

Article

Novel Integrative Mobilizable Elements (IMEs) in *Vibrio* Chromosomes Linked to Translucent Post-Larvae Disease (TPD)

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Abstract

Translucent post-larvae disease (TPD), caused by specific *Vibrio parahaemolyticus* strains, poses a global threat to shrimp aquaculture. A key virulence factor in these strains is the *vhv* gene cluster, which encodes toxins central to TPD pathogenesis. While plasmid-borne *vhv* genes have been extensively studied, the chromosome-borne *vhv* genes in TPD-associated strains remain poorly characterized. In this study, we systematically analyzed integrative mobilizable elements (IMEs) on the small secondary chromosomes of TPD-associated *V. parahaemolyticus*. Seven nearly identical novel IMEs harboring full-length *vhv* clusters were identified from seven independent strains. These IMEs encode a tyrosine recombinase for site-specific chromosomal integration, yet lack core conjugative transfer machineries including origin-of-transfer (*oriT*), relaxase, type IV coupling protein (T4CP), and IV secretion system (T4SS), consistent with the definition of non-autonomous IMEs. Each IME carries diverse functional cargo genes, covering five virulence factors (including previously unreported colonization and chemotaxis genes specific to TPD mobile elements), one pollutant-degrading enzyme, and two distinct anti-phage defense systems (*PsyrTA* and *Lamassu-Fam*), thereby potentially boosting the host's fitness and pathogenicity in aquaculture environments. This study provides evidence for the critical role of IMEs in mediating the horizontal transfer of chromosomal *vhv*, offering new insights into TPD epidemiology and a foundation for developing targeted strategies to mitigate the disease's impact on shrimp aquaculture.

Keywords: integrative mobilizable element (IME); translucent post-larvae disease (TPD); chromosome-borne *vhv* genes

Key Contribution: Seven unique integrase-bearing but conjugation-deficient IMEs are identified in TPD-related *V. parahaemolyticus*. These distinct mobile elements carry abundant virulence, metabolic and anti-phage genes that boost bacterial adaptability, uncovering a novel vector facilitating the spread of shrimp disease toxins.



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1. Introduction

Vibrio species represent a major limiting factor for sustainable shrimp farming globally, causing epidemics, mass shrimp deaths, and significant economic losses [1,2]. Translucent post-larvae disease (TPD), also known as highly lethal *Vibrio* disease (HLVD) or glass post-larvae disease (GPD), first broke out in shrimp farms along the southern coast of China in late 2019 and quickly spread to major shrimp farming areas in Southeast Asia [3–5].

TPD specifically infects early-stage shrimp post-larvae; infected individuals exhibit transparent bodies, empty digestive tracts, and atrophied hepatopancreas [5,6]. Clinically, the cumulative mortality rate can reach 90% within 24–48 h [6]. The presence of TPD severely impacts the aquaculture industry, necessitating in-depth research into the molecular genetic mechanisms driving pathogen virulence and the spread of virulence genes [4]. The key pathogenic factor of *Vp*_{TPD} (*Vibrio parahaemolyticus* causing TPD) is the *Vibrio* high virulent protein (VHVP) encoded by the *vhv* gene cluster, which belongs to the Tc toxin family [7]. The complete *vhv* gene locus consists of three structural subunits: *vhv*-1, *vhv*-2, and *vhv*-3, which synthesize the toxin components VHVP-1 (TcA), VHVP-2 (TcB), and VHVP-3 (TcC), respectively [8]. Among these subunits, the *vhv*-2 gene product, VHVP-2, can directly invade the epithelial cells of the midgut and hepatopancreas of shrimp, inducing cell necrosis, tissue sloughing, and ultimately leading to rapid organism death [9,10].

Studies have shown that the pathogenicity of *Vp*_{TPD} strains is closely related to horizontally acquired genetic elements, with plasmids playing a central role [8,10,11]. These strains carry two to five plasmids, on which key pathogenic factors, such as *vhv* genes, extended secretion systems (e.g., type IV and type VI secretion systems), and a wider range of antibiotic resistance genes are typically located [8,9]. Comparative genomics studies have confirmed that horizontally transferred plasmid genes are central to the high virulence phenotype of *Vp*_{TPD} isolates [12]. Further linking *vhv* genes to extended secretion systems and antibiotic resistance highlights their role in pathogen adaptation and pathogenicity.

