

## Identification of a Novel Parvovirus in the Arctic Wolf (*Canis lupus arctos*)

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### Abstract

A novel virus, temporarily named “Arctic wolf parvovirus” (AWPV), was discovered in a pharyngeal metagenomic library derived from an Arctic wolf (*Canis lupus arctos*) in China. The genome sequence was assigned GenBase accession number C\_AA071902.1. AWPV has a genome comprised of 4,920 base pairs with a nucleotide composition of 36.4% A, 23.4% T, 18.2% G, and 22.0% C, with a GC content of 40.2%. Its structure resembles parvoviruses, containing two open reading frames: the nonstructural (NS) region encoding replication enzymes and the structural (VP) region encoding capsid protein. Pairwise sequence comparison and phylogenetic analysis suggest AWPV may represent a novel species within the genus *Protoparvovirus*. This discovery enhances our understanding of mammalian virus ecology and potential future infectious diseases.

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**Keywords:** parvovirus, virome, metagenomics, Arctic wolf, novel virus

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### Introduction

The family *Parvoviridae* comprises round, non-enveloped viruses with linear, single-stranded DNA genomes ranging from 4–6 kb (Cotmore et al. 2019). They have been detected in nearly all major groups of vertebrates, as well as in both proto- and deuterostome invertebrates (Pénzes et al. 2020). In 1975, the family *Parvoviridae* was recognized, and in 1993 it was divided into two subfamilies, *Parvovirinae* and *Densovirinae*, to classify viruses that infect either vertebrate or invertebrate hosts (Cotmore et al. 2019). According to the International Committee for the Taxonomy of Viruses (ICTV) classification principles, the family *Parvoviridae* comprises three subfamilies, 28 genera, and 175 species. Parvoviruses have been reported in numerous countries from a wide range of hosts, including but not limited to mammals such as humans (Phan et al. 2012), mice (Janus and Bleich 2012), canines (Decaro and Buonavoglia 2012), and chimpanzees (Adlhoch et al. 2012), as well as arthropods such as crickets (Meng et al. 2013), and birds such as ducks (Liu et al. 2020),

red-crowned cranes (Wang et al. 2019), and pigeons (Bergmann and Kiupel 1982).

Parvoviral genomes are characterized by long inverted terminal repeats (LTRs) located at both the 5' and 3' ends. These can adopt hairpin-like structures and play a role in the viruses' expression and transcription strategies (Brown 2010). They encode two open reading frames (ORFs): ORF1 encodes non-structural proteins NS1 and NS2, while ORF2 encodes structural proteins VP1 and VP2 (de Oliveira Santana et al. 2022). Parvoviruses can be classified as belonging to the same species if their NS1 proteins share over 85% amino acid sequence identity (Pénzes et al. 2020). Similarly, a genus can be defined as a cluster of species that form a single branch and have a minimum of 35–40% amino acid sequence identity with coverage of over 80% between any two members (Pénzes et al. 2020).

The viruses known to infect carnivores (order *Carnivora*) are distributed across various genera within two subfamilies. These include the genus *Chaphamaparvovirus* of the subfamily *Hamaparvovirinae*, as well as the genera *Amdoparvovirus*, *Bocaparvovirus*, *Dependoparvovirus*,

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and *Protoparvovirus* within the subfamily *Parvovirinae*. Within the genus *Protoparvovirus*, which comprises 19 species, including 15 officially recognized and four proposed, notable members include *Protoparvovirus carnivoran1* (containing both canine parvovirus 2 (CPV-2) and feline panleukopenia virus (FPV)), *Protoparvovirus carnivoran3* (canine bufavirus), and *Protoparvovirus carnivoran4* (fox parvovirus), all of which have been detected in carnivores (Ndiana et al. 2021). Among these, CPV-2, belonging to the genus *Protoparvovirus* of the family *Parvoviridae*, stands out as a highly contagious viral pathogen primarily affecting canids, especially dogs. It induces severe gastrointestinal disease, characterized by symptoms such as vomiting, diarrhea, anorexia, and dehydration (Decaro and Buonavoglia 2012; Decaro et al. 2020). CPV-2 exhibits a broad host range, including dogs, foxes, and wolves, and is predominantly transmitted via the fecal-oral route (Kurucay et al. 2023). Upon infection, CPV-2 initially targets the pharynx before entering the bloodstream within days, colonizing the intestines and bone marrow. This colonization leads to severe leukopenia and may result in viremia, which can progress to myocarditis (Tuteja et al. 2022; Kurucay et al. 2023).

The Arctic wolf (*Canis lupus arctos*), a subspecies of the gray wolf, inhabits the Arctic regions of North America and Eurasia. Adapted to extreme cold, Arctic wolves have a thick, insulating coat and are well-suited to the harsh, icy environment in which they live. They primarily hunt caribou, muskoxen, and other large herbivores, and their social structure is adapted to the severe climate, often forming small, tight-knit packs that are capable of covering large distances in search of food. Understanding the Arctic wolf's ecology and habitat is crucial as it provides insight into how the virus might have adapted to this specific host. The Arctic wolf's unique environmental pressures and dietary habits could influence the virus's evolution and adaptation.

