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Virome specific to tick genus with distinct ecogeographical distribution

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Abstract

Background The emergence of tick-borne pathogens poses a serious threat to both human and animal health. There remains controversy about virome diversity in relation to tick genus and ecogeographical factors.

Results We conducted the meta-transcriptomic sequencing of 155 pools of ticks encompassing 7 species of 3 genera collected from diverse geographical fauna of Ningxia Province, China. Two species of *Dermacentor* genus were distributed in the predominantly grassland areas of the central and eastern regions, with the lowest viral diversity. Two species of *Hyalomma* ticks were found in the predominantly desert areas of the northern regions, with intermediate viral diversity. Three species of *Haemaphysalis* ticks were concentrated in the predominantly forested areas of the southern regions, exhibiting the highest viral diversity. We assembled 348 viral genomes of 63 species in 14 orders, including 26 novel viruses. The identified viruses were clearly specific to tick genus: 22 virus species were exclusive to *Dermacentor*, 12 to *Hyalomma*, and 27 to *Haemaphysalis*.

Conclusions The associations between tick genera and geographical distribution, viral richness, and composition provide new insights into tick-virus interactions, offering clues to identify high-risk regions for different tick-borne viruses.

Keywords Tick, Virome, RNA virus, Ecogeographical distribution

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Introduction

Ticks (superfamily: *Ixodoidea*) are obligate, hematophagous ectoparasites distributed worldwide. They serve as competent vectors for a diverse array of pathogenic viruses, bacteria, and protozoa that infect humans, domesticated livestock, and wildlife [1]. Currently, there are 105 tick-borne viruses (TBVs) capable of successful host jumps, with 59 of these known to be pathogenic to humans. The emergence of tick-borne pathogens poses a serious threat to both human and animal health, garnering global attention [2, 3]. In the past decade, at least 12 new tick-borne viruses associated with human infections have been reported, such as Alongshan Virus, Songling Virus, Yezo Virus, Wetland Virus, Jingmen Virus, and Xue-Cheng Virus identified by the mainland China [4–9]. Consequently, there is a limited understanding of the

prevalence of pathogenic TBVs across most tick species despite the potential for spillover events associated with many tick-associated viruses.

With the rapid advancement of sequencing technology, especially next-generation sequencing (NGS), a plethora of unknown viruses have been unearthed from ticks [10, 11], highlighting the complexity of the tick virome and the inadequacy of our understanding. Although previous studies have investigated the viromes of multiple tick species [12, 13], the variations in viruses among ticks remain largely unexplored. Different tick genus and species inhabit various ecological environments and a wide range of animal hosts, suggesting a diverse array of viruses they may carry [14–16]. While tick species are distributed across various regions and habitats, the extent to which the distribution of tick-borne viruses is influenced by tick species and environmental factors necessitates further investigation. Therefore, conducting multi-species tick virome studies in different habitats can not only elucidate differences in viromes among tick species but also contribute to understanding the biological basis of tick-borne virus transmission [12].

We conducted investigations in Ningxia, northwestern China (located between longitude 104°17′–107°39′ E and latitude 35°14′–39°23′ N), characterized by its complex terrain, diverse ecology, abundant flora and fauna, and rapid growth in animal husbandry [17]. In this study, the ecological factors influencing the distribution of tick species were explored. Subsequently, based on meta-transcriptomic analysis, we conducted comparative virome studies on seven species of free-living ticks collected across the territory of Ningxia. The objectives were to understand the composition of viral communities in these seven tick species, identify novel tick-borne viruses, elucidate the genetic evolution characteristics of viruses, compare the differences in virus carriage between different tick species and genera, and ultimately provide scientific support for the prevention and control of tick and tick-borne virus infections.

Methods

Sample collection

Ticks were collected in Ningxia Province, China, during the spring of 2022 and 2023. Sampling locations were selected based on the ecological environment, including forests, grasslands, farmlands, deserts, and Gobi. Ticks were collected by dragging a 1-square-meter standard flannel flag over the vegetation. The latitude and longitude of each sampling location were recorded. Entomologists identified the species, sex, and developmental stage of each tick. Free-living adult ticks were used for subsequent meta-transcriptome analysis and divided into pools based on tick species, sex, and sampling location.

Comparative analysis of different tick species and ecological factors

ArcGIS software (v10.7) was used to perform spatial autocorrelation analysis (Global Moran's I) and inverse distance weighting (IDW) for ticks, showing the spatial autocorrelation and spatial distribution of different tick species. Based on the IDW-predicted regional divisions, a comparison of ecogeographical factors in each region was conducted, including Digital Elevation Model (DEM), annual precipitation, NDVI, annual average relative humidity, annual sunshine duration, annual evaporation, annual average ground temperature, annual average temperature, and annual average pressure extracted from the Institute of Geographic Sciences and Natural Resources Research (https://www.resdc.cn/). The Kruskal–Wallis test was performed with R software to evaluate the statistical differences in ecological factors among different tick species.

RNA preparation and sequencing

Total RNA was extracted using the AllPrep DNA/RNA Mini-kit (Qiagen) from 155 pools of free-living ticks. Each pool only included the ticks of the same species and same sex collected from the same site. The number of ticks in each pool varies depending on the size and weight of the tick species (Supplementary Table S1). The process was as follows: the ticks were homogenized using an RLT solution in a mortar treated with liquid nitrogen. Homogenate was incubated with Qiagen protease K (Qiagen) at room temperature for 10 min and centrifuged at 15,000×*g* for 30 s. The homogenized lysate was transferred to AllPrep DNA spin column and centrifuged at 8000×*g* for 30 s. The flowing liquid was used for RNA purification according to the manufacturer's instructions.

The purified RNA was quantified using Qubit 4.0 fluorometer, and RNA quality was assessed using an Agilent Bioanalyzer 2200 (Agilent). The ribosomal RNA was removed using RiBo-Zero Gold rRNA removal reagents (Illumina). Paired-end (2×150 bp) sequencing (RNA-seq) was conducted on an Illumina NovaSeq 6000 platform at Novogene Tech.

Analyses of tick virome diversity

Low-quality and short reads in raw sequencing reads were removed using AfterQC (v2.3.3) [18]. The remaining clean reads were compared with *Hy. asiaticum* genome (GCA_013339685 BIME_Hyas_1.3), *D. silvarum* genome (GCA_013339745 BIME_Dsil_1.4), and *Hae. longicornis* genome (GCA_013339765 BIME_HaeL_1.3) using Bowtie2 (v2.3.5.1) [19] to remove reads associated with the ticks. The quality-controlled reads were compared to the NCBI nucleotide sequence database by Kraken2 (v2.1.2)

[20], and the virus abundance of each family was measured as the number of mapped reads per million viral reads in the library (RPM). Alpha diversity was measured at the viral order level using the Richness, Shannon index, and Simpson index using the Rhea alpha diversity script [21] in the R software. We further analyzed the reads from complete viral sequences assembled in this study on order level (Supplementary Fig. S1). Statistical differences in α-diversity among groups of different tick species or genus were assessed by the Kruskal–Wallis test and Wilcoxon rank-sum test. Based on the Bray–Curtis distance index, the similarity matrix was calculated for different groups, and principal coordinates analysis (PCoA) was used to analyze the β-diversity of virome among tick species. All statistical analyses were performed in R.

Viral contig assembly and annotation

The quality-controlled reads were de novo assembled using Trinity (v2.13.2) [22]. All assembled contigs were compared to the NCBI non-redundant protein database (nr) using Diamond BLASTx (v2.0.13) [23] and to the NCBI nucleotide sequence database (nt) using blastn (v2.12.0+) [24] to identify virus-related contigs. The *E*-value was set at 1×10^{-5}. The potential open reading frames (ORFs) of viral contigs were compared with reference sequences downloaded from NCBI using Geneious prime v2023.0.4 (https://www.geneious.com). The conserved RNA-dependent RNA polymerase (RdRp) domains present in the contigs were annotated and compared with the Conserved Domain Database (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi).

To address the potential issue of identical or near-identical viral sequences observed across multiple libraries, we implemented the following steps to rule out possible contamination. First, we estimated the read ratio between the highest-abundance library and other lower-abundance libraries on the same sequencing chip. If the ratio was below the index-hopping threshold for the sequencing platform, the reads from the lower-abundance libraries were considered as potential cross-contamination during library preparation and were excluded from further analysis [25]. To mitigate the risk of index-hopping, we set a threshold of 0.1% as the maximum allowable rate based on previous studies [26, 27].

