Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2026.232

RESEARCH ARTICLE

Metagenomic Analysis of Mosquito-Borne Viruses in the Chinese Border Region at the China-North Korea-Russia Tri-border Area

Baoquan Huang[1†], Jixu Li[2†], Jian Chen[3], Shengwei Ji[1], Eloiza May Galon[4], Xu Gao[1], Chenghui Li[1], Qichao Cui[1], Guolu Yin[1], Zhe Liu[1], Ying Xu[1] and Shujiang Xue[1*]

[1]Department of Veterinary Medicine, Agricultural College of Yanbian University, Yanji, China; [2]Yanbian Korean Autonomous Prefecture Center for Disease Control and Prevention; [3]Yanbian Animal Disease Prevention and Control Center; [4]Department of Basic Veterinary Sciences, College of Veterinary Medicine and Biomedical Sciences, Cavite State University, Indang, Cavite, Philippines 4122.
†Baoquan Huang, Jixu Li contributed equally to this work.
*Corresponding author: sjxue@ybu.edu.cn

ARTICLE HISTORY (26-678)

Received: June 07, 2026
Revised: August 26, 2026
Accepted: August 29, 2026
Published online: October 19, 2026

Key words:
Aedes vexans nipponii
China–Russia–DPRK border
Mosquito-borne viruses
Songling virus
Viral metagenomics

ABSTRACT

The Tumen River basin, located at the tri-border region of China, North Korea, and Russia, is a hotspot for mosquito-borne diseases. However, research regarding mosquitoes as vectors and their viruses remains understudied. This study aims to elucidate the composition and distribution patterns of mosquito-borne viruses in this basin. From April to July 2025, 6,310 mosquitoes were collected in the four areas of the Tumen River basin by using mosquito traps. Viral metagenomics were confirmed by PCR and Sanger sequencing to identify the virus. We identified six mosquito viruses: Songling virus (SGLV), Daeseongdong virus 2 (DSDV2), Hubei mosquito virus 2 (HMV2), Yongsan picorna-like virus 3 (YPLV3), Yongsan picorna-like virus 4 (YPLV4), and Yichang virus (YCV). PCR results show that the minimum infection rates (MIR) for SGLV in Longjing mosquitoes were 25.02 per 1,000 mosquitoes; in Hunchun, the MIRs for YPLV3, YPLV4, YCV, HMV2, and DSDV2 were 1.61, 0.80, 1.61, 29.74, and 7.23 per 1,000 mosquitoes, respectively; and the MIR for YCV detected in Helong was 2.61 per 1,000 mosquitoes. This paper reports the first case of a new zoonotic virus strain in China, SGLV, and determines that it is highly concentrated in local mosquito species. The detection of SGLV RNA in field-collected mosquitoes reflects viral presence in the local ecological setting, although whether this represents transient blood-meal remnants, mechanical carriage, or active infection remains an unverified hypothesis. Phylogenetic analysis revealed high sequence homology of SGLV and DSDV2 with isolates reported from other Eurasian countries, suggesting a broad geographic and transcontinental distribution of these viral lineages. The cross-host transmission potential of HMV2 and co-circulation with *picorna-like* viruses suggest the need for continued surveillance of these emerging insect-associated viruses. The above results have reduced the knowledge gap in the distribution patterns and genetic diversity of new arboviruses and provided support for building an effective cross-border surveillance system.

To Cite This Article: Huang B, Li J, Chen J, Ji S, Galon EM, Gao X, Li C, Cui Q, Yin G, Liu Z, Xu Y and Xue S, 2026. Metagenomic analysis of mosquito-borne viruses in the chinese border region at the China-North Korea-Russia tri-border area. Pak Vet J, 46(10): 2618-2627. http://dx.doi.org/10.29261/pakvetj/2026.232

INTRODUCTION

Mosquitoes occur worldwide. Environmental-climatic changes, the world's trade and travel have spread vector mosquitoes in recent years (Kraemer *et al.*, 2019). Many mosquitoes transmit pathogens of medical and veterinary relevance; over 300 mosquito species are known for virus vectors (Socha *et al.*, 2022). Every year, over 700 million people are infected with mosquito-borne diseases around the world, and almost 700,000 dies from these diseases (Franklinos *et al.*, 2019). Besides the well-known illnesses of dengue fever and Japanese encephalitis, viral metagenomics and high-throughput sequencing have overcome the limitations of traditional cell-culture-based

assays to discover various emerging and neglected insect-borne viruses in nature (Bassi *et al.*, 2022). Recent metagenomic studies have identified thousands of new invertebrate RNA viruses, significantly expanded "viral dark matter" (Shi *et al.*, 2016), and recovered overlooked pathogens (Banna virus, Tahyna virus) as well as novel flaviviruses, nairoviruses and parvoviruses from field-collected blood-feeding mosquitoes across different regions (Xia *et al.*, 2018a). These viruses have good cross-host spillover abilities and warrant continued surveillance to better understand their ecological dynamics and potential cross-species transmission barriers (Gould *et al.*, 2017).

The Tumen River System is a relatively large cross-border river in China that passes through the China-DPRK-Russia Triple Border Area. A relatively stable continental monsoon climate has good biodiversity and is suitable for the development and transmission of mosquito-borne viruses (Min *et al.*, 2026). As a cross-border ecological corridor for trilateral cooperation, frequent cross-border human-goods movement requires characterization of local mosquito-borne virus epidemiology and spatial distribution (Li *et al.*, 2022). Migratory wildlife and intensively farmed livestock are typical examples of cross-species pathogen carriers that provide abundant mosquito blood meals and thus accelerate virus transmission cycles (Liu *et al.*, 2019). At the same time, occupational exposure of local farmers/herders and an increase in cross-border tourism have raised the risk of cross-border mosquito-borne diseases (Msellemu *et al.*, 2024).

SGLV is an emerging tick-borne virus belonging to the genus *Orthonairovirus* within the family *Nairoviridae* (order *Bunyavirales*). In 2021, it was first isolated from a tick-bitten patient in Heilongjiang, China (Ma *et al.*, 2021). The virus has been found in the China-DPRK border area (Li *et al.*, 2024) and in Xinjiang rodents (Ji *et al.*, 2023). SGLV infection causes fever and headache, etc., and has significant zoonotic disease potential among these poorly understood tick-borne viruses (Wang *et al.*, 2019). Assuming that mosquitoes may acquire SGLV through co-habitation or mechanical blood-feeding, this study included mosquitoes for surveillance to examine their potential role in SGLV cross-border transmission.

YPLV3 is a novel insect-associated positive-sense single-stranded RNA virus belonging to the order *Picornavirales*, which was first identified from *Aedes vexans nipponii* mosquitoes in South Korea (Sanborn *et al.*, 2019). In this study, we detected a related picorna-like sequence designated as Yongsan picorna-like virus 4 (YPLV4). Emerging surveillance demonstrates that diverse picorna-like viruses naturally circulate within various mosquito taxa and aquatic environments worldwide, highlighting an underappreciated evolutionary diversity in vector populations (Zell *et al.*, 2017; Guimarães *et al.*, 2025b).

DSDV2 is a novel insect-specific virus belonging to the family *Permutotetraviridae*, and in 2013, it was first found in *Culex* mosquitoes from Paju City, Gyeonggi Province, South Korea (Hang *et al.*, 2016). The virus has been widely distributed among mosquitoes in South Korea and East Asia, and thus can be naturally transmitted by these vectors. Genetic and evolutionary studies of this virus can help us understand how insect-specific viruses (or permutotetra-like viruses) originated and whether they are capable of interspecies transmission.