Horizontal gene transfer (HGT) driven by diverse mobile genetic elements (MGEs) dominates the adaptive evolution of bacterial pathogens, including plasmids, genomic islands (GIs), integrative conjugative elements (ICEs), transposons and integrative mobilizable elements (IMEs) [13]. IMEs are divided into two categories: autonomous IMEs, carrying *oriT* sequences and relaxase genes to support self-mobilization; and non-autonomous IMEs, which only encode site-specific recombinases and lack all core conjugative modules [14]. Non-autonomous IMEs cannot complete intercellular transfer independently; instead, they hijack the full conjugative machinery of co-resident conjugative plasmids or ICEs to achieve HGT of their cargo genes [14–16]. This trans-mobilization strategy enables widespread dissemination of virulence determinants, metabolic loci and anti-phage defense systems across bacterial populations, profoundly reshaping strains' environmental adaptability and pathogenic potential [12,17].

In this research, investigating the chromosomal localization of *vhv* genes in TPD-associated *Vibrio* strains holds great promise for unraveling the molecular mechanisms underlying the acquisition and spread of virulence traits. By analyzing the distribution, structure, and cargo genes of the genetic elements carrying *vhv*, we can gain insights into their contribution to the pathogenicity, antibiotic resistance, and ecological fitness of these strains. This research will not only enhance our understanding of TPD epidemiology but also provide a foundation for developing targeted strategies to mitigate the impact of this emerging disease on shrimp aquaculture.

2. Materials and Methods

2.1. Data Collection and Annotation

The complete nucleotide sequence of *vhv* (i.e., *Tc*, including *TcA*, *TcB*, and *TcC*; source: NCBI CP152366.1) was used to perform BLAST alignment in the NCBI database (search date: 7 June 2025). The core nucleotide database (core_nt) was selected as the search set, and the Megablast algorithm was chosen to optimize for highly similar homologous sequences. Matched results with a query coverage greater than 80% were selected. Subsequently, the complete bacterial genomes (Assembly level: Complete) corresponding to these matched sequences were downloaded. The basic information of the selected bacterial genomes

was summarized, and genome annotation was performed using Bakta (version 1.11.3, open-source, <https://github.com/oschwengers/bakta>, accessed on 26 August 2025) [18].

2.2. Identification and Localization of IMEs

To characterize the *vhv*-positive chromosomes, we first selected chromosomes ranging from 1.8 to 2.3 Mbp (of similar length) for comparison. The pyANI-plus (version 0.0.1, open-source, <https://github.com/pyani-plus/pyani-plus>, accessed on 26 August 2025) was used to analyze the similarity of chromosome sequences similar in length to *vhv*-positive chromosomes [19]. Co-localization analysis aimed to investigate the spatial association of the *vhv* genes with their associated IMEs. The analysis included the 3 following components: *vhv*-negative chromosomes, *vhv*-positive chromosomes, and the *vhv* gene itself. To visualize the co-localization patterns of genetic elements, the BRIG (version 0.95, open-source, <https://github.com/happykhan/BRIG>, accessed on 26 August 2025) was employed [20]. Additionally, compared *vhv*-positive chromosomes with closely related *vhv*-negative chromosomes identified by the clinker (version 0.0.31, open-source, <https://github.com/gamcil/clinker>, accessed on 26 August 2025) [21]. This alignment-based approach enabled mapping of insertion sites between two flanking open reading frames (ORFs).

To precisely map the insertion sites of the IMEs, we performed detailed sequence comparisons between *vhv*-positive chromosomes and length-matched *vhv*-negative sequences. Specifically, we extracted the following regions from both chromosome types: from *vhv*-positive chromosomes, the *rbgA* gene with its 5' upstream non-coding region and the EAL domain-containing gene with its 3' downstream non-coding region; from *vhv*-negative chromosomes, the contiguous genomic segment spanning *rbgA* to the EAL domain-containing gene. The three types of sequences underwent MUSCLE alignment, and regions of high sequence similarity were identified. These regions were subsequently validated using the Repeat Finder plugin in Geneious Prime (version 2024.0.7, New Zealand). The intervening sequences between these repeats were defined as the refined IMEs and subjected to downstream analysis.