The viruses detected in this study were classified according to the report of the International Committee on Taxonomy of Viruses (ICTV) (https://talk.ictvonline.org/ictvreports/ictv_online_report/). If the species demarcation criteria remain unclear in the report, a novel virus was proposed if the sequence holds > 90% amino acid similarity of the RdRp with known viruses [28, 29]. Moreover, the new viral genome was confirmed by checking read coverage and continuity using Bowtie2. All putative novel viruses were provisionally named "Ningxia", plus the genus name or the family name, excluding "viridae" characters according to their taxonomy.

Phylogenetic analysis

The amino acid or nucleotide of RdRp in viral sequences was aligned with related reference downloaded from GenBank using MAFFT (v7.490) [30], and ambiguously aligned regions were removed using TrimAl (v1.4) [31]. Maximum likelihood (ML) phylogenetic trees were constructed using the IQ-TREE (v2.2.2.3) algorithm [32] with 1000 bootstrap replicates based on the sequence alignments. Visualize the tree using the ggtree [33], phangorn [34], treeio [35], and ggplot2 [36] packages in R and identify the midpoint as the root of the phylogenetic tree.

Prevalence and abundance of viruses for tick species

The positive rate with a 95% confidence interval (CI) of each tick-associated RNA virus was calculated using R. The association among the viruses identified in this study tick species, and NDVI was visualized using the networkD3 package (https://github.com/christophergandrud/networkD3) in R. We used Bowtie2 to map the clean reads to assembled viral contigs. Read counts for each viral RNA were obtained from the mapping results and normalized within each sample (reads per million/viral reads) for comparison. Finally, the relative abundance of reads for each virus family was represented by the font size of their names.

Results

Three tick genera inhabiting distinct geographical regions

From 2022 to 2023, we conducted a comprehensive survey of ticks in Ningxia, China. We pooled free-living ticks according to collection sites, tick species, and gender, extracted total RNA, and finally constructed 155 sequencing libraries for meta-transcriptome analysis. These libraries originated from seven tick species of three genera, including *Hyalomma scupense* (12), *Hyalomma asiaticum* (18), *Dermacentor nuttalli* (54), *Dermacentor silvarum* (39), *Haemaphysalis longicornis* (6), *Haemaphysalis qinghaiensis* (15), and *Haemaphysalis japonica* (11) (Supplementary Table S1). Ticks used for meta-transcriptome analysis covered various land-use types in Ningxia, including farmland, forest, grassland, desert, and Gobi (Fig. 1a). Each tick genus was found in unique habitats, with *Hyalomma* ticks predominantly distributed in arid areas such as deserts and the Gobi, characterized by low elevation, low Normalized difference vegetation index (NDVI), and limited precipitation. *Dermacentor* ticks were mainly found in farmland and grasslands, while *Haemaphysalis* ticks were exclusively

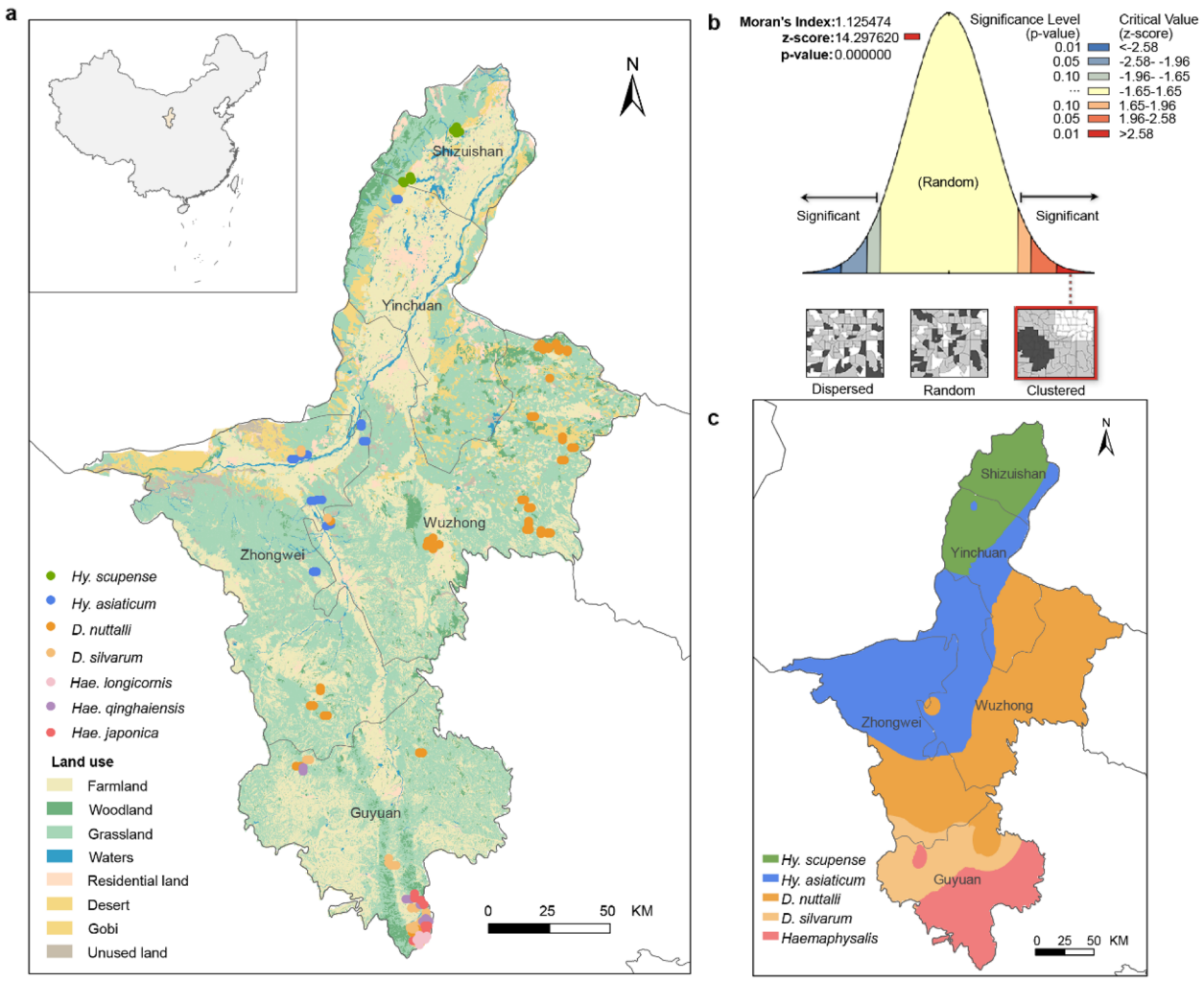


Fig. 1 Geographic distribution of tick collections and spatial correlation in Ningxia, China. **a** Distribution of sample collection. Land use is shown in different colors on the map. The color of the points represents the tick species. **b** Spatial autocorrelation analysis among seven tick species in Ningxia. **c** Spatial clustering of tick species based on inverse distance weighting (IDW) analysis. The colors on the map represent clusters of tick species or genus. *Hyalomma scupense*, *Hyalomma asiaticum*, *Dermacentor nuttalli*, *Dermacentor silvarum*, and *Haemaphysalis* spatial clustering regions are represented by green, blue, orange, yellow, and red, respectively

discovered in mountainous regions with higher elevation, higher NDVI, and greater precipitation (Fig. 1a and Supplementary Table S2).

To understand the spatial distribution patterns of the seven tick species, spatial autocorrelation analysis was conducted. The results showed a Global Moran's Index of 14.3 ($P < 0.001$), indicating a high degree of spatial autocorrelation in the distribution of tick species in Ningxia (Fig. 1b). Further, using Inverse Distance Weight (IDW) to discriminate the distribution range for each tick species, it was found that the northernmost part of Ningxia was mainly inhabited by *Hy. scupense*, while the central and western regions were dominated by *Hy. asiaticum*. The predicted *D. nuttalli* distribution was in the Middle East, accounting for 28.7% of the total area of Ningxia, with 18,949 km^2. The distribution area of *D. silvarum* was primarily in the central and southern parts, with the smallest predicted area of 4698 km^2. The three species of *Haemaphysalis* were found to overlap in the southernmost region (Fig. 1c). Based on the IDW-predicted regional divisions, a comparison of ecogeographical factors in each region was conducted, revealing significant differences in all factors, including elevation, annual precipitation, NDVI, annual average relative humidity, annual sunshine duration, annual evaporation, annual average ground temperature, annual average temperature, and annual average pressure (Kruskal–Wallis test, all $P < 0.001$) (Supplementary Table S3), indicating that

the distribution of each tick species is influenced by a combination of geographical and ecological factors [37]. In general, the distinct geographical clustering of tick genera was observed. *Hyalomma* ticks predominantly occurred in northwestern Ningxia, *Haemaphysalis* in the central and eastern parts, and *Dermacentor* primarily in the southern regions.