HMV2 is a newly discovered insect-specific virus that was first identified in 2016 in *Culex* mosquitoes from Hubei Province, China (Shi *et al.*, 2016). Viruses have been found in mosquitoes across many parts of China, including Hubei and Yunnan; therefore, regional natural circulation of vectors such as *Culex* mosquitoes may have occurred (Hameed *et al.*, 2021; Wu *et al.*, 2023). At present, only insects are hosts for this virus.

YCV is a new mesonivirus belonging to the family *Mesoniviridae* (order *Nidovirales*). In 2017, it was first isolated and identified from *Culex mosquito* samples in Yichang City, Hubei Province, China (Wang *et al.*, 2017). Related mesoniviruses have been found in many Hemiptera insects and the environment across central China, and it is believed that they can form a natural cycle among insect communities linked to agricultural ecosystems (Agboli *et al.*, 2023).

Given the above reasons, it has been determined that an in-depth study of the community structure and spatial distribution of mosquito-borne viruses in the Tumen River Basin is required (Liu *et al.*, 2025). To address the research gap mentioned above, viral metagenomics was used in this study to identify and characterize all viruses in mosquito samples from the border area of the basin comprehensively. To provide a scientific basis for the prevention and control of mosquito-borne infectious diseases in the area and reduce the risk of cross-border transmission of such diseases effectively (Guimarães *et al.*, 2025a; Zhang *et al.*, 2026).

MATERIALS AND METHODS

Mosquito Collection: From April to July 2025, 6,310 mosquitoes were collected in the four areas of the Tumen River basin by using mosquito traps. A Stereomicroscope was used to observe the morphology, and sequencing of the mitochondrial cytochrome oxidase subunit I (COI) gene was employed for identification (Chan *et al.*, 2014). Morphological and COI identification concordance was 96 per cent (96/100). All mosquitoes were divided into 636 pools according to the collection site and species (10 individuals per pool); for rare species with fewer than 10 individuals (e.g., Aedes flavopictus, Culex bitaeniorhynchus, and Aedes nipponicus), individuals of the same species from the same collection site were grouped into a single pool or processed based on the number available and stored at -80°C.

Mosquito Processing: Each pool received 400μL of DMEM and four 3mm zirconium beads, and was homogenized at 60Hz for 5min. Centrifuge the homogenate at 13,400×g for 10min in a 4°C centrifuge, and then sequentially filter the supernatant through 0.45μm and 0.22μm filters. Added clarified filtrate (150μL) to a sterile tube. Using the QIAamp Viral RNA Mini Kit as instructed by the manufacturer, extract viral RNA. Measure RNA Purity and Concentration using an SMA 1000 UV-spectrophotometer.

Library Construction and Sequencing: Supernatants from the 636 pools were divided into four groups

according to geographic origin. First-strand viral cDNA was synthesized by SuperScript reverse transcriptase with random primers (100pmol/μL), and then converted to double-stranded cDNA using the Klenow fragment. Single-strand PCR amplification was carried out with barcoded random primers (Table 1). Purify the PCR products with the QIAquick PCR Purification Kit and elute in 50μL of TE buffer (100mM Tris-HCl, 10mM EDTA, pH8.0) for subsequent experiments (Meng *et al.*, 2019).

Table 1: Primers for metagenomic analysis

Primers type	Primers number	Number (5'-3')
Anchored Random Primers	RT1	GCCGGAGCTCTGCAGATATCNNNNNN
	RT2	GTATCGCTGGACACTGGACCNNNNNN
	RT3	CATCACATAGGCGTCCGCTGNNNNNN
	RT4	CGCAGGACCTCTGATACAGGNNNNNN
	RT5	CCGAGGTTCAAGCGAGGTTGNNNNNN
	RT6	GGTGGGCGTGTGAAATCGACNNNNNN
	RT7	CGACCCTCTTATCGTGACGGNNNNNN
	RT8	CGTCCAGGCACAATCCAGTCNNNNNNN
Barcode Primers	Primer 1	GCCGGAGCTCTGCAGATATC
	Primer 2	GTATCGCTGGACACTGGACC
	Primer 3	CATCACATAGGCGTCCGCTG
	Primer 4	CGCAGGACCTCTGATACAGG
	Primer 5	CCGAGGTTCAAGCGAGGTTG
	Primer 6	GGTGGGCGTGTGAAATCGAC
	Primer 7	CGACCCTCTTATCGTGACGG
	Primer 8	CGTCCAGGCACAATCCAGTC

Bioinformatics Analysis: Four sets of labelled and purified PCR products were sent to Nanjing Novazene Biotechnology Co., Ltd. for high-throughput viral metagenomic sequencing on the Illumina NovaSeq 6000 platform. The initial sequencing data was in the FASTQ format. Use fastp (v0.20.0) for filtering and quality control of raw sequencing data to obtain valid sequences. The quality-controlled valid sequences were aligned with the host reference sequence using minimap2 software to eliminate host-derived contaminating sequences (Li, 2018). The remaining reads were assembled by MEGAHIT software (Li *et al.*, 2015), and only contigs of 300bp or longer were retained. Kaiju (Menzel *et al.*, 2016) and DIAMOND BLASTp were employed to align the assembled reads and contigs with the NCBI NR database and the RefSeq Viral database, and an E-value threshold of 1×10^{-5} was set (Buchfink *et al.*, 2015, Camacho *et al.*, 2009). The sequence with the highest BLAST similarity in each group was selected for the following analysis (Shao *et al.*, 2020).

To obtain the full-length viral genome sequence, primers were designed using Primer5.0 software (Primer Biosoft International, USA); then, RT-PCR was employed to fill in the sequence gaps between contigs, and the amplified products were verified by Sanger sequencing (Sanger *et al.*, 1977). MAFFT (v7.490) was used to generate multiple sequence alignments of the conserved genomic region (partial S segment or RdRp amino acid sequences). The best-fit nucleotide/amino acid substitution models were automatically selected by ModelFinder. Then, using the Maximum Likelihood (ML) method in IQ-TREE (v2.2.0), 1,000 ultrafast bootstrap replicates were carried out to build phylogenetic trees (Nguyen *et al.*, 2015). The specific substitution models and sequence alignment lengths were: SGLV (Fig. 2): partial S segment nucleotide alignment (325 bp) under the GTR+F+I+G4 model; YCV (Fig. 3): RdRp nucleotide alignment (536 bp) under the GTR+F+R3 model; DSDV2 (Fig. 4): RdRp amino acid alignment (181 aa) under the LG+I+G4 model; HMV2 (Fig. 5): RdRp nucleotide alignment (641 bp) under the GTR+F+G4 model; and YPLV3/YPLV4 (Fig. 6): RdRp nucleotide alignment (607 bp) under the GTR+F+R2 model.