Herein, we present the genome characterization of a novel parvovirus tentatively named as Arctic wolf parvovirus (AWPV) identified in a pharyngeal sample collected from an Arctic wolf (*Canis lupus arctos*). The discovery of this new genome expands our understanding of the diversity of parvoviruses. Our analysis suggests that AWPV could potentially be classified as a new species in the genus *Protoparvovirus*.

## Experimental

### Materials and Methods

**Metagenome assembly.** While studying potential pathogenic viruses in mammals, an available library – SRR12366691 – was downloaded from the Sequence

Read Archive (SRA) database. This library, deposited by Du and coworkers from Hainan Medical University, with Biosample accession number SAMN15700777, was collected from an Arctic wolf in Xi'an, China, in 2018. The method for processing the samples has been described (Zhang et al. 2022). The SRA file format was transformed to fastq format utilizing Pfastq-dump v0.1.6 (<https://github.com/inutano/pfastq-dump>), and the elimination of host sequences was executed using Bowtie2 v2.4.5 (Langmead and Salzberg 2012; Langmead et al. 2019). The potential primer sequences present in the raw reads were removed by applying Trim Galore v0.6.5 ([https://www.bioinformatics.babraham.ac.uk/projects/trim\\_galore](https://www.bioinformatics.babraham.ac.uk/projects/trim_galore)). Afterwards, the resultant files were subjected to quality control using the options '--phred33 --length35 --stringency3 --fastqc'. PRINSEQ-lite v0.20.4 (-derep 1) (Schmieder and Edwards 2011) was employed to mark duplicated reads. An in-house pipeline was utilized to assemble this library. Single-end reads were assembled using MEGAHIT v1.2.9 (Li et al. 2016) with default parameters. After the assembly process, containers with a sequence length greater than 1,500 bp were kept. Following the above-mentioned steps, the outcomes were imported into Geneious Prime v2022.0.1 (<https://www.geneious.com>) to be manually sorted and confirmed.

**Search for novel vertebrate-associated viruses.** The contigs obtained were subjected to alignment against the non-redundant protein (nr) database (downloaded in February 2023) using the BLASTx program implemented in DIAMOND v2.0.15 (Buchfink et al. 2021), with a cut-off E-value of  $< 10^{-5}$ . Taxonomic identification was performed using the built-in rma2info program in MEGAN6 (Gautam et al. 2022). To assemble the viral sequences of interest, the contigs that showed significant BLASTx similarity to related viruses were selected and merged in Geneious Prime. Putative open reading frames (ORFs) were predicted using Geneious Prime with default parameters (Minimum size: 400) (Kearse et al. 2012). These predictions underwent validation by comparison with ORFs identified in closely related viruses. Annotations of these ORFs were accomplished through comparisons with the Conserved Domain Database (CDD). As a result, a putative novel parvovirus exhibiting complete genome organization was identified.

**Phylogenetic analysis.** To infer phylogenetic relationships, reference protein sequences related to parvovirus were downloaded from the NCBI GenBank database. The protein sequences were aligned using the alignment program in Geneious Prime. Subsequently, the resulting alignment was optimized further by utilizing MUSCLE in MEGA v7.0 (Kumar et al. 2016) and MAFFT v7.3.1, which employed the E-INS-I algorithm (Kuraku et al. 2013). MrBayes v3.2 (Ronquist et al. 2012) was utilized to construct Bayesian inference

trees. A Markov chain was executed for a maximum of 1 million generations, with sampling occurring every 50 generations. The first 25% of Markov chain Monte Carlo (mcmc) samples were discarded as burn-in. In addition, Maximum Likelihood trees were constructed using MEGA v7.0 (Kumar et al. 2016) software to verify all Bayesian inference trees. The Sequence Demarcation Tool v1.2 (Muhire et al. 2014) was used to conduct color-coded pairwise identity matrix analysis comparing the novel parvovirus to other members of *Parvoviridae*. The identity score for each pair of sequences is computed as  $1 - M/N$ , where  $M$  is the number of mismatched nucleotides, and  $N$  is the total number of columns along the alignment where neither sequence has a gap character (Muhire et al. 2014).

**Prediction of potential genome recombination events.** The Recombination Detection Program v4.39 (RDP4) software was used to analyze the genomic alignments of both the reference and AWPV strains. It was done through various algorithms including RDP, GENECONV, Chimaera, MaxChi, BootScan, and SiScan, in order to detect possible recombination events (Martin et al. 2015).

**Prediction of spatial structure.** The three-dimensional structure of the viral structural protein identified in this study was predicted using ColabFold (Mirdita et al. 2022). SWISS-MODEL (Waterhouse et al. 2018) was employed to compare and screen models with comparable spatial structures from the PDB database. PyMOL v2.0 (The PyMOL Molecular Graphics System, Schrödinger, LLC) was utilized to visualize the results.

**Data availability.** The novel parvovirus sequence obtained in this study has been deposited in the GenBank database under accession number C\_AA071902.1.