Significant difference in virome diversity among tick genera

In this study, a total of 5.6×10^9 paired-end reads of 100–150 bp were generated from 155 sequencing libraries. After removing background gene reads of ticks, the median clean reads for *Hae. qinghaiensis* was the highest at 15.61×10^6, followed by *Hae. japonica* (median of 14.61×10^6), *Hy. scupense* (median of 12.76×10^6), *Hy. asiaticum* (median of 10.85×10^6), *D. silvarum* (median of 6.33×10^6), *Hae. longicornis* (median of 6.33×10^6), and *D. nuttalli* (median of 6.06×10^6), and the proportion of viral reads of the remaining total reads in each library ranged from 0.03 to 11.59%, with a median of 0.09% (Supplementary Table S1). Subsequently, virus reads were classified into 85 virus families after excluding phages and revealed significant differences in the prevalence and abundance of viruses in each family among different tick genera (Fig. 2a). These virus families included 17 families of double-stranded DNA (dsDNA) viruses, 8 families of single-stranded DNA (ssDNA) viruses, 8 families of dsRNA viruses, 31 families of positive-sense single-stranded RNA (ssRNA(+)) viruses, and 21 families of negative-sense single-stranded RNA (ssRNA(−)) viruses. Most virus families in *Dermacentor* ticks showed lower prevalence and abundance compared to those in *Hyalomma* and *Haemaphysalis* ticks. The family *Artiviridae*, which was known as vertebrate-associated virus, exhibited higher abundance and prevalence in both species of *Dermacentor*. Another vertebrate-associated virus family *Phenuiviridae* had a high prevalence and abundance in *D. silvarum* and *Hae. longicornis*. The families *Sedoreoviridae*, *Flaviviridae*, *Orthomyxoviridae*, and *Rhabdoviridae* showed relatively high prevalence in *Hyalomma* and *Haemaphysalis* ticks. The family *Nairoviridae* was specific to the genus *Haemaphysalis* regardless of species. Among the fungal-related virus families, families *Narnaviridae* and *Botourmiaviridae* showed higher prevalence in *Haemaphysalis* compared to other tick genera.

We evaluated the distribution of viruses at the order level to compare the differences in viral composition among samples of seven tick species. Beta diversity analysis conducted through Principal Coordinates Analysis (PCoA) revealed significant differences in virome among different tick species. Notably, two species of the genus *Dermacentor* formed distinct clusters in the virome compared to samples from the other two tick genera (Fig. 2b), which is consistent with the genus-level results (Supplementary Fig. S2a). We assessed α-diversity among samples of the three tick genera using Richness, Shannon index, and Simpson index, and found significant differences (Kruskal–Wallis test, all P values < 0.001) (Fig. 2c, Supplementary Fig. S2b and c). The viral diversity of *Dermacentor* ticks was significantly lower than the other two genera (Wilcoxon rank-sum test, Richness ($P<0.001$), Shannon index ($P<0.001$), and Simpson index ($P<0.001$)), while the Richness of *Hyalomma* ticks carrying viruses was significantly lower than those of *Haemaphysalis* ticks (Wilcoxon rank-sum test, $P=0.0014$). Further comparisons revealed significant differences in viral diversity among tick species (Kruskal–Wallis test, all P values < 0.001) (Fig. 2d, Supplementary Fig. S2d and e). *D. nuttalli* had the lowest richness of viruses (Wilcoxon rank-sum test, all P values < 0.001), and there was no significant difference between the two species of the genus *Hyalomma*, while *Hae. longicornis* had significantly lower viral richness than *Hae. qinghaiensis* ($P=0.0012$) and *Hae. japonica* ($P=0.0021$). The analysis based on the reads from complete viral sequences assembled in this study on order level further validated the virome diversity (Supplementary Fig. S1).

Taxonomy and evolutionary of RNA viruses

We de novo assembled 34,311 virus-related contigs from 155 libraries, obtaining 348 virus genomes containing RdRp. Among these, 284 sequences were complete or nearly complete and have been deposited in GenBank (Supplementary Table S4). According to the classification criteria provided by the International Committee on Taxonomy of Viruses (https://talk.ictvonline.org/ictv-reports/ictv_online_report/), a total of 14 orders and 63 species were identified, comprising 4 dsRNA viruses, 14 ssRNA(−) viruses, and 45 ssRNA(+) viruses (Fig. 3a, Supplementary Table S5). Among the 63 species, 37 belong to known viral genera within 15 families, while 26 ssRNA(+) viruses from 5 families were newly discovered in this study (Supplementary Fig. S3).

For the dsRNA viruses, we constructed the phylogenetic tree of the order *Ghabrivirale* using representative viral sequences from 19 families and 23 genera and found that our sequences were genetically closest to the family *Artiviridae*, which were all reported in different kinds of arthropods. Therefore, we have tentatively named them Ningxia arti-like viruses to indicate their origin from the arthropod tick. According to the latest species classification criteria for this virus order by the ICTV, viruses with more than 70% amino acid identity are considered the same species. Our sequences belong to three distinct virus species. Although they cluster in different