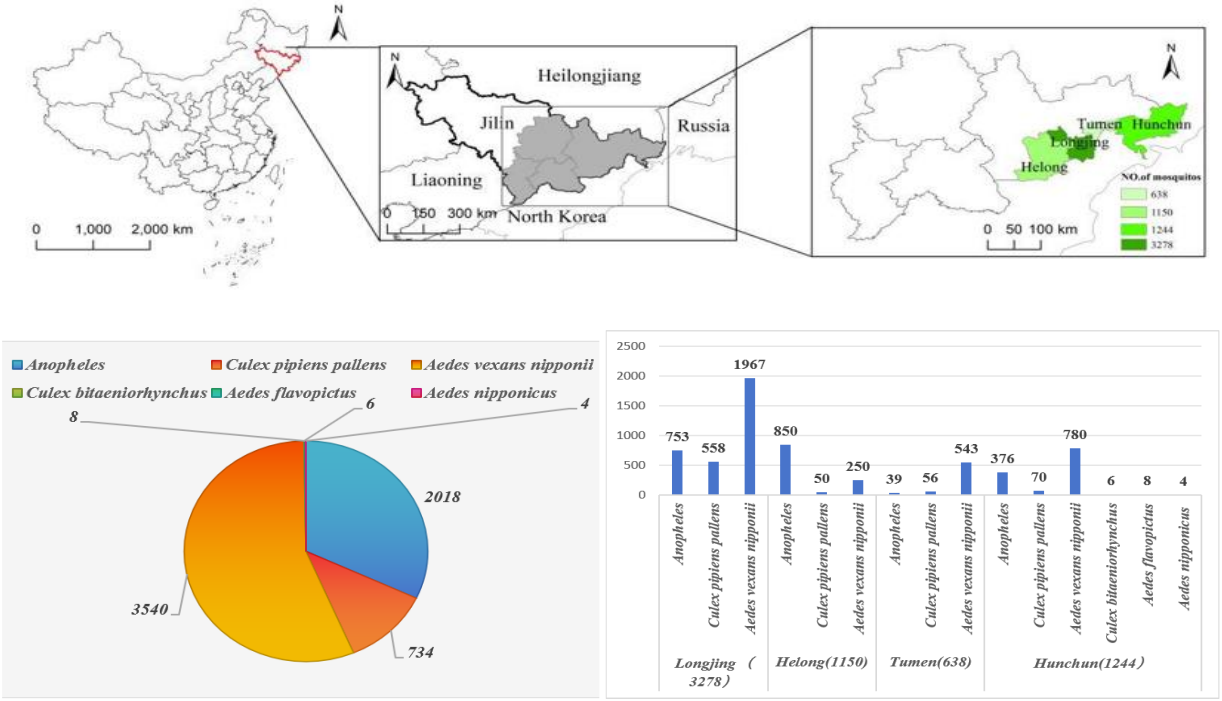


Fig. 1: Overview of mosquito sample collection. Sampling locations in Longjing, Helong, Tumen, and Hunchun (A). The proportion (B) and quantity (C) of different mosquito species used in the study are represented by pie charts and bar charts, respectively. The map was constructed using ArcGIS v10.8.2 software. The basemap shapefile was downloaded from the Resource and Environment Science and Data Center of China (http://www.resdc.cn/).

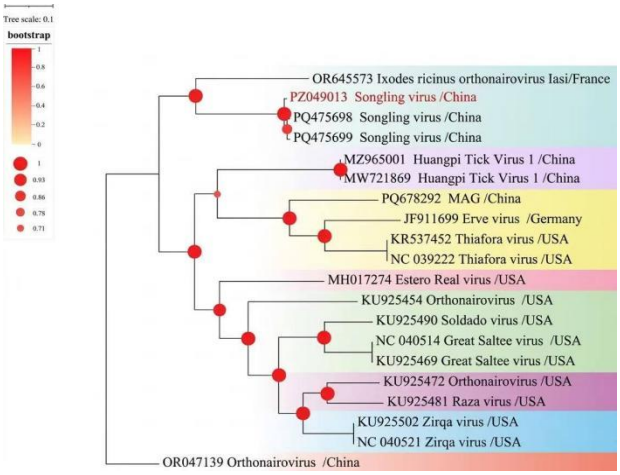


Fig. 2: Maximum likelihood phylogenetic tree of identified Songling virus (SGLV, accession no. PZ049013) based on partial S segment sequences. The phylogenetic tree was constructed using IQ-TREE (v2.2.0) based on the Maximum Likelihood (ML) method under the best-fit GTR+F+I+G4 substitution model (determined by ModelFinder). Bootstrap support values derived from 1,000 ultrafast bootstrap replicates are indicated on the nodes by colored circles ranging from 0.71 to 1.00 (as shown in the legend). The sequence identified in this study (PZ049013) is highlighted in bold red. Scale bar indicates 0.1 nucleotide substitutions per site.

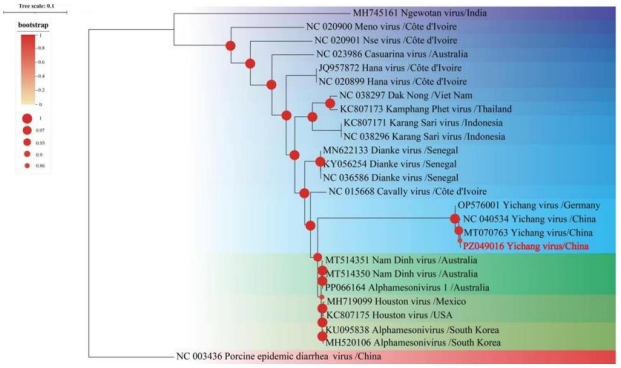


Fig. 3: Maximum likelihood phylogenetic tree of identified Yichang virus (YCV, accession no. PZ049016) based on RdRp sequences. The phylogenetic tree was constructed using IQ-TREE (v2.2.0) based on the Maximum Likelihood (ML) method under the best-fit GTR+F+R3 substitution model (determined by ModelFinder). Bootstrap support values derived from 1,000 ultrafast bootstrap replicates are indicated on the nodes by colored circles ranging from 0.86 to 1.00 (as shown in the legend). The sequence identified in this study (PZ049016) is highlighted in bold red. Scale bar indicates 0.1 nucleotide substitutions per site.

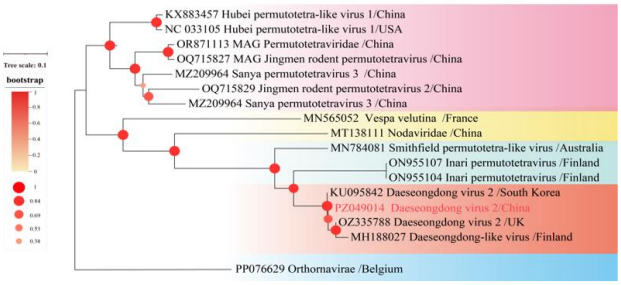


Fig. 4: Maximum likelihood phylogenetic tree of identified Daeseongdong virus 2 (DSDV-2, accession no. PZ049014). The phylogenetic tree was constructed using IQ-TREE (v2.2.0) based on the Maximum Likelihood (ML) method under the best-fit LG+I+G4 substitution model (determined by ModelFinder). Bootstrap support values derived from 1,000 ultrafast bootstrap replicates are indicated on the nodes by colored circles ranging from 0.38 to 1.00 (as shown in the legend). The sequence identified in this study (PZ049014) is highlighted in bold red. Scale bar indicates 0.1 amino acid substitutions per site.

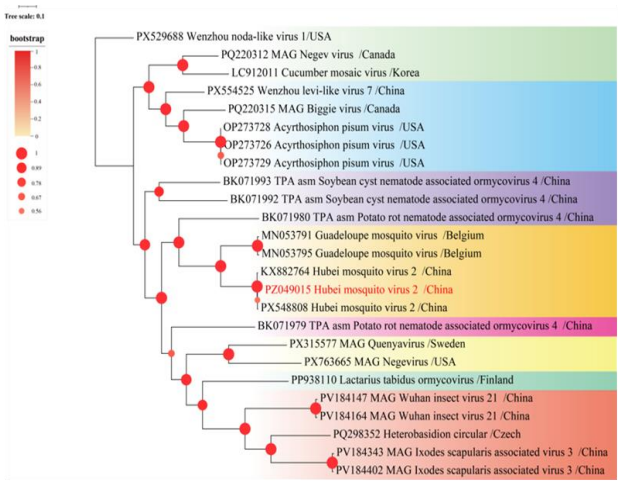


Fig. 5: Maximum likelihood phylogenetic tree of identified Hubei mosquito virus 2 (HMV2, accession no. PZ049015) based on RdRp sequences. The phylogenetic tree was constructed using IQ-TREE (v2.2.0) based on the Maximum Likelihood (ML) method under the best-fit GTR+F+G4 substitution model (determined by ModelFinder). Bootstrap support values derived from 1,000 ultrafast bootstrap replicates are indicated on the nodes by colored circles ranging from 0.56 to 1.00 (as shown in the legend). The sequence identified in this study (PZ049015) is highlighted in bold red. Scale bar indicates 0.1 nucleotide substitutions per site.

