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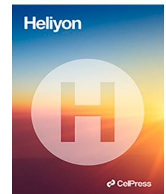

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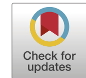

Diversity of viral communities in faecal samples of farmed red foxes

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ABSTRACT

Emerging and existing viruses from various human and animal samples have been studied and analyzed using viral metagenomics, which has proven to be an effective technique. Foxes, as a kind of significant economic animal, are widely raised in China. Viruses carried by foxes may potentially infect humans or other animals. There are currently very few studies of faecal virome in farmed foxes. Using viral metagenomics, we evaluated the faecal virome of twenty-four foxes collected from the same farm in Jilin Province, China. Some sequences more closely related to the families *Parvoviridae*, *Picornaviridae*, *Smacoviridae*, *Anelloviridae*, and *Herpesviridae* were detected in the faecal sample. The main animal viruses that infect farmed red foxes were parvovirus and picornavirus. Five smacovirus strains were found and provided evidence for genetic diversity in the genus *Smacoviridae*. In addition, some viruses infecting avian species or rats were detected in this study. The study helped us better understand faecal virome in farmed red foxes and assisted in the surveillance and prevention of viral diseases in these animals.

1. Introduction

Red fox (scientific name: *Vulpes*) is a member of the Canidae family that shows high ecological adaptability [1], and its fur has high economic value. Foxes have been raised as important economic animals in recent years. However, intensive farming poses an inescapable risk of infectious disease transmission. Viral diseases are an important factor affecting the fox breeding industry. Many viruses can cause diseases in foxes, such as parvovirus, astrovirus, canine distemper virus (CDV), canine adenovirus, and porcine pseudorabies virus (PRV) [2-5]. Besides them, some viruses found in foxes have been clustered into the zoonotic group, such as influenza virus, rabies virus (RV), neurotropic arbovirus (TBEV) and Getah virus [6-8]. Due to frequent contact between foxes and farmers, these zoonotic viruses could easily spread to humans and cause disease [9-11]. There has been no systematic study of viruses that infect farmed foxes.

With the rapid development of next-generation sequencing technologies (NGS), metagenomics has become a powerful tool for analyzing biodiversity, population structure, evolutionary relationships, and potential biological implications [12]. Viral metagenomics has been used to investigate the virus in wild foxes [13-15], but limited virome data is available for farmed foxes. Following

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the domestication of some wild fox species, the likelihood of spillover effects in contact with humans and domestic animals will increase, so ongoing research into the viral composition of valuable economic animals is warranted. In the present study, viral metagenomics was used to study the faecal virome of farmed red foxes on a farm in Jilin Province, China. Our results showed different viral compositions, including the families of *Anelloviridae, Parvoviridae, Smacoviridae, Circoviridae, Genomoviridae, Herpesviridae, Picornaviridae, Picobirnaviridae, Flaviviridae, Iridoviridae, and Poxviridae*. The study will advance our understanding of the faecal virome of farmed red foxes and contribute to further surveillance and prevention of viral disease.

2. Materials and methods

2.1. Sample collection and preparation

The purpose of this research was to investigate the virome of farmed foxes. From a single red fox farm in China's Jilin province, 24 faecal samples from adult foxes were randomly taken. Using disposable sterile materials, specialists collected all samples and transported them on dry ice. Resuspended samples were vigorously vortexed in a 2 ml Dulbecco's Phosphate-buffered Saline (DPBS) for 10 min before freeze-thawing three times. After centrifugation (10 min at 15,000×*g*, 4 °C), supernatants from each sample were then collected in a new 1.5 ml centrifuge tube and stored at −80 °C.

2.2. Viral nucleic acid extraction

The 24 supernatants were randomly and evenly grouped to form two sample pools.

Eukaryotic and some bacterial cell-sized particles were removed from each sample pool using a 0.45 μm filter (Millipore) (5 min at 8,000×*g*, 4 °C), and DNase (Turbo DNase from Thermo Fisher; Baseline-ZERO from Epicentre; benzonase from Novagen) and RNase (Fermentas) kits were used to digest unprotected nucleic acid in filtrates enriched with viral particles [16,17]. QIAamp viral RNA Minikit (Qiagen) was used to extract the remaining total viral RNA and DNA according to the manufacturer's protocol.

2.3. Library construction and bioinformatics analysis

Reverse transcription reactions with enriched viral RNA from the respective pools were performed using reverse transcriptase (Super-Script IV, Invitrogen) and Random Hexamer Primers, and then double-strand DNA synthesis using Klenow fragment polymerase (New England Biolabs). Using the Nextera XT DNA Sample Preparation Kit (Illumina), two libraries were constructed and then sequenced on the Miseq Illumina platform with 250 bases paired ends.

