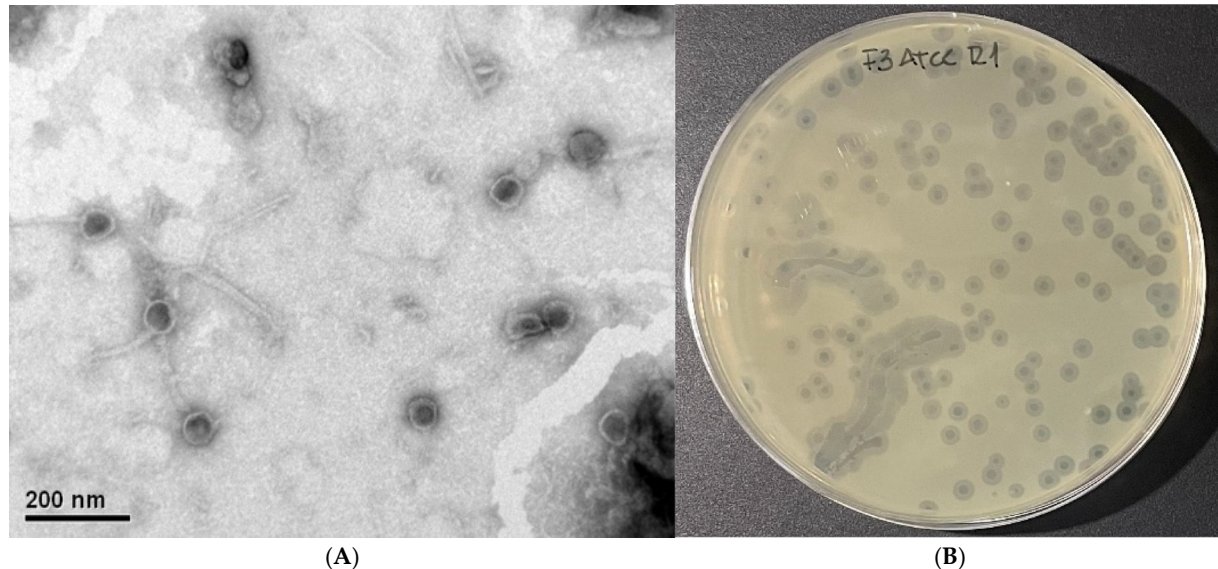
The search for genes encoding antibiotic resistance factors was performed using Resfinder 4.6.0 [33] and the resistance gene identifier in the Comprehensive Antibiotic Resistance Database (CARD) [34].

To identify putative Acrs, we used PaCRISPR [35] and AcRanker [36] with default parameters.

## 3. Results and Discussion

*Escherichia coli* is generally a harmless member of the human microbiota, yet certain pathogenic lineages can cause severe infections and often display resistance to commonly used antibiotics [1,2]. This growing challenge has renewed interest in bacteriophages as potential therapeutic tools. In the present work, we report the isolation and comprehensive phenotypic and genomic characterization of a new *E. coli*-infecting phage, designated

BorMax. Screening of wastewater samples resulted in the recovery of a single lytic bacteriophage, which produced clear plaques on the corresponding host strain and was purified by three successive rounds of plaque isolation prior to further characterization. TEM analysis revealed a tailed morphology featuring an icosahedral head and long tail (Figure 1A). BorMax produced circular lysis plaques with a diameter of 1.5 mm (Figure 1B).