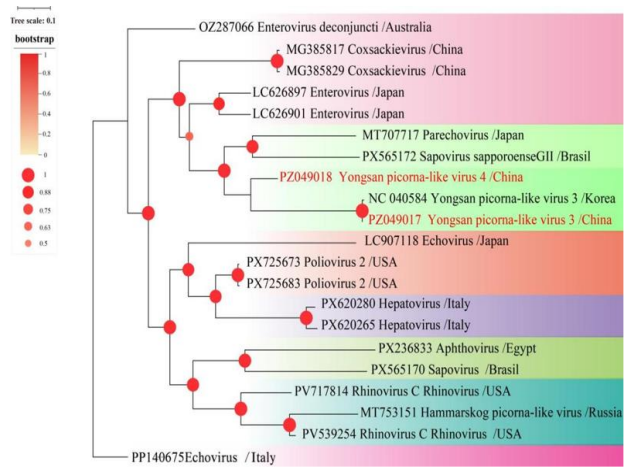


Fig. 6: Maximum likelihood phylogenetic tree of identified Yongsan picorna-like virus 3 (YPLV3, accession no. PZ049017) and Yongsan picorna-like virus 4 (YPLV4, accession no. PZ049018) based on RdRp sequences. The phylogenetic tree was constructed using IQ-TREE (v2.2.0) based on the Maximum Likelihood (ML) method under the best-fit GTR+F+R2 substitution model (determined by ModelFinder). Bootstrap support values derived from 1,000 ultrafast bootstrap replicates are indicated on the nodes by colored circles ranging from 0.50 to 1.00 (as shown in the legend). The sequences identified in this study (PZ049017 and PZ049018) are highlighted in bold red. Scale bar indicates 0.1 nucleotide substitutions per site.

PCR Detection of Pathogens in Samples: To confirm the results of metagenomic sequencing and determine the distribution of SGLV, DSDV2, HMV2, YPLV3, YPLV4 and YCV, RT-PCR was performed using the primers listed in Table 2 (Xia *et al.*, 2018b). Positive samples were subjected to 1% agarose gel electrophoresis and confirmed by sequencing.

Statistical Analysis: IBM SPSS Statistics 26.0 was used for the above statistical analysis. Categorical variables are displayed as absolute frequencies (n) and percentages (%). Fisher's exact test was used to compare categorical

variables because of a small sample size and low expected cell counts in some mosquito species and regional groups (Kim, 2017). Given the exploratory nature of this virome surveillance, P-values were not adjusted for multiple comparisons and should be interpreted as exploratory indicators (with $P<0.05$ considered statistically significant). The two indices calculated in this paper are presented below: Pool Positivity Rate: Calculated as (Number of positive pools/Total number of pools tested)×100%. It is the proportion of infected mosquito pools. Minimum Infection Rate (MIR): Calculated as (Number of positive pools/Total number of individual mosquitoes tested)×1,000. This is a lower-bound estimate of the vector population infection rate in infected individuals per 1,000 mosquitoes and the site-specific MIR was calculated using the total number of mosquitoes collected at each site as the denominator to assess the overall viral carriage burden at the mosquito community level across different geographic regions. This way assumes that at least one infected person exists in every positive pool and is a relatively conservative estimate suitable for vector surveillance data analysis in low-prevalence areas. The 95% confidence intervals (95% CIs) of the Minimum Infection Rates (MIR) were calculated using the exact binomial method (Clopper-Pearson method), and the total number of mosquitoes tested was taken as the base for the calculation. All confidence intervals are per 1,000 mosquitoes and aligned with the MIR baseline.

RESULTS

Sample Characteristics: A total of 6,310 mosquitoes were collected in the Tumen River basin, including 2,018 Anopheles mosquitoes, 3,540 Aedes vexans nipponii, 734 Culex pipiens pallens, 8 Aedes flavopictus, 6 Culex bitaeniorhynchus, and 4 Aedes nipponicus. Aedes vexans nipponii was the dominant mosquito species. Geographically, 3,278 mosquitoes were collected in Longjing City, 1,150 in Helong City, 1,244 in Hunchun City, and 638 in Tumen City (Fig. 1A-C). The mosquitoes were divided into 636 pools for subsequent analysis (Table 3).

Taxonomic Classification: Based on sampling locations, the homogenates were divided into four mixtures for NGS analysis, mosquito samples were divided into four libraries based on their geographic origin (Longjing: 330 pools; Helong: 115 pools; Hunchun: 126 pools; Tumen: 65 pools). A total of 73GB of raw nucleotide data was generated, of which 98.47 to 98.86% were of high quality (>Q20). Each library generated approximately 67,401,498-77,435,866 clean reads, identifying 45,338-52,979 viral reads, accounting for about 0.067%-0.079% of the total reads. Following de novo assembly, a total of 7,828 viral contigs were obtained. Analysis using Kaiju and DIAMOND BLASTP revealed the presence of six viruses: SGLV, DSDV2, HMV2, YPLV3, YPLV4, and YCV. All nucleotide sequences obtained in this study have been submitted to the NCBI database under GenBank accession numbers: PZ049013, PZ049014, PZ049015, PZ049016, PZ049017, and PZ049018.

Viral Prevalence and Infection Rates: SGLV, YPLV3, and YPLV4 were exclusively detected in Aedes vexans nipponii. In contrast, HMV2 exhibited broader tropism, being identified in Anopheles, Aedes vexans nipponii, and Culex pipiens pallens. Out of the 636 pools, 82, 9, 37, 2, 1 and 5 were positive for SGLV, DSDV2, HMV2, YPLV3, YPLV4, and YCV, respectively. Overall screening of 6,310 mosquitoes found MIRs for the six viruses ranging from 0.16 to 13.00 per 1,000 mosquitoes, and SGLV had the highest rate (13.00, 95% CI: 10.35-16.10). Species-stratified analysis showed that only Aedes vexans nipponii carried all six viruses, and SGLV had the highest pool positivity rate. Anopheles harboured only DSDV2, HMV2 and YCV; Culex pipiens pallens carried only HMV2. Viral spread varied among the areas despite their closeness (Tables 4-6).

Notably, SGLV was exclusively found in a high proportion of Aedes vexans nipponii from Longjing. Five viruses (DSDV2, HMV2, YPLV3, YPLV4 and YCV) were also found in Hunchun. Therefore, Hunchun represents a localized area with high mosquito-associated viral diversity, warranting further investigation into the ecological dynamics and potential exposure risks of these viruses.