The 250 bp paired-end reads were debarcoded using Illumina's vendor software. Data was processed using an in-house analysis pipeline running on a 32-node Linux cluster, and reads were treated as duplicates if bases 5 to 55 were identical, with only one random copy of duplicates kept. The Phred quality score of 10 as the threshold was used to trim tails with low sequencing quality, and adaptors were clipped using VecScreen's default parameters, which are NCBI BLASTn with specific adjustment parameters. Using Bowtie2 (v2.2.4), bacterial nucleotide sequences were mapped from the BLAST NT database and subtracted, and cleaned reads were de-novo assembled by SOAPdenovo2 using Kmer size 63 with default settings [18]. With an E-value cutoff of 10^{-5}, the assembled contigs, along with singlets, were aligned to an in-house viral proteome database using BLASTx (v.2.2.7). The virus BLASTx database was compiled using NCBI virus reference proteome (*https://ftp.ncbi.nih.gov/refseq/release/viral/*) (accessed on December 20, 2021) to which viral protein sequences were added from NCBI nr FASTA file (based on annotation taxonomy in the Virus Kingdom) [17]. Non-viral protein sequences from the NCBI nr FASTA file (based on annotation taxonomy excluding Virus Kingdom) were used to remove false positive viral hits with an E-value cutoff of 10^{-5}. Viral contigs without significant BLASTx similarity were searched for viral protein families in the vFam database [19] using HMMER3 to detect distant viral protein similarities [20-22]. Additionally, MEGAN (v 6.22.2), a metagenomic annotation tool, was used to assign each sequence present in metagenomic data to different taxa using the NCBI taxonomic database.

2.4. Phylogenetic analysis

The amino acid (aa) sequences of reference strains belonging to different virus groups were downloaded from the NCBI GenBank database to infer phylogenetic relationships. Sequence alignment was performed using MUSCLE with the default settings in MEGA software (version 10.1.8) [23]. The alignment of sites with gaps greater than 50% was temporarily removed, and MrBayes (version 3.2.7) was used to construct Bayesian inference trees [24]. During MrBayes analysis, "lset nst = 6 rates = invgamma" were used for phylogenetic analysis based on nucleotide sequences, which set the evolutionary model to the GTR substitution model with gamma-distributed rate variation across sites and a proportion of invariable sites ("GTR + I+Γ"), while we set "prset aamodelpr = mixed" for the phylogenetic analysis using amino acid sequences, which allows the program to utilize the 10 built-in amino acid models, and the number of generations was increased to one million until the standard deviation of split frequencies is below 0.01, sampled every 50 generations, and the first 25% of Markov chain Monte Carlo (MCMC) samples were discarded as burn-in. All Bayesian inference trees were further validated using the maximum likelihood trees constructed by the MEGA software (version 10.1.8).

Table 1
Distribution of metagenomic reads and contigs of viral sequence from farmed foxes faecal.

	Library 11					Library 12				
Potential virus family	Number of sequence reads	Number of contig (s)	Mean contig length (nt)	Minimum contig length (nt)	Maximum contig length (nt)	Number of sequence reads	Number of contig (s)	Mean contig length (nt)	Minimum contig length (nt)	Maximum contig length (nt)
Parvoviridae	387	33	632	178	3177	714	59	515.7	172	2456
Picornaviridae	654	63	572.9	249	2119	273	46	465.2	196	1339
Smacoviridae	124	10	644.2	256	1356	230	19	502.2	259	1864
Herpesviridae	51	11	353.6	251	457	182	27	375.5	230	648
Anelloviridae	100	10	770.2	314	1607	137	8	820.1	254	3521
Genomoviridae	27	4	458	250	713	74	10	548.6	254	1149
Iridoviridae	17	2	255	251	259	38	4	476.5	337	601
Picobirnaviridae	35	5	391.4	278	471	33	3	421	331	478
Poxviridae	11	1				26	7	328.3	300	362
Flaviviridae						16	2	419	372	466
Circoviridae	65	6	461.2	249	786					

2.5. Nucleotide sequence accession number

The resulting virus genome and fragments were deposited in GenBank with accession numbers: ON872775 – ON872784. The raw sequence reads were stored in the Sequence Read Archive (SRA) of the GenBank database under accession numbers: SRR19759933 and SRR1759659 (Table. S1).

3. Result

3.1. Overview of viral metagenomic

A total of 236,783 raw reads from the two pools were obtained and classified using Megan.6. With the Evalue cutoff of $<10^{-5}$, a total of 181,221 sequences (76.5%) demonstrated significant sequence identities for known viruses, the majority of which could be attributed to bacteriophage sequences (98.2%) with a considerable fraction of the family *Microviridae*, *Siphoviridae*, and *Podoviridae* (Table.S2). Sequences from the following families of viruses that could infect eukaryotes were detected: *Parvoviridae*, *Picornaviridae*, *Smacoviridae*, *Herpesviridae*, *Anelloviridae*, *Genomoviridae*, *Iridoviridae*, *Picobirnaviridae*, *Poxviridae*, *Flaviviridae*, and *Circoviridae*. Members of these families (including *Poxviridae*, *Herpesviridae*, *Iridoviridae*, *Genomoviridae,* and *Flaviviridae*) were disregarded due to insufficient reads. *De novo* assembly within the families *Parvoviridae* and *Picornaviridae* produced the 92 and 109 viral contigs (Table .1), respectively, which account for the major part of viruses observed infecting eukaryotes in this study.