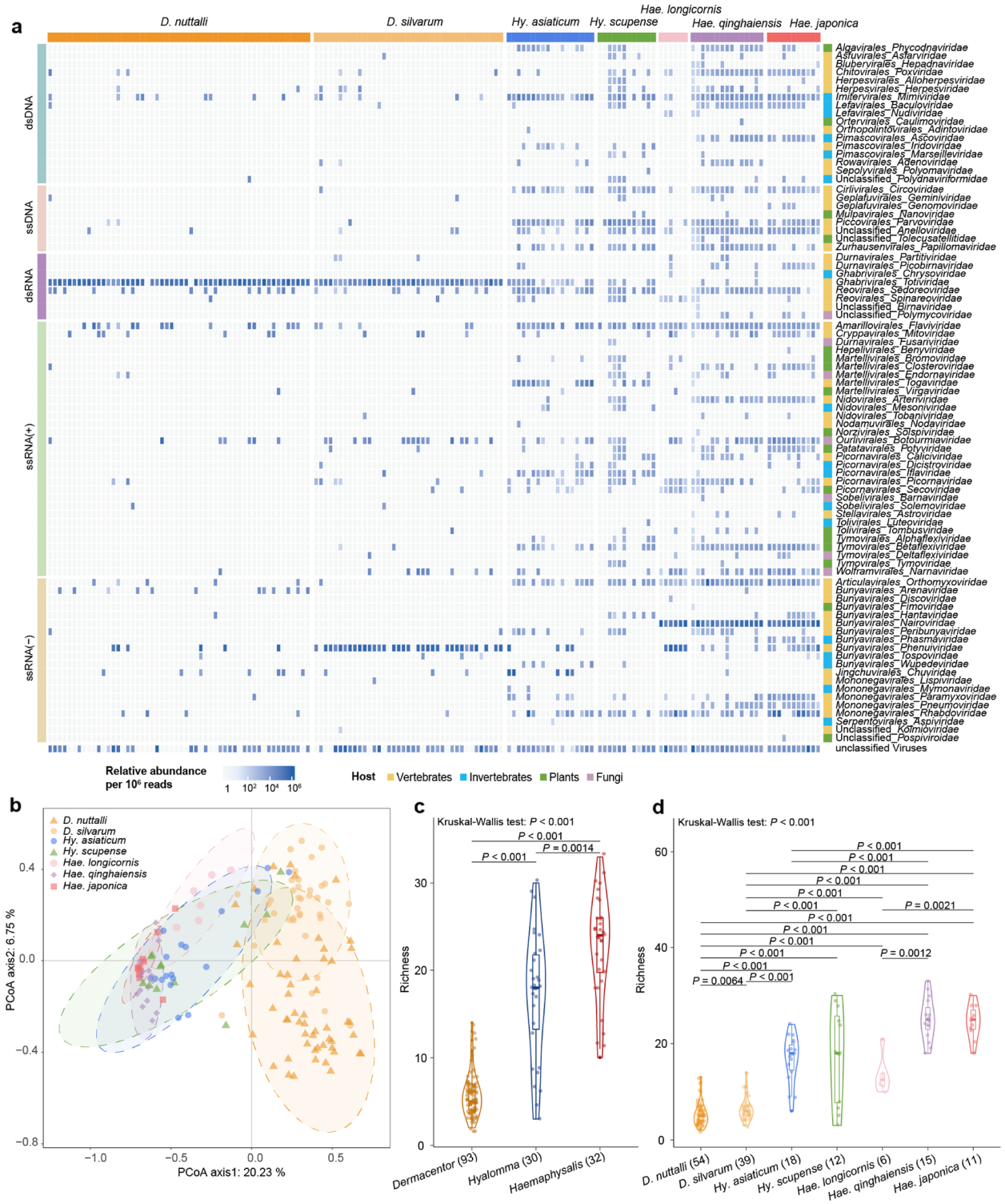


Fig. 2 Virome diversity among tick species. **a** Relative abundance of viral families detected in each library. Each cell in the heat map represents the normalized number of reads belonging to the viral family. **b** Between-group clustering of viromes among seven tick species by Principal Coordinate Analysis of Bray–Curtis dissimilarities, with axes 1 (6.75%) and 2 (20.23%). **c** Comparison of the viral richness among tick genera. **d** Comparison of the viral Richness among tick species. Boxplot elements: center line, median; box limits, upper and lower quartiles; whiskers (error bars), the highest and lowest points within the 1.5 interquartile range of the upper and lower quartiles. The *P* value was calculated using a two-sided Wilcoxon rank-sum test for pairwise comparisons and a Kruskal–Wallis test for multiple comparisons

branches from previously reported *Totiviridae* sp. [12], we chose to adopt the terminology of the *Artiviridae* family and have temporarily named them Ningxia arti-like virus 1–3. Ningxia arti-like virus 1 was found in 58 *D. nuttalli* and 44 *D. silvarum* ticks, previously reported in the genus *Dermacentor*. Ningxia arti-like virus 2 was detected in nine samples of *Hy. asiaticum* ticks, respectively, were previously reported in the genus *Hyalomma*. Ningxia arti-like virus 3 has been reported in two species of the genus *Haemaphysalis* and was found in seven *Hae. japonica* and 12 *Hae. qinghaiensis* ticks in this study. In summary, these three viruses closely related to the family *Artiviridae* exhibited tick-genus-specific distributions. Furthermore, a sample of *D. nuttalli* contained *Partitiviridae* sp., a virus previously reported in three tick genera (Supplementary Fig. S4).

For the 14 ssRNA(−) viruses, tick genus specificity was generally observed. Volzhskoe tick virus, Bole Tick Virus 1, and *Alpharicinrhavirus bole* discovered in the genus *Hyalomma*, clustered together with viruses previously reported in *Hyalomma* ticks (Fig. 3c–e). *Phenuiviridae* sp. has been previously found in the genus *Dermacentor*; in this study, it was detected in two species of *Dermacentor* with no sequence divergence between these tick species (Fig. 3d). Seven ssRNA(−) viruses were detected in three species of *Haemaphysalis* ticks, confirming their tick genus specificity, as previously observed only in *Haemaphysalis* ticks (Fig. 3e–i). *Mivirus boleense* and Taishun Tick Virus were reported predominantly in *Hyalomma* ticks both in this study and previous studies, while *Uukuvirus dabieshanense* was predominantly reported in *Haemaphysalis* ticks, with only a few sequences reported in other tick genera [38–40] (Supplementary Fig. S4).

Among the 45 ssRNA(+) viruses, 19 were known viruses and 26 were novel viruses. Among the 19 known species, Bole hyalomma asiaticum virus 1 and Bole tick virus 4 were detected in the genus *Hyalomma*, consistent with previous reports [28, 41] (Fig. 4a, b). Among them, Bole tick virus 4 was detected in the skin tissue of a patient bitten by a tick [42]. In this study, *Iflaviridae* sp. and Xinjiang tick-associated virus 1 were found in the genus *Dermacentor*, as reported previously [12] (Fig. 4c). *Flaviviridae* sp. was primarily found in the genus *Dermacentor* in both this study and previous studies. This study was the first to report the presence of Hubei sobemo-like virus 15 in *Hae. japonica*, which was previously found in *Hae. longicornis* [43]. Additionally, we identified 7, 2, 1, and 1 known viruses in the families *Botourmiaviridae*, *Mitoviridae*, *Narnaviridae*, and *Virgaviridae* respectively (Fig. 4d–g), most of them showing tick genus-specific.

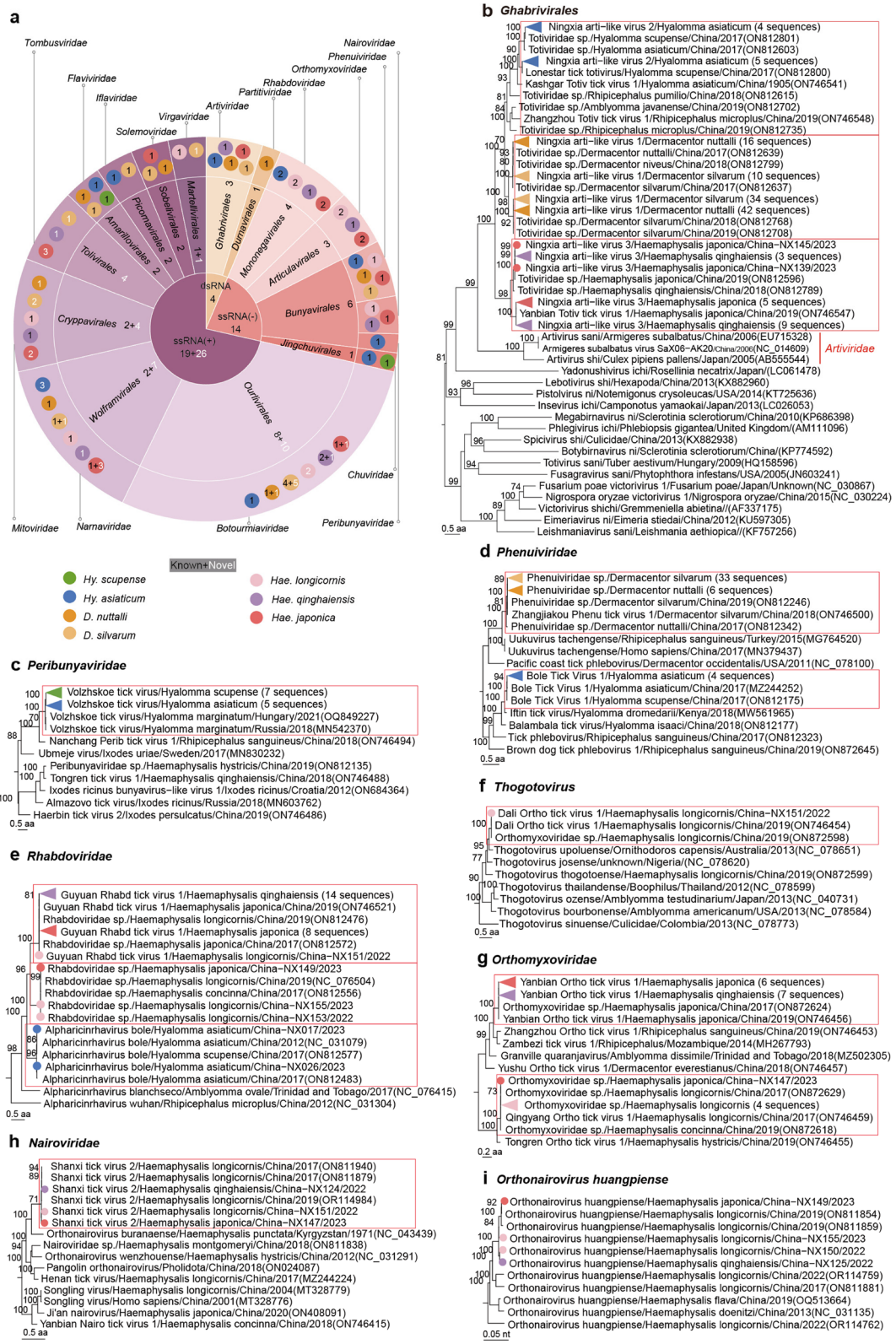
Among the 26 novel ssRNA(+) virus species, 10, 7, 4, and 4 belonged to the families *Botourmiaviridae* (Fig. 4d), *Narnaviridae* (Fig. 4f), *Mitoviridae* (Fig. 4e), *Tombusviridae*, respectively (Fig. 4e, h), with one belonging to the family *Virgaviridae* (Fig. 4g). The amino acid similarity in the RdRp for these viruses was less than 90% compared to their closest virus species (Supplementary Table S6). Most of their viral genome structures were similar to those of viruses within the same genus or family (Supplementary Fig. S3), with the exception of Ningxia tombus-like virus 2, which exhibits structural differences compared to its closest sequences. The most similar virus to the novel Ningxia luteovirus was Cheeloo luteovirus 2 (OR148390) [43], detected from *Hae. longicornis*, with an RdRp amino acid similarity of 86.27%. Except for Ningxia duamitovirus 1, Ningxia tombus-like virus 3, and Ningxia virga-like virus, most of the other viruses clustered together with arthropod-borne or tick-borne viruses within their respective families, forming distinct branches.