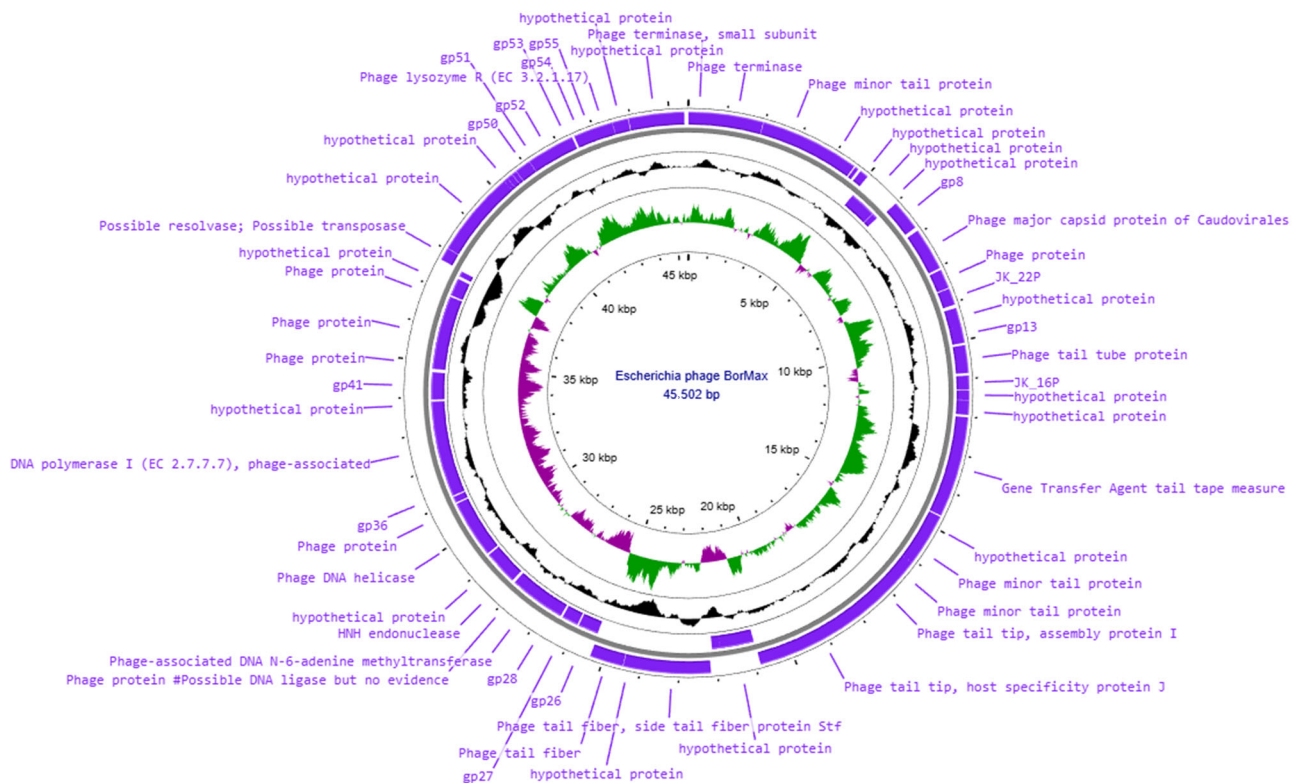


**Figure 1.** Transmission electron micrograph of phage BorMax (A) and lysis plaques of phage BorMax on the lawn of *E. coli* ATCC 25922 (B).

The assembled genome of the novel phage BorMax is a dsDNA molecule with a length of 45,502 bp and GC content of 53.45% (Figure 2). It was deposited in the GenBank database of NCBI (accession no. PX586628). It consists of 70 predicted ORFs organized into the classical modular architecture of lytic Caudoviricetes infecting *Escherichia coli*, without tRNAs predicted. The absence of integrases, repressors, excisionases, or any lysogeny-related genes confirms that BorMax is strictly lytic—an essential requirement for therapeutic applications. The genome encodes a complete set of structural proteins, including the terminase small and large subunits, the major capsid protein, multiple minor tail components, and a long tape measure protein consistent with a siphovirus-like flexible tail. Notably, BorMax possesses an expanded tail tip region containing proteins I and J, together with multiple tail fiber proteins, suggesting interactions with diverse bacterial surface receptors. In this regard, variable tail fibers are a characteristic feature of *Dhillonvirus*, and this structural diversity is consistent with the divergent host ranges observed within the group [37].

