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Original Article

Detection and Genetic Analysis of Songling Virus in *Haemaphysalis concinna* near the China-North Korea Border

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Abstract

Background: Songling virus (SGLV) is a spherical, enveloped, fragmented, negative-stranded RNA virus belonging to the genus *Orthonairovirus* in the *Nairoviridae* family. SGLV is transmitted by ticks and can cause disease in humans. This study identified and characterized SGLV in *Haemaphysalis concinna* ticks collected in 2023 in the Yanbian Korean Autonomous Prefecture (China) near the China-North Korea border.

Methods: A real-time quantitative polymerase chain reaction (RT-qPCR) was used to screen for SGLV nucleic acid in ticks. Baby hamster kidney (BHK-21) and African green monkey kidney (Vero) cells were used to isolate strains of SGLV from nucleic acid-positive samples through three successive passages. Next-generation sequencing and phylogenetics methods were used to characterize the SGLVs.

Results: Of the 1659 ticks collected from 6 towns in the Yanbian Korean Autonomous Prefecture near the China-North Korea border, positive SGLV nucleic acid results were identified in 19 *H. concinna* tick pools from Helong and Longjing towns. This discovery led to the extraction of 17 SGLV genome sequences. Homology analysis that compare the newly discovered L, M, and S segments of SGLV strain HLJ1202 revealed nucleotide similarities ranging from 95.5%–97.1%, 91.9%–98.9%, and 98.3%–99.2%, respectively, and amino acid similarities ranging from 95.7%–97.4%, 97.1%–98.8%, and 98.2%–98.9%, respectively. Six distinct clades, characterized by specific geographic locations and host organisms, were identified on the Maximum Likelihood tree of the L segment. The YB129 and YB150 isolates demonstrated SGLV nucleic acid replication across three successive passages in Vero cells, as evidenced by the decrease in RT-qPCR Ct values.

Conclusion: This study marks the initial identification of SGLV in *H. concinna* within the Yanbian Korean Autonomous Prefecture.

Keywords: Songling virus, *Haemaphysalis concinna*, Identification, China-North Korea border

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INTRODUCTION

The genus *Orthonairovirus*, which belongs to the family *Nairoviridae*, encompasses significant tick-transmitted pathogens, such as Crimean-Congo hemorrhagic fever virus (CCHFV) and Nairobi sheep disease virus [NSDV] [1-4]. Orthonairoviruses are primarily transmitted by ticks (Ixodidae

and Argasidae) and naturally infect birds, small mammals (including bats, rodents, lagomorphs, moles, shrews, and hedgehogs), and ungulates [5]. Occasionally, these infections spill over to humans, causing illnesses ranging from mild fever to potentially fatal hemorrhagic fever [6-8]. Nine distinct genogroups of Orthonairoviruses have been identified, each with unique structural characteristics and ecologic associations [6,9]. Among these genogroups, significant attention has focused on CCHF and the Tamdy group of the genus due to the association with diseases in humans and animals. The CCHF group includes tick-borne pathogens, such as CCHFV and NSDV [3,4,10-12]. The Tamdy group has also been studied, with Tacheng tick virus 1 (TcTV-1), Tamdy virus (TAMV), and Songling virus (SGLV) reported to cause non-lethal diseases and symptoms, including fevers and headaches, in humans [13-16].

SGLV is a new member of the Tamdy group within the genus Orthonairovirus. SGLV poses pathogenicity to humans with primary clinical manifestations that include headaches, fevers, depression, fatigue, and dizziness. Initial detection of SGLV occurred in patients in China in 2021 [15]. Subsequently, the virus nucleic acid was detected in ticks in the Greater and Lesser Khingan Mountains in northeast China, and in great gerbils in Xinjiang [15,17-19]. SGLV encompasses typical characteristics of orthonairoviruses. Specifically, the genome RNA is approximately 18.8 kb in length (S segment: ≈1.7 kb; M segment: ≈4.9 kb; and L segment: ≈12.2 kb) and characterized by the genus-specific 3′segment terminus, AGAGUUUCU, and the 5′segment terminus, AGAAACUCU. These RNAs encode three different proteins: the S segment encodes a structural nucleoprotein; the M segment encodes the viral glycoprotein precursor; and the L segment encodes the large protein with its RNA-directed RNA polymerase domain in the virus-complementary sense RNA. SGLV virions are enveloped spheres (80–120 nm in diameter) spiked with heterodimeric glycoprotein projections consisting of the cleavage products of the glycoprotein precursor [15]. Despite elucidating the molecular biology of SGLV, a comprehensive understanding of the biology, variants and evolution, geographic distribution, potential reservoirs, and pathogenic potential of SGLV is lacking, which has led to inadequate prevention and control of SGLV.