Virus distribution exhibits tick genus specificity

We further summarized the relationship between 63 tick-associated RNA viruses and tick genera in relation to NDVI (Fig. 5). The results showed that 61 of these viruses exhibited genus specificity: 22 virus species were specific to the genus *Dermacentor*, 12 to the genus *Hyalomma*, and 27 to the genus *Haemaphysalis*. Among the 22 *Dermacentor*-specific viruses, six were detected in both *D. nuttalli* and *D. silvarum*. Ningxia arti-like virus 1 had the highest prevalence rates in *D. nuttalli* and *D. silvarum*, at 88.89% (95% confidence interval [CI] 80.5–97.27%) and 92.31% (95% CI 83.94–100%), respectively, with relatively high abundance. In contrast, *Phenuiviridae* sp. showed a significantly higher prevalence in *D. silvarum* (84.62%) compared to *D. nuttalli* (11.11%). The most abundant virus families in *Dermacentor* ticks were *Phenuiviridae* and *Artiviridae* (Supplementary Fig. S5). Of the

(See figure on next page.)

Fig. 3 Phylogenetic analysis of dsRNA and ssRNA(−) viruses. **a** Taxonomic distribution of RNA viruses discovered in this study. **b** Phylogeny of viruses in the order *Ghabrivirales* based on RdRp protein. **c** Phylogeny of viruses in the family *Peribunyaviridae* based on RdRp protein. **d** Phylogeny of viruses in the family *Phenuiviridae* based on RdRp protein. **e** Phylogeny of viruses in the family *Rhabdoviridae* based on RdRp protein. **f** Phylogeny of viruses in the genus *Thogotovirus* based on PB1 protein. **g** Phylogeny of viruses in the family *Orthomyxoviridae* based on PB1 protein. **h** Phylogeny of viruses in the family *Nairoviridae* based on RdRp protein. **i** Phylogenetic tree of *Orthonairovirus huangpiense* based on RdRp gene. Viruses of different tick species detected in this study are represented by different colors. The sequences in the red box are the same virus species



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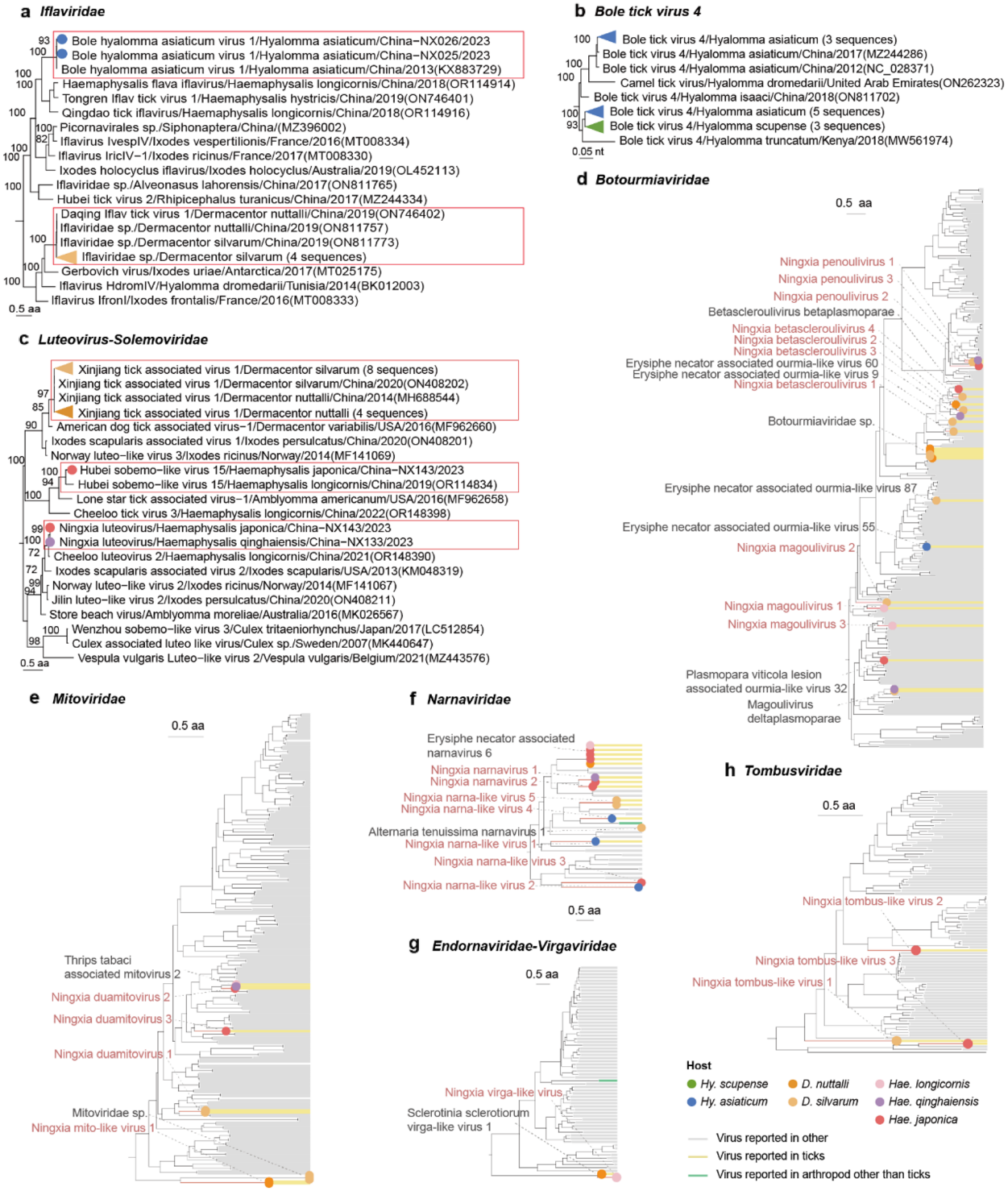

Fig. 4 Phylogenetic analysis of ssRNA(+) viruses. **a** Phylogeny of viruses in the family *Iflaviridae* based on RNA-dependent RNA polymerase (RdRp) protein. **b** Phylogenetic tree of Bole tick virus 4 based on RdRp gene. **c** Phylogeny of viruses between the genus *Luteovirus* and family *Solemoviridae* based on RdRp protein. The colors of tip points represent the tick species. The sequences in the red box are the same virus species. Family-level phylogenetic trees of *Botourmiaviridae* (**d**), *Mitoviridae* (**e**), *Narnaviridae* (**f**), *Endornaviridae-Virgaviridae* (**g**), and *Tombusviridae* (**h**) are based on RdRp proteins. The phylogenetic branches and text of novel viruses in this study are highlighted in red. Tree tips are colored according to host types: yellow, virus reported in ticks; green, virus reported in arthropods other than ticks; gray, virus reported in others

