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Functional Shifts in the Gut DNA Virome in a Long-Distance Migratory Shorebird During the Pre-Migratory Fattening

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ABSTRACT

Migration represents one of the most energetically demanding phases in the life cycle of long-distance migratory birds. Pre-migratory fattening is a critical preparatory stage characterized by hyperphagia, rapid fat accumulation, organ remodelling, and immune modulation. Although the gut microbiome has been recognized as a key contributor to these physiological adaptations, the role of the gut virome remains poorly understood. In this study, the diversity, functional potential, and temporal dynamics of the gut DNA virome in a trans-hemispheric migratory shorebird, the Hudsonian godwit (*Limosa haemastica*), were assessed during pre-migratory fattening. Adult individuals were maintained under controlled aviary conditions for 15 weeks during the preparation for northbound migration, and faecal samples were collected at two distinct physiological time points: at the beginning and the end of pre-migratory fattening. Shotgun metagenomic sequencing revealed 798 high-quality viral operational taxonomic units (vOTUs), the majority of which were bacteriophages (92%). Potential functional annotation identified auxiliary metabolic genes (AMGs) associated with nucleotide metabolism, redox balance, and host adaptation. Although overall gut virome diversity did not differ between stages, significant changes in potential functional profiles of phages were observed, especially during the final stage of fattening when energy demands are at their highest. In addition to bacteriophages, we report two divergent adenoviruses potentially associated with the *Siadenovirus* and *Aviadenovirus* genera. These findings suggest that dynamic viral communities may play underrecognized roles in supporting host physiology during energetically costly life stages.

1 | Introduction

Migration is a critical life-history trait in many species, involving large-scale, seasonal movements between breeding and non-breeding areas that demand extensive physiological, immunological, and ecological adaptations (Dingle 1991; Newton 2003; Dingle 2006; Shaw and Couzin 2013; Zhang et al. 2019). Among vertebrates, long-distance migratory birds exemplify the extreme energetic demands of seasonal migration (Kersten and Piersma 1986; Schmaljohann et al. 2022), requiring profound physiological and behavioural adjustments to optimize energy acquisition, storage, and utilization (Kersten and Piersma 1986; Hedenström 2008; Guglielmo 2018; Eikenaar et al. 2021). A key preparatory stage for migration is the pre-migratory fattening, characterized by hyperphagia and rapid fat accumulation, which are required to meet the energetic demands of the forthcoming migration (Kersten and Piersma 1986; Lindström and Piersma 1993). This phase involves modifications in foraging behaviour, gut morphology, and metabolic pathways (Piersma 1990; Lindström and Piersma 1993; Piersma and van Gils 2011). Increasing evidence suggests that the gut microbiome, which includes bacteria, archaea, fungi, phages, viruses, and transposons among other components (Berg et al. 2020), plays a crucial role in supporting these physiological changes by enhancing energy extraction, regulating lipid metabolism, and modulating immune function (Kohl et al. 2021; Hird 2017; Grond et al. 2019; Davidson et al. 2020; Trevelline et al. 2023). However, less is known about the changes in trans-kingdom interactions (Zhang et al. 2021; Bodawatta et al. 2022), particularly the viral components of the gut microbiome, and their potential modification of functionality during this critical life stage.

Physiological adjustments associated with migration significantly influence the structure and function of the avian gut microbiome (Grond et al. 2023). Seasonal shifts in habitat, diet, and immune regulation along the migratory route crossing diverse ecological landscapes can lead to substantial gut microbial turnover and restructuring (Risely et al. 2017; Turjeman et al. 2020; Zhang et al. 2021). In particular, the bacterial component of the gut microbiome is known to play an active role in preparing migratory birds for the extreme metabolic demands of long-distance flight (Grond et al. 2023; Trevelline et al. 2023). Studies in shorebirds and other migrants show that bacterial communities undergo compositional and functional shifts during stopover and fattening, often favouring taxa involved in energy harvest, lipid metabolism, and the production of short-chain fatty acids that enhance host energetic efficiency (Davidson et al. 2020; Grond et al. 2023; Trevelline et al. 2023; Capilla-Lasheras and Risely 2025). Such microbial adjustments may facilitate hyperphagia, rapid nutrient assimilation, and gut morphological flexibility, key elements of pre-migratory fat accumulation (Lindström and Piersma 1993; Piersma and van Gils 2011). Despite the growing evidence of bacterial roles in migration physiology, the viral component of the avian gut microbiome remains comparatively understudied.

Unlike bacteria, viruses lack universal marker genes, meaning that virome research relies almost exclusively on shotgun metagenomic or metatranscriptomic approaches. Although powerful, these methods are comparatively expensive, technically demanding, and relatively sensitive to contamination, requiring substantial challenges for virome characterization (Paez-Espino et al. 2016; Roux et al. 2017). In addition, viral genomes are exceptionally diverse, rapidly evolving, and poorly represented in reference databases, which limits taxonomic assignment and functional inference, especially in wild, non-model hosts such as migratory birds (Yang et al. 2025). The gut virome, encompassing bacteriophages (phages), diet-associated viruses, and eukaryotic viruses infecting the host, represents a dynamic and integral part of the microbiome (Wylie et al. 2012; Virgin 2014; Carding et al. 2017). Phages, in particular, can influence bacterial abundance, turnover, and metabolic pathways through predation, lysogeny, and gene transfer, thereby influencing microbial functions closely related to host physiology (Papaianni et al. 2020; Ji et al. 2025). In this context, the DNA virome, dominated by double-stranded DNA bacteriophages, which account for more than 90% of currently described phages, represents a robust and informative proxy for assessing the functional potential of gut viral communities (Paez-Espino et al. 2016; Roux et al. 2019). Integrating viral and bacterial perspectives is therefore essential for understanding how gut microbial communities support the physiological remodelling required for migration. Energetically demanding stages such as pre-migratory fattening may act as major drivers of virome dynamics, either by selecting for viral taxa that support efficient microbial metabolism or by disrupting existing community structures (Grond et al. 2019; Ramírez-Martínez et al. 2018; Shan et al. 2022; Wille et al. 2021).