2.3. Novelty of the IMEs Analysis

To evaluate the novelty of the IMEs, a comparative analysis was conducted with the ICEberg (version 3.0, developed by Microbial Bioinformatics Group, State Key Laboratory of Microbial Metabolism, Shanghai Jiao Tong University, 200030, China, open-source, <https://tool2-mml.sjtu.edu.cn/ICEberg3/>) (accessed on 26 August 2025) (a database of bacterial integrative and conjugative elements) using two methods: Mash and MultigeneBlast [22]. The similarity among the seven newly identified IMEs was analyzed using pyANI-plus v0.0.1 [19]. To gain deeper insights into the protein relationships between the newly identified IMEs and known IMEs (both predicted and experimentally validated) from ICEberg 3.0, a network analysis of the protein sequences from these IMEs was conducted. The protein sequences of the IMEs were clustered using the easy-cluster subcommand of mmseqs2 (--min-seq-id 0.5, -c 0.8). Subsequently, protein-protein association networks were visualized using Cytoscape (version 3.9.1, open-source, developed at University of California San Diego, La Jolla, CA, USA, <https://cytoscape.org>) [23].

2.4. Characterization of IMEs Composition

To comprehensively characterize the diverse functional capabilities and genetic architectures of the investigated IMEs, origin-of-transfer (*oriT*) regions of bacterial MGEs were identified using *oriTfinder2* with default parameters (open-source, <https://bioinfo-mml.sjtu.edu.cn/oriTDB2/oriTfinder.php>) (accessed on 26 August 2025) [24]. Additionally, the accessory genes of the IMEs were characterized by screening against six specialized

databases: (1) Antibiotic resistance genes: Predicted using the online Resistance Gene Identifier (RGI) with criteria set to perfect, strict and loose; partial genes were included using a 95% identity threshold. (2) Virulence factors: Screened against the Virulence Factor Database (VFDB full dataset) using BLASTn in Geneious Prime (E-value $\leq 1 \times 10^{-5}$). (3) Metal resistance genes: Screened against BacMet2 using BLASTx (E-value $\leq 1 \times 10^{-5}$). (4) Degradation genes: Screened against mibPOPdb using BLASTx (E-value $\leq 1 \times 10^{-5}$). (5) Symbiosis genes: Screened against ICEberg 3.0 using BLASTx (E-value $\leq 1 \times 10^{-5}$). (6) Defense systems: Identified within the IMEs using DefenseFinder (open-source, <https://defensefinder.mdmlab.fr/>) [25–27]. For genes that matched multiple homologous entries with identical coordinates, only one representative annotation was reported in the final tally.

3. Results

3.1. Bacterial and Chromosomal Screening

BLASTn search using the complete *vhv* gene (NCBI CP152366.1) retrieved 24 sequences (query coverage > 80%) derived from 20 bacterial strains, including 17 *V. parahaemolyticus* and 3 *V. campbellii* (Table S1). Among these, each strain possesses 2 chromosomes and 1–5 plasmids, with 7 *V. parahaemolyticus* of the chromosomes carrying the *vhv* genes. Notably, 7 independent *V. parahaemolyticus* strains harbored intact *vhv* gene clusters only on chromosomes of 1.8–2.3 Mbp in length, while remaining positive strains carried *vhv* on plasmids; no single strain simultaneously contained both chromosomal and plasmid-borne *vhv*. For structural comparison, 13 length-matched (1.8–2.3 Mbp) *vhv*-negative chromosomes were selected for comparison (length range: 1.8–2.3 Mbp; Figure 1). Pairwise average nucleotide identity (ANI) analysis via pyANI-plus revealed that similarity ranged from 84% to 98% (7 vs. 13, Figure S1, Table S2).