## Results

**Overview of the pharyngeal metagenomic library of the Arctic Wolf.** This library was sequenced using the Illumina HiSeq® 2500 platform (Illumina, Inc., USA), generating 28,946,890 raw reads. Following quality control, 28,945,811 clean reads were obtained and assembled into 736 contigs greater than 1,500 bp in length. Upon searching these contigs against the nr database, 260 contigs exhibited the highest identity with viral proteins (Table SI). Eleven viral families were identified, with the family *Myoviridae* being the most prevalent, accounting for 28.85% of all viral contigs, followed by *Siphoviridae* (18.08%), *Podoviridae* (12.31%), *Ackermannviridae* (11.54%), *Demerecviridae* (6.54%), *Autographiviridae* (6.15%), *Microviridae* (4.23%), *Herleviridae* (3.08%), *Astroviridae* (0.77%), *Parvoviridae* (0.38%) and *Leviviridae* (0.38%) (Table SI). However, most of them are already documented. Moreover,

20 contigs were identified, which could not be confidently assigned to any viral taxonomic group (E-value  $> 10^{-5}$ ). This finding suggests the potential existence of novel viruses within these contigs or the prevalence of non-coding regions in the assembled sequences.

A BLASTx search was conducted to assess the host of the library (SRR12366691) used in this study. We compared the contigs assembled from the NGS data of this library, particularly those containing potential mammalian sequence reads, against the complete mitochondrial proteome database downloaded from GenBank. The results indicated that most mammalian matches originated from the Arctic wolf (Table SII).

**Identification of a novel parvovirus.** This study identified a large contig containing non-structural and structural proteins, sharing high amino acid sequence identities of 76.30% and 62.21%, respectively, with parvovirus. Subsequently, the contig was further assembled using Geneious Prime, ultimately resulting in the acquisition of a complete genome sequence of the parvovirus. This novel strain of parvovirus, isolated from the Arctic wolf, was tentatively named Arctic wolf parvovirus (AWPV). AWPV has a genome size of 4,920 bp, characterized by a GC content of 40.20% and a nucleotide composition of 36.36% A, 23.43% T, 18.21% G, and 21.99% C. The typical organizational pattern of this viral genome is shown in Fig. 1A. Specifically, AWPV possesses a partial 5' non-translated region (68 bp), a complete NS1 open reading frame (620 aa), a complete virus protein (VP) 1 open reading frame (739 aa), a complete virus protein (VP) 2 open reading frame (581 aa), and a partial 3' non-translated region (214 bp). The NS1 protein contains several conserved motifs that are related to ATP- or GTP-binding, including a Walker A loop  $^{408}\text{GPASTGKS}^{415}$  [GXXXXGK (T/S)], a Walker B loop at  $^{448}\text{IWVEE}^{453}$  (xxxxEE), and a Walker B' loop at  $^{465}\text{KAICSGQSIRIDQK}^{478}$  (KxxxxGxxxxxxK). In addition, we identified a conserved replication initiator motif  $^{132}\text{KLHIHVLLHH}^{141}$  (xxHxHxxxxx) in the NS1 protein. By aligning with other protoparvovirus sequences, potential splicing signals for expressing VP1 were identified. In the N-terminal of VP1, the phospholipase A<sub>2</sub> (PLA2) motif was identified, which contains the expected calcium-binding (YLGPG) site and catalytic residues (Fig. 1A). A total of 2,508 reads matched this genome, with a read length of 250 bp, resulting in an approximate sequencing depth of 127X. Specifically, the NS1 gene region (nucleotide positions 69–1928) has 650 reads mapped, while the VP1 and VP2 gene regions (nucleotide positions 1941–3665 and 1934–4706) have 1,951 reads mapped. The coverage map in Fig. 1A shows an even distribution across the genome, with certain regions, such as nucleotide positions 2400–2800, exhibiting higher coverage and regions, such as positions 1–600, exhibiting lower coverage.

**Phylogenetic analysis.** Representative sequences with high identity to the NS1 and VP1 proteins of AWPV, as well as other parvoviruses, were included in subsequent analysis. Based on the phylogenetic analysis of NS1 and VP1 protein sequences, AWPV was positioned between the parvoviruses identified in California sea lions (Altan et al. 2020) and foxes (Canuti et al. 2021; Lanave et al. 2023), forming a distinct branch (Fig. 1B). Based on the distance matrix analysis of NS1 and VP1 proteins, it was determined that AWPV shares less than 77% identity with the protein sequences of other parvovirus members. This suggests that AWPV should be classified as a new species of the genus *Protoparvovirus* (Fig. 2 and Table SIII). In addition, the RDP4 software did not indicate any significant evidence of recombination signals.

**Predicting the spatial structure of the AWPV's VP.** The structure and biophysical properties of *Protoparvovirus* capsids are essential for their survival in the natural environment and entry into host cells (Ros et al. 2017). To predict and contrast the similarity of the spatial structure of AWPV's VP with the structure dictated by present known sequences, the sequence exhibiting the greatest degree of similarity to AWPV's VP, which is the *Newlavirus* strain ITA/2016/51.20-153 (UZZ82241), was retrieved from the GenBank database. The spatial structure of sequences encoding VP was predicted using ColabFold. Furthermore, SWISS-MODEL was utilized to search and obtain the virus model (6x2k.1) of *Tusavirus* (Mietzsch et al. 2020), which exhibited the highest similarity to AWPV. The PyMOL software was used to import all PDB files and conduct pairwise comparisons. The Root Mean Square Deviation (RMSD) is an indicator of the overall similarity between two spatial structures. Typically, an RMSD of less than 2 Å suggests a significant degree of similarity. The AWPV identified in this study did not show significant similarity in the VP spatial structure with the *Newlavirus* strain ITA/2016/51.20-153 and *Tusavirus* (RMSD = 2.888 and 4.458), indicating that AWPV and other related viruses may have different characteristics in mediating cell attachment during infection (Fig. 3).