3.2. Viruses belonging to the anelloviridae

Anellovirus sequences were discovered in both libraries (100 reads in library # 11, 137 reads in library # 12), where a nearly complete genome and a complete genome of anelloviruses were obtained and respectively named ANE11 (mean coverage: 7.5) and ANEL12 (mean coverage: 12.3). The nearly or complete genome of ANEL11 and ANEL12 was 1,889 nt and 1,964 nt, respectively. The genome organization of both viruses presented the typical feature of gyroviruses, with three partially overlapping ORFs in the same direction. For ANEL11, the three ORFs encoded three proteins: VP1 (507–1889 nt, 461 aa), VP2 (4–696 nt, 232 aa) and VP3 (131–505 nt, 125 aa), while the length of VP1, VP2, and VP3 of ANEL12 were 460 aa, 233 aa, and 125 aa, respectively (Fig. 1A). The VP1, VP2 and VP3 of these two viruses shared the highest nt sequence identity of 99.6%, 99.0% and 98.7%, respectively. Chicken anemia virus (CAV, GenBank no. M55918) was the first isolated single-stranded circular DNA virus of the genus *Gyrovirus* in which the genome has been thoroughly analyzed [25]. At the complete genome level, ANEL11 shared 51% nt sequence identity with CAV strain Cux-1 and ANEL12 shared 50.3% nt identity.

ANEL11 and ANEL12 were examined for genetic relationships with other anelloviruses using the VP1 aa sequence phylogenetic tree. The results showed that 10 species of the genus *Gyrovirus* were clearly delineated in the VP1 phylogenetic tree. ANEL11 and ANEL12 clustered with one avian gyrovirus 2 strain NX1506-1 (GenBank no. KX708508) and formed a clade (Fig. 1B). Sequence

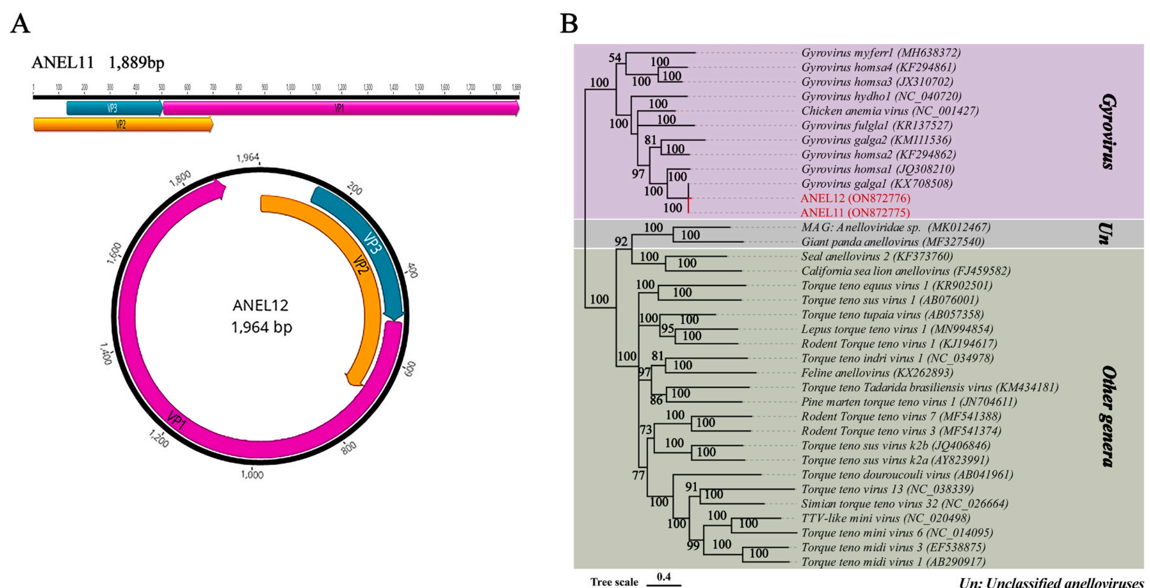

Fig. 1. The genomic organization and phylogenetic analysis of anelloviruses detected in domestic red foxes. (A) The genomic organization of ANEL11 and ANEL12. The viral encoding proteins of ANEL11 and ANEL12 were separately marked with different colors. The arrow represented the direction of gene coding. (B) The phylogenetic analysis is based on the amino acid sequences of VP1 of ANEL11, ANEL12, and different reference strains. ANEL11 and ANEL12, identified in this study, were highlighted using the red font. Un, Unclassified anelloviruses.

analysis showed that ORF1 of ANEL11 and ANEL12 shared the highest nucleotide sequence identity (98.2% of ANEL11, 95.2% of ANEL12) with avian gyrovirus 2 isolate NX1506-1 (GenBank no. KX708508). According to the species classification criteria in the family *Anelloviridae*, the virus belongs to that specific species if it shares >69% pairwise identification of the complete ORF1 coding region nucleotide sequences with that of any member assigned to a currently classified species [26], so ANEL11 and ANEL12 are classified as the species *Gyrovirus galga1*.

3.3. Viruses belonging to the parvoviridae

Sequence reads corresponding to the family *Parvoviridae* were identified from all libraries (387 reads in library #11, 714 reads in library #12). Two nearly complete genomes of parvovirus were obtained from the two libraries and named DJF11 (mean coverage: 6.8) (library #11) and DJF12 (mean coverage: 10.3) (library #12), respectively. The nearly complete genomes of DJF11 and DJF12