The Yanbian Korean Autonomous Prefecture, which is situated in China's Jilin province and borders North Korea and Russia, lies between latitudes 41 and 44 degrees north. This geographic region encompasses the Changbai Mountains and the Tumen River system. During the spring and summer months, the forests and grasslands host a dense population of ticks, rendering the Yanbian Korean Autonomous Prefecture a natural hotspot for tick-borne viruses and posing high risks to humans and animals [20-22]. We screened for ticks carrying the SGLV virus collected from the Yanbian Korean Autonomous Prefecture in 2023, performed virus isolation, and conducted genetic characterization analysis of the virus to provide fundamental data for the prevention and control of tick-borne diseases in the region.

MATERIALS AND METHODS

Tick collection

From March to July 2023 the corduroy dragging method was used to collect free-living ticks in a forested and grassy area within the Yanbian Korean Autonomous Prefecture (Jilin Province, China) near the China-North Korea border. The captured ticks were identified using morphologic keys and subsequently stored at −80°C for further study [23].

RT-qPCR detection of viral RNA from ticks

The tick samples were mixed into a pool of 10 adult and 30 nymph ticks according to collection time, location, species, sex, and tick status. The pooled tick samples were homogenized in 1 ml of ice-cold minimal essential medium (MEM; Gibco, Grand Island, NY, USA) using a TissueLyser II (Qiagen, Hilden, Germany). The homogenates were centrifuged and the resultant supernatants were passed through sterile 0.22-μm filters (Merck Millipore, Billerica, MA, USA). The total viral DNA/RNA was extracted from supernatants using the Tianlong CqEx-DNA/RNA Mini kit (Tianlong, Xian, China). The specific RT-qPCR primers targeting the S segment of SGLV used in this study were as follows [24]: forward, 5′-ATGGCACCTGTGTATGAG-3′; reverse, 5′-AGGCTTTCGTACTCCTTG-3′; and probe, 5′-ATCGTCAGGAGAAGCTTCG-3′. The sensitivity and specificity of RT-qPCR were also determined. RT-qPCR reactions were performed using the AgPath-ID™ One-step RT-PCR kit (Thermo Fisher Scientific, Carlsbad, CA, USA). The reaction mixture consisted of 2× RT-PCR buffer (12.5 μL), 25× RT-PCR mix enzyme (1 μL), upstream and downstream primers (10 μmol/L [0.5 μL each]), probe (10 μmol/L, 0.25 μL), and 1 μL of tick sample DNA/RNA extract. The reaction volume was adjusted to 25 μL using DEPC water. The mixture was then placed in a CFX96 automatic RT-PCR instrument (Bio-Rad, Hercules, CA, USA) under the following conditions: 45°C for 10 min; 95°C for 3 min; and 45 cycles at 95°C for 15 seconds and 60°C for 30 seconds (acquisition of the fluorescence signal). A Ct value <35 was considered positive. During qPCR, a plasmid with the target DNA served as the positive control, tick homogenate without SGLV nucleic acid was the negative control, and DEPC water was the blank control.

Virus isolation

BHK-21 and Vero cells were cultured in 24-well plates at 37°C in 5% CO_2 until 70%–80% confluency was reached. BHK-21 cells were cultured in MEM supplemented with 10% FBS (Gibco) and 1% penicillin-streptomycin [PS] (Gibco), while Vero cells were cultured in medium 199 (Gibco, Grand Island, NY, USA) supplemented with 10% FBS and 1% PS. Supernatants filtered from 19 SGLV nucleic acid-positive *Haemaphysalis concinna* tick pools were inoculated onto monolayers of 24-well Vero and BHK-21 cells, followed by culture with 2% FBS and 1% PS. Additionally, each 24-well cell plate included negative control wells that were inoculated with blank

MEM. These control wells served to assess any non-specific effects or contamination arising from the experimental procedures [25]. The cells in each well were carefully observed for cytopathic effects (CPEs) throughout the experiments. Passages C1–C3 viral RNA levels were assessed by RT-qPCR, as described above.

Next-generation sequencing

The preprocessing of nucleic acid was performed as detailed in a previous study [26]. The nucleic acid concentration of the extracts was determined using Nanodrop (Thermo Fisher Scientific). Reverse transcription was performed using the SuperScript IV First-Strand Synthesis System kit (Thermo Fisher Scientific) with the following parameters based on the manufacturer's recommendations for optimal cDNA synthesis efficiency: 23°C for 10 min; 50°C for 20 min; 80°C for 10 min; and 37°C for 20 min after adding 1 μL of RNase H. The REPLI-g® Mini-kit (Qiagen) was used for whole-genome amplification with the following parameters on the manufacturer's recommendations used for common DNA amplification: 30°C for 90 min followed by 65°C for 3 min.

The DNA libraries were prepared through a series of steps, including DNA fragmentation, end repair, adapter ligation, and PCR amplification (Illumina, San Diego, CA, USA). To maintain the integrity and quality of the sequencing libraries, the PCR cycles were limited to ≤12. This cautious approach minimized the risk of PCR-induced biases and ensured the generation of high-quality sequencing libraries. Additionally, the size of each library was assessed by agarose gel electrophoresis and quantification was performed using qPCR.