Table 2: Primers and probes used in the present study

Virus	Type	Genome locus	Primer/probe	Sequence (5′→3′)	Amplicon size (bp)	Reference
SGLV	RT-PCR	segment S	F	TCCTGTCAACCCTCACCTACT	325	This study
			R	ATCGCATTCCTCCACTCAA		
DSDV2	RT-PCR	RdRp	F	CATACTCACGCACCATGGATACTT	543	This study
			R	ACATAGTTTTGTAGAGGGGCACC		
YPLV3	RT-PCR	RdRp	F	ACGATCTTGGCACCCTTTG	520	This study
			R	TGTTAGACGGTCCCTTGAGC		
HMV2	RT-PCR	RdRp	F	CCTAATTCAAAGCGAACAAATGTAC	641	This study
			R	ACGGTACTTCGATCTTATGCTTCTC		
YPLV4	RT-PCR	RdRp	F	GGTGAGAATCGAGAGATGAATCC	607	This study
			R	CATCTTTCTTCATCGCGTCATTA		
YCV	RT-PCR	RdRp	F	ACTCGACTCGTAACAGGAACCC	536	This study
			R	CACGGTAAACACGTTGACACAGT		

Table 3: Mosquitos species collected in Tumen River basin area, China

Collection site	*Anopheles*	*Culex pipiens pallens*	*Aedes vexans nipponii*	*Culex bitaeniorhynchus*	*Aedes flavopictus*	*Aedes nipponicus*	Total
Helong	85(850)	5(50)	25(250)	–	–	–	115(1150)
Hunchun	38(376)	7(70)	78(780)	1(6)	1(8)	1(4)	126(1244)
Tumen	4(39)	6(56)	55(543)	–	–	–	65(638)
Longjing	76(753)	57(558)	197(1967)	–	–	–	330(3278)
Total	203(2018)	75(734)	355(3540)	1(6)	1(8)	1(4)	636(6310)

Values are presented as Number of pools (Number of individual mosquitoes).

Table 4: Regional variations in the prevalence of six mosquito-borne viruses detected in six mosquito species in the Tumen River Basin

Viruses	Pool positivity rate (%)						P-value
	Anopheles (N=203, n=2018)	*Culex pipiens pallens* (N=75, n=734)	*Aedes vexans nipponii* (N=355, n=3540)	*Culex bitaeniorhynchus* (N=1, n=6)	*Aedes flavopictus* (N=1, n=8)	*Aedes nipponicus* (N=1, n=4)	
SGLV	0	0	82(23.10%)	0	0	0	P<0.001
DSDV2	2(0.99%)	0	7(1.97%)	0	0	0	0.476
HMV2	4(1.97%)	2(2.67%)	31(8.73%)	0	0	0	0.010
YPLV3	0	0	2(0.56%)	0	0	0	0.716
YPLV4	0	0	1(0.28%)	0	0	0	1.000
YCV	3(1.48%)	0	2(0.56%)	0	0	0	0.308

Values are presented as positive pools (pool positivity rate %). N indicates the total number of tested pools; n indicates the total number of mosquitoes collected. Mosquitoes were pooled by species with up to 10 individuals per pool. Rare species with limited sample sizes (*Culex bitaeniorhynchus, Aedes flavopictus, and Aedes nipponicus*) were pooled based on available numbers. Negative findings in rare species (n=4-8) do not indicate a lack of viral susceptibility. P-values were calculated using Fisher's exact test due to small sample sizes and expected cell counts below 5. As this is an exploratory study, raw P-values are reported without multiple-comparison correction.

Table 5: Regional differences in the prevalence of six mosquito-borne viruses across four regions of the Tumen River Basin

Viruses	Pool positivity rate (%)				
	Longjing(N=330)	Helong (N=115)	Tumen (N=65)	Hunchun (N=126)	P-value
SGLV	82(24.85%)	0	0	0	P<0.001
DSDV2	0	0	0	9(7.14%)	P<0.001
HMV2	0	0	0	37(29.37%)	P<0.001
YPLV3	0	0	0	2(1.59%)	0.039
YPLV4	0	0	0	1(0.79%)	0.198
YCV	0	3(2.61%)	0	2(1.59%)	0.021

Values are presented as positive pools (pool positivity rate %). N indicates the total number of tested pools. Mosquitoes were pooled by species with up to 10 individuals per pool. P-values were calculated using Fisher's exact test due to sparse data and low expected cell counts across collection sites. As this is an exploratory study, raw P-values are reported without multiple-comparison correction.

Table 6: Minimum infection rates (MIR per 1,000 mosquitoes) of six mosquito-borne viruses across different collection sites in the Tumen River basin, China

Collection site	SGLV		DSDV2		HMV2	
	Positive pools (n)	MIR (95% CI) (per 1,000)	Positive pools (n)	MIR (95% CI) (per 1,000)	Positive pools (n)	MIR (95% CI) (per 1,000)
Helong	0	-	0	-	0	-
Hunchun	0	-	9	7.23(3.31-13.73)	37	29.74(21.08-40.83)
Tumen	0	-	0	-	0	-
Longjing	82	25.02(19.94-30.98)	0	-	0	-
Total	82	13.00(10.35-16.10)	9	1.43(0.65-2.71)	37	5.86(4.13-8.07)
Collection site	YPLV3		YPLV4		YCV	
Helong	0	-	0	-	3	2.61(0.54-7.62)
Hunchun	2	1.61(0.19-5.81)	1	0.80(0.02-4.48)	2	1.61(0.19-5.81)
Tumen	0	-	0	-	0	-
Longjing	0	-	0	-	0	-
Total	2	0.32(0.04-1.14)	1	0.16(0.00-0.88)	5	0.79(0.26-1.85)

Minimum Infection Rate (MIR) is calculated as: (Number of positive pools / Total number of mosquitoes tested)×1,000. Both MIR and its corresponding 95% Confidence Interval (CI) are expressed per 1,000 mosquitoes. The site-specific MIR is calculated based on the total number of individual mosquitoes collected per site to reflect overall community-level viral prevalence. For the single permissive vector *Aedes vexans nipponii* in Longjing, the species-specific MIR for SGLV was 41.69 per 1,000 mosquitoes (95% CI: 33.37-51.44). 95% CIs were calculated using the exact binomial method (Clopper-Pearson) based on total individual mosquitoes tested. Dashes ("-") indicate that the parameter could not be calculated due to zero positive pools.

Phylogenetic Analysis: Based on a partial S-segment phylogenetic analysis, the evolutionary position and taxonomic status of the SGLV Yanbian strain (PZ049013) in the genus Orthonairovirus (family Nairoviridae, order Bunyavirales) were elucidated (Fig. 2). The phylogenetic tree shows that the SGLV Yanbian strain is closely related to the Ixodes ricinus orthonairovirus Iasi strain (OR645573) from France and belongs to a separate monophyletic group with high bootstrap support (>0.93). They have very little genetic distance, with as high as 99.13% nucleotide identity, and are thus considered geographical variants of the same virus. The SGLV Yanbian strain is closely related to Chinese Songling virus isolates (PQ475698/PQ475699) and Huangpi Tick Virus 1 (MZ965001), with nucleotide identity of 99.57% and 99.13%, respectively, and thus belongs to the same evolutionary group of Orthonairovirus.

Phylogenetic analysis placed the YCV strain (PZ049016) in a large evolutionary cluster of known mesoniviruses (family Mesoniviridae), such as Nam Dinh and Houston viruses. It clustered closely (bootstrap>0.82) with three Yichang virus strains: OP576001 (Germany), NC040534 (China), and MT070763 (China), sharing 98.99, 98.59, and 99.20% nucleotide homology, respectively. Minimal genetic distance indicates that the Yanbian strain is a Yichang virus isolate. It is about 85%–87% similar to the Nam Dinh virus clade (e.g., MT514351) and is closely related to it within the genus (Fig. 3). It has a relatively clear genetic boundary and high intra-group homogeneity, and is thus considered a species of YCV Yanbian strain.