We used the Hudsonian godwit (*Limosa haemastica*) as a model species to explore the composition and diversity of the gut virome during two critical stages of the non-breeding season: the initial and final stages of the pre-migratory fattening period. Hudsonian godwits are long-distance migratory shorebirds that breed in high-latitude regions of North America, particularly in Alaska and Northern Canada, and winter in southern South America, with an exceptionally well-documented annual cycle (Senner et al. 2014). Each year, the Alaskan population migrates over 10,000 km, including an extraordinary non-stop northbound flight from the Chiloé Archipelago (Chile) to inland North America (Linscott et al. 2022), before continuing to breeding areas in Alaska (Senner et al. 2014; Swift et al. 2020). During the non-breeding season, individuals remain in Chiloé from October to early April, undergoing the pre-migratory fattening before departure (Gutiérrez et al. 2019). Here, we aimed to characterize the temporal dynamics of the gut virome during a critical phase of avian migration, the pre-migratory fattening, to assess its functional potential, mediated through interactions with predicted bacterial hosts, and its role in shaping metabolic preparedness for migration.

2 | Methodology

2.1 | Animal Capture and Housing

Hudsonian godwits were captured in January 2021 during high tide using cannon nets at Caulín Bay, Chiloé Island, Chile (41°49′S, 73°63′W). Upon capture, individuals were weighed and measured (bill length, head-bill length, tarsus length, and wing length) using standardized protocols (Gherardi-Fuentes et al. 2020). Age class was estimated based on moult patterns (Pyle 2008), and sex was later confirmed molecularly

(Gherardi-Fuentes et al. 2020). A total of 16 adult individuals (eight males and eight females) were selected and transported to an avian experimental facility. The aviary consisted of four isolated rooms (4×3 m each) designed for temporary housing shorebirds for experimental purposes. Each room was separated and equipped with access to fresh seawater pools, controlled room temperature (mean: 21°C; range: 20°C–23°C), relative humidity (mean: 80%), and natural photoperiod through skylight windows. Birds were fed *ad libitum* with a standardized commercial pelletized diet (Nutra Parr 60, Skretting) formulated to contain 49% protein, 22% lipids, and 12.6% carbohydrates on a dry-matter basis, a macronutrient profile that closely approximates that reported for nereidid polychaetes, the primary natural prey of godwits (Duijns et al. 2013; Dorgham et al. 2015; Micael and Navedo 2018).

2.2 | Experimental Protocol and Sample Collection

Four individuals were randomly assigned to each room, with a balanced sex ratio in each group, and housed for 15 weeks. Of these, six animals (three females and three males) were included in the study, without being subjected to any experimental procedures. Birds were acclimated for 4 weeks (January) during which minimal handling occurred except for weekly weighing, aviary maintenance, and cleaning. Individuals were initially offered natural prey, and the commercial pelletized diet was progressively introduced and fully replaced the natural prey within the first 2 weeks. After acclimatization, godwits were handled weekly for weight recording and faecal sample collection from February to April 2021. Faecal samples were collected at two distinct physiological stages associated with the northbound pre-migratory fattening (Figure 1): The *initial-fattening* stage was defined as the time immediately after acclimation and before a significant weight gain in early February (experimental week 5–6). The average body mass was 260.5 g (range: 163.5–310 g). The *final-fattening* stage corresponded to the period closest to the northbound migration in late March (experimental weeks 11–12), when body condition peaked. Godwits in this stage have significantly increased in weight after an intense feeding activity (hyperphagia), and are usually obese, reflecting the substantial fat accumulation. The average body mass was 335.6 g (range: 276.5–434.5 g), representing a 40%–69% increase. Individual faecal samples were weekly collected by placing the animals in individual enclosures lined with sterile plastic until defecation occurred. Fresh faeces were transferred to cryovials without transport media using a sterile wooden spatula, snap-frozen in liquid nitrogen, and then stored at −80°C until further processing.

2.3 | Nucleic Acid Extraction and Metagenomic Sequencing

Twenty-four faecal samples (0.25 g each; two samples per individual per stage) were homogenized in sterile phosphate-buffered saline (PBS, 1:5 w/v), freeze-thawed three times, and centrifuged at 12,000×g for 5 min. Total DNA was extracted from the supernatant using the PowerSoil DNA Isolation Kit (Qiagen), following the manufacturer's instructions. After DNA extraction, twelve pooled DNA samples were obtained by combining two samples per individual per stage. For each individual, DNA samples pooled within a given stage were collected 1 week apart to increase DNA yield while maintaining temporal consistency. The pooled genomic DNA was subsequently purified and concentrated using the Genomic DNA Clean & Concentrator-10 kit (Zymo Research). Twelve pooled DNA samples were quantified using a Qubit DNA assay (Thermo Fisher Scientific)