Clustering of the index-coded samples was performed on a cBot Cluster Generation System using a Novaseq 6000 S4 Reagent kit (Illumina) according to the manufacturer's instructions. After cluster generation, the DNA libraries were sequenced on an Illumina NovaSeq 6000 platform and 150 bp paired-end reads were generated. To avoid reads with artificial bias (i.e., low-quality paired reads, which primarily result from base-calling duplicates and adaptor contamination), the following types of reads were removed: (i) reads with 3nt unidentified nucleotides (Ns); (ii) reads containing the adaptor; and (iii) reads with ≥20% bases with a phred quality ≤5. All downstream bioinformatics analyses were based on high-quality clean data, which were retained after these steps.

Virus genome determination

The virus genome determination was performed using CLC Genomics Workbench (v20.0.4; Qiagen Bioinformatics, Redwood, CA, USA). High-quality sequencing data were imported and aligned against the reference genome (SGLV strain HLJ1202, GI: NC079000-2) using the Resequencing Analysis tool. Mapping parameters were selected to achieve a balance between sensitivity and specificity, which is crucial for accurately identifying viral sequences. The parameters used for mapping were as follows: (a) match score set to 1; (b) mismatch cost set to 10; (c) insertion cost set to 3; (d) insertion open cost set to 6; (e) insertion extend cost set to 1; (f) deletion open cost set to 6; (g) deletion extend cost set to 1; (h) length fraction set to 0.5; and (i) similarity fraction set to 0.6.

The parameter settings utilized for the consensus sequence extraction tool included a low coverage threshold set to 0, which ensured that only regions with adequate sequencing coverage contributed to the consensus sequences. In regions lacking sufficient coverage, 'N' ambiguity symbols were inserted to denote uncertainty. Additionally, a noise threshold of 0.1 was implemented to mitigate the impact of sequencing errors and minimize noise in the consensus sequences. These settings were selected to facilitate the extraction of precise and dependable consensus sequences by accounting for coverage depth and reducing the influence of sequencing artifacts.

Phylogenetic analysis

A thorough screening of sequences available in GenBank was performed considering the abundance of sequences and the length of gene fragments. The dataset for phylogenetic analysis encompassed the region extending from position 6639 to 7438 of the L segment in the reference genome, HLJ1202 (Supplementary Materials S1). Multiple sequence alignments were conducted using Geneious Prime (v11.0.15, 64 bit; Dotmatics, Auckland, New Zealand). A phylogenetic tree was constructed by Mega 7.0 based on the maximum likelihood (ML) method using the Kimura 2-parameter model as the substitution model. Bootstrap analysis with 1000 replicates was performed to assess the reliability of the trees and evaluate the robustness of the inferred phylogenetic relationships. The resulting tree files were subsequently visualized and annotated using the Chiplot online website (www.chiplot.online, accessed on 27 December 2023). MegAlign v7.1.0 (DNAStar, Madison, WI, USA) was used to evaluate nucleotide and amino acid homology within viral sequences.

RESULTS

Tick sample collection

A total of 1659 ticks were collected across 6 towns near the China-North Korea border in Yanbian city, China from March to July 2023 (Fig 1). These ticks belonged to the following 5 species: *Ixodes persulcatus* (115 ticks); *Dermacentor silvatrum* (481 ticks); *H. concinna* (607 ticks); *H. japonica* (155 ticks); and *H. longicornis* (301 ticks; Table 1).

RT-qPCR detection of SGLV in ticks

A total of 19 *H. concinna* tick pools (YB129, YB131, YB132, YB133, YB140, YB143, YB149, YB150, YB153, YB161, YB162, YB263, YB273, YB278, YB299, YB389, YB402, YB407, and YB431) tested positive for SGLV nucleic acids based on RT-qPCR analysis. The results of the positive,