To clarify the taxonomic position of the new DSDV2 (PZ049014) found in this study, a phylogenetic tree was built based on the amino acid sequences of the RNA-dependent RNA polymerase (RdRp) of representative viruses of the Permutotetraviridae family (Fig. 4). Phylogenetic analysis was conducted to determine the evolutionary relationships and homologies of Permutotetra-like viruses. As shown in the phylogenetic tree, it has high homology with the three strains of Daeseongdong virus 2: KU095842 (South Korea), OZ335788 (UK), and MH188027 (Daeseongdong-like virus/Finland). They formed a single, closely grouped branch with high confidence (bootstrap>0.84), and the

nucleotide homologies were 98.99, 99.24, and 98.48% respectively. DSDV2 was in the same cluster and thus considered a variant of Daeseongdong virus 2. The branch lengths of the cluster in terms of genetic distance were very short. As a result, it has been determined that this new virus is closely related to the known Daeseongdong virus 2 strains and has shown relatively little genetic variation across East Asia and Europe. Finally, a close cluster of the Yanbian strain indicates that this virus exists in China.

To classify the Yanbian strain of HMV2 (PZ049015), a phylogenetic tree was built based on RdRp nucleotide sequences from representative RNA viruses isolated from mosquitoes, nematodes and other arthropods (Fig. 5). HMV2 Yanbian strain clustered tightly (bootstrap>0.89) with two strains of Hubei mosquito virus 2 (KX882764 and PX548808), and showed nucleotide homologies of 98.96% and 99.38%, respectively. It has a very short branch length and is thus closely related to the Hubei mosquito virus 2 strain. It can be seen from the above that the strains in Hubei, Fujian and the area of this study are genetically very similar.

Notably, the phylogenetic tree includes several viral lineages associated with different hosts: Wenzhou noda-like virus, Cucumber mosaic virus (a plant virus), and Acyrthosiphon pisum virus (an aphid virus) are located above; those below are Lactarius tabidus ormycovirus 1 (a fungal virus), Wuhan insect virus 21, and Ixodes scapularis associated virus 3 (a tick-borne virus). All the different origins of these hosts suggest that viruses in this group have wide natural host ranges, including insects, nematodes, fungi, plants and ticks; thus, they provide rich materials for studying cross-host transmission and evolutionary adaptation.

Phylogenetic analysis was performed to determine the relative locations of the Yanbian strains YPLV3 (PZ049017) and YPLV4 (PZ049018) in the family tree (Fig. 6). Both isolates belong to the same large clade of members in the order Picornavirales. Among the above clades, the YPLV3 and YPLV4 branches are closely related to the Parechovirus (MT707717) and Sapovirus (PX565172) clade, forming a parallel sister group with the overlying Coxsackievirus/Enterovirus clade.

Specifically, the YPLV3 Yanbian strain (PZ049017) clustered closely with the Yongsan picorna-like virus 3 reference strain from South Korea (NC_040584) in an independent monophyletic branch at a high confidence level (bootstrap>0.88). The two have a small genetic distance and are 98.59% nucleotide-homologous. Notably, the new YPLV4 Yanbian strain (PZ049018) did not belong to this YPLV3 subcluster directly, but formed a strongly supported sister branch at the base. Sequence divergence and phylogenetic positioning demonstrate that this isolate represents a distinct, unclassified lineage within the order Picornavirales, phylogenetically separated from the canonical YPLV3 lineage.

DISCUSSION

The Tumen River basin is at a tri-border intersection of China, North Korea and Russia, and is thus a high-risk area for cross-border transmission of mosquito-borne diseases. However, the original data on viral diversity, spread patterns and transmission risks in this basin are unavailable. 6,310 mosquitoes were sampled systematically from the four sites in the basin (Longjing, Helong, Hunchun and Tumen) for viral metagenomics. Six viruses were found: SGLV, DSDV2, HMV2, YPLV3, YPLV4 and YCV. Specify the desired host, minimum infection rate (MIR) and phylogenetic position of each virus to conduct an all-around characterization of the mosquito-borne viral community in this watershed, as done previously. It will help to resolve the surveillance gap in the border Area and provide a foundation for the later construction of a regional monitoring and surveillance system along China's northeastern border.

The discovery of SGLVs has broad ecological consequences. SGLV is a new zoonotic disease, and it was once thought that only ticks transmitted it (Ma *et al.*, 2021). The minimum infection rate (MIR) of the locally dominant species Aedes vexans nipponii in this study was 23.16 per 1,000 mosquitoes (corresponding to a species-specific MIR of 41.69 per 1,000 within Aedes vexans nipponii in Longjing, or 23.16 per 1,000 across all Aedes vexans nipponii collected in the basin). Phylogenetic analysis showed that the SGLV Yanbian strain is highly homologous to the European Ixodes ricinus orthonairovirus Iasi strain (OR645573) (Bratuleanu *et al.*, 2023), and it is believed to have spread widely across Eurasia.

Given the relatively high frequency of detection of this typical tick-borne viral RNA in mosquitoes, an environmental transmission problem has been raised; though it does not yet prove an expansion of biological vector competency beyond ticks. We believe this is because the dominant mosquitoes and ticks have significant ecological niche overlaps on wild vertebrate hosts (e.g., rodents and birds) in complex temperate forest-wetland ecosystems. The detection of SGLV RNA in whole-mosquito homogenates may merely reflect the ingestion of viremic blood meals from co-occurring vertebrate hosts rather than active viral replication or tissue dissemination. While mechanical carriage or alternate transmission pathways represent intriguing ecological hypotheses given the high detection rate in local Aedes vexans nipponii, mosquitoes cannot be designated as competent vectors or biological reservoirs without rigorous experimental validation. Whether mosquitoes play any biological or mechanical role in the maintenance of SGLV remains an open question that requires future laboratory vector-competence assays and viral isolation. However, it has not been determined whether Aedes vexans nipponii is a competent vector for SGLV to date.

Whether mosquitoes are vectors or not for transmitting these viruses must be confirmed in a laboratory experiment. Finally, the isolation of the Iasi strain from hard ticks and its close evolutionary relationship with these ticks strongly suggest long-term coevolution between the virus and tick vectors, maintaining a highly conserved spatio-temporal transmission niche (Celina and Černý, 2024). Furthermore, Aedes vexans nipponii in the Tumen River watershed were the sole species in which YPLV3, YPLV4, and DSDV2 were detected. This observation aligns with the high relative abundance of Aedes vexans

nipponii in the local mosquito population, though direct ecological modeling is required to confirm whether vector density acts as the primary driving factor. This trend is in line with the idea that "the dominant regional vector determines the local viral epidemiological profile" (Liu *et al.*, 2023). At the same time, many other viruses that affect insects have been identified around the world (Wu *et al.*, 2023). The above pathogen ecological features are mainly due to the absolute ecological dominance of Aedes vexans nipponii in this area. Given that it has a humid subtropical monsoonal climate and is relatively warm in the summer, it is suitable for the cross-host and cross-vector transmission of various insect-borne viruses. Finally, all viral strains have been systematically identified based on highly conserved polymerase or nucleoprotein sequences in accordance with the ICTV's international consensus guidelines, and their taxonomy has been verified accurately (Gorbalenya *et al.*, 2020).

This study documents the first mainland China detection of multiple viruses, including YPLV3, YPLV4, and DSDV2, which significantly expands their known geographic ranges and suggests potential connectivity among regional viral pools. Notably, the SGLV isolate exhibited 99.13% nucleotide homology with the Iasi strain of Ixodes ricinus orthonairovirus (Bratuleanu *et al.*, 2023). Previously, YPLV3 was detected only in Aedes vexans nipponii in South Korea (Sanborn *et al.*, 2019), and DSDV2 sporadically in South Korea, the UK, and the USA (Hang *et al.*, 2016; Pilgrim *et al.*, 2026), without mainland China records.