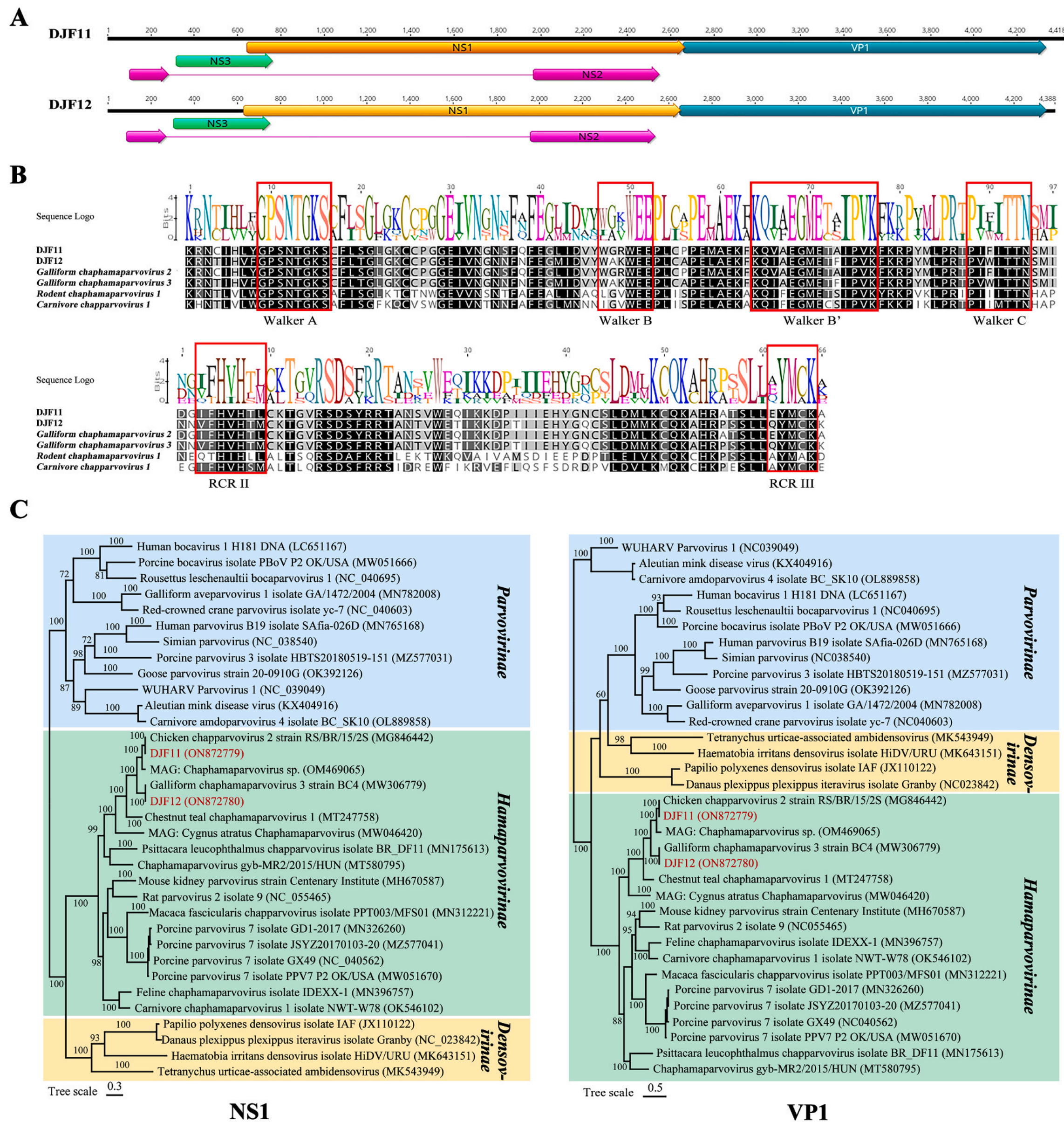

Fig. 2. The genomic organization and phylogenetic analysis of the parvoviruses detected in domestic red foxes. (A) The genomic organization of the DJF11 and DJF12 genes was identified in this study. The viral encoding proteins of DJF11 and DJF12 were marked with different colors. (B) The comparison of aa sequences of helicase motifs in members of the genus *Chaphamaparvivirus*. The conserved rolling circle replication (RCRs) and Walker motifs of SF3 helicases were shown in the red square. (C) The phylogenetic analysis of parvoviruses identified in this study is based on the amino acid sequences of NS1 and VP1. The DJF11 and DJF12 identified in this study were marked with red font.

were 4,418 nt, and 4,380 nt in length, respectively, and both contain two complete ORFs (ORF1 and ORF2). Both of DJF11 and DJF12 ORF1 were 2,022 nt in length, but their ORF2 were 1,674 and 1,701 nt, respectively. The ORF1 and ORF2 encoded a non-structural protein (NS1) and a structural protein (VP1), respectively. Besides the two ORFs, two additional ORFs overlapping the NS1 ORF were detected and encoded two nonstructural proteins (Fig. 2A). The conserved Walker and rolling circle replication (RCR) motifs typical of parvoviral helicases were present in the NS1 protein, which were similar with the reference strains (GenBank no. MG846442, MW306779, NC_040,843, and MH893826) (Fig. 2B).