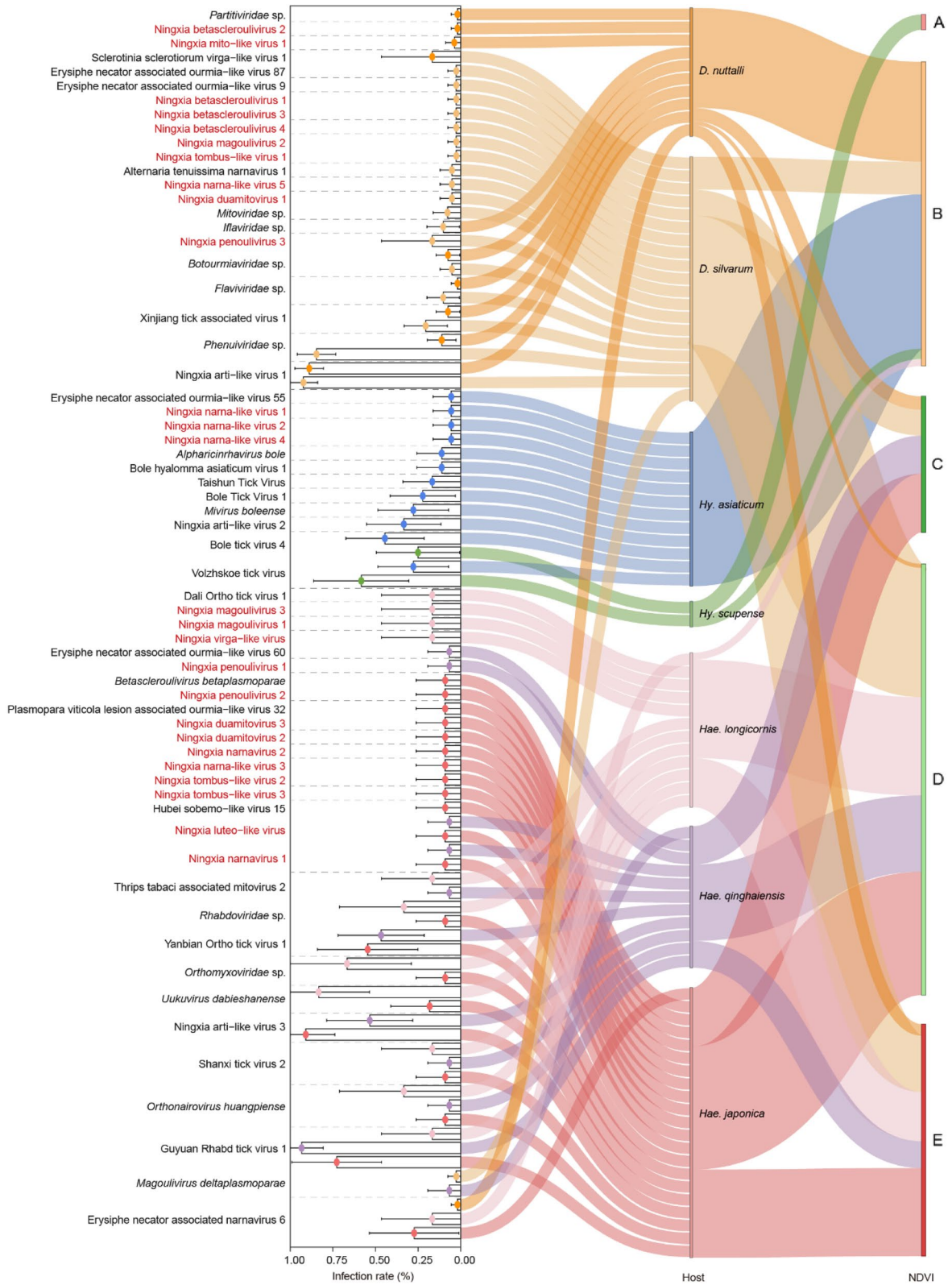

Fig. 5 Association between prevalence of viruses tick species and normalized difference vegetation index. A Sankey diagram to visualize the associations among the prevalence of viruses identified in this study tick species and normalized difference vegetation index (NDVI) where they were located. The classification criteria of NDVI are as follows: level A < 10%; 10% ≤ level B < 30%; 30% ≤ level C < 50%; 50% ≤ level D < 70%; level E ≥ 70%. The novel viruses discovered in this study are highlighted in red. Bar chart elements: whiskers (error bars), 95% confidence interval

remaining 17 viruses, 4 were detected in *D. nuttalli* and 13 in *D. silvarum*. When classifying the sample collection sites by NDVI, it was found that *D. nuttalli* samples were mostly distributed in areas with NDVI level B, while *D. silvarum* samples were mainly found in areas with NDVI levels C and D.

Among the 13 *Hyalomma*-specific viruses, 11 were found only in *Hy. asiaticum*, with prevalence rates below 28%. *Hy. asiaticum* ticks were exclusively collected from areas with an NDVI level of B. Unlike *Hy. asiaticum*, which carried more viruses, *Hy. scupense* only harbored two viruses (Bole tick virus 4 and Volzhskoe tick virus) that were also detected in *Hy. asiaticum*. The infection rate of Volzhskoe tick virus in *Hy. scupense* was significantly higher than in *Hy. asiaticum* ($P=0.003$). The most abundant virus families in *Hyalomma* were *Flaviviridae* and *Iflaviridae* (Supplementary Fig. S5).

Among the 28 *Haemaphysalis*-specific viruses, 12 were detected in multiple tick species. Shanxi tick virus 2, *Orthonairovirus huangpiense*, and Guyuan Rhabd tick virus 1 were found in all three *Haemaphysalis* species, with Guyuan Rhabd tick virus 1 detected in 71.87% of *Haemaphysalis* ticks (23/32), and the highest prevalence in *Hae. qinghaiensis* at 93.33% (95% CI 80.71–105.96%). The highest number of species-specific viruses was found in *Hae. japonica* (10), followed by *Hae. longicornis* (4), and *Hae. qinghaiensis* (2). *Haemaphysalis* ticks were primarily distributed in areas with higher NDVI levels (C–E). Unlike *Dermacentor* and *Hyalomma*, the most abundant virus families in *Haemaphysalis* were *Nairoviridae* and *Rhabdoviridae* (Supplementary Fig. S5).

Additionally, we found two ssRNA(+) viruses that exhibited cross-genera infections. *Magoulivirus deltaplasmoparae* was detected in only one sample each of *D. silvarum* and *Hae. qinghaiensis*, while Erysiphe necator associated narnavirus 6 had a lower infection rate in *Dermacentor* compared to *Haemaphysalis*, indicating that cross-genera infections of tick-associated RNA viruses were rare.

We further compared the virus variation among pools within the same tick genus and found that the virus families carried by each pool of *Dermacentor* ticks were quite similar. Except for viruses shared by both *Dermacentor* species, *D. silvarum* carried more viruses than *D. nuttalli*. In the genus *Hyalomma*, *Hy. asiaticum* carried remarkably more viruses, while two viruses found in *Hy. scupense* were almost present in every pool. The virus composition in *Haemaphysalis* ticks was more complex, with greater variation in the virus species across each pool (Supplementary Fig. S5). When we investigated the possible association between virus populations and regions, we found that the same tick genus in different regions carried the same viruses, while different tick genera within the same region carried different viruses, further supporting the tick genus-specificity of the viruses (Supplementary Fig. S6).

Discussion

This study conducted a survey of tick viromes in the ecologically and geographically diverse Ningxia Province of northwestern China, characterized by diverse host types. We identified 63 RNA viruses, including 26 novel viruses, thereby enriching the diversity database of tick-borne viruses. Different tick genera, including *Dermacentor*, *Haemaphysalis*, and *Hyalomma* harbored distinct tick-specific viruses distributed across their respective ecological habitats. Since all tick samples were free-living individuals, this study basically reflects the true viral carriage characteristics of ticks themselves. These findings suggest that the composition of tick viromes is a result of the combined effects of tick genera, ecology, and geographical factors, not only reflecting co-evolutionary relationships between ticks and viruses but also providing foundational data for assessing risk areas of ticks and tick-borne viral infections.