VICTOR and taxMyPhage analyses classified BorMax within the genus *Dhillonvirus*, a group that currently lacks an assigned subfamily, family, and order within the class Caudoviricetes. The genus *Dhillonvirus* comprises a group of strictly lytic bacteriophages, established following the recent ICTV-driven reorganization of tailed phage taxonomy [38]. The genus *Dhillonvirus* was originally established in 2014 under the name *HS78likevirus*, within the former family Siphoviridae. In 2015, this genus was formally renamed as *Dhillonvirus*. Subsequent reclassifications aligned with the major revision of tailed phage taxonomy: in 2018, the genus retained its rank, but its lineage was updated as part of the restructuring of Caudovirales; in 2019 and again in 2021, its placement was revised following the dissolution of the traditional families Siphoviridae, Myoviridae, and Podoviridae, ultimately assigning *Dhillonvirus* to the class Caudoviricetes [38]. Since then—including the most recent 2024 ICTV release—the genus has remained unchanged. Currently, *Dhillonvirus* comprises

49 recognized species, representing a cohesive group of strictly lytic phages with genomes of approximately 40–45 kb and a highly conserved modular organization, including structural, replication, and lysis cassettes, the latter typically encoding a lysozyme-type endolysin. *Dhillonvirus* phages predominantly infect Enterobacterales, particularly *Escherichia coli* and *Shigella* spp., and are frequently isolated from wastewater and other environmental sources. Although this genus encompasses nearly 50 recognized species, many remain poorly characterized beyond initial genome descriptions. This limited phenotypic and functional information underscores the importance of characterizing new isolates in this group to improve our understanding of their diversity, biology, and potential applications.



**Figure 2.** Genome map of novel phage BorMax obtained using Proksee. Open reading frames (ORFs), GC content and GC skew are indicated.

BorMax also carries a rich repertoire of replication and DNA metabolism genes, including a phage-associated DNA polymerase I, helicase, endonucleases, a putative DNA ligase, and an N6-adenine methyltransferase likely involved in evasion of host restriction systems. A functional lysis module containing a canonical endolysin (lysozyme R) supports efficient cell wall degradation at the end of infection. Bacteriophage endolysins are enzymes that degrade the bacterial cell wall and have emerged as promising antimicrobial agents [39]. They act rapidly, show high specificity, and remain effective against multidrug-resistant bacteria. Unlike traditional antibiotics, endolysins cause direct enzymatic lysis and can be applied externally as purified proteins, making them attractive candidates for developing new antibacterial therapies. In this regard, nearly half of the ORFs encode small hypothetical proteins, a common feature of tailed phages and reflective of the high level of genetic novelty typical of phage genomes [8].

VIRIDIC revealed that BorMax shares intergenomic similarity (IS) values below 77 with all known members of the genus *Dhillonvirus* (Table 1). Its closest relative in *Dhillonvirus* is the *Shigella*-infecting phage StarDew [40], with an observed intergenomic similarity of 76.62. Phage starDew was isolated from wastewater treatment facilities in the western United

States using *S. boydii* as the host. Notably, StarDew was only described at the genomic level, and no additional phenotypic or functional characterization was reported, leaving aspects such as host range, infection kinetics, structural biology, and potential biocontrol or therapeutic relevance uninvestigated.