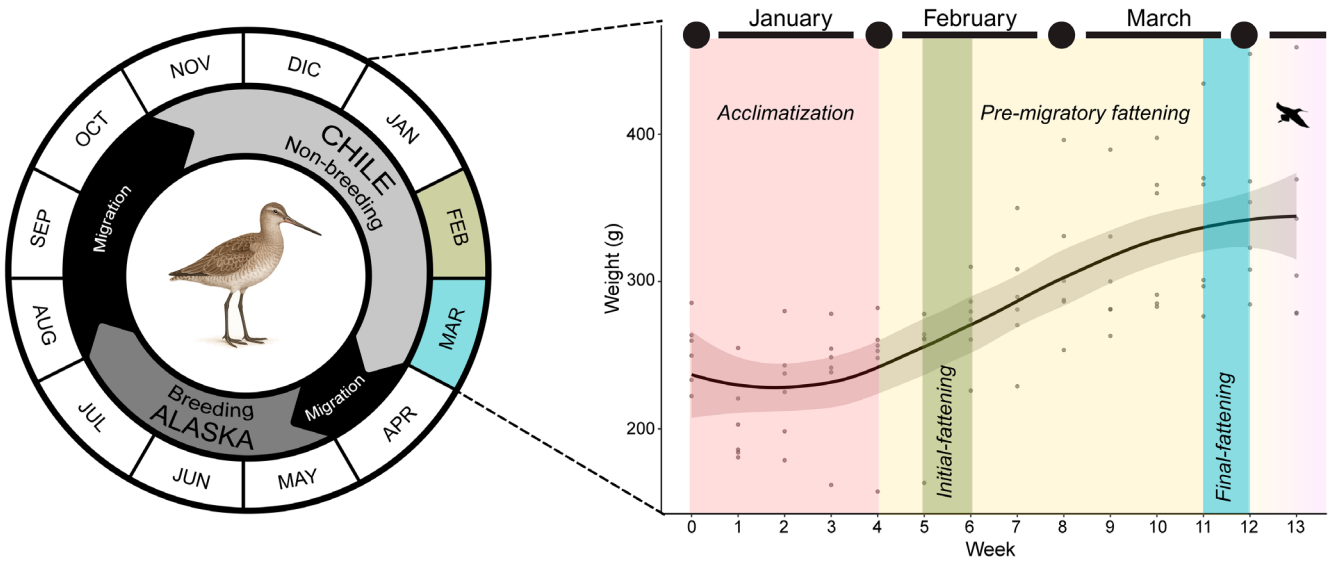

FIGURE 1 | Schematic migration cycle of Hudsonian godwit in the left (based on Navedo and Ruiz 2020; Swift et al. 2020). During the non-breeding season, pre-migratory fattening has been focused on, including the experimental protocol for migration preparation. The plot displays the weights of the captured godwits over time, including the acclimatization period (red shading), the pre-migratory fattening period (yellow shading), and the departure period (purple shading). A smoothed predicted polynomial regression model line (black) with a 95% confidence interval (grey shading) illustrates the overall pattern of weight change. The sampled groups are marked with a green shade in the *initial-fattening* stage and a blue shade in the *final-fattening* stage, indicating the exact pooling week sampled.

and assessed for fragment size distribution using a Fragment Analyser (Agilent Technologies) before being sequenced using DNBSEQ PE150 with 20Gb per library (BGI Genomics, China) to explore viral communities.

2.4 | Quality Control, Assembly, and Viral Mining

The read quality of the metagenomic data was assessed using FastQC (Andrews 2010). Adapter trimming and quality filtering were conducted using Trimmomatic v0.38 (Bolger et al. 2014) with the following parameters: SLIDINGWINDOW:4:15 and MINLEN:75. The resulting high-quality reads were *de novo* assembled using MEGAHIT (Li et al. 2015) with default parameters, retaining contigs ≥2000 bp. Viral contigs were identified with geNomad (Camargo et al. 2024) and filtered to remove unclassified and <4000 bp sequences. The novelty and completeness of viral operational taxonomic units (vOTUs) were assessed with CheckV (Nayfach et al. 2021). Clustering of vOTUs was performed using MMseqs2 (Steinegger and Söding 2017) with thresholds of >95% identity and >85% coverage. The longest representative contig per cluster was defined as a vOTU (Roux et al. 2019). To assess the viral contamination level from reagents used during the laboratory process (Holmes 2019), all identified vOTUs were aligned through Blast+ (Camacho et al. 2009) against libraries containing approximately 800 contaminant viral sequences previously reported by Asplund et al. (2019) and Duan et al. (2024). Read mapping to vOTUs was performed using Bowtie2 (Langmead and Salzberg 2012). Raw read counts associated with each vOTU were first normalized using the Trimmed Mean of M-values (TMM) implemented in the EdgeR package v4.4.0 (Robinson et al. 2010; Chen et al. 2025) using R v4.3.2, to account for differences in library size across samples. Subsequently, normalized counts were converted to Transcripts Per Million (TPM) mapped reads (Li et al. 2010) using SAMtools (Danecek et al. 2021) to adjust by contig coverage (≥75%) and nucleotide identity threshold (≥95%) (Roux et al. 2017), using BBMap (Bushnell 2014). The identified vOTUs were grouped by the highest taxonomic rank supported for the majority of contigs for further analysis.

2.5 | Viral Functional Annotation

The functional annotation of viral genomes was performed in a separate FASTA file using DRAM-v (Shaffer et al. 2020) using the phage vOTUs. Open reading frames (ORFs) were predicted using Prodigal (Hyatt et al. 2010) in contigs >2500 bp, and annotated against the KEGG, Pfam, MEROPS, and NCBI Viral RefSeq databases via MMseqs2. Putative auxiliary metabolic genes (AMGs) were identified and quantified across phage vOTUs. Functional abundances were normalized using the same workflow described above for general mapped vOTUs.

2.6 | Phage Host Prediction

The prediction of bacterial hosts of potentially functional phage was performed using iPHoP (Roux et al. 2023), which integrates multiple computational approaches to predict host taxonomy at the genus level for a broad range of uncultivated phages while minimizing false discovery rate. Analyses were performed using a 75% confidence score.

2.7 | Statistical Analysis

All statistical analyses were performed using R v4.3.2. A structured phyloseq object was used to integrate and analyse abundance data and sampling metadata in the *phyloseq* v1.46.0 (McMurdie and Holmes 2013) and *vegan* v2.6-8 (Oksanen et al. 2025) packages. Alpha diversity measurements (observed richness and Shannon index) were calculated at the vOTU level using the *microbiome* package v1.24.0 (Lahti and Shetty 2017). Generalized linear models (GLM) and generalized linear mixed models (GLMM) were fitted using *lme4* v1.1-36 (Bates et al. 2015) and *lmerTest* v3.1-3 (Kuznetsova et al. 2017) to estimate statistical differences in Shannon diversity between stages (*initial-fattening* and *final-fattening*), including sex as a fixed effect, and individual ID and aviary room as random effects. The best-fit model selection was estimated using *MuMIn* v1.48.4 (Barton 2024), and ANOVA was used to compare the most significant models with and without random effects. A negative binomial model was fitted for each vOTU, and a differential abundance testing (Wald test) was performed, implemented in the *DESeq2* package v1.50.2 (Love et al. 2014). Beta diversity was evaluated using Bray-Curtis distances between stages and visualized with non-metric multidimensional scaling (NMDS). Statistical differences between stages and including sex as a variable were tested using permutational multivariate analysis of variance (PERMANOVA) after 9999 permutations using the *adonis2* function in *vegan*. A similarity percentage (SIMPER) test, also available in *vegan*, was used to identify vOTUs that contributed most to the observed between-group differences. All data were visualized using *ggplot2* v3.5.1 (Wickham 2016).