## Discussion

Metagenomics has revolutionized our understanding of viral diversity, greatly expanding the number of identified viral families, genera, and species beyond traditional methods. This is measured through sequence diversity and evolutionary distances (Greninger 2018). Additionally, metagenomics enables the detection of novel viruses and the characterization of viromes in diverse samples, which enhances our knowledge of virus-host interactions and their potential public health

implications, particularly for emerging and re-emerging infectious diseases.

The Arctic wolf, also known as the white wolf or polar wolf, is a subspecies of the gray wolf in the family *Canidae* and is distributed in the northern regions of Eurasia, northern Canada, and northern Greenland. In recent years, there have been reports about Arctic wolves being infected with protoparvovirus, but most are related to CPV-2. For example, in 2019, Stilwell and others identified CPV-2 in a female Arctic wolf that died at the age of six weeks due to systemic canine distemper virus (CDV) infection (Stilwell et al. 2019). In this study, we recovered a novel virus belonging to the genus *Protoparvovirus* from the Arctic wolf pharyngeal metagenomic library. The virus, tentatively named AWPV, has a genome length of 4,920 bp, and its best match in the GenBank database shows a nucleotide sequence identity of 75.76% and coverage of 70%. The Bayesian phylogenetic trees constructed based on NS1 and VP1 amino acid sequences exhibited overall similar topology, with AWPV forming a unique branch located between the recently identified parvoviruses from California sea lions (California sea lion parvovirus, MN982959) and foxes (Newlaviruses, ON959793-96), and AWPV having a relatively distant relationship with CPVs. The California sea lion parvovirus was derived from a 2–3-year-old subadult emaciated female California sea lion found stranded and deceased in 2010 (Altan et al. 2020). The NS1 and VP1 proteins of AWPV were found to be 75.20–76.10% and 63.70–70.50% identical to the Newlaviruses strains. The Newlaviruses were detected and identified in the retropharyngeal lymph node samples from carcasses of foxes in Southern Italy and stool samples from foxes in Canada (Canuti et al. 2021; Lanave et al. 2023). The genetic distance between AWPV and canine parvoviruses was significantly more significant, with identity percentages of less than 42%, indicating a more distant relationship (Table SIII). Due to the possibility of this Arctic wolf being captive, we cautiously speculate that AWPV may have originated from interactions with other captive animals.

The discovery of AWPV raises important questions about its potential impacts on the health of Arctic wolves and other species, including humans. Protoparvoviruses are known to cause various diseases in animals, often leading to severe outcomes such as gastrointestinal illness and immune suppression (Yeşil et al. 2019; Başıç et al. 2021; Vargas-Bermudez et al. 2023; Yeo et al. 2023). Given the phylogenetic relationship of AWPV with other known parvoviruses, it is crucial to explore its pathogenic potential and transmission dynamics. Arctic wolves, both in the wild and captivity, could serve as reservoirs for this virus, potentially affecting other wildlife and possibly humans, especially those in close contact with these animals.