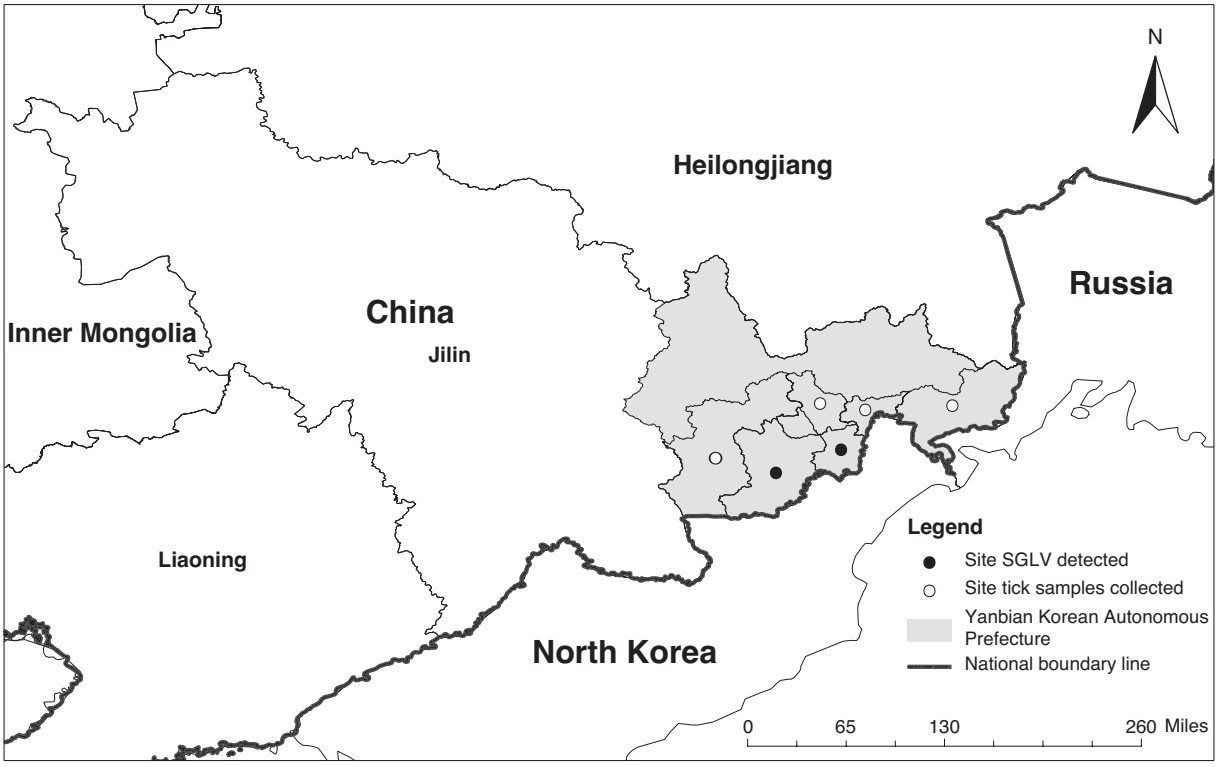

FIGURE 1 | The collection sites of tick samples near the China-North Korea border. The gray-shaded area in the figure represents the Yanbian Korean Autonomous Prefecture. Hollow circles indicate the prefectures where tick samples were collected, while solid circles indicate where SGLV was detected.

TABLE 1 | Information of tick specimen collection in Yanbian, China in 2023.

Location	*Ixodes persulcatus*		*Dermacentor silvatrum*		*Haemaphysalis concinna*			*Haemaphysalis japonica*			*Haemaphysalis longicornis*			Total
	Adult tick		Adult tick		Adult tick		Nymph tick	Adult tick		Nymph tick	Adult tick		Nymph tick	
	♀	♂	♀	♂	♀	♂		♀	♂		♀	♂		
Yanji City	0	3	150	98	1	1	0	3	3	0	0	0	0	259
Tumen City	16	18	24	8	25	28	0	41	18	0	2	3	0	183
Hunchun City	9	14	4	4	0	1	0	0	1	0	3	2	171	209
Helong City	19	18	6	1	170	192	2	16	37	20	0	0	0	481
Longjing City	3	3	3	1	97	82	5	2	1	4	0	0	120	321
Antu County	4	8	103	79	0	3	0	5	4	0	0	0	0	206
Total	51	64	290	191	293	307	7	67	64	24	5	5	291	1659

Note: ♀ Indicates a female, ♂ indicates a male.

negative, and blank controls in each independent test were reliable. Among the positive pools, 11 were collected from Helong City (longitude, 128°94′N; latitude, 42°08′E) and 8 were from Longjing City (longitude, 129°40′N; latitude, 42°45′E; Fig 1). Of the positive pools, 8 and 11 were male and female ticks, respectively. The Ct values for these positive samples ranged from 25.50–29.88 (Table 2).

Virus isolation

All 19 supernatants of newly detected SGLV nucleic acid-positive ticks were inoculated onto BHK-21 and Vero cells. No CPEs were noted through C1–C3 passages of BHK-21 and Vero cells under light microscopy. RT-qPCR was performed to determine if SGLV replication occurred in three passages of BHK-21 and Vero cells after inoculation.

The YB129 and YB150 samples exhibited noticeable growth of SGLV nucleic acid on Vero cells but not on BHK-21 cells, while no growth was observed for samples YB131, YB132, YB133, YB140, YB143, YB149, YB153, YB161, YB162, YB263, YB273, YB278, YB299, YB389, YB402, YB407, and YB431 on BHK-21 or Vero cells.

TABLE 2 | Detection of Songling virus in ticks by RT-qPCR.