2.8 | Viral Discovery

Eukaryotic viral sequences were identified within vOTUs using BLASTn and translated and annotated using Geneious Prime 2025.0.3 (www.geneious.com) against related reference sequences. Translated sequences from a region of interest, following ICTV parameters, were aligned using MAFFT v7.49 with the E–INS–I algorithm (Katoh and Standley 2013). Poorly aligned regions, identified by gaps or ambiguous alignments, were removed using trimAL v1.4 (Capella-Gutiérrez et al. 2009). Maximum-likelihood trees were inferred in IQ-TREE (Nguyen et al. 2015) using 1000 ultrafast bootstrap replicates, approximate likelihood-ratio tests, and ModelFinder Plus for the amino acid best-fit-model selection.

3 | Results

3.1 | Metagenomic Sequencing and Viral Identification

The sequencing of six individuals' faecal samples per stage (12 pools) generated 1.93 GB of metagenomic data, with an average read length of 150 bp, an average GC content of 42.33%, and an average Q30 value of 94.03%. Reads were assembled

into 540,509 contigs > 2000 bp in length (Table S1). From this assembly, 73,449 contigs (2000–137,339 bp) were identified as vOTUs, of which 853 (0.012%) were classified as complete or as high- (> 90% completeness), medium- (50%–90% completeness), or low-quality (< 50% completeness) genomes, following CheckV's quality framework on estimated completeness thresholds. The remaining undetermined contigs or that had no viral genes detected were excluded from downstream analysis. We found no evidence of viral contamination in our dataset of vOTUs identified, with no sequences matched with ≥ 95% identity. The retained vOTUs represented a broad viral taxonomic range, including four viral realms, four kingdoms, and eight classes (Table S2). Although 90% of the vOTUs could not be resolved beyond the Class level, eight Orders and 16 Families were assigned in the 10% of the remained identified vOTUs. After quality filtering and clustering, a total of 798 vOTUs (733 *Caudoviricetes*, 53 *Revtraviricetes*, and 12 classified as "Other contigs"; Table S2) were retained and used for mapping and normalized relative abundance. Since it is highly challenging to differentiate endogenous and exogenous origins in *Revtraviricetes*, only *Caudoviricetes*, commonly known phages, were used for functional and host prediction analyses. At the same time, potential eukaryotic viruses were searched separately using the "Other contigs" file.

3.2 | Viral Functional Annotation

Functional analyses of the 733 *Caudoviricetes* vOTUs revealed 91 putative AMGs across 42 phages confidently identified, associated with 31 pivotal signalling pathways (Table S3). These potentially functional phages included a diverse array of metabolic and enzymatic genes that optimize host manipulation and viral replication. Particularly, genes encoding functional elements involved in nucleotide and amino acid metabolism, energy regulation, and redox balance. Additionally, glycosyl hydrolases and membrane-binding domains, regulatory proteins, and tRNA-modifying enzyme were identified.

3.3 | Phage Host Prediction

Host prediction for 26 potentially functional phages identified 176 bacterial host candidates across 30 genera, with a prediction confidence score ranging from 75% to 99.3% (Table S4). The most frequently assigned bacterial families included *Enterobacteriaceae* (26.7%), *Moraxellaceae* (23.3%), *Lactobacillaceae* (17.6%), *Pseudomonadaceae* (14.2%), *Streptococcaceae* (6.3%), and *Leptospiraceae* (5.6%), followed by the remaining families, each contributing < 5% (6.3%).

3.4 | Viral Alpha and Beta Diversity

Alpha diversity across all vOTUs showed no significant differences in viral diversity between the *initial-fattening* evaluated by the observed richness (mean: 178.333; 95% CI: 77.437–279.229) and Shannon index (mean: 2.279; 95% CI: 1.051–3.508) and the *final-fattening* stages evaluated by the observed richness (mean: 158; 95% CI: −9.26–325.26; df = 5, $t = -0.342$, $p = 0.747$) and Shannon index (mean: 2.081; 95% CI: 0.681–3.48; df = 5, $t = 0.419$, $p = 0.693$). Observed richness and Shannon differences between stages remained non-significant when adding fixed (stage, sex) or random effects (individual ID, aviary room) to the generalized linear (mixed) models. Similarly, no significant differences were found in beta diversity between stages, either visually in the NMDS based on the Bray-Curtis distance or statistically using PERMANOVA, including (df = 3, $F = 1.05$, $p = 0.401$) or not (df = 1, $F = 0.875$, $p = 0.584$) sex as a variable.

Focusing on the *Caudoviricetes* vOTUs, the observed richness (df = 5, *t*-value = −0.342, $p = 0.747$), Shannon index (df = 5, *t*-value = −0.341, $p = 0.747$), and beta diversity (df = 1, $F = 0.961$, $p = 0.484$) were not significantly different between stages, sex, or random effects (individual ID, aviary room). However, when focusing specifically on potentially functional phages, even though the observed richness did not differ ($\text{mean}_{\textit{initial-fattening}}$: 10, 95% CI: 5.354–14.646; $\text{mean}_{\textit{final-fattening}}$: 11.4, 95% CI: 3.971–18–829; df = 4.223, $t = 0.852$, $p = 0.439$), the Shannon index significantly increased during the *final-fattening* (mean: 1.203; 95% CI: 0.175–2.231) compared with the *initial-fattening* stage (mean: 0.074; 95% CI: 0.018–0.129; df = 5.131, t = 3.432, $p = 0.018$; Figure 2A), with the best-fit model including just individual ID as a random effect (ΔAIC > 2, weight = 0.752). ANOVA confirmed the significance of including the variable as a random effect in the final model (df = 1, Res.Dev. = 2.756, $p = 0.0007$). The abundance of 16 potentially functional phages was significantly higher in the *final-fattening* stage, with $\log_2$ fold changes ranging from 4 to 30 (Figure 2B), and was considered a primary contributor to the observed increase in alpha diversity between stages.

Although NMDS ordination based on the Bray-Curtis distance revealed no clear clustering by stage (Figure S1), PERMANOVA analysis revealed significant differences in potentially functional phages composition between *initial-fattening* and *final-fattening* stages (df = 1, $R^2 = 0.418$, $F = 6.481$, $p = 0.004$), and when including sex variable as an interaction (df = 3, $R^2 = 0.567$, $F = 3.064$, $p = 0.019$). Stratified analyses revealed that the effect of stage was more pronounced in males ($R^2 = 0.887$), whereas in females the effect was weaker ($R^2 = 0.325$); however, these within-sex comparisons were not statistically significant ($p = 0.1$ in both cases). Sixteen potentially functional phages (Table S5) were considered as significant ($p < 0.05$) contributors to the observed dissimilarities between stages, all of which, except 'Caudoviricetes_779', increased in abundance during the *final-fattening* stage (Figure 3). Overall, these potentially functional phages explained 72.6% of the compositional differences between stages, with individual contributions ranging from 0.007% to 42.84% (Table S5). Notably, the potentially functional phage with the highest contribution to dissimilarity (42.84%) increased during the *initial-fattening* stage.