Phylogenetic analysis was carried out based on NS1 and VP1 aa sequences including reference sequences from subfamilies *Densovirinae*, *Hamaparvovirinae*, and *Parvovirinae*. The results showed that DJF11 and DJF12 clustered with other viruses of the subfamily *Hamaparvovirinae* in the NS1 and VP1 phylogenetic trees. DJF11 clustered with one chicken chapparvovirus 2 strain RS/BR/15/2S (GenBank no. MG846442) formed a clade, while DJF12 clustered with one galliform chaphamaparvovirus 3 strain BC4 formed a clade (GenBank no. MW306779) (Fig. 2C). Sequence analysis showed that NS1 of DJF11 shared the highest aa sequence identity of 99.11% with strain RS/BR/15/2S, and VP1 of DJF11 had the highest aa identity of 98.20% with the same strain. Alignment with the strain RS/BR/15/2S showed that NS1 and VP1 in DJF11 had only 6 aa and 10 aa mutations (data not shown). NS1 and VP1 of DJF12 shared the highest aa sequence identity (99.0% and 97.7%) with strain BC4, respectively. Viruses within a species usually encode NS1 protein that exhibits >85% aa sequence identity, according to the International Committee on Taxonomy of Viruses (ICTV) classification criteria for species in the family *Parvoviridae* [27]. Therefore, DJF11 and DJF12 belong to the subfamily *Hamaparvovirinae*, genus *Chaphamaparvovirus*, species *Galliform Chaphamaparvovirus 2* and *Galliform Chaphamaparvovirus 3*, respectively. This is the first time these viruses have been detected in farmed fox faecal samples.

3.4. Smacovirus and smaco-like viruses detected here

Five complete genomes of smacoviruses were obtained from these two libraries. Two of them from library # 11 were named

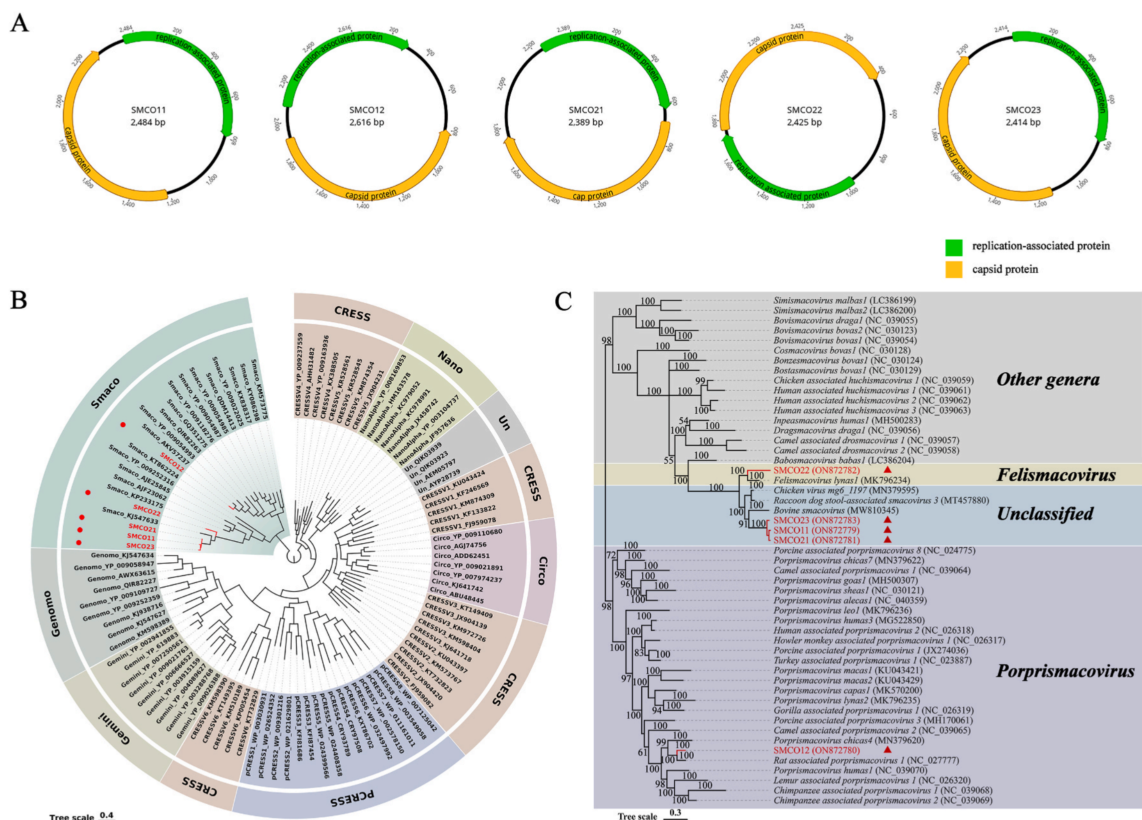

Fig. 3. Schematic genome organization and phylogenetic analysis of five smacoviruses. (A) Genome organizations of SMCO11, SMCO12, and SMCO21-23. The replication-associated protein was marked with green and the capsid protein with yellow. The arrow represented the direction of gene coding. (B) The phylogenetic tree was constructed based on the Rep protein of reference strains of CRESS-DNA virus (*Circoviridae, Genomoviridae, Geminiviridae, Nanoviridae, Bacilladnaviridae, and Smacoviridae*), unclassified CRESS-DNA virus (CRESSV1-6), Bacterial plasmids (pCRESS1-8), and five viruses identified in this study. The viruses identified in this study were shown in red font. (C) Phylogenetic analysis of Rep from various smacovirus genera (*Bovismacovirus*, *Cosmacovirus*, *Dragsmacovirus*, *Drosmacovirus*, *Huchismacovirus*, *Porprismacovirus*, *Inpeasmacovirus*, *Bostasmacovirus*, *Bonzesmacovirus*, *Simismacovirus*, *Babosmacovirus*, *Felismacovirus*, and Unclassified *Smacoviridae*). The viruses detected in this study were highlighted using the red font.