Strain/isolated	Collection time	Collection location	Tick species	Sex	Number	RT-qPCR (Ct)
YB129	2023.5.13	Helong City	*Haemaphysalis concinna*	♀	10	27.57
YB131	2023.5.13	Helong City	*Haemaphysalis concinna*	♀	10	26.56
YB132	2023.5.13	Helong City	*Haemaphysalis concinna*	♀	10	28.97
YB133	2023.5.13	Helong City	*Haemaphysalis concinna*	♀	10	25.5
YB140	2023.5.13	Helong City	*Haemaphysalis concinna*	♀	10	27.24
YB143	2023.5.13	Helong City	*Haemaphysalis concinna*	♀	10	29.23
YB149	2023.5.13	Helong City	*Haemaphysalis concinna*	♂	10	26.84
YB150	2023.5.13	Helong City	*Haemaphysalis concinna*	♂	10	27.4
YB153	2023.5.13	Helong City	*Haemaphysalis concinna*	♂	10	27.98
YB161	2023.5.13	Helong City	*Haemaphysalis concinna*	♂	10	26.17
YB162	2023.5.13	Helong City	*Haemaphysalis concinna*	♂	10	27.39
YB263	2023.5.19	Longjing City	*Haemaphysalis concinna*	♀	10	27.1
YB273	2023.5.19	Longjing City	*Haemaphysalis concinna*	♀	10	27.47
YB278	2023.5.19	Longjing City	*Haemaphysalis concinna*	♀	10	29.79
YB299	2023.5.19	Longjing City	*Haemaphysalis concinna*	♀	10	27.67
YB389	2023.5.19	Longjing City	*Haemaphysalis concinna*	♀	10	27.87
YB402	2023.5.19	Longjing City	*Haemaphysalis concinna*	♂	10	29.84
YB407	2023.5.19	Longjing City	*Haemaphysalis concinna*	♂	10	28.72
YB431	2023.5.19	Longjing City	*Haemaphysalis concinna*	♂	10	29.88

Note: ♀ Indicates a female, ♂ indicates a male.

The Ct values for passages C1–C3 were 26.14, 24.36, and 22.43 for sample YB129, respectively, and 25.70, 24.59, and 20.84 for sample YB150, respectively (Fig 2).

SGLV genome determination

The 19 SGLV genome sequences obtained by next-generation sequencing, total read count mapped, consensus length, and average coverage of each strain based on L, M, and S segments are listed in Supplementary Materials S2. The SGLV L segment had mapped read counts from 162–44,046, consensus length from 8682–11,931bp, consensus length (%) from 72.34%–99.42%, and average coverage from 1.82–488.98X. The SGLV M segment had mapped read counts from 112–35,064, consensus length from 3055–4321bp, consensus length (%) from 70.47%–99.68%, and average coverage from 2.58–851.32X. The SGLV S segment had mapped read counts from 121–16009, consensus length from 1823–1878bp, consensus length (%) from 96.92%–99.84%, and average coverage from 8.19–1158.94X (Supplementary Materials S2).

Homology analysis of SGLV nucleotide and amino acid sequences

Analysis of nucleotide and amino acid homology, as well as phylogenetic analysis, was omitted for strains with genome coverage <80%. The analysis of nucleotide homology in strains YB129, YB131, YB132, YB133, YB140, YB143, YB149, YB150, YB153, YB161, YB162, YB263, YB273, YB278, YB299, YB389, and YB431 compared to the reference genome strains HLJ1202, NE-TH1, NE-TH2, and YC585 within the L segment (Fig 3A) revealed a similarity range from 95.5% (HLJ1202) to 98.2% (NE-TH2). The corresponding amino acid homology for these sequences exhibited similar variability, spanning from 95.6% (NE-TH2) to 100.00% (YC585). In like manner, nucleotide homology analysis of the SGLV M gene (Fig 3B) displayed a similarity range from 91.9% (HLJ1202) to 98.7% (YC585), while the amino acid homology within this segment had a similarity range from 97.1% (HLJ1202) to 100.0% (NE-TH2). Nucleotide homology analysis spanned from 98.3% (HLJ1202) to 99.8% (YC585) similarity for the SGLV S gene (Fig 3C) and the corresponding amino acid homology ranged from 98.2% (HLJ1202) to 100.0% (YC585) similarity (Supplementary Materials S3).

Phylogenetic analysis

Divergence existed among sequences originating from various geographic locations and host organisms based on the analysis of genetic sequences represented by the ML tree (Fig 4). Six distinct clades emerged from this analysis. Clades 1 and 2 were characterized by a predominant