3.5 | Novel Eukaryotic Viruses Discovery

Two contigs were most closely related to members of the *Adenoviridae*. One sequence was a 26,484 nt contig with 75% query coverage and 78.2% identity to a *Siadenovirus* (*Siadenovirus* sp.; MN480433), and the second sequence was a 35,135 nt contig with 4% query coverage and 71.8% identity to an

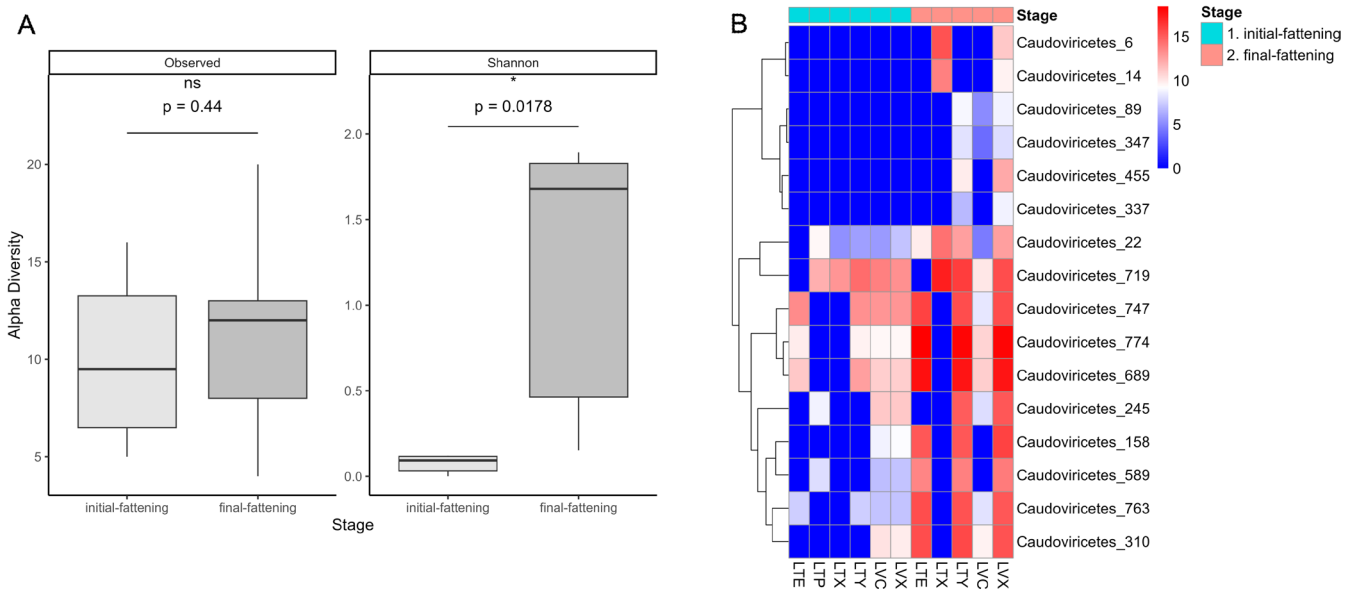

FIGURE 2 | (A) Boxplots evidencing the potentially functional *Caudoviricetes* significant differences in alpha diversity between stages (*initial-fattening* and *final-fattening*) when considering Shannon index, but not in observed richness. (B) Heatmap showing 16 potentially functional phages responsible for differences in alpha-diversity between stages, considering their log2FoldChange. On top, the stages are visualized in light blue, the *initial-fattening* and pink, the *final-fattening* stage. Dendrograms (vertical left) represent hierarchical clustering based on Euclidean distances of log-transformed abundances, illustrating similarity among samples (names in horizontal bottom) and among viral contigs (names in vertical right). The $\log_2$ fold change goes from 0 in dark blue up to 20 in dark red.

Aviadenovirus (Pacific black duck *Aviadenovirus*; MT894382). BLAST-based annotation predicted 17 open reading frames (ORFs) for the *Siadenovirus* and 15 ORFs for the *Aviadenovirus* with amino acid similarities of 60% and 40%, respectively.

Phylogenetic analyses based on DNA polymerase amino acid sequences (1106 aa for the *Siadenovirus* and 1205 aa for the *Aviadenovirus*) with other *Siadenovirus* and *Aviadenovirus* reference sequences (Figure 4) revealed that the *Siadenovirus* sequence from this study (PX216771) clustered closely with a virus previously isolated in a common murre (*Uria aalge*, *Charadriiformes*), a boreal and Arctic seabird. In contrast, the *Aviadenovirus* sequence from Hudsonian godwits (PX216770) formed a distinct, previously undescribed clade, suggesting the presence of a novel and highly divergent adenoviral lineage (Figure 4). No differences among stages, sex, or aviary were evidenced for adenovirus vOTUs found.

4 | Discussion

Over the past decade, growing attention has been directed to the gut virome's role in shaping host physiology, immunity, and metabolism across taxa (Caldwell 2016; Fry et al. 2023; Gil et al. 2023). In this study, we characterized the DNA gut virome of Hudsonian godwits (*Limosa haemastica*), a trans-hemispheric migratory shorebird, during pre-migratory fattening, a period marked by hyperphagia, rapid fat deposition, weight gain, and extensive metabolic remodelling in preparation for non-stop migration. Our findings support evidence that microbial and viral community dynamics are closely associated with the host's physiological state (Grond et al. 2023; Capilla-Lasheras and Risely 2025). Seasonal life-history events such as avian migration provide powerful natural models for investigating how viral communities are restructured in response to seasonal shifts in host metabolism.