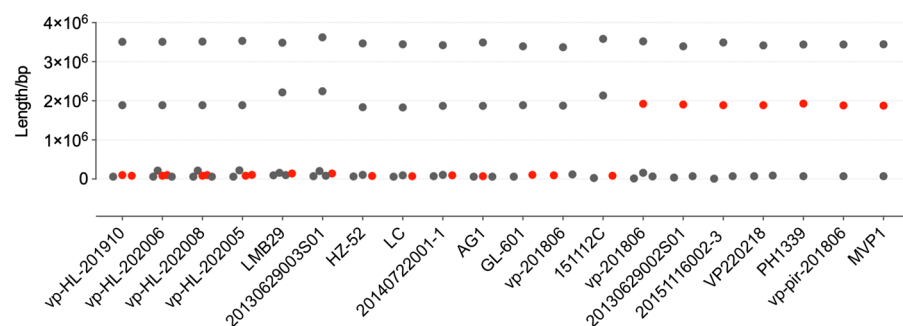


Figure 1. Length distribution of genomic elements carrying *vhv* genes in *Vibrio* strains. Scatter plot comparing sequence lengths of chromosomes and plasmids across 20 *Vibrio* strains (x-axis). Each point represents a genomic element. Red points: *vhv*-positive elements; gray points: *vhv*-negative elements. The *vhv*-positive chromosomes ($n = 7$) cluster within a distinct size range (1.8–2.3 Mbp), highlighting their structural divergence from other genomic elements.

3.2. Identification of IMEs Associated with *vhv*

BRIG ring visualization (Figure 2A) revealed large differential genomic regions unique to all seven *vhv*-positive chromosomes, which were completely absent in all 13 negative control chromosomes; the full *vhv* toxin cluster was embedded within these strain-specific differential intervals. Clinker global alignment further localized the consistent insertion site of differential fragments between two universally conserved chromosomal ORFs: upstream *rbgA* and downstream EAL domain-containing gene (Figure 2B).