Virome analysis identified significant differences in viral prevalence and abundance among tick genera, with *Haemaphysalis* displaying the highest viral diversity, followed by *Hyalomma* and *Dermacentor*. This pattern may be attributed to higher vegetation indices in regions inhabited by *Haemaphysalis* (Figs. 1 and 5), which typically support greater biodiversity and diverse host species for ticks to feed on [44]. Most ticks are multi-host organisms, and the high host diversity increases opportunities for *Haemaphysalis* ticks to acquire and transmit multiple viruses. Arboviruses can be transmitted horizontally through blood-feeding and vertically from infected female ticks to their offspring [45–47], potentially leading to the formation of unique viral compositions across different tick genera following long-term adaptation (Fig. 5).

Genetic evolutionary analysis further confirms that most viruses exhibit specificity to tick genera, with only occasional low-probability events of cross-genus transmission. During the growth and development of ticks, viral filtering may occur, selecting viruses beneficial for their evolution. Many RNA viruses undergo long-term co-divergence with their hosts, seldom crossing species barriers [48]. For instance, vertebrate viruses from *Picornaviridae*, *Paramyxoviridae*, and *Hepeviridae* are believed to have co-diverged with their hosts over extended periods [28]. In contrast, other viruses like flaviviruses appear to frequently transmit across species, often mediated by arthropod vectors and characterized by short infection durations [49]. Our samples are

free-living ticks and thus reflect viruses that have completed their transmission cycle, not yet acquiring viruses from subsequent feeding stages. These tick-genus-specific viruses have never been observed in other animals, indicating their long-term evolutionary adaptation. Viruses must overcome evolutionary and ecological barriers to successfully cross species boundaries and establish in new hosts, suggesting that successful cross-species transmission is relatively rare [50, 51].

The discovery of 26 new viruses expands our understanding of tick viromes, suggesting many viruses remain undiscovered within tick populations. These newly identified viruses are ssRNA(+) viruses belonging to the families *Botourmiaviridae*, *Narnaviridae*, *Mitoviridae*, *Tombusviridae*, and *Virgaviridae*. Arthropods are considered primary reservoirs of diverse viruses [38, 52], including those affecting vertebrates and plants. Because ticks often climb onto plants while seeking hosts, they probably acquire and transmit plant-related viruses [53]. Identifying these new viruses is critical for enhancing our understanding of the potential threats posed by tick-borne diseases and devising strategies to mitigate their impact.

In this virome study conducted in a region with limited tick and tick-borne disease surveillance, we identified 26 novel viral species. The pathogenicity of these newly discovered viruses remains uncertain and warrants further investigation. However, based on existing knowledge of known pathogens, certain viruses may pose potential health risks, including those from the genera of *Thogotovirus*, *Orthonairovirus*, and *Orthomyxovirus* [7, 54, 55], all of which are widely distributed across three tick species of the genus *Haemaphysalis* (Fig. 3f–i). We also detected Bole Tick Virus 1 of *Phlebovirus* genus and Bole tick virus 4 of *Flavivirus* genus [42] with pathogenic potential from two tick species of the genus *Hyalomma* (Fig. 3d and Fig. 4b). Given these findings, it is essential to strengthen surveillance of human infections in the region, particularly in areas inhabited by *Haemaphysalis* and *Hyalomma* ticks, in order to enable early detection and targeted prevention of potential emerging tick-borne diseases, thus minimizing the risk of public health threats.

In summary, our study results not only reveal the relationships among ecological diversity, tick genera, and tick-associated RNA viruses but also further delineate the differences in viral carriage among different tick genera, highlighting the pronounced genus-specificity of tick-associated RNA viruses. Ultimately, this provides a scientific basis for the prevention and control of tick-borne viruses. These insights enhance our understanding of the ecological dynamics influencing tick distribution and viral diversity, emphasizing the importance of considering habitat and host interactions in strategies for preventing tick-borne diseases.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40168-025-02061-6.

Additional file 1: Supplementary Table S1. Basic information of each library for sequencing in this study. Supplementary Table S2. Distribution of environmental factors for each tick species. Supplementary Table S3. Environmental factors predicted by IDW interpolation for tick species or genus. Supplementary Table S4. Viral sequences of this study deposited in GenBank. Supplementary Table S5. Information of viruses detected in this study. Supplementary Table S6. Novel viruses identified in this study. Supplementary Figure S1. Alpha diversity of viromes at the order level among tick genera or species by using only complete virus genomes. Supplementary Figure S2. Alpha diversity of viromes at the genus level among tick genera or species. Supplementary Figure S3. Genome structure of novel viruses detected in this study. Supplementary Figure S4. Phylogenetic analysis of tick-genus-specific viruses reported in other tick genera. Supplementary Figure S5. Heatmap plot of relative abundance for virus species detected in this study. Supplementary Figure S6. Combination of tick spatial clustering map based on inverse distance weighted (IDW) analysis and virus species detected in this study.

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Authors' contributions
W.-C.C., and Z.-H.L. designed and supervised the research. D.T., N.W., W.-Y.G., Z.-T.L., W.-J.Z., B.-H.W., Q.-S.W., H.W., Y.-F.W., X.-Y.S., and X.-M.C. collected samples. D.T., N.W., W.-Y.G., Y.-T.L., W.-J.Z., Y.S., N.J., and J.-J.F. prepared materials for sequencing. D.T., R.-Z.Y., Y.-Y.L., and N.W. set up the database. D.T., R.-Z.Y., and Y.-Y.L. performed genome analysis and interpretation. D.T., R.-Z.Y., and Y.-Y.L. prepared figures and tables. W.-C.C., Z.-H.L., D.T., and R.-Z.Y. wrote the paper.

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Data availability
The sequencing data are available from the NCBI Sequence Read Archive (SRA) (SRR30280935–SRR30281088) under BioProiect PRJNA1136696 (temporarily available from the Reviewer link: https://dataview.ncbi.nlm.nih.gov/object/PRJNA1136696?reviewer=evfc3kh2bn0qd3ount82f3qesq), and the assembled virus sequences have been deposited in the GenBank with the accession no. PP944994–PP945476 (Supplementary Table 4).