Previous research on migratory shorebird microbiomes has largely focused on gut bacteria (Risely et al. 2017; Zhang et al. 2021; Grond et al. 2023; Trevelline et al. 2023), leaving the gut virome underexplored, particularly at South American wintering sites and stopovers. The gut microbiome plays a vital role during migration, which imposes extreme metabolic, nutritional, and immune challenges (Guglielmo and Williams 2003; Wikelski et al. 2003; Altizer et al. 2011; Risely et al. 2017; Grond et al. 2019). While no significant differences in alpha or beta diversity were observed for the full set of vOTUs or for Caudoviricetes between the *initial-* and *final-fattening* stages, a distinct pattern emerged when focusing on potentially functional phages. These phages showed a significant increase in alpha diversity during the *final-fattening* stage, and significant compositional differences between stages of potentially functional phages indicate a restructuring of the phage community associated with migration preparation. Most of the phages driving these differences increased in abundance prior to departure, consistent with the metabolic demands during this period. In contrast, the dominance of a single phage ('Caudoviricetes_779') during *initial-fattening* suggests that while most viral changes reflect increased metabolic demands before migration, some may represent transient colonization or early microbial turnover events. Furthermore, the overlapping functional profiles observed among phages from both stages, especially those enriched in the *final-fattening* stage, suggest a potential functional redundancy within the phage community. Such redundancy may buffer host physiological transitions, maintaining a stable microbiome function (Rodriguez-Valera et al. 2009).

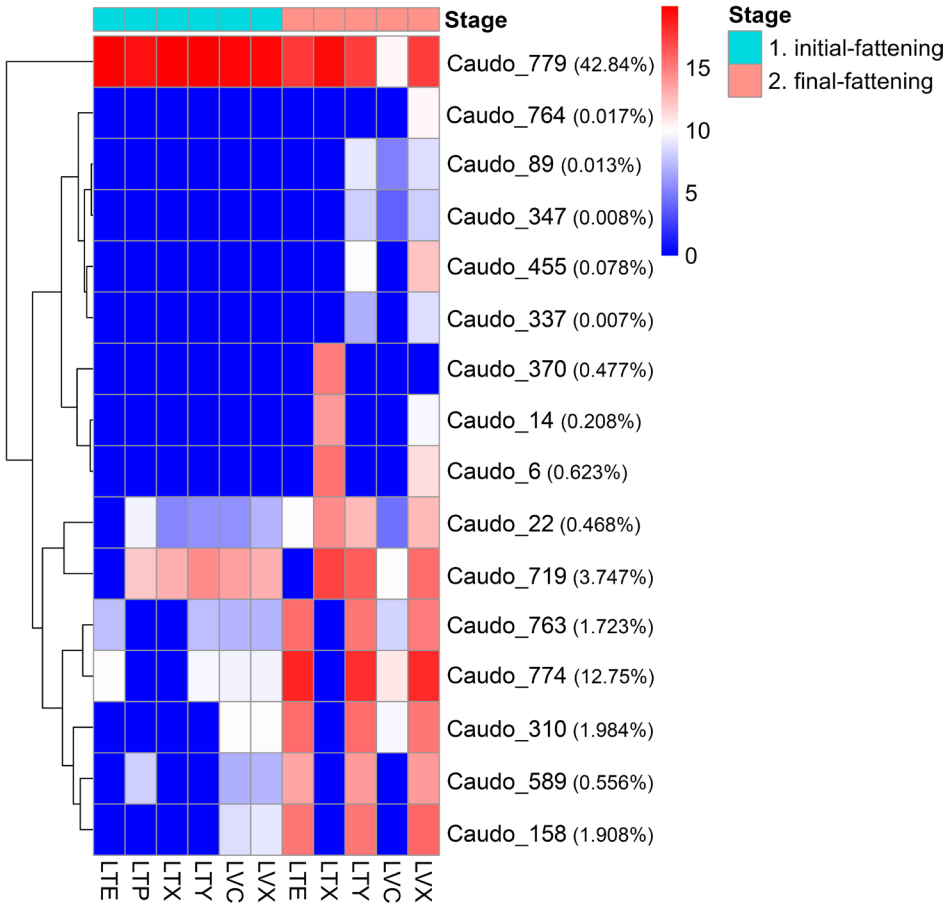

FIGURE 3 | Heatmap showing the log-transformed abundances of the 16 potentially functional phages, and their dissimilarity contribution in parenthesis, that account for significant differences in beta-diversity between stages (*initial-fattening* and *final-fattening*). The stages are indicated at the top, with light blue representing the *initial-fattening* stage and pink the *final-fattening* stage. Dendrograms (vertical left) represent hierarchical clustering based on Euclidean distances of log-transformed abundances, illustrating similarity among samples (names in horizontal bottom) and among viral contigs (names in vertical right). The log-transformed abundances range from 0 in dark blue to 20 in dark red.

Our results suggest that while the gut virome's overall taxonomic structure remains stable, its potentially functional phage component becomes enriched during the *final* stage of fattening, likely reflecting shifts in host metabolism, immune regulation, and microbial resource availability induced by the pre-migratory hyperphagia and fat accumulation (Guglielmo 2018; Eikenaar et al. 2021). Increased bacterial diversity has been reported in migratory birds and mammals under dietary shifts, stress, or other environmental changes, allowing a degree of metabolic flexibility (Reyes et al. 2010; Norman et al. 2015; Liang and Bushman 2021). The enrichment of potentially functional phages during *final-fattening* suggests they may modulate gut microbiome function by turning over bacterial hosts or mobilizing metabolic genes (Al-Shayeb et al. 2020; Shaffer et al. 2020). This is consistent with previous findings of pre-migration increases bacterial diversity, likely driven by physiological, dietary, and environmental changes (Capilla-Lasheras and Risely 2025). Such enhanced bacterial richness expands the range of available hosts, which may, in turn, promote phage proliferation and diversification, thereby increasing phage lytic activity. Thus, these processes not only boost viral diversity but also have the potential to influence microbial community structure and function (Silveira and Rohwer 2016).