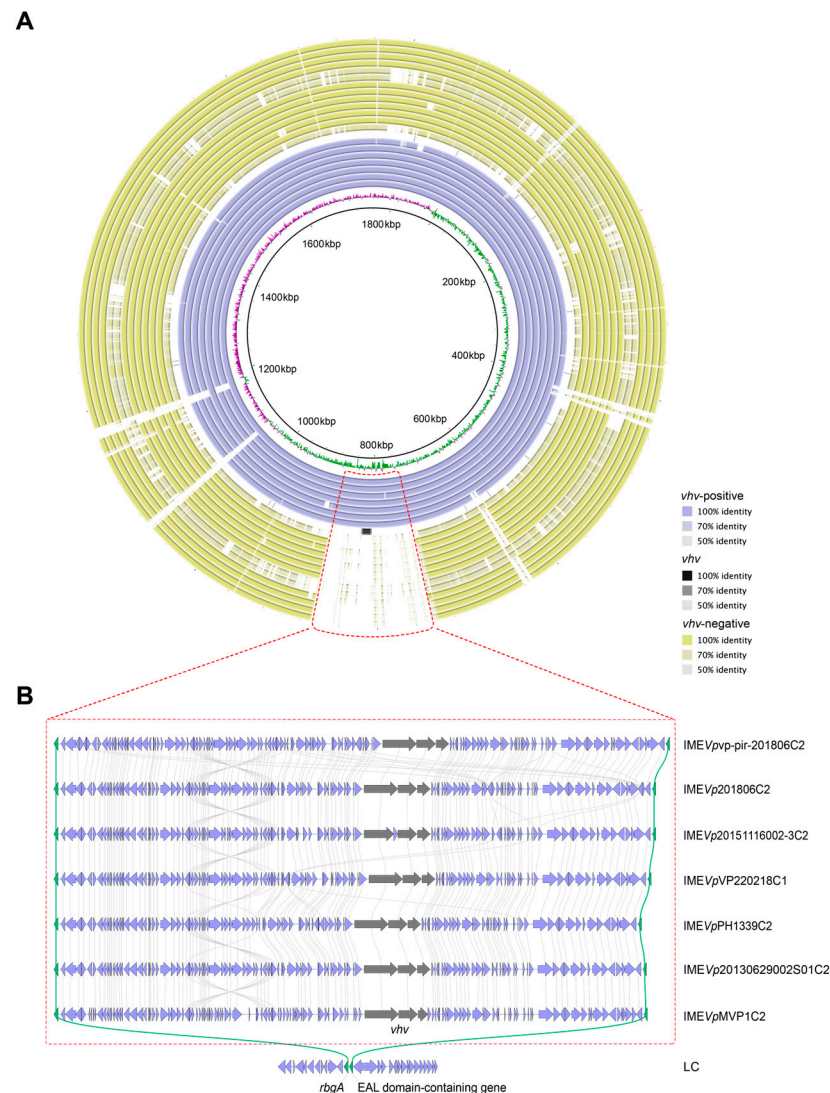


Figure 2. Identification of *vlv*-associated IMEs in *Vibrio* chromosomes. **(A)** BRIG co-localization analysis of *vlv*-positive chromosomes, *vlv*, and reference genomes (23 concentric rings, inner to outer): Ring 1: Genomic position scale (bp). Ring 2: GC skew. Rings 3–9: *vlv*-positive chromosomes (7 strains, light purple). Ring 10: *vlv* gene in black. Ring 11–23: Reference *vlv*-negative chromosomes (13 strains, light yellow). Red dashed boxes highlight divergent MGE-rich regions exclusive to *vlv*-positive chromosomes. **(B)** Precise mapping of IME insertion sites. Clinker-based alignment of *vlv*-positive chromosomes (top) against closely related *vlv*-negative chromosomes (bottom). Gray and green ribbons denote conserved synteny; dashed boxes highlight IME insertion sites flanked by the *rbgA* gene (left) and an EAL domain-containing gene (right). IMEs (>130 kbp) integrate between these conserved ORFs across all *vlv*-positive strains.

Comparative sequence analysis between *vlv*-positive and *vlv*-negative chromosomes (using LC chromosome 2 as a representative) revealed conserved direct repeats flanking the insertion sites in all 7 strains (Figure S2). We designated the repeat proximal to *rbgA* as the putative *attL* site, located within its 5' upstream non-coding region and extending into the gene's 5' end, and the repeat proximal to the EAL domain-containing gene as the putative *attR* site, found in the 3' downstream non-coding sequence of this gene. The corresponding unoccupied locus in the *vlv*-negative chromosome was designated as putative *attB* (Figure 3). To operationally define the IMEs for downstream analysis, their sequences were delineated based on the unique regions of *vlv*-positive chromosomes, excluding the flanking non-coding sequences, with the precise boundaries provided in (Table S3).

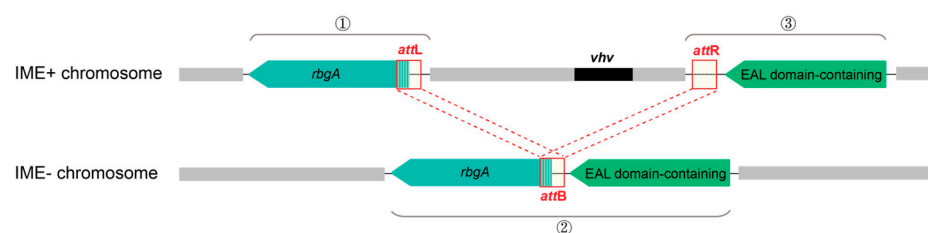


Figure 3. Structural model of IME integration. Structural model of locus disruption by IME integration. Top section: Sequences from *vhv*-positive chromosomes (IME+). Bottom section: Sequences from *vhv*-negative chromosomes (IME-). Gray shading indicates omitted genes. Red boxes highlight repeat sequences (*att* sites) identified through manual inspection.

3.3. Novelty of the IMEs

To compare with the ICEberg 3.0 database, no similar MGEs to the seven IMEs were identified, confirming these IMEs as novel genetic elements. ANI analysis via pyANI-plus showed similarity exceeding 99.6% among the seven IMEs (Table S4), with only minor variations in accessory gene content. Cytoscape network analysis revealed distinct differences in protein similarity clustering between the newly identified IMEs and those (all predicted and experimental IMEs) in the ICEberg 3.0 database. The newly identified IMEs exhibit sequence homology only in their integrase genes with entries in the ICEberg 3.0 database; no significant homology was detected for any other sequences (Figure 4).