**Table 1.** Intergenomic comparison between phage BorMax and its closest relatives.

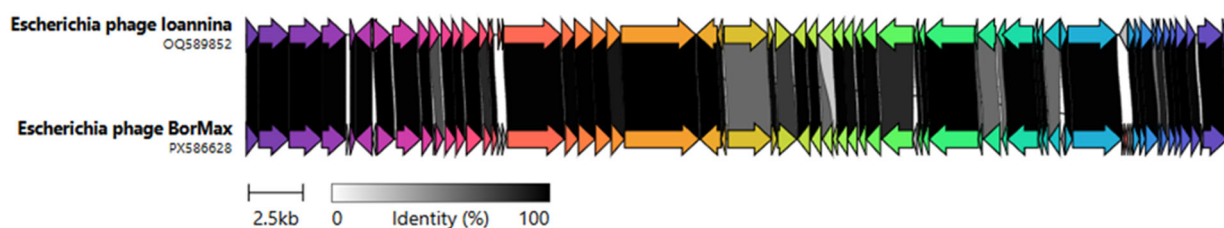
|              | IS with BorMax | Genome Size (bp) | GenBank Accession Number | Host                                     | Reference |
|--------------|----------------|------------------|--------------------------|------------------------------------------|-----------|
| BorMax       | --             | 45,502           | PX586628                 | <i>E. coli</i> ATCC 25922                | This work |
| Ioannina     | 92.35          | 45,270           | OQ589852                 | <i>E. coli</i> ATCC 25922                | [41]      |
| starDew      | 76.62          | 44,715           | OL615010                 | <i>Shigella boydii</i>                   | [40]      |
| Rolling      | 76.01          | 46,017           | MN850575                 | <i>E. coli</i> MG1655                    | [42]      |
| EK99P-1      | 75.44          | 44,332           | KM233151                 | <i>Salmonella</i> sp. and <i>E. coli</i> | [43]      |
| dv011D5      | 75.33          | 45,204           | MT478993                 | <i>E. coli</i>                           | --        |
| glutonyev152 | 75.28          | 44,825           | LR597646                 | <i>E. coli</i>                           | [44]      |
| bov221       | 75.06          | 44,612           | MT884014                 | <i>E. coli</i>                           | [45]      |
| BF9          | 75.03          | 44,963           | MW822006                 | <i>E. coli</i>                           | [46]      |

The most similar phage to BorMax detected in GenBank but not yet validated as a member of the genus *Dhillonvirus* according to ICTV criteria, is phage Ioannina, with an observed IS of 92.35 (Figure 3). Both phages were isolated using *E. coli* ATCC 25922 as the host; however, Ioannina was recovered from wastewater in Greece [41]. The Ioannina genome consists of a 45,270 bp linear dsDNA molecule encoding 61 predicted CDSs. Vasileiadis et al. [41] reported that Ioannina forms a distinct cluster within the *Dhillonvirus* genus, which at the time included only the CtFRY1 metagenome. Although BorMax and Ioannina share high overall genomic similarity and synteny, BorMax exhibits several genomic features that were not reported, searched for, or detected in the Ioannina study. Notably, BorMax encodes 70 CDSs, compared to the 61 CDSs described for Ioannina, including additional minor tail proteins, tail tip components, and replication-associated genes such as a phage-associated DNA polymerase I, multiple HNH endonucleases, and an N6-adenine methyltransferase. Divergence in these accessory genomic regions suggests functional differentiation, potentially affecting host interaction, replication dynamics, or anti-defense strategies. Together, these observations highlight the genomic plasticity within the *Dhillonvirus* lineage and support the interpretation of BorMax as a novel species (Figure 4).