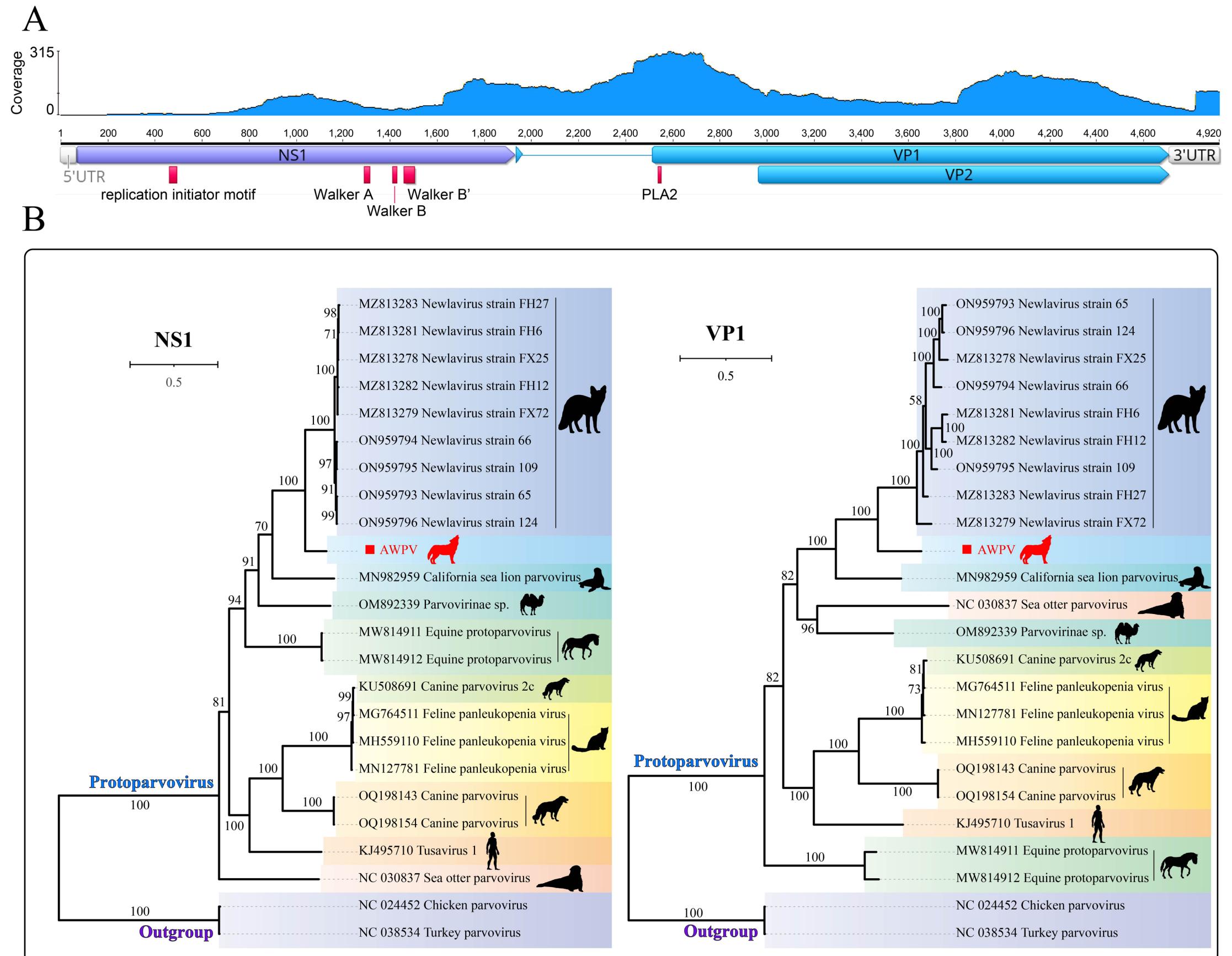


Fig. 1. Genome and phylogenetic analysis of a novel parvovirus.

A) Coverage map and organization of the AWPV genome; B) phylogenetic analysis of AWPV. The Bayesian inference tree has been constructed based on protein sequences obtained from the NS1 and VP1 regions of AWPV. The virus discovered in this study is denoted in red within the tree.