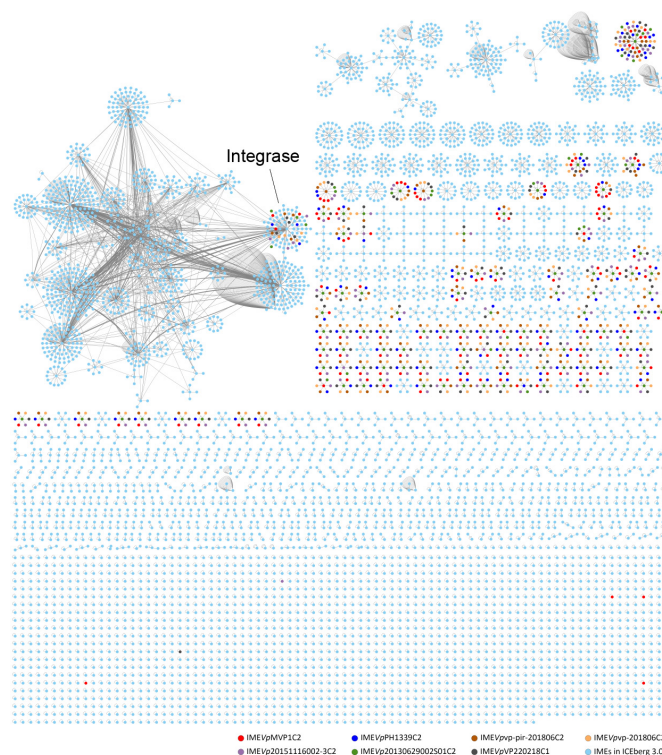


Figure 4. Visualization of protein association networks in IMEs. Nodes represent protein clusters, and edges connect proteins with significant sequence similarity.

3.4. Characterization of IMEs

All seven *vhv*-bearing chromosomal fragments belong to a novel clade of non-autonomous IMEs, with precise lengths ranging from 130 to 138 kbp (Table S3). Each IME encodes a single tyrosine-type site-specific recombinase required for chromosomal integration, yet lacks core autonomous conjugation machinery: transfer origin *oriT*, relaxase,

T4CP, and T4SS (Tables S5–S11). This genetic architecture devoid of self-transfer modules strictly matches the canonical definition of non-autonomous IMEs [14].

Whole-element functional annotation with Bakta v1.11.3 identified a core conserved gene repertoire shared across all seven IMEs, including an intact fucose degradation operon (*fucA-fucP*), two paralogous copies of *adhB* and *xylE*, and single copies of *chuR_1*, *srmB* and *dgt* [28]. Multi-database virulence screening recovered five distinct virulence-associated cargo genes within these IMEs: the core cytotoxic toxin subunit *TccC* (*vhh-3*), a well-validated primary virulence determinant of TPD-causing *V. parahaemolyticus*; T6SS effector delivery components *VgrG1* and *AtsA* that mediate epithelial invasion in aquatic *Vibrio* pathogens [29]; and colonization/chemotaxis regulatory factors *AcfB/TcpI* and *CheD*. Notably, *AcfB/TcpI* and *CheD* have only been functionally characterized in *Vibrio cholerae* in prior research [30,31].

One gene encoding iron-dependent alcohol dehydrogenase was annotated within the IME cargo, which shares high sequence similarity with halogenated pollutant detoxification genes *pcpE* and *linF* [32]. Two distinct anti-phage defense operons were further characterized via DefenseFinder prediction: the *PsyrTA* Type II toxin–antitoxin (TA) system and the broad-spectrum *Lamassu-Fam* antiviral system [33]. As experimentally validated in previous genomic and plaque assays, *PsyrTA* mediates abortive infection upon phage invasion and stabilizes large mobile elements during host cell division via toxin–antitoxin addiction [34], while the SMC-containing *Lamassu-Fam* module recognizes and degrades invasive foreign phage dsDNA to restrict viral replication [33]. Functional screening across six specialized databases identified no genes related to antibiotic tolerance, metal resistance, or host–symbiosis interaction within the seven IME sequences.