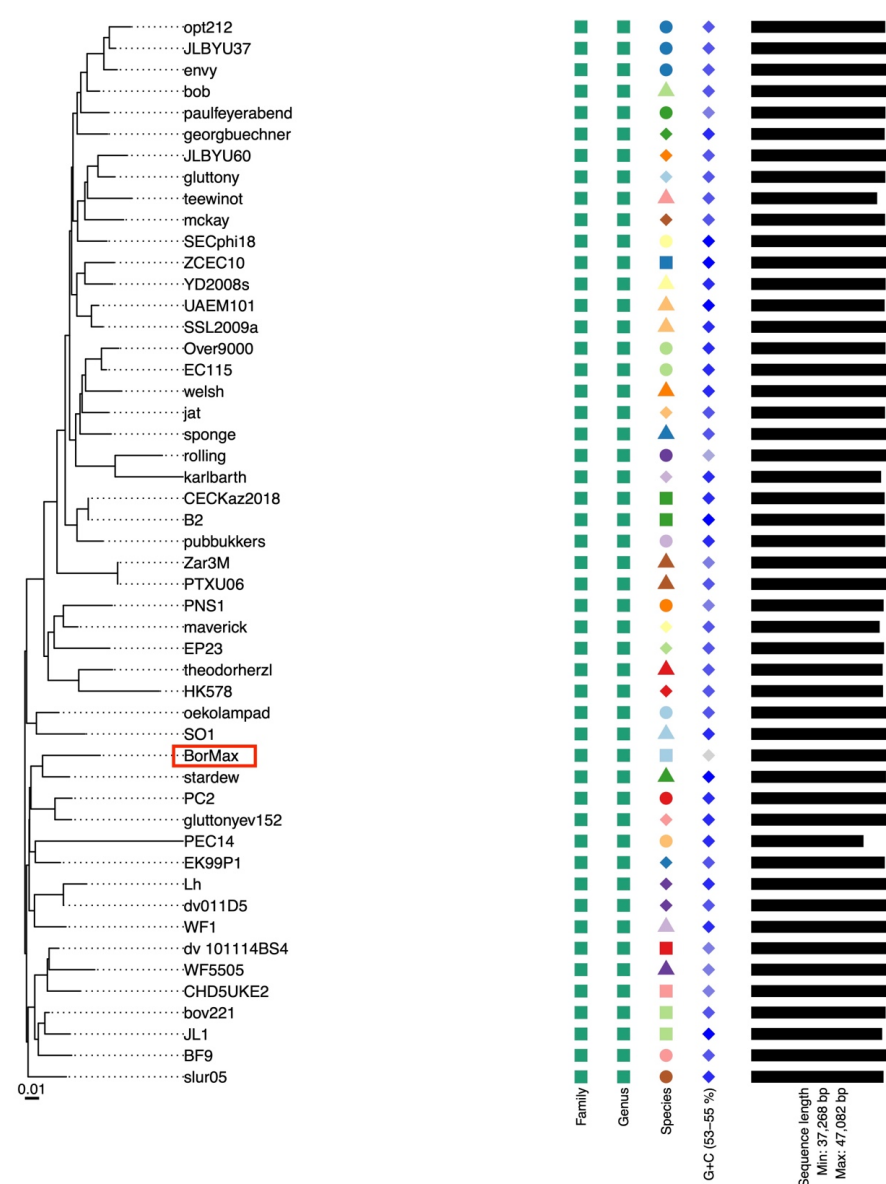
Host range testing revealed that Ioannina was highly specific. It lysed *E. coli* ATCC 25922, and 11 *E. coli* isolates. It was unable to infect *Klebsiella pneumoniae*, *P. aeruginosa*, *Yersinia enterocolitica* and *Acinetobacter baumannii*. Likewise, our results indicated that BorMax was able to infect ATCC 25922 and ten more *E. coli* strains and unable to infect *S. enterica* and *P. aeruginosa*. In this regard, it has been widely described that bacteriophages possess strict host specificity, typically infecting select strains within a species [13]. Nine BorMax-susceptible strains were classified as MDR, as they exhibit non-susceptibility to at least one agent in three different antimicrobial classes [47]. Notably, these isolates were recovered from UTI and produce ESBLs, underscoring the clinical importance of BorMax as a potential alternative to conventional antibiotics for managing infections caused by MDR *E. coli* (Table 2).

PaCRISP identified seven proteins in the BorMax genome as putative Acr candidates, whereas AcRanker predicted sixteen potential Acr-like proteins. Notably, four coding sequences (CDS 8, CDS 30, CDS 53, and CDS 59) were consistently detected by both prediction tools (Table 3). These proteins are annotated as hypothetical, lack identifiable conserved domains, and are relatively small in size, a characteristic commonly observed among reported Acrs [48]. The agreement between two independent bioinformatic approaches suggests that these loci may represent candidate Acr-like elements in BorMax. To our

knowledge, Acr systems have not been systematically examined in phages assigned to the genus *Dhillionvirus*. Although experimental validation is required, these predictions provide a basis for future studies aimed at assessing the potential role of these proteins in phage–host interactions and their possible relevance to the biological properties of BorMax.



**Figure 3.** Genomic comparison of phage BorMax and its closest relative, phage Ioannina. Arrow colors represent the gene clusters encoding homologous proteins, and the connecting lines indicate proteins that share >80% sequence identity.