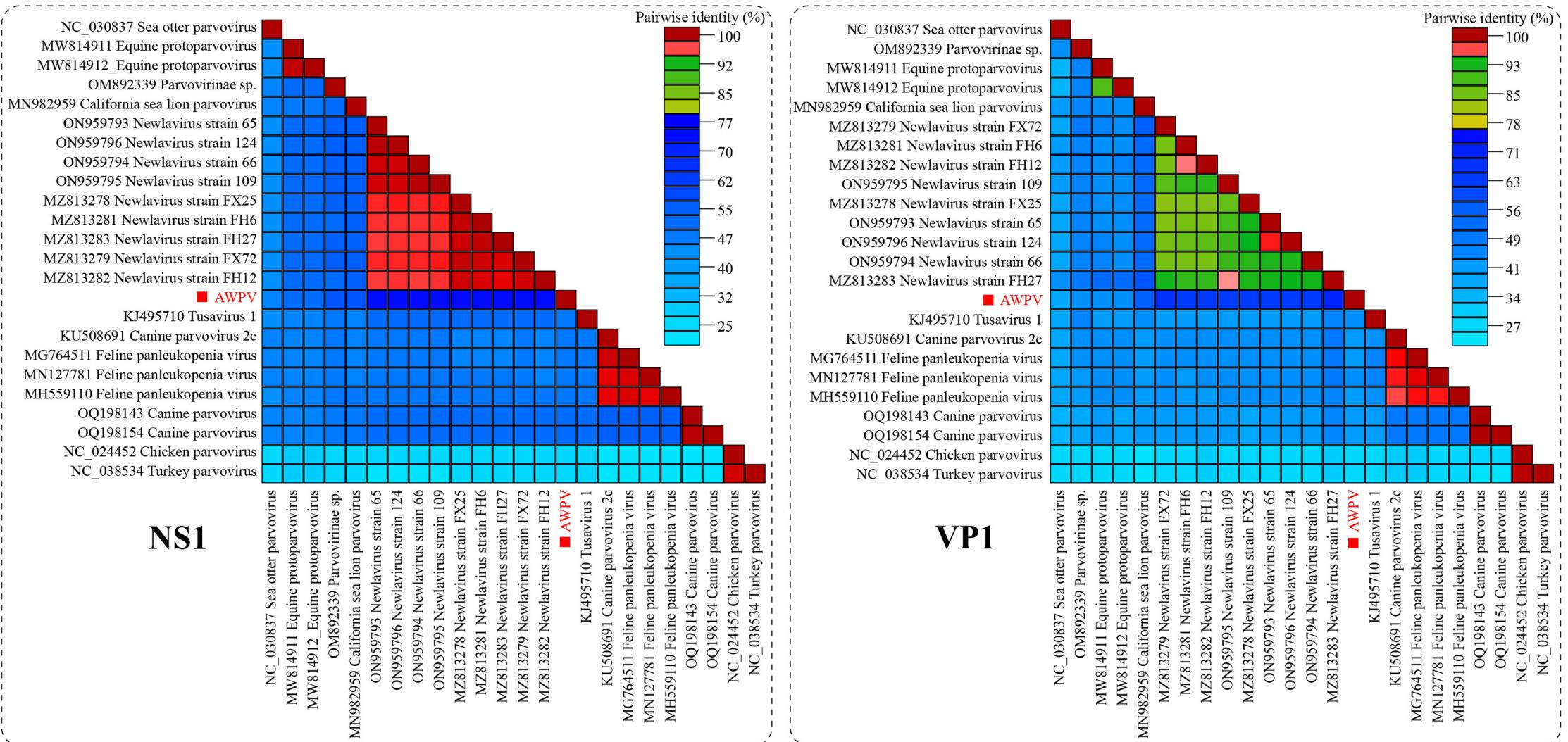


Fig. 2. Pairwise identity matrix analysis of AWPV.  
Pairwise sequence comparison produced with VP protein sequences within the Bayesian consensus tree.

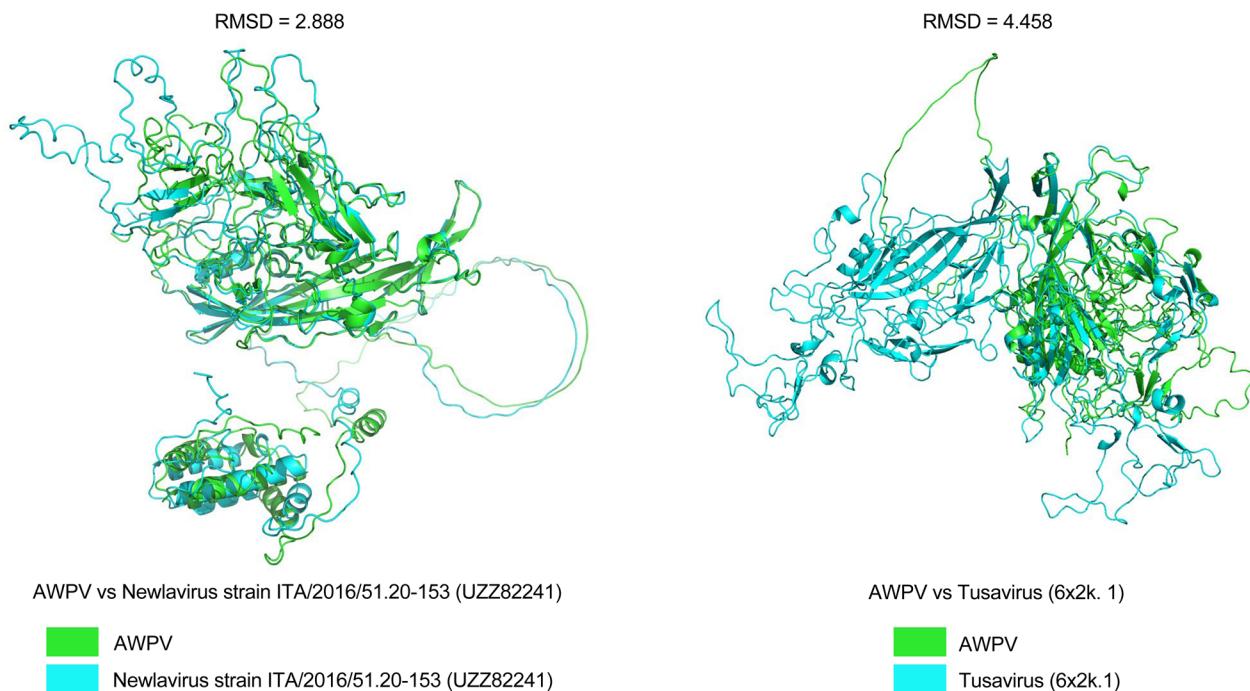


Fig. 3. The structural model visualization of the VP protein was done through PyMOL v2.0 software using the PDB files and pairwise alignment.

The emergence of AWPV in Arctic wolves could signify a broader ecological and epidemiological impact, necessitating vigilant surveillance and further research to understand its implications for ecosystem health and zoonotic potential.

To fully understand the implications of AWPV, future research should investigate its role in the ecology of Arctic wolves. This includes studying how the virus affects their health, behavior, and population dynamics through long-term monitoring of wild and captive populations. Additionally, given the phylogenetic relationship of AWPV with other known parvoviruses, it is crucial to explore its potential for cross-species infections. Research should determine whether AWPV can infect other wildlife species, domestic animals, or even humans, particularly those in close contact with Arctic wolves. Further studies on the pathogenesis and transmission dynamics of AWPV are necessary to understand how the virus spreads and causes disease. Finally, investigating the genomic evolution of AWPV can reveal how the virus adapts to its host and environment. Comparative genomics can identify genetic changes that may influence its virulence and transmissibility.