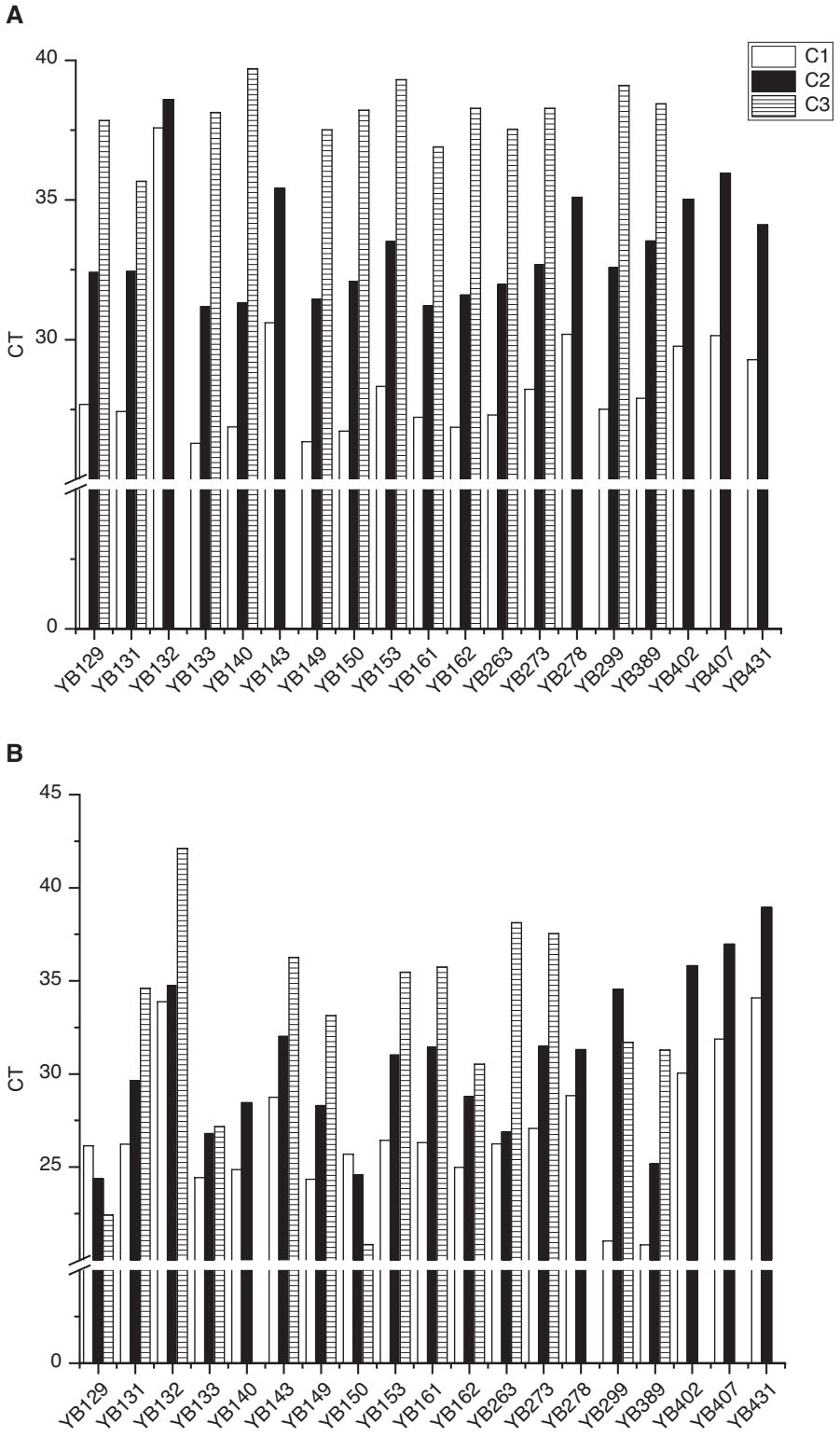

FIGURE 2 | Viral RNA level of Songling virus on BHK-21 and Vero cells C1–C3 passages after positive supernatants were inoculated. A. Ct values of C1–C3 passages in BHK-21 cell supernatants. B. Ct values of C1–C3 passages in Vero cell supernatants. The X-axis represents the isolated ID and the Y-axis represents the Ct value with distinct hatching indicating C1–C3 passages. Because the concentration of nucleic acid was lower than the lower limit of the instrument detection, the C3 passage of YB132, YB143, YB278, YB402, YB407, and YB431 in BHK-21 cells and the C3 passage of YB140, YB278, YB402, YB407, and YB431 in Vero cells had no Ct values.

representation of sequences from Heilongjiang and Inner Mongolia province, where both human and *H. longicornis* serve as hosts. In contrast, clades 4-6 exhibited a prevalence of sequences sourced from Jilin province, with *H. concinna* identified as the primary host organisms. Notably, clade 3 stood out because clade 3 was shown to