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Moming A, Bai Y, Wang J, Zhang Y, Tang S, Fan Z, et al. The known and unknown of global tick-borne viruses. Viruses. 2024;16(12):1807.
2. Sonenshine D, Roe R. Biology of Ticks. New York: Oxford Univ; 2013.
3. Babayan SA, Orton RJ, Streicker DG. Predicting reservoir hosts and arthropod vectors from evolutionary signatures in RNA virus genomes. Science. 2018;362(6414):577–80.
4. Wang ZD, Wang B, Wei F, Han SZ, Zhang L, Yang ZT, et al. A new segmented virus associated with human febrile illness in China. N Engl J Med. 2019;380(22):2116–25.
5. Ma J, Lv XL, Zhang X, Han SZ, Wang ZD, Li L, et al. Identification of a new orthonairovirus associated with human febrile illness in China. Nat Med. 2021;27(3):434–9.
6. Zhang MZ, Bian C, Ye RZ, Cui XM, Yao NN, Yang JH, et al. A series of patients infected with the emerging tick-borne Yezo virus in China: an active surveillance and genomic analysis. Lancet Infect Dis. 2024;S1473–3099(24):00616–9.
7. Zhang XA, Ma YD, Zhang YF, Hu ZY, Zhang JT, Han S, et al. A new *Orthonairovirus* associated with human febrile illness. N Engl J Med. 2024;391(9):821–31.
8. Jia N, Liu HB, Ni XB, Bell-Sakyi L, Zheng YC, Song JL, et al. Emergence of human infection with Jingmen tick virus in China: a retrospective study. EBioMedicine. 2019;43:317–24.
9. Zhang MZ, Bian C, Ye RZ, Cui XM, Chu YL, Yao NN, et al. Human infection with a novel tickborne *Orthonairovirus* species in China. N Engl J Med. 2025;392(2):200–2.
10. Brinkmann A, Dinçer E, Polat C, Hekimoğlu O, Hacıoğlu S, Földes K, et al. A metagenomic survey identifies Tamdy orthonairovirus as well as divergent phlebo-, rhabdo-, chu- and flavi-like viruses in Anatolia. Turkey Ticks Tick Borne Dis. 2018;9(5):1173–83.
11. Ma R, Zhao M, Wang H, Hou R, Qin K, Qian Y, et al. Virome of giant panda-infesting ticks reveals novel bunyaviruses and other viruses that are genetically close to those from giant pandas. Microbiol Spectr. 2022;10(4):e0203422.
12. Ni XB, Cui XM, Liu JY, Ye RZ, Wu YQ, Jiang JF, et al. Metavirome of 31 tick species provides a compendium of 1,801 RNA virus genomes. Nat Microbiol. 2023;8(1):162–73.
13. Harvey E, Rose K, Eden JLo, Abeyasuriya T, Shi M, Doggett SL, Holmes EC. Extensive diversity of RNA viruses in Australian ticks. J Virol. 2019;93(3):e01358-18.
14. Gilbert L. The impacts of climate change on ticks and tick-borne disease risk. Annu Rev Entomol. 2021;66:373–88.
15. Hart TM, Dupuis AP 2nd, Tufts DM, Blom AM, Starkey SR, Rego ROM, et al. Host tropism determination by convergent evolution of immunological evasion in the Lyme disease system. PLoS Pathog. 2021;17(7):e1009801.
16. Douam F, Gaska JM, Winer BY, Ding Q, von Schaewen M, Ploss A. Genetic dissection of the host tropism of human-tropic pathogens. Annu Rev Genet. 2015;49:21–45.
17. Li Z, Sun X, Huang Z, Zhang X, Wang Z, Li S, et al. Changes in nutrient balance, environmental effects, and green development after returning farmland to forests: A case study in Ningxia. China Sci Total Environ. 2020;735:139370.
18. Chen S, Huang T, Zhou Y, Han Y, Xu M, Gu J. AfterQC: automatic filtering, trimming, error removing and quality control for fastq data. BMC Bioinformatics. 2017;18(Suppl 3):80.
19. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat Methods. 2012;9(4):357–9.
20. Wood DE, Lu J, Langmead B. Improved metagenomic analysis with Kraken 2. Genome Biol. 2019;20(1):257.
21. Lagkouvardos I, Fischer S, Kumar N, Clavel T. Rhea: a transparent and modular R pipeline for microbial profiling based on 16S rRNA gene amplicons. PeerJ. 2017;5:e2836.
22. Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, et al. Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat Biotechnol. 2011;29(7):644–52.
23. Buchfink B, Reuter K, Drost HG. Sensitive protein alignments at tree-of-life scale using DIAMOND. Nat Methods. 2021;18(4):366–8.
24. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, et al. BLAST+: architecture and applications. BMC Bioinformatics. 2009;10:421.
25. Cobbin JC, Charon J, Harvey E, Holmes EC, Mahar JE. Current challenges to virus discovery by meta-transcriptomics. Curr Opin Virol. 2021;51:48–55.
26. Li Q, Zhao X, Zhang W, Wang L, Wang J, Xu D, et al. Reliable multiplex sequencing with rare index mis-assignment on DNB-based NGS platform. BMC Genomics. 2019;20(1):215.
27. Effects of Index Misassignment on Multiplexing and Downstream Analysis (Illumina, 2017); https://www.illumina.com/content/dam/illumina-marketing/documents/products/whitepapers/index-hopping-white-paper-770-2017-004.pdf?linkId=36607862
28. Shi M, Lin X-D, Tian J-H, Chen L-J, Chen X, Li C-X, et al. Redefining the invertebrate RNA virosphere. Nature. 2016;540(7634):539–43.
29. Shi W, Shi M, Que T-C, Cui X-M, Ye R-Z, Xia L-Y, et al. Trafficked Malayan pangolins contain viral pathogens of humans. Nat Microbiol. 2022;7(8):1259–69.
30. Rozewicki J, Li S, Amada KM, Standley DM, Katoh K. MAFFT-DASH: integrated protein sequence and structural alignment. Nucleic Acids Res. 2019;47(W1):W5-w10.
31. Capella-Gutiérrez S, Silla-Martínez JM, Gabaldón T. trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. Bioinformatics. 2009;25(15):1972–3.
32. Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol. 2015;32(1):268–74.
33. Yu G, Lam TT, Zhu H, Guan Y. Two methods for mapping and visualizing associated data on phylogeny using ggtree. Mol Biol Evol. 2018;35(12):3041–3.
34. Schliep KP. Phangorn: phylogenetic analysis in R. Bioinformatics. 2011;27(4):592–3.
35. Wang LG, Lam TT, Xu S, Dai Z, Zhou L, Feng T, et al. Treeio: An R Package for phylogenetic tree input and output with richly annotated and associated data. Mol Biol Evol. 2020;37(2):599–603.
36. Wickham H. ggplot2: elegant graphics for data analysis. Springer; 2016.
37. Doi K, Kato T, Tabata I, Hayama SI. Mapping the potential distribution of ticks in the western kanto region, Japan: predictions based on land-use, climate, and wildlife. Insects. 2021;12(12).
38. Li CX, Shi M, Tian JH, Lin XD, Kang YJ, Chen LJ, et al. Unprecedented genomic diversity of RNA viruses in arthropods reveals the ancestry of negative-sense RNA viruses. Elife. 2015;4.
39. Xu L, Guo M, Hu B, Zhou H, Yang W, Hui L, et al. Tick virome diversity in Hubei Province, China, and the influence of host ecology. Virus Evol. 2021;7(2).
40. He L, Xu W, Zeng C, Li Y, Lin R, Xie X, et al. Prevalence and genetic diversity of Dabieshan tick virus in Shandong Province. China J Infect. 2022;85(1):90–122.
41. Shi M, Lin XD, Vasilakis N, Tian JH, Li CX, Chen LJ, et al. Divergent viruses discovered in arthropods and vertebrates revise the evolutionary history of the flaviviridae and related viruses. J Virol. 2016;90(2):659–69.
42. Zhang J, Zheng YC, Chu YL, Cui XM, Wei R, Bian C, et al. Skin infectome of patients with a tick bite history. Front Cell Infect Microbiol. 2023;13:1113992.
43. Ye RZ, Li YY, Xu DL, Wang BH, Wang XY, Zhang MZ, et al. Virome diversity shaped by genetic evolution and ecological landscape of *Haemaphysalis longicornis*. Microbiome. 2024;12(1):35.
44. Moura MR, Oliveira GA, Paglia AP, Pires MM, Santos BA. Climate change should drive mammal defaunation in tropical dry forests. Glob Chang Biol. 2023;29(24):6931–44.
45. Woelk CH, Holmes EC. Reduced positive selection in vector-borne RNA viruses. Mol Biol Evol. 2002;19(12):2333–6.
46. Shi C, Zhao L, Atoni E, Zeng W, Hu X, Matthijnssens J, et al. Stability of the virome in lab- and field-collected *Aedes albopictus* mosquitoes across different developmental stages and possible core viruses in the publicly available virome data of Aedes Mosquitoes. mSystems. 2020;5(5).
47. Coffey LL, Vasilakis N, Brault AC, Powers AM, Tripet F, Weaver SC. Arbovirus evolution in vivo is constrained by host alternation. Proc Natl Acad Sci U S A. 2008;105(19):6970–5.
48. Jackson AP, Charleston MA. A cophylogenetic perspective of RNA-virus evolution. Mol Biol Evol. 2004;21(1):45–57.
49. Kitchen A, Shackelton LA, Holmes EC. Family level phylogenies reveal modes of macroevolution in RNA viruses. Proc Natl Acad Sci U S A. 2011;108(1):238–43.

50. Geoghegan JL, Senior AM, Holmes EC. Pathogen population bottlenecks and adaptive landscapes: overcoming the barriers to disease emergence. Proc Biol Sci. 2016;283(1837).
51. Drosten C, Geoghegan JL, Duchêne S, Holmes EC. Comparative analysis estimates the relative frequencies of co-divergence and cross-species transmission within viral families. PLOS Pathog. 2017;13(2).
52. Neri U, Wolf YI, Roux S, Camargo AP, Lee B, Kazlauskas D, et al. Expansion of the global RNA virome reveals diverse clades of bacteriophages. Cell. 2022;185(21):4023–37 e18.
53. Jeger MJ. The epidemiology of plant virus disease: towards a new synthesis. Plants (Basel). 2020;9(12).
54. Lledó L, Giménez-Pardo C, Gegúndez MI. Epidemiological study of Thogoto and Dhori virus infection in people bitten by ticks, and in sheep, in an area of Northern Spain. Int J Environ Res Public Health. 2020;17(7):2254.
55. Xue L, Chang T, Li Z, Wang C, Zhao H, Li M, et al. Cryo-EM structures of Thogoto virus polymerase reveal unique RNA transcription and replication mechanisms among orthomyxoviruses. Nat Commun. 2024;15(1):4620.

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