SMCO11 (mean coverage: 9.4) and SMCO12 (mean coverage: 6.2), while the remaining three from library # 12 were named SMCO21 (mean coverage: 27.1), SMCO22 (mean coverage: 8.1), and SMCO23 (mean coverage: 41.8) respectively. The complete genomes of these five viruses are 2,389 to 2,616 nt in length and have two ORFs encoding the putative Rep and Cap proteins separately. As shown in Fig. 3A, the five virus genomes had two types of gene structures, and except for SMCO12, which contains two bidirectional ORFs, the other four viruses (SMCO11, SMCO21, SMCO22, and SMCO23) contain two ORFs in the same direction. The nucleotide sequence identity among them was 39.7%~68.4%. Amino acid sequences analysis based on the Rep protein showed that SMCO11 shared the highest aa identity of 95.02% with the syrmaticus ellioti CRESS-DNA virus sp. isolate eph225sma2 (GenBank no. MW182789), SMCO12 shared the highest aa identity of 82.61% with the Rat stool-associated circular ssDNA virus isolate Mu/10/1799 (GenBank no. KP860907), SMCO21 shared the highest aa identity of 96.14% with the CRESS virus sp. isolate 16806 × 66_211 (GenBank no. MH111087), SMCO22 shared the highest aa identity of 90.94% with the Smacoviridae sp. isolate w3chi090cir1 (GenBank no. MT138076), while SMCO23 shared the highest identity of 95.75% to the Smacoviridae sp. isolate wbp226sma2 (GenBank no. MT138085).

The CRESS-DNA viruses associated with eukaryotic hosts have been classified into six families by ICTV, namely *Circoviridae, Genomoviridae, Geminiviridae, Nanoviridae, Bacilladnaviridae,* and *Smacoviridae* [28]. To investigate the genetic relationship of these five viruses with other CRESS-DNA viruses, phylogenetic trees were constructed based on the Rep protein. As shown in Fig. 3B, the five viruses in this study clustered with other smacoviruses. We further explored the evolutionary relationships of these five viruses by constructing phylogenetic trees with other smacoviruses of different genera (*Bovismacovirus, Cosmacovirus, Dragsmacovirus, Drosmacovirus, Huchismacovirus, Porprismacovirus, Inpeasmacovirus, Bostasmacovirus, Bonzesmacovirus, Simismacovirus, Babosmacovirus,* and *Felismacovirus*) (Fig. 3C). Phylogenetic analysis showed that SMCO12 and SMCO22 were delineated in the genus *Porprismacovirus* and *Felismacovirus*, respectively. SMCO12 formed a clade with *Rat associated porprismacovirus 1* (GenBank no. NC_027777) and shared 81.5% aa identity of Rep protein, while SMCO22 formed a clade with *Felismacovirus lynas1* (GenBank no. MK796234) and shared 65.4% aa identity of Rep protein. According to the ICTV criteria for smacoviruses, the 40% aa sequence identity of Rep with strong phylogenetic support was proposed as a genus-level demarcation threshold [28]. The SMCO12 and SMCO22 belonged to the genus