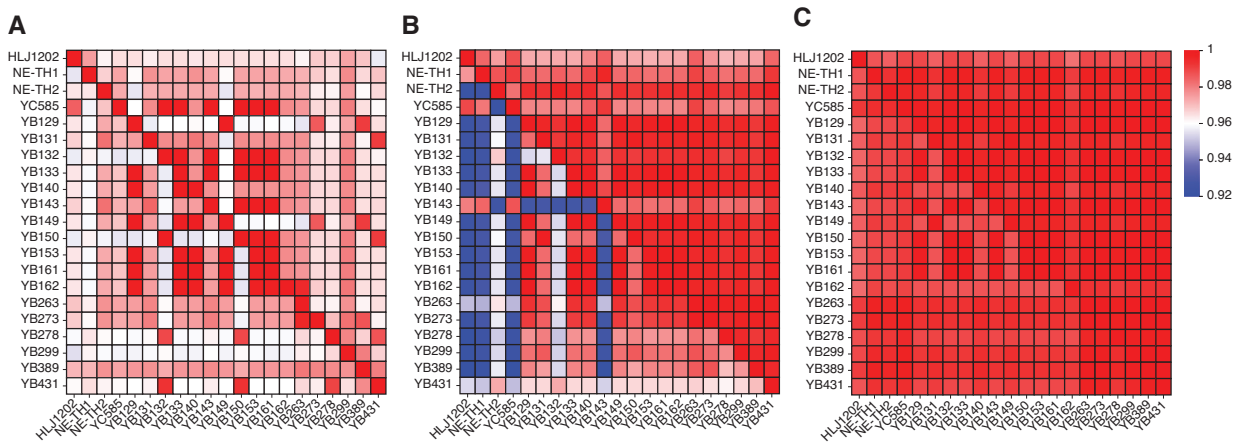

FIGURE 3 | Percentage identity was determined from pairwise comparisons of amino acid sequences (above the diagonal) and nucleotide sequences (below the diagonal) of the SGLVs. Nucleotide and amino acid homology analysis was performed based on Songling virus L segment (A), M segment (B), and S segment (C).

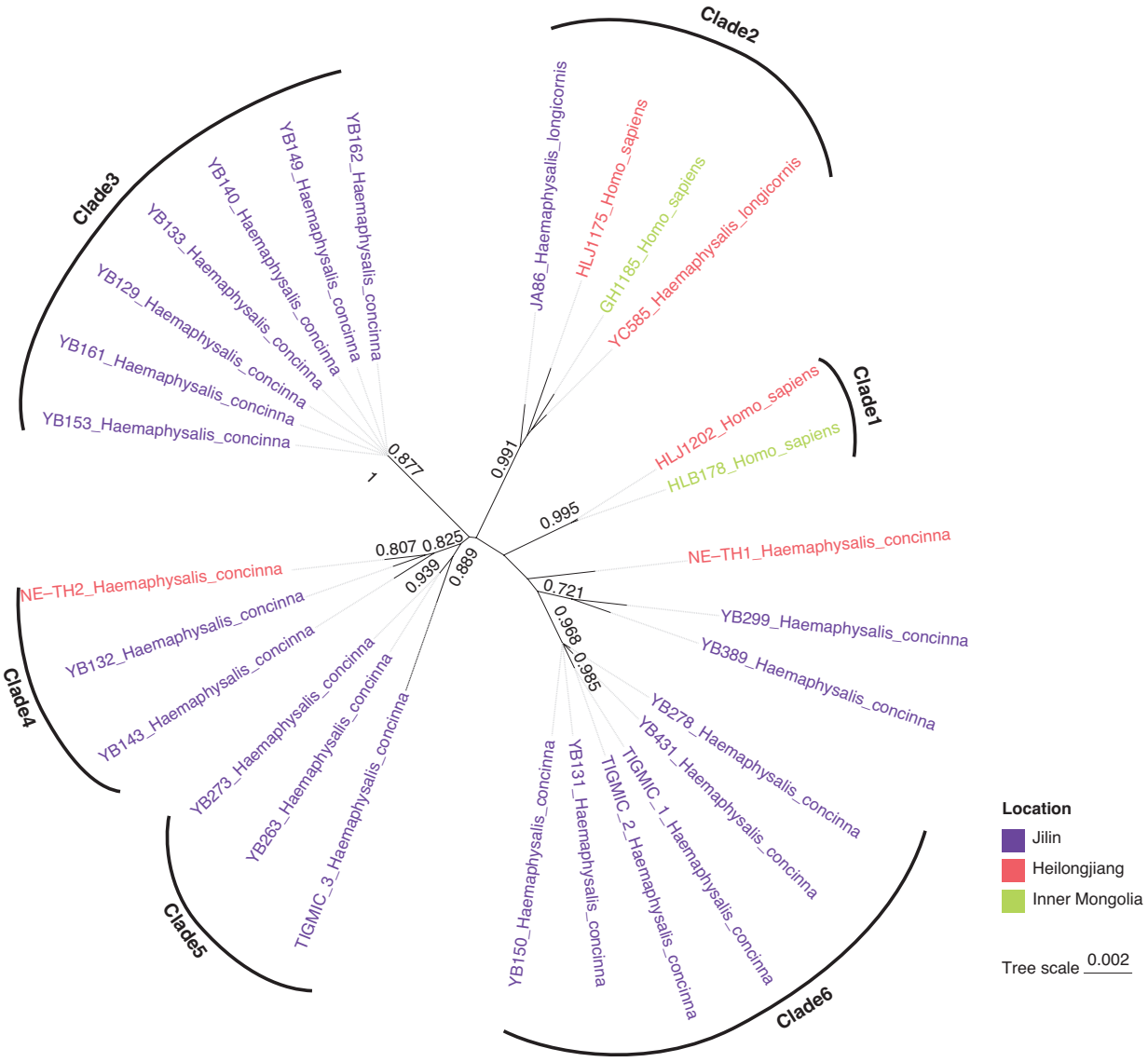

FIGURE 4 | Maximum likelihood tree generated from molecular phylogenetic analysis using nucleic acid sequences of SGLV L segments (Strain HLJ1202, position 6639–7438). Numbers on branches represent the percentage of 1000 bootstrap replicates supporting their existence. Branches with <60% bootstrap support have been condensed.

be predominantly composed of sequences originating specifically from Longjing city within Jilin province.