Although the potentially functional phages could not be classified beyond the class level (Camargo et al. 2024; Yang et al. 2025), most of them were significantly enriched during the *final-fattening* stage encoding AMGs associated with nucleotide biosynthesis, energy regulation, glycosyl hydrolase activity, and redox balance. Host prediction analyses further suggested that these phages likely infect bacteria such as *Enterobacteriaceae*, *Moraxellaceae*, *Lactobacillaceae*, *Pseudomonadaceae*, and *Streptococcaceae* families known to contribute to nutrient metabolism and gut microbial function, particularly relevant during periods of high energetic demand such as pre-migratory fattening. Despite some contigs being incomplete and should be interpreted with caution, these potential functions may enhance phage replication efficiency during this physiologically demanding period by modulating the bacterial host metabolic pathways, such as glycolysis, the tricarboxylic acid cycle, and oxidative stress response, thereby increasing the availability of essential resources to sustain viral entry and replication under high metabolic pressure (Brum et al. 2016; Al-Shayeb et al. 2020). Similar functional changes have been observed in diet-induced mammal obesity models, where gut phage communities, particularly *Caudoviricetes*, shift significantly in response to nutritional state (Howard et al. 2024), and in environmental viromes,

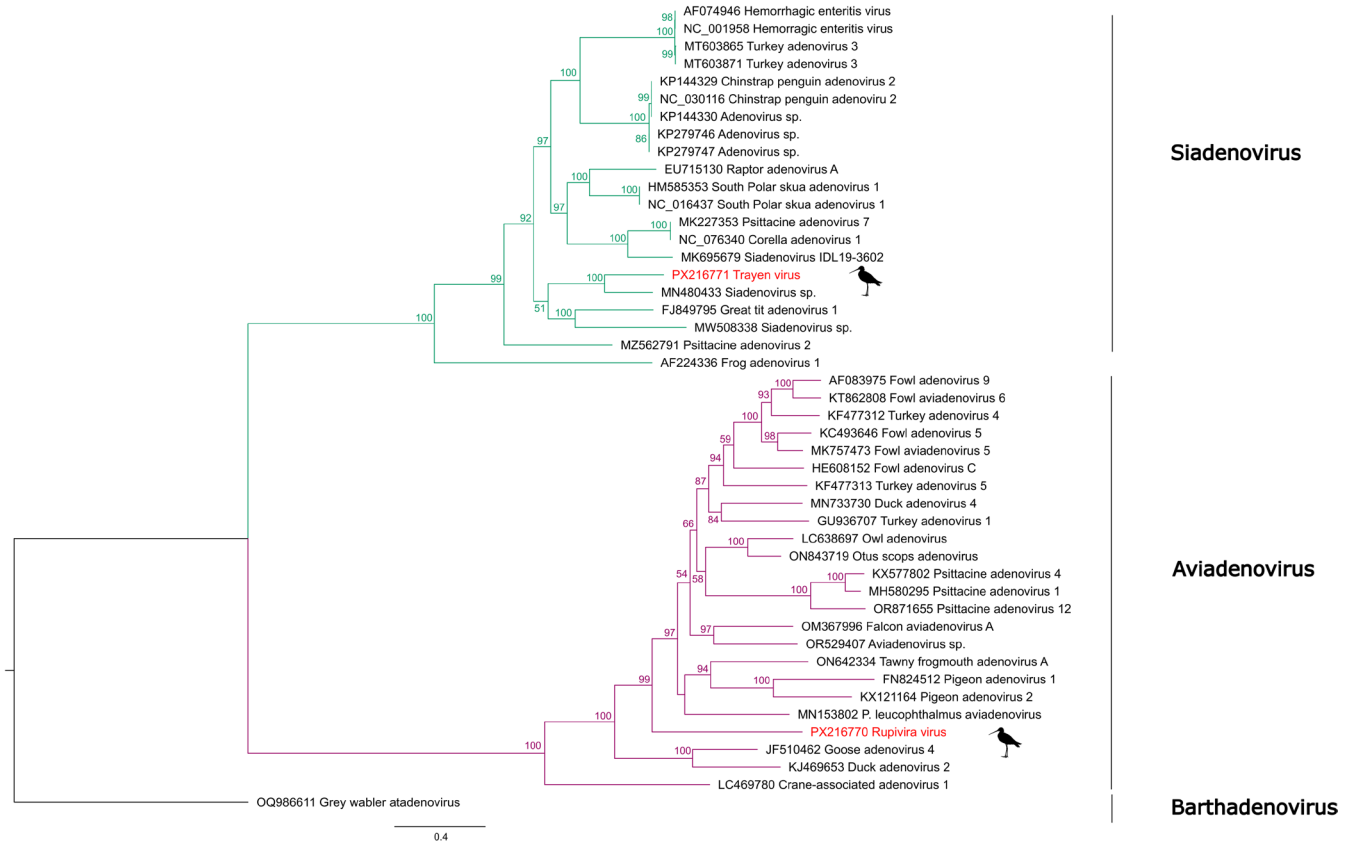

FIGURE 4 | Phylogenetic tree inferred for representative members of adenovirus. The green clades correspond to *Siadenoviruses*, and the purple ones to *Aviadenoviruses*. Grey warbler atadenovirus was set as the outgroup. The sequences identified in this study have been named for clarity, are highlighted in red text and are accompanied by a silhouette of *Limosa haemastica*. Scale bar corresponds to the number of substitutions per site. Tip names include the GenBank strain names and associated accession numbers.

where AMGs modulate bacterial host metabolism and physiology according to their habitat (Luo et al. 2022). Such functions are particularly relevant during pre-migratory fattening in birds, a stage characterized by rapid mobilization and storage of energy, tissue remodelling, and increased oxidative load (Guglielmo 2018; Eikenaar et al. 2021). The enrichment of phage functions related to redox balance and oxidative stress modulation is consistent with previous studies in shorebirds showing elevated antioxidant capacity, rather than oxidative damage, during fattening (Gutiérrez et al. 2019). This suggests that molecular strategies to mitigate oxidative stress in migratory birds may be supported by microbial functional contributions. Genes involved in membrane-binding and tRNA modification further suggest a potential phage adaptive strategy to maintain viral activity under stress such as rapid bacterial host turnover, an event of pre-migratory physiology (Paez-Espino et al. 2016; Liu et al. 2017). Such phage-mediated modulation of microbial metabolism may, therefore, contribute to their avian host fitness by host energy acquisition and gut microbiome stability during migration preparation (Gutiérrez et al. 2019; Grond et al. 2023).