4. Discussion

IMEs represent a widespread yet understudied class of non-autonomous MGEs that contribute significantly to bacterial genome evolution. Unlike self-transmissible ICEs, IMEs lack the core conjugative machinery (e.g., *oriT*, relaxase, T4CP, and T4SS) and rely on these functions from co-resident conjugative elements for horizontal dissemination [14]. IMEs integrate multi-functional gene clusters encompassing metabolic pathways, stress response modules and virulence effectors, which collectively reshape host ecological competitiveness and pathogenic potential [12,14,17].

Here, we identified and characterized a cohort of novel IMEs harboring the critical *vhh* toxin genes in the chromosomes of TPD-associated *V. parahaemolyticus*. These elements possess a tyrosine integrase and conserved direct repeats at their boundaries (putative *attL* and *attR* sites) but lack essential conjugative modules. This genomic structure firmly aligns them with the established definition of non-autonomous IMEs [14]. The identification of these features suggests a site-specific integration mechanism between the conserved *rbgA* and EAL domain-containing genes, with the direct repeats facilitating potential excision and circularization [35]. This model is further supported by the recent characterization of Tn5253, an ICE that site-specifically integrates into the *rbgA* gene, downstream of a conserved 11 bp sequence across diverse streptococcal and enterococcal hosts [36]. The conservation of *rbgA*, as an integration hotspot for distinct MGEs, underscores its functional importance and suggests a shared evolutionary strategy for stable chromosomal maintenance. However, the precise boundaries of these IMEs, and consequently their exact size and ability to circularize, remain partially ambiguous without direct experimental validation of the attachment sites.

A striking feature of these *vhh*-carrying IMEs is their substantial size, ranging from 130 to 138 kbp (Table S3). This dimension is remarkable when contrasted with the typical size range of known ICEs. A systematic analysis of 200 ICEs by Cury et al. reported an

average length of only 52.4 kbp, with a maximum observed value of 155 kbp [37]. It is generally believed that large MGEs impose a significant fitness cost on their hosts, often leading to their purging unless counterbalanced by strong selective advantages [37]. The stable maintenance of these large IMEs in *V. parahaemolyticus* challenges the notion that “large MGEs are difficult to retain long-term” and suggests they may possess mechanisms to mitigate this cost, potentially through the beneficial functions of their cargo genes, thereby offering new insights into the size distribution and host co-evolution of mobile elements.

Furthermore, these large IMEs are phylogenetically distinct from all previously documented elements. Comprehensive comparisons with the ICEberg 3.0 database, supported by both Mash and protein-clustering analyses, confirmed their novelty and the absence of close homologs.

Beyond harboring the core *vhv* toxin, these IMEs constitute multifunctional genetic units that significantly augment host fitness through a diverse repertoire of cargo genes. The functional arsenal of these IMEs spans several key adaptive categories. From a metabolic perspective, the intact *fucA–fucP* fucose degradation operon enables efficient catabolism of marine polysaccharide detritus in sediment and aquaculture water [28]. Duplicated *adhB* and *xylE* genes further expand the strain’s spectrum of oxidizable alcohol and lignin-derived carbon substrates [38]. One annotated iron-dependent alcohol dehydrogenase shares high sequence similarity with *pcpE* and *linF*, two characterized enzymes that drive detoxification of halogenated aquaculture pollutants [39,40]. With regard to virulence and pathogenesis, a suite of genes directly contributes to pathogenicity, including established determinants for adhesion (e.g., *AcfB/TcpI*), toxin delivery (e.g., *VgrG1*, *AtsA*), chemotaxis (*CheD*), and critical in vivo survival factors such as iron acquisition (*chuR_1*) [29–31]. The IMEs also equip the host with defense and detoxification mechanisms, encompassing anti-phage systems (*PsyRTA* and *Lamassu-Fam*) [33,34,41–43] and a gene for degrading persistent pollutants, annotated as an iron-containing alcohol dehydrogenase with homology to *pcpE/linF* [39,40]. Furthermore, genes involved in fundamental cellular processes such as RNA remodeling (*srmB*) and nucleotide metabolism (*dgt*) are present, likely supporting core physiological functions and host fitness [44,45]. This diverse functional repertoire collectively enhances host pathogenicity, defense, and metabolic adaptability, thereby providing a strong selective advantage that promotes the stable maintenance and inheritance of these large IMEs [14,46].