DISCUSSION

The Tamdy group virus within the *Orthonairovirus* genus is associated with domestic ungulates (camels, cattle, sheep, and goats) and small mammals (rodents) in their ecologic settings [6]. As a newly identified member of the Tamdy group, SGLV has undergone limited scientific scrutiny, resulting in gaps in our understanding of its distribution, reservoir hosts, and biological characteristics. Initially reported in northeast China in 2021 as a novel tick-borne virus, SGLV has been linked to human diseases. Further investigations have revealed the presence of SGLV nucleic acid in *H. concinna* ticks and great gerbils in the northeastern and northwestern region of China [15,17-19]. This study focused on virus testing of ticks collected from six towns in the Yanbian Korean Autonomous Prefecture (Jilin Province, China), which is situated near the China-North Korea border. Our investigation encompassed several dominant tick species in the region. Notably, only *H. concinna* ticks collected in Helong and Longjing towns tested positive for SGLV. The observed natural infection rate of SGLV in *H. concinna* was 3.1% (19 of 607), while other tick species examined in the current study showed no natural infection rate. The ixodid tick, *H. concinna*, has been confirmed to be a competent vector of viruses endemic to wide areas of Europe and Asia [27]. The emergence of *H. concinna* as a significant vector near the China-North Korea border for the transmission of SGLV underscores the specificity of SGLV transmission dynamics within tick populations. However, it is noteworthy that previous study have documented the natural infection rate of SGLV in various tick species, as follows: *Ixodes crenulatus* (5.5%); *I. persulcatus* (0.8%); *H. concinna* (1%); and *H. longicornis* [1.6%] [15]. Further research is warranted to elucidate the specific mechanisms underlying SGLV transmission by *H. concinna* and its potential implications for public health. The genetic analysis, as depicted in the maximum likelihood tree (Fig 3), underscores the intricate dynamics of viral transmission influenced by geography and host organisms. Six distinct clades highlight significant genetic differentiation within the studied population, which emphasizes the importance of tick species and ecologic factors in shaping viral diversity and facilitating regional viral spread. Understanding the mechanisms governing these spatial and host-specific distribution patterns is crucial for deciphering viral transmission dynamics and designing effective control strategies. Overall, the findings of the current study shed light on the intricate interplay between geographic factors, host diversity, and viral genetic diversity, highlighting the need for continued surveillance and research efforts to better understand and mitigate the threat posed by emerging infectious diseases.

Ma et al. reported that CPEs were observed in infected SMMC-7721 (hepatocellular carcinoma) cells but not in Vero (African green monkey kidney) and BHK-21 (baby hamster kidney) cells, and detected viral particles in BHK-21, Vero, and SMMC-7721 cell lines using an indirect immunofluorescence assay [15]. Of the 19 SGLV strains tested in the current study, only strains YB129 and YB150 exhibited viral growth and nucleic acid replication in Vero cell over three passages without CPEs, which is consistent with the findings in a previous study. The remaining 17 strains showed no signs of nucleic acid replication, indicating the extremely low efficiency in culturing the virus in BHK-21 and Vero cells.

Our study had several limitations that should be acknowledged. First, the investigation was restricted to ticks collected from a specific town in the Yanbian Korean Autonomous Prefecture (Jilin Province) near the China-North Korea border. As a result, the findings may not be fully representative of the broader regional or nationwide situation regarding virus prevalence in ticks. Second, the study period was limited to 1 year, spanning from spring-to-summer, potentially overlooking seasonal variations in virus circulation and evolutionary dynamics. Furthermore, the limited availability of genome sequences in GenBank, coupled with incomplete metadata regarding location, host, collection time, and clinical implications, hinders a comprehensive understanding of SGLV natural distribution and transmission dynamics. To address these limitations and gain a more comprehensive insight into the epidemiology and ecology of SGLV, future research efforts should expand the geographic scope of surveillance, collect additional disease-related data, and extend the duration of the study period.

CONCLUSION

A comprehensive investigative study was conducted involving SGLV in ticks collected from the Yanbian Korean Autonomous Prefecture (Jilin Province, China), which significantly contributes to our understanding of the SGLV geographic distribution and vector preference, and emphasizes the importance of tick species and ecologic factors in shaping viral diversity. Further research should address the acknowledged limitations to enhance our knowledge of SGLV molecular epidemiology and ecology in the region.

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CONFLICT OF INTEREST

Huanyu Wang is an Editorial Board Member of Zoonoses (italic). He was not involved in the peer-review or handling of the manuscript. The other authors have no other competing interests to disclose.

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