In conclusion, our study identified a novel parvovirus, AWPV, from the metagenomic library of Arctic wolves. AWPV represents a distinct species within the genus *Protoparvovirus*, exhibiting less than 77% identity with other known parvovirus members. Phylogenetic analysis positioned AWPV between parvoviruses detected in California sea lions and foxes, forming a distinct branch. This study underscores the impor-

tance of metagenomic approaches in identifying novel viruses and highlights the need for further investigation into the ecology, epidemiology, and pathogenesis of AWPV in carnivore populations.

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#### Availability of data and material

The novel parvovirus sequence obtained in this study have been deposited in GenBase database under accession number C\_AA071902.1.

#### Author contributions

ZD and HW collected data and wrote this article, QL, MS, HC, RZ and ZD assisted in data processing and proofreading. All authors reviewed the manuscript.

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#### Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

#### Literature

Adlhoch C, Kaiser M, Loewa A, Ulrich M, Forbrig C, Adjogoua EV, Akoua-Koffi C, Couacy-Hymann E, Leendertz SA, Rietschel W, et al. Diversity of parvovirus 4-like viruses in humans, chimpanzees,

- and monkeys in hunter-prey relationships. *Emerg Infect Dis*. 2012 May;18 (5):859–862. <https://doi.org/10.3201/eid1805.111849>
- Altan E, Delaney MA, Colegrave KM, Spraker TR, Wheeler EA, Deng X, Li Y, Gulland FMD, Delwart E.** Complex virome in a mesenteric lymph node from a Californian sea lion (*Zalophus Californianus*) with polyserositis and steatitis. *Viruses*. 2020 Jul;23(12):793. <https://doi.org/10.3390/v12080793>
- Başçı S, Bakırtaş M, Yiğenoglu TN, Uncu Ulu B, Batgi H, Yıldız J, Dal MS, Kızıl Çakar M, Altuntas F.** The outcome of diffuse large B cell lymphoma patients in adolescent and young adult age group. *J Adolesc Young Adult Oncol*. 2021 Aug;10 (4):483–487. <https://doi.org/10.1089/jayao.2020.0145>
- Bergmann V, Kiupel H.** [Inclusion body enteritis in pigeons caused by adenovirus and parvovirus] (in German). *Arch Exp Veterinarmed*. 1982;36 (3):445–453.
- Brown KE.** The expanding range of parvoviruses which infect humans. *Rev Med Virol*. 2010 Jul;20 (4):231–244. <https://doi.org/10.1002/rmv.648>
- Buchfink B, Reuter K, Drost HG.** Sensitive protein alignments at tree-of-life scale using DIAMOND. *Nat Methods*. 2021 Apr;18 (4):366–368. <https://doi.org/10.1038/s41592-021-01101-x>
- Canuti M, Bouchard , Rodrigues B, Whitney HG, Hopson M, Gilroy C, Stenson G, Dufour SC, Lang AS, Verhoeven JTP.** Newlavirus, a novel, highly prevalent, and highly diverse protoparvovirus of foxes (*Vulpes spp.*). *Viruses*. 2021 Sep;13 (10):1969. <https://doi.org/10.3390/v13101969>
- Cotmore SF, Agbandje-McKenna M, Canuti M, Chiorini JA, Eis-Hübler AM, Hughes J, Mietzsch M, Modha S, Ogliastro M, Pérez JJ, et al.; Ictv Report Consortium.** ICTV virus taxonomy profile: *Parvoviridae*. *J Gen Virol*. 2019 Mar;100 (3):367–368. <https://doi.org/10.1099/jgv.0.001212>
- de Oliveira Santana W, Silveira VP, Wolf JM, Kipper D, Echeverrigaray S, Canal CW, Truyen U, Lunge VR, Streck AF.** Molecular phylogenetic assessment of the canine parvovirus 2 worldwide and analysis of the genetic diversity and temporal spreading in Brazil. *Infect Genet Evol*. 2022 Mar;98:105225. <https://doi.org/10.1016/j.meegid.2022.105225>
- Decaro N, Buonavoglia C, Barrs VR.** Canine parvovirus vaccination and immunisation failures: Are we far from disease eradication? *Vet Microbiol*. 2020 Aug;247:108760. <https://doi.org/10.1016/j.vetmic.2020.108760>
- Decaro N, Buonavoglia C.** Canine parvovirus – A review of epidemiological and diagnostic aspects, with emphasis on type 2c. *Vet Microbiol*. 2012 Feb;155 (1):1–12. <https://doi.org/10.1016/j.vetmic.2011.09.007>
- Gautam A, Felderhoff H, Bağci C, Huson DH.** Using AnnoTree to get more assignments, faster, in DIAMOND+MEGAN microbiome analysis. *mSystems*. 2022 Feb;7 (1):e0140821. <https://doi.org/10.1128/msystems.01408-21>
- Greninger AL.** A decade of RNA virus metagenomics is (not) enough. *Virus Res*. 2018 Jan;244:218–229. <https://doi.org/10.1016/j.virusres.2017.10.014>