Prior genomic research on TPD virulence transmission has focused solely on plasmid-located *vhv* operons [8,11], while the genetic carriers of chromosomal *vhv* remained uncharacterized. This work identified a novel group of 130–138 kbp non-autonomous IMEs integrated onto the 1.8–2.3 Mbp secondary chromosomes of pathogenic *V. parahaemolyticus*, each carrying intact full-length *vhv* toxin cassettes. Unlike single toxin-carrying mobile elements, these IMEs are sophisticated composite modules integrating metabolic, virulence and anti-phage functional genes, which act as critical evolutionary drivers shaping the pathogenic diversity of aquaculture-derived *Vibrio*. Our genomic data put forward a computationally predicted alternative pathway for the horizontal spread of TPD core virulence factors separate from plasmids. However, all inferences regarding IME-mediated horizontal gene transfer are merely bioinformatics predictions. Further experiments will be conducted to verify the transfer capabilities of these IMEs and to further elucidate their actual epidemiological transmission patterns in shrimp farming environments.

5. Conclusions

While virulence factors of TPD have historically been attributed to plasmids, this study reveals the chromosomal presence of the key *vhv* toxin gene within a group of seven closely related IMEs in *V. parahaemolyticus*. These elements were found inserted in a site-specific

manner between conserved chromosomal loci (*rbgA* and EAL domain-containing genes) and flanked by direct repeat sequences, a structure indicative of integration events and potential mobility. Although they carry diverse cargo genes, the absence of key conjugative transfer modules indicates an inability to self-mobilize, and their mechanism of horizontal transfer remains to be experimentally demonstrated. This discovery elucidates the genetic basis for the chromosomal localization of *vhv* and suggests that IMEs could act as key vectors in the horizontal dissemination of TPD-related virulence determinants. The identification of these IMEs opens new avenues for hypothesizing their role in pathogen evolution and disease dynamics in aquaculture systems. Further experimental validation is necessary to confirm the mobility and integration mechanisms of these IMEs. If functional, targeting IME dynamics could inform novel strategies for controlling TPD outbreaks.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/fishes11080441/s1>, Figure S1: Heatmap of chromosomes similarity analysis (1.8–2.3 Mbp); Figure S2: Sequence alignment of IME insertion sites; Table S1: Bacterial whole-genome sequences harboring the *vhv* genes; Table S2: The ANI values between 1.8–2.3 Mbp chromosome sequence; Table S3: Extraction sites of 7 IMEs; Table S4: The ANI values between 7 IMEs; Table S5: Annotation of IMEVp201806C2; Table S6: Annotation of IMEVpPH1339C2; Table S7: Annotation of IMEVp20130629002S01C2; Table S8: Annotation of IMEVpvp-pir-201806C2; Table S9: Annotation of IMEVpVP220218C2; Table S10: Annotation of IMEVp20151116002-3C2; Table S11: Annotation of IMEVpMVP1C2.

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Institutional Review Board Statement: This study is a purely bioinformatics-based investigation and did not involve any animal experiments, animal handling, animal sampling, or the collection of biological specimens. All sequence data analyzed in this study were obtained from publicly available databases, including the National Center for Biotechnology Information (NCBI). No new biological materials were collected, and no live animals were used or manipulated for the purposes of this research. Therefore, this study did not involve procedures requiring approval from an Institutional Animal Care and Use Committee (IACUC) or any other ethics committee.

Data Availability Statement: All sequences analysed in this study were obtained from the National Center for Biotechnology Information database, with all utilized sequence accessions summarized in Table S1.

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

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