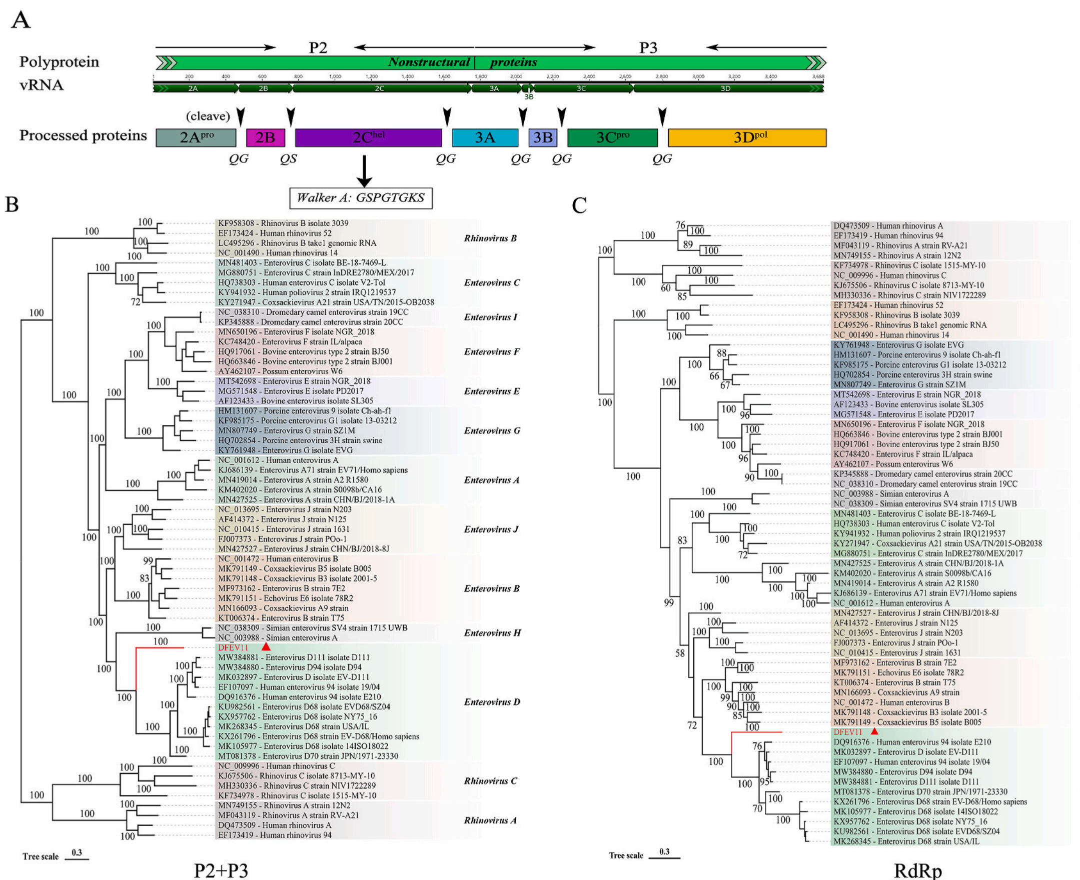

Fig. 4. The genome organization and phylogenetic analysis of enterovirus (A) The partial genome structure of P2 and P3 region and putative cleavage sites of enterovirus D-like virus were shown. (B) The phylogenetic tree based on the nucleotide sequences of the partial P2 and P3 of DFEV11, the reference strains of 11 *Enterovirus* species and 3 *Rhinovirus* species. (C) The phylogenetic tree based on the RdRp nucleotide sequences of DFEV11. The virus detected in this study was marked with red triangle.

Porprismacovirus and *Felismacovirus*, respectively. Otherwise, SMCO11, SMCO21, and SMCO23, together with the Bovine smacovirus strain 68-Smacoviridae-2 (GenBank no. MW810345) formed a clade and shared 60.4%, 62.2%, and 61.5% aa sequence identity of Rep protein with it, respectively, so all of them belonged to the unclassified smacovirus.

3.5. An enterovirus D-like strain detected in this study

An incomplete enterovirus genome was identified and named DFEV11 (mean coverage: 12.2) in Library #11. The incomplete enterovirus genome is 3,688 nt in length, including partial P2 and P3 regions. The aa sequence of P2+P3 has 72.62% identity with enterovirus D94 (GenBank no. DQ916376). The P2+P3 sequence was cut into several nonstructural proteins, including partial 2A, 2B (99 aa), and 2C (328 aa), 3A (92 aa), 3B (22 aa), 3C (183 aa), and partial 3D, respectively. 2A and 3C proteins were proteases involved in the cleavage of polyprotein [29]. The putative cleavage sites were QG (2A/2B), QS (2B/2C), QG (2C/3A), QG (3A/3B), QG (3B/3C), and QG (3C/3D), respectively. The 2C protein contained a conserved nucleotide phosphate-binding motif, also known as the Walker A motif GSPGTGKS (GXXGXGK [S/T]), which belonged to the *P*-loop NTPase domain superfamily (Fig. 4A). 3A protein was a critical component of the enterovirus replication complex [30,31]. Orthologous proteins 2C, 3C, and 3D were conserved in all picornaviruses. The incomplete 3D protein in this study also contained a conserved RdRp domain. Based on RdRp aa sequence alignment, DFEV11 showed 81.59%~84.58% aa sequence identity with other enterovirus D strains (Fig.S1).

The VP1 sequence variant is used to classify enterovirus serotypes. It has been proposed that enteroviruses should be categorized as belonging to the same serotype if their VP1-coding sequence shares at least 75% nucleotide similarity (>85% aa sequence similarity) [32]. Because the whole gene sequence was not available, we investigated the genetic relationship based on the P2+P3 nucleotide sequences of DFEV11. The results showed that DFEV11, as a separate branch, was clustered with 11 enterovirus D strains (Fig. 4B). Furthermore, RNA-dependent RNA-polymerase (RdRp) was used to examine evolutionary relationships among picornaviruses [33]. Similarly, phylogenetic analysis using RdRp nucleotide sequences showed that DFEV11 remained a separate branch, clustering with 11 enterovirus D strains (Fig. 4C). Although DFEV11 fell outside the enterovirus D clade, RdRp sequence analysis showed that DFEV11 shared a high level of nucleotide and amino acid identity with other enterovirus species in this genus, implying that DFEV11 was closely related to the enterovirus D strain.

4. Discussion

Viral metagenomics is an attractive tool for discovering broad-based pathogens and highly mutated viruses. Since the 20th century, identifying new pathogens has significantly impacted infectious diseases, microbiology, and human health [34-36]. Zoonotic diseases are diseases transmitted from animals to humans through direct contact, food, water, and the environment. It was estimated that rough 61% of emerging infectious diseases are zoonoses, caused mainly by viruses [37,38]. Diversification of market demands, such as the fur industry, petting zoos, and specialty food products, accelerates the spread of numerous undiscovered pathogens. Domestic animals may act as amplifiers for wild pathogens; therefore, changes in viral community diversity in livestock farms should be monitored more closely.