Although members of the related viral groups are generally geographically restricted, sequence analysis shows that they are closely related to overseas strains. We believe that the above spatial distribution patterns may have been promoted by regional ecological corridors, such as the 'East Asia–Australasia Flyway', where migratory birds or transport facilities can passively spread infectious arthropods, such as ticks, across borders (Hubálek, 2004). However, as no samples from neighboring North Korea or Russia were included in this study, the cross-border dissemination hypothesis remains unverified and requires further validation through cooperative transboundary sampling. RdRp gene analysis (Gorbalenya *et al.*, 2020) shows that these cross-regional strains have extremely high sequence homology and have not undergone substantial regional genetic differentiation during spatial spread. Nucleic acid tests alone have identified SGLV and YPLV4 in blood-feeding Aedes vexans nipponii for the first time. YCV has also been found in Anopheles and Aedes vexans nipponii. Since previous records of YCV in China were primarily reported from Culex mosquitoes (Wang *et al.*, 2017) and other insects in Central China (Chang *et al.*, 2020), its detection in Anopheles and Aedes vexans nipponii in Northeast China significantly expands the known geographical distribution and mosquito association profile of this virus. Based on phylogenetic analysis, these sequences are closely related to arbovirus lineages of blood-feeding insects. In addition, the geographically distant isolates formed subclusters based on their origins and displayed spatial genetic variation (Shi *et al.*, 2016). At the same time, close relatives of the virus at different places around the world have been identified; their transmission paths have not yet been determined. The HMV2 phylogenetic tree includes sequences derived from diverse sources, such as insects, nematodes, fungi and plants, and has formed an intricate ecological network involving cross-species host jumps over a long period (Liu *et al.*, 2023).

Although the above results have extended knowledge on the geographical distribution and mosquito associations of viruses, nucleic acid detection alone cannot provide specific public health risk assessments. To confirm whether the detected viruses are indeed caused by mosquitoes and not due to environmental contamination or ingestion of blood-meal residues, gold-standard experiments need to be conducted. In the future, live-virus isolation, whole-genome sequence alignment, functional analysis of mutation sites and mosquito infection models will be added to confirm replication and transmission ability (Kramer and Ciota, 2015).

A relatively high proportion of SGLV in the local dominant mosquitoes suggests a possible risk of viral spillover into the human population (Wang *et al.*, 2019). The discovery of other viruses in blood-feeding mosquitoes raises questions regarding potential zoonotic interfaces and thus requires all-weather public health risk assessment (Weaver and Reisen, 2010). Analysis has been carried out on the epidemic development of various mosquito-borne diseases in the Tumen River Basin since their introduction by foreigners (Hubálek, 2004). Virus isolation confirmation, vector transmission ability tests and baseline infection rates in humans and animals were conducted to provide a foundation for targeted surveillance and quarantine at border ports (Kramer and Ciota, 2015).

This study has some deficiencies. First, metagenomic sequencing and phylogenetic analysis of conserved genomic fragments cannot determine whether the detected nucleic acids originated from replicating infectious particles, and the full genomic characteristics and recombination events are also unknown. Strictly comply with the standardized international arbovirus surveillance protocols. Dual-orthogonal Kaiju and DIAMOND BLASTp algorithms were used for sequence alignment and validation, and phylogenetic analysis excluded false positives. Since ICTV has specifically defined the grouping of RNA viruses based on conserved sequences (Gorbalenya *et al.*, 2020), our results on species identification, taxonomy, distribution in different areas, and hosts should also be taken as reliable. Second, there is no supporting data from host animals, human serology and environmental samples to fully characterize the transmission cycle and actual human infection risk. Additionally, the small sample sizes for rare mosquito species (n=4-8) preclude definitive conclusions regarding their susceptibility or vector role. Nonetheless, the first goal is to clarify the baseline mosquito-borne viral community in this area (Xiao *et al.*, 2018) and thus provide a foundation for vector-borne disease prevention. This standardized cross-border surveillance system has set up a risk stratification mechanism to define specific scopes for follow-up monitoring and thus avoided redundant research. Finally, only metagenomic sequencing and RT-PCR were employed; neither demonstrated active replication or infectious virus production, nor confirmed true vector competence. Without viral isolation or transmission assays,

our results show possible carriage rather than confirmed biological vectoring. Future lab-to-lab transmission studies are required.

Conclusions: This study provides the first baseline data on the composition and distribution of mosquito-borne viruses in the Chinese border area of the Tumen River basin (China-North Korea-Russia tri-border area). We found six RNA viruses, and among them, SGLV had a relatively high minimum infection rate for the main local species Aedes vexans nipponii. Metagenomics and phylogenetics showed that the local viral strains were closely related to those found in the neighboring area and other places. Although the above results provide useful baseline surveillance data on vector-associated viral diversity in this strategic border area, active replication, actual vector competence and potential transboundary dissemination routes need to be verified through coordinated cross-border sampling and laboratory infection experiments. Future studies should expand the scope of surveillance under the 'One Health' approach to better understand the ecology of regional arboviruses and public health risks in Northeast China.

Authors contributions: BH, JL and SX planned, executed the research. All statistical analyses were conducted by BH. Eloiza May Galon, JC, SJ, XG, CL, QC, ZL, GY,and YX provided help to execute this project through materials and finances and also in the editing of the draft. All the members were actively involved in write up and editing of the manuscript.

Funding: This study was supported by grants from the Jilin Province Science and Technology Development Plan Item (20250601056RC), the Natural Science Foundation of Jilin Province (YDZJ202201ZYT616), the National Natural Science Foundation of China (32360886), and the Department of Education of Jilin Province (JJKH20250418).

Institutional Review Board Statement: No human or animal subjects or materials were included in this study; therefore, no Institutional Review Board (IRB) or Institutional Animal Care and Use Committee (IACUC) approval was required.

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Biosafety: All sample processing was performed in a Biosafety Level 2 (BSL-2) laboratory in accordance with institutional biosafety guidelines.

Generative AI Statement: ChatGPT was used solely for routine language polishing, grammar correction, and improving sentence readability. No AI tools were used to generate scientific data, perform metagenomic, or interpret research findings.

Acknowledgments: Not applicable.

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

REFERENCES

Agboli E, Schulze J, Jansen S, *et al*., 2023. Interaction of mesonivirus and negevirus with arboviruses and the RNAi response in *Culex tarsalis*-derived cells. Parasites & Vectors 16:361.

Bassi C, Guerriero P, Pierantoni M, *et al*., 2022. Novel virus identification through metagenomics: a systematic review. Life 12:2048.

Bratuleanu BE, Temmam T, Chaintoutis M, *et al*., 2023. Diversity of viruses in *Ixodes ricinus* in Europe including novel and potential arboviruses. Transboundary and Emerging Diseases 2023:6661723.

Buchfink B, Xie C and Huson DH, 2015. Fast and sensitive protein alignment using DIAMOND. Nature Methods 12:59-60.

Camacho C, Coulouris G, Avagyan V, *et al*., 2009. BLAST+: architecture and applications. BMC Bioinformatics 10:421.

Celina S and Černý J, 2024. Genetic background of adaptation of Crimean-Congo haemorrhagic fever virus to the different tick hosts. PLOS ONE 19:e0302224.

Chan A, Chiang LP, Hapuarachchi HC, *et al*., 2014. DNA barcoding: complementing morphological identification of mosquito species in Singapore. Parasites & Vectors 7:569.