In addition to the phage-dominated gut virome, we identified two adenoviruses, one *Siadenovirus*, and other *Aviadenovirus*. Although in-aviary acquisition cannot be excluded, field-based origin remains the most likely alternative, as the birds encounter diverse viral communities along their migratory routes before entering captivity (Altizer et al. 2011; Wille et al. 2019). Adenoviruses are generally considered to show strong host-specificity, often co-evolving with their vertebrate hosts over evolutionary timescales (Davison et al. 2003; Wellehan et al. 2004), particularly within *Aviadenovirus*, *Atadenovirus*, and *Siadenovirus* genera (Davison et al. 2003; Wellehan et al. 2004; Athukorala et al. 2022). In avian species, this co-evolutionary signal is particularly strong, with avian adenoviruses frequently clustering by host order or family, suggesting long-term viral persistence within avian lineages. However, there is also evidence of occasional host-switching events (Athukorala et al. 2022). In our study, the *Siadenovirus*-like sequence shared a common ancestor with a sequence of a strain isolated from the common murres (*Uria aalge*; GenBank accession MN480433), a seabird species within the same order (Charadriiformes), consistent with the pattern of taxon-specific viral lineages. Conversely, the deeply divergent *Aviadenovirus*-like sequence found in Hudsonian godwits likely represents a novel lineage and implies long-term viral divergence, potentially shaped by host-specific adaptation. This hidden viral diversity and highly divergent adenoviruses in migratory birds highlight the need for broader virological surveillance, particularly in remote stopover and overwintering sites that are currently underrepresented in viral datasets.

This study was conducted under controlled and standardized aviary conditions, which minimized environmental variability (e.g., temperature, humidity, water flow) and enabled a

more precise assessment of host-virome interactions during pre-migratory fattening. Nevertheless, several limitations should be acknowledged. The relatively small sample size, an inherent constraint when working with captive wildlife, particularly migratory shorebirds, limits the extent to which our findings can be generalized to broader ecological contexts. To particularly mitigate this limitation, individuals were randomly assigned to aviary rooms with balanced sex ratios, and a deep sequencing strategy (≈20 Gbp per library) was employed, reducing potential housing effects and allowing high-resolution characterization of virome composition within hosts. As part of the controlled and standardized experimental design, godwits were maintained on a captive diet that closely matched the macronutrient profile of their natural prey and lacked additives such as pre- or probiotics. Although diet is a key determinant of gut microbial composition, including its viral component, dietary effects on microbial communities are often moderate to small unless major shifts in macronutrient composition occur (David et al. 2014; Howard et al. 2024). Nonetheless, it is important to recognize that captive diets may introduce microbial and viral exposures that differ from those found in natural prey communities, potentially influencing virome structure and dynamics (Li et al. 2010; Howard et al. 2024). While controlled conditions cannot replicate ecological complexity of natural environments, they provide a valuable and complementary framework for examining gut virome and microbiome responses to well-defined physiological transitions in migratory birds.

In conclusion, our findings provide new insights into the temporal dynamics of gut virome diversity and potential functional capacity during fattening in a long-distance migratory bird. Given the consistent physiological remodelling that occurs during pre-migratory fattening (Buehler et al. 2008; Piersma and van Gils 2011; Gutiérrez et al. 2019), the functional potential of viral communities may be a critical yet underappreciated component of this physiologically demanding process. Therefore, our results emphasize the importance of the gut virome as a dynamic and responsive component of the host-associated microbiome, playing a complementary and potentially synergistic role alongside bacterial communities in influencing host physiology during life-history transitions such as migration.

Author Contributions

Designed research: J.G., J.G.N., C.V. Performed research: J.G., J.V.-A., C.M., J.G.N., C.V. Formal analysis: J.G., M.W., E.C.-N., S.G.-L., C.V. Funding acquisition: J.G., C.V. Methodology: J.G., J.V.-A., C.M., J.G.N., M.W., S.G.-L., E.C.-N., C.V. Supervision: J.G.N., M.W., E.C.-N., C.V. Validation: J.G., M.W., S.G.-L., E.C.-N., C.V. Writing – original draft: J.G. Writing – review and editing: J.G., J.V.-A., C.M., J.G.N., M.W., S.G.-L., E.C.-N., C.V.

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Disclosure

Samples (faeces of *Limosa haemastica*) were collected in Chiloé, Chile, in accordance with Chilean national regulations and in compliance with the Convention on Biological Diversity and the Nagoya Protocol. Sampling was authorized by the Chilean Agricultural and Livestock Service (SAG; Resolution No. 5932/2016 and 7625/2018), and the Institutional Animal Care and Use Committee (CICUA) of Universidad Austral de Chile (Protocol No. 355/2019). Benefits from this research accrue from the sharing of data and results via public repositories and scientific publication. No additional benefit-sharing arrangements apply beyond data accessibility and coauthorship.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The raw sequencing reads used in this study are available at the NCBI Sequence Read Archive database (BioProject PRJNA1306318). The R script and databases are available at Dryad repository (DOI: 10.5061/dryad.tb2rbp0g9).

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Sequencing and assembly statistics for each library. Columns indicate the name of each library (ID), number of raw reads (reads), reads retained after trimming (trimmed), GC content (%), Q30 percentage, number of assembled contigs (contigs), and minimum (min length) and maximum contig lengths (max length). **Table S2:** Taxonomic annotation of retained viral operational taxonomic units (vOTUs). Taxonomic classification is provided from realm to family for each vOTU. **Table S3:** Functional annotation of Caudoviricetes vOTUs based on DRAM-v analysis. The table reports putative auxiliary metabolic genes (AMGs) identified across Caudoviricetes vOTUs, including pathway assignments and functional categories. Columns correspond to DRAM-v output fields. **Table S4:** iPHoP-based host prediction for viral operational taxonomic units (vOTUs). The table reports predicted bacterial hosts for each vOTU, including taxonomic assignments, confidence scores, and supporting evidence as provided by iPHoP. Columns correspond to iPHoP output fields. **Table S5:** PERMANOVA-based contribution of vOTUs to beta-diversity dissimilarities between stages. For each vOTU, the table includes average abundance, overall dissimilarity explained, percentage contribution, ratio, rank order (ord), cumulative contribution (cusum), and *p*-values. **Figure S1:** NMDS of Caudoviricetes vOTUs with a potentially associated function showing no spatial ordination between stages (initial-fattening in light blue and final-fattening in pink) during the pre-migratory fattening in Hudsonian godwits. Samples from the same individual are connected by a dashed line across stages.