In this study, faecal samples from a red fox farm in Jilin Province, China were analyzed using high-throughput sequencing to predict the viral community composition of farmed red foxes. Canidae, such as wolves, foxes, raccoon dogs and dogs, are the natural hosts of the rabies virus (RV), canine distemper virus (CDV), and canine parvovirus (CPV) [13,39,40]. However, RV and CDV were not detected in this study, possibly because of standardized vaccination procedures and the change in living habits which may lead to differences in the viral composition in domestic and wild foxes. In previous studies, a great proportion of viral reads were mapped to circovirus in juveniles and picobirnavirus in wild adult foxes [15]. Similarly, astroviruses were also identified from faecal samples of wild red foxes [41]. However, the majority virus communities in this study were parvoviruses and picornaviruses, which also reflected the unique distribution of red fox virus communities in different areas under rearing methods compared with wild foxes.

Following rigorous assembly parameter setting and Polymerase Chain Reaction (PCR) validation, several virus strains were identified. Parvovirus is small, non-enveloped virus with linear, single-stranded DNA genomes of about 4–6 kb in size. Some members of the family *Parvoviridae* can cause diseases that range from subclinical to lethal alone, while some require co-infection with helper viruses from other families [27,42,43]. Two chaphamaparvoviruses discovered in this study were clustered with other galliform chaphamaparvoviruses. In addition, two anelloviruses were firstly detected in this study and clustered together with avian-associated gyrovirus strains. Since chicken flesh was a staple of the farmed fox's diet, this might be the explanation for where these viruses came from. Meanwhile, animal research is needed to confirm whether amino acid deletions or mutations of the DJF11 and DJF12 capsid proteins impact host range and pathogenicity.

Members of the family *Smacoviridae* have small, circular, single-stranded DNA genomes encoding replication-associated proteins (Rep) and capsid proteins (Cap), with a genome length of approximately 2.3–2.9 kb. Although these viruses are thought to infect eukaryotes, their actual host remains unconfirmed [44]. In this study, five smacoviruses were detected. Phylogenetic analysis showed that SMCO12 and SMCO22 were clustered with rat and lynx-associated virus strains, respectively, and were assigned to two distinct recognized genera (GenBank no. NC_027777, no. MK796234). SMCO11, SMCO21, and SMCO23 were clustered with unclassified smacoviruses and belonged to samco-like viruses. Unlike members of the family *Smacoviridae*, the Rep and Cap encoding in their genome were in the same orientation rather than in the bidirectional orientation, suggesting that these smaco-like viruses may represent a new genus. In addition, the smacovirus from lynx was thought to be a common felid gut microbiome associated virus in previous study [45]. Two smacoviruses belonging to the genus *Porprismacovirus* and *Felismacovirus* were first detected in faecal samples from healthy farmed red foxes. In this study, we suspected that smacoviruses may be the common viruses associated with the fox gut

microbiome. We cannot also rule out the possibility that these five smacoviruses, particularly SMCO12, arose from foodborne consumption due to environmental complexity.

Enterovirus, a member of the family *Picornaviridae*, has a genome of about 7.3 kb and is a non-enveloped, single-stranded, positive-sense RNA virus. The P1 region encodes the structural polypeptide and further cleaves it into four structural proteins (VP1~VP4). The P2 and P3 regions encode replication-associated nonstructural proteins and cleave into three nonstructural proteins (2A~2C) and four nonstructural proteins (3A~3D), respectively. Based on sequence diversity, enteroviruses (EVs) are currently divided into 15 species, including *Rhinovirus A-C* and *Enterovirus A-L*. An enterovirus D-like strain (known as DFEV11) was detected in red fox faeces samples in this study. Enteroviruses have been shown to be responsible for varying degrees of disease and may infect multiple mammals, including humans and non-human primates [46]. However, foxes did not show clinical symptoms during sample collection, indicating that DFEV11 may not be pathogenic for red foxes.

In addition, viruses from other families have also been found, such as *Genomoviridae*, *Herpesviridae*, *Iridoviridae*, *Picobirnaviridae*, and *Poxviridae*. However, relatively complete genetic information cannot be obtained, which may be caused by the loss of some gene fragments in the process of library enrichment. Despite the inherent limitions of sample size and sequencing method, the research also demonstrates to some extent the relationship between viral community diversity and the environment, which eventually provides valuable information for monitoring the health of domestic animals.

5. Conclusion

Fecal virome in farmed foxes are examined in this study, which dramatically increases our understanding of viral diversity in samples from members of the Canidae family. Information from this study may help prevent viral diseases in farmed foxes and help monitor the health of these animals.

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Ethics statements

Studies involving animal subjects

Regarding the use of animals, the Jiangsu University Ethics Committee reviewed and approved the animal study, and it complied with Chinese ethics regulations and laws. Written informed consent to participate in this study was sought from the owners of their animals.

Studies involving human subjects

No human studies were involved in this research.

Data availability statement

These datasets are available in online repositories. A list of repository names and accession numbers can be found in the supplementary material.

Author contribution statement

Shixing Yang and Wen Zhang conceived and designed the experiments. Dianqi Zhang, Yan Wang, and Xu Chen performed the experiments. Dianqi Zhang, Yumin He, Min Zhao, Xiang Lu, Juan Lu, Wen Zhang, Yan Wang, Likai Ji, and Xiaochun Wang analyzed and interpreted the data. Wen Zhang, Shixing Yang, Likai Ji, and Quan Shen contributed reagents, materials, analysis tools or data. Dianqi Zhang wrote the paper, and all authors substantially reviewed and revised the manuscript.

Declaration of competing interest

The authors state that there are no competing interests.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.heliyon.2023.e12826.

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