**Figure 4.** Phylogenetic tree of phage BorMax within the genus *Dhillionvirus*, constructed using the Virus Classification and Tree Building Online Resource (VICTOR) based on whole-genome sequence analysis. Bootstrap values are shown at branch nodes, and branch lengths indicate genomic distance. The results support the classification of BorMax as a novel species.

**Table 2.** Characteristics of *E. coli* isolates sensitive to phage BorMax.

| Antibiotic Resistance Profile |       |                     |                   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-------------------------------|-------|---------------------|-------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Strains                       | EOP   | Sample              | Enzymatic Profile | GEN | SXT | AMP | CFR | CAZ | TZP | FEP | CIP | AMK | CTX | CXM | NIT | ETP | MEM | IPM |
| <i>E. coli</i> 1              | 0.001 | Urine               | NDM               | S   | S   | R   | S   | R   | R   | R   | R   | R   | R   | R   | I   | R   | R   | R   |
| <i>E. coli</i> 9              | 0.001 | Urine               | BLEE              | S   | S   | R   | S   | S   | R   | S   | S   | S   | S   | R   | I   | S   | S   | S   |
| <i>E. coli</i> 13             | 0.10  | Urine               | BLEE              | S   | R   | R   | R   | S   | S   | S   | R   | S   | R   | R   | S   | S   | S   | S   |
| <i>E. coli</i> 19             | 0.001 | Urine               | BLEE              | R   | S   | R   | R   | S   | S   | S   | R   | S   | R   | R   | S   | S   | S   | S   |
| <i>E. coli</i> 29             | 0.001 | Urine               | BLEE              | S   | R   | R   | R   | R   | S   | I   | I   | S   | R   | R   | S   | S   | S   | S   |
| <i>E. coli</i> 35             | 0.10  | Urine               | BLEE              | S   | R   | R   | R   | S   | S   | S   | R   | S   | R   | R   | S   | S   | S   | S   |
| <i>E. coli</i> 36             | 0.01  | Urine               | BLEE              | S   | R   | R   | R   | S   | S   | S   | R   | S   | R   | R   | S   | S   | S   | S   |
| <i>E. coli</i> 38             | 0.26  | Urine               | BLEE              | S   | R   | R   | R   | S   | S   | S   | I   | S   | R   | R   | S   | S   | S   | S   |
| <i>E. coli</i> 57             | 0.26  | Peristomy secretion | NDM               | R   | R   | R   | R   | R   | R   | R   | R   | R   | R   | R   | R   | R   | R   | R   |

Antimicrobial response classification is reported as Susceptible (S), Intermediate (I), or Resistant (R) according to standardized breakpoints. Abbreviations: GEN, gentamicin; SXT, cotrimoxazole (trimethoprim-sulfamethoxazole); AMP, ampicillin; CFR, cefadroxil; CAZ, ceftazidime; TZP, piperacillin/tazobactam; FEP, cefepime; CIP, ciprofloxacin; AMK, amikacin; CTX, cefotaxime; CXM, cefuroxime; NIT, nitrofurantoin; ETP, ertapenem; MEM, meropenem; IPM, imipenem.

**Table 3.** Putative Acr-encoding genes detected in phage BorMax.

| CDS | PaCRISP Score | AcRanker Score (Rank) | Size (aa) | Sequence                                                                                                       |
|-----|---------------|-----------------------|-----------|----------------------------------------------------------------------------------------------------------------|
| 8   | 0.56          | −4.54 (11)            | 53        | MLTFNNGIAEVKLDNGVFYFWHKLERWVPCQDQKLALRQMEDADARGELYNH                                                           |
| 30  | 0.75          | −4.17 (6)             | 85        | MKDLTLKFENKAAFAAFKAQLNWDENEALQDDILLDVIGNVYEGDEDNLVAVE-GYFVNVRILDDHGINFEQYAVVLDSPLRQWA                          |
| 53  | 0.64          | −3.83 (3)             | 109       | MNINEAIEVLSNAIRGETGKSWYYTNAVGTLLFEVSRLSRVEKELTAKLELLNG-TAASKLQKHNEEMEEYKKQVIRLREEGKSWAMIAELTGINQSTVRSWVRNTKTAK |
| 59  | 0.65          | −4.50 (8)             | 101       | MRALIWNYTMAVAKLYEAKHTNGNADDARQAVILAQQGIMNAFDDMFASLDELEPLR-VAICEYAMAFARERLAYRFGIGKTAVANIARIQAQRELHAILAK         |