Chang T, Liang S, Zhang Z, *et al*., 2020. First report of a mesonivirus and its derived small RNAs in an aphid species *Aphis citricidus* (Hemiptera: Aphididae), implying viral infection activity. Journal of Insect Science 20:14.

Franklinos LHV, Jones KE, Redding DW, *et al*., 2019. The effect of global change on mosquito-borne disease. The Lancet Infectious Diseases 19:e302-e312.

Gorbalenya AE, Krupovic M, Mushegian A, *et al*., 2020. The new scope of virus taxonomy: partitioning the virosphere into 15 hierarchical ranks. Nature Microbiology 5:668-674.

Gould E, Pettersson JHO, Higgs S, *et al*., 2017. Emerging arboviruses: why today? One Health 4:1-13.

Guimarães LO, Ribeiro GO, Couto RS, *et al*., 2025a. Exploring mosquito virome dynamics within São Paulo Zoo: insights into mosquito-virus-environment interactions. Frontiers in Cellular and Infection Microbiology 14:1496126.

Guimarães LO, Almeida AR, Ramos ESF, *et al*., 2025b. Evolutionary features of new picorna-like viruses in *Culex* (Melanoconion) mosquitoes. Current Research in Parasitology & Vector-Borne Diseases 8:100333.

Hameed M, Wahaab A, Shan T, *et al*., 2021. A metagenomic analysis of mosquito virome collected from different animal farms at Yunnan-Myanmar border of China. Frontiers in Microbiology 11:591478.

Hang J, Klein TA, Kim HC, *et al*., 2016. Genome sequences of five arboviruses in field-captured mosquitoes in a unique rural environment of South Korea. Genome Announcements 4:e01644-15.

Hubálek Z, 2004. An annotated checklist of pathogenic microorganisms associated with migratory birds. Journal of Wildlife Diseases 40:639-659.

Ji N, Wang N, Liu G, *et al*., 2023. Tacheng tick virus 1 and Songling virus infection in great gerbils (*Rhombomys opimus*) in northwestern China. Journal of Wildlife Diseases 59:138-142.

Kim HY, 2017. Statistical notes for clinical researchers: Chi-squared test and Fisher's exact test. Restorative Dentistry & Endodontics 42:152-155.

Kraemer MUG, Reiner RC, Brady OJ, *et al*., 2019. Past and future spread of the arbovirus vectors *Aedes aegypti* and *Aedes albopictus*. Nature Microbiology 4:854-863.

Kramer LD and Ciota AT, 2015. Dissecting vectorial capacity for mosquito-borne viruses. Current Opinion in Virology 15:112-118.

Li D, Liu CM, Luo RB, *et al*., 2015. MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. Bioinformatics 31:1674-1676.

Li D, Li J, Wang R, *et al*., 2024. Detection and genetic analysis of Songling virus in *Haemaphysalis concinna* near the China-North Korea border. Zoonoses 4:22. https://doi.org/10.15212/ZOONOSES-2024-0004

Li H, 2018. Minimap2: pairwise alignment for nucleotide sequences. Bioinformatics 34:3094-3100.

Li JX, Zhang S, Liang W, *et al*., 2022. Survey of tick species and molecular detection of selected tick-borne pathogens in Yanbian, China. Parasite 29:38.

Liu H, Zhang X, Li LX, *et al*., 2019. First isolation and characterization of Getah virus from cattle in northeastern China. BMC Veterinary Research 15:320.

Liu Q, Cui F, Liu X, *et al*., 2023. Association of virome dynamics with mosquito species and environmental factors. Microbiome 11:101.

Liu Z, Ji S, Chang Q, *et al*., 2025. Surveillance of tick-borne viruses in the border regions of the Tumen River Basin: co-circulation in ticks and livestock. PLOS Neglected Tropical Diseases 19:e0013500.

Ma J, Lv XL, Zhang X, *et al*., 2021. Identification of a new *orthonairovirus* associated with human febrile illness in China. Nature Medicine 27:434-439.

Meng F, Ding M, Tan Z, *et al*., 2019. Virome analysis of tick-borne viruses in Heilongjiang Province, China. Ticks and Tick-borne Diseases 10:412-420.

Menzel P, Ng KL and Krogh A, 2016. Fast and sensitive taxonomic classification for metagenomics with Kaiju. Nature Communications 7:11257.

Min P, Song J, Meng Y, *et al*., 2026. Investigation and correlation analysis of pathogens carried by ticks and cattle in Tumen River Basin, China. Veterinary Sciences 13:78.

Msellemu D, Tanner M, Yadav R, *et al*., 2024. Occupational exposure to malaria, leishmaniasis and arbovirus vectors in endemic regions: a systematic review. Current Research in Parasitology & Vector-Borne Diseases 6:100185.

Nguyen LT, Schmidt HA, von Haeseler A, *et al*., 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution 32:268-274.

Pilgrim J, Widlake E, Wilson R, *et al*., 2026. Mosquito viromes across England and Wales reveal hidden arbovirus signals and limited ecological structuring. Frontiers in Microbiology 17:1749228.

Sanborn MA, Klein TA, Kim HC, *et al*., 2019. Metagenomic analysis reveals three novel and prevalent mosquito viruses from a single pool of *Aedes vexans nipponii* collected in the Republic of Korea. Viruses 11:222.

Sanger F, Nicklen S and Coulson AR, 1977. DNA sequencing with chain-terminating inhibitors. Proceedings of the National Academy of Sciences 74:5463-5467.

Shao L, Pang Z, Fu H, *et al*., 2020. Identification of recently identified tick-borne viruses (Dabieshan tick virus and SFTSV) by metagenomic analysis in ticks from Shandong province, China. Journal of Infection 81:973-978.

Shi M, Lin XD, Tian JH, *et al*., 2016. Redefining the invertebrate RNA virosphere. Nature 540:539-543.

Socha W, Kwasnik M, Larska M, *et al*., 2022. Vector-borne viral diseases as a current threat for human and animal health-One Health perspective. Journal of Clinical Medicine 11:3026.

Wang Y, Xia H, Zhang B, *et al*., 2017. Isolation and characterization of a novel mesonivirus from *Culex* mosquitoes in China. Virus Research 240:130-139.

Wang ZD, Wang B, Wei F, *et al*., 2019. A new segmented virus associated with human febrile illness in China. The New England Journal of Medicine 380:2116-2125.

Weaver SC and Reisen WK, 2010. Present and future arboviral threats. Antiviral Research 85:328-345.

Wu Z, Liu J, Feng X, *et al*., 2023. Identification and molecular characteristics of a novel single-stranded RNA virus isolated from *Culex tritaeniorhynchus* in China. Microbiology Spectrum 11:e0053623.

Xia H, Wang Y, Shi C, *et al*., 2018a. Comparative metagenomic profiling of viromes associated with four common mosquito species in China. Virologica Sinica 33:59-66.

Xia H, Wang Y, Atoni E, *et al*., 2018b. Mosquito-associated viruses in China. Virologica Sinica 33:5-20.

Xiao P, Li C, Zhang Y, *et al*., 2018. Metagenomic sequencing from mosquitoes in China reveals a variety of insect and human viruses. Frontiers in Cellular and Infection Microbiology 8:364.

Zell R, Delwart E, Gorbalenya AE, *et al*., 2017. ICTV virus taxonomy profile: *Picornaviridae*. Journal of General Virology 98:2421-2422.

Zhang N, Atoni E, Nyaruaba R, *et al*., 2026. Host and geography shape microbial communities in Kenyan mosquitoes: insights from metatranscriptomics. mSystems 11:e0142725.