Several genomic features of BorMax support its consideration as a potential candidate for future phage-based applications, including its strictly lytic lifestyle, the absence of lysogeny-associated, virulence, and antimicrobial resistance genes, and its activity against MDR clinical *E. coli* isolates. These attributes fulfill essential biosafety criteria commonly proposed for therapeutic phages. However, important limitations must also be acknowledged. The host range of BorMax appears restricted to a subset of *E. coli* strains, and quantitative infection parameters such as adsorption rates and burst size were not evaluated. In addition, the emergence of bacterial resistance during prolonged exposure was not assessed. Therefore, while the present study establishes a solid genomic and biological framework, further experimental work will be required to evaluate the therapeutic robustness and stability of BorMax under clinically relevant conditions.

#### 4. Conclusions

In this study, we report the complete genomic characterization of BorMax, a novel *Dhillionvirus* infecting multidrug-resistant *Escherichia coli*. Comparative analyses confirmed its classification as a new species within the genus and revealed a typical modular genome architecture consistent with strictly lytic *Dhillionviruses*. Notably, BorMax lacks genes associated with lysogeny, virulence, and antimicrobial resistance, while encoding multiple putative anti-CRISPR proteins, some of which were consistently predicted by both PaCRISPR and AcRanker. These genomic attributes support the safety and novelty of BorMax and contribute to expanding the current knowledge on *Dhillionvirus* diversity, genome evolution, and host interaction mechanisms.

The genomic uniqueness and anti-CRISPR repertoire of BorMax highlight its value as a model for studying phage–host dynamics in *E. coli* and as a potential candidate for future biotechnological or therapeutic exploration. Further studies focused on functional validation and biological performance will help to clarify its applicability in antimicrobial development.

**Author Contributions:** Conceptualization, B.P. and M.S.; methodology, B.P., D.Z. and M.S.; software, B.P. and M.S.; validation, B.P. and D.R.; formal analysis, B.P., D.R. and E.Y.S.-V.; investigation, B.P.; resources, B.P.; data curation, B.P.; writing—original draft preparation, B.P. and M.S.; writing—review and editing, B.P., D.R., E.Y.S.-V. and M.S.; visualization, B.P.; supervision, B.P.; project administration, B.P.; funding acquisition, B.P. All authors have read and agreed to the published version of the manuscript.

**Funding:** This project received funding from the grant “Proyecto Interno, Vicerrectoría de Investigación y Postgrado, Universidad de las Américas” PIR 2025-5.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The sequencing data for bacteriophage BorMax are available in GenBank under accession no. PX586628.

**Acknowledgments:** We thank to the Centro de Espectroscopía y Microscopía (CESMI) for their invaluable assistance in providing electron microscopy images.

**Conflicts of Interest:** The authors declare no conflicts of interest.

## Abbreviations

The following abbreviations are used in this manuscript:

|      |                                                |
|------|------------------------------------------------|
| MDR  | Multidrug Resistant                            |
| AMR  | Antimicrobial Resistance                       |
| TEM  | Transmission Electron Microscopy               |
| ARG  | Antimicrobial Resistance Genes                 |
| Acr  | Anti-CRISPR                                    |
| CDS  | Coding Sequences                               |
| UTI  | Urinary Tract Infection                        |
| ESBL | Extended-spectrum $\beta$ -lactamases          |
| WHO  | World Health Organization                      |
| MGE  | Mobile Genetic Elements                        |
| DLA  | Double Layer Agar                              |
| PFU  | Plaque-Forming Units                           |
| PEG  | Polyethylene Glycol                            |
| MOI  | Multiplicity of Infection                      |
| CLSI | Clinical & Laboratory Standards Institute      |
| ICTV | International Committee on Taxonomy of Viruses |

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