- Janus LM, Bleich A.** Coping with parvovirus infections in mice: Health surveillance and control. *Lab Anim*. 2012 Jan;46 (1):14–23. <https://doi.org/10.1258/la.2011.011025>
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, et al.** Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*. 2012 Jun;28 (12):1647–1649. <https://doi.org/10.1093/bioinformatics/bts199>
- Kumar S, Stecher G, Tamura K.** MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol Biol Evol*. 2016 Jul;33 (7):1870–1874. <https://doi.org/10.1093/molbev/msw054>
- Kuraku S, Zmasek CM, Nishimura O, Katoh K.** aLeaves facilitates on-demand exploration of metazoan gene family trees on MAFFT sequence alignment server with enhanced interactivity. *Nucleic Acids Res*. 2013 Jul;41 (W1):W22–W28. <https://doi.org/10.1093/nar/gkt389>
- Kurucay HN, Tamer C, Muftuoglu B, Elhag AE, Gozel S, Cicek-Yildiz Y, Demirtas S, Ozan E, Albayrak H, Okur-Gumusova S, et al.** First isolation and molecular characterization of canine parvovirus-type 2b (CPV-2b) from red foxes (*Vulpes vulpes*) living in the wild habitat of Turkey. *Virol J*. 2023 Feb;20 (1):27. <https://doi.org/10.1186/s12985-023-01988-2>
- Lanave G, Ndiana LA, Pellegrini F, Diakoudi G, Di Martino B, Sgroi G, D'Alessio N, Vasinioti V, Camero M, Canuti M, et al.** Detection at high prevalence of newlavirus (protoparvovirus) in the carcasses of red foxes. *Virus Res*. 2023 Jan;323:198971. <https://doi.org/10.1016/j.virusres.2022.198971>
- Langmead B, Salzberg SL.** Fast gapped-read alignment with Bowtie 2. *Nat Methods*. 2012 Mar;9 (4):357–359. <https://doi.org/10.1038/nmeth.1923>
- Langmead B, Wilks C, Antonescu V, Charles R.** Scaling read aligners to hundreds of threads on general-purpose processors. *Bioinformatics*. 2019 Feb;35 (3):421–432. <https://doi.org/10.1093/bioinformatics/bty648>
- Li D, Luo R, Liu CM, Leung CM, Ting HF, Sadakane K, Yamashita H, Lam TW.** MEGAHIT v1.0: A fast and scalable metagenome assembler driven by advanced methodologies and community practices. *Methods*. 2016 Jun;102:3–11. <https://doi.org/10.1016/j.ymeth.2016.02.020>
- Liu J, Yang X, Hao X, Feng Y, Zhang Y, Cheng Z.** Effect of goose parvovirus and duck circovirus coinfection in ducks. *J Vet Res*. 2020 Jul;64 (3):355–361. <https://doi.org/10.2478/jvetres-2020-0048>
- Martin DP, Murrell B, Golden M, Khoosal A, Muhire B.** RDP4: Detection and analysis of recombination patterns in virus genomes. *Virus Evol*. 2015 May;1 (1):vev003. <https://doi.org/10.1093/ve/vev003>
- Meng G, Zhang X, Plevka P, Yu Q, Tijssen P, Rossmann MG.** The structure and host entry of an invertebrate parvovirus. *J Virol*. 2013 Dec;87 (23):12523–12530. <https://doi.org/10.1128/jvi.01822-13>
- Mietzsch M, McKenna R, Väistönen E, Yu JC, Ilyas M, Hull JA, Kurian J, Smith JK, Chipman P, Lasanajak Y, et al.** Structural characterization of cuta- and tusavirus: Insight into protoparvoviruses capsid morphology. *Viruses*. 2020 Jun;12 (6):653. <https://doi.org/10.3390/v12060653>
- Mirdita M, Schütze K, Moriawaki Y, Heo L, Ovchinnikov S, Steinberger M.** ColabFold: Making protein folding accessible to all. *Nat Methods*. 2022 Jun;19 (6):679–682. <https://doi.org/10.1038/s41592-022-01488-1>
- Muhire BM, Varsani A, Martin DP.** SDT: A virus classification tool based on pairwise sequence alignment and identity calculation. *PLoS One*. 2014 Sep;9 (9):e108277. <https://doi.org/10.1371/journal.pone.0108277>
- Ndiana LA, Lanave G, Desario C, Berjaoui S, Alfano F, Puglia I, Fusco G, Colaianni ML, Vincifori G, Camarda A, et al.** Circulation of diverse protoparvoviruses in wild carnivores, Italy. *Transbound Emerg Dis*. 2021 Jul;68 (4):2489–2502. <https://doi.org/10.1111/tbed.13917>
- Pérez JJ, Söderlund-Venermo M, Canuti M, Eis-Hübler AM, Hughes J, Cotmore SF, Harrach B.** Reorganizing the family *Parvoviridae*: A revised taxonomy independent of the canonical approach based on host association. *Arch Virol*. 2020 Sep;165 (9):2133–2146. <https://doi.org/10.1007/s00705-020-04632-4>
- Phan TG, Vo NP, Bonkoungou IJ, Kapoor A, Barro N, O’Ryan M, Kapusinszky B, Wang C, Delwart E.** Acute diarrhea in West African children: Diverse enteric viruses and a novel parvovirus genus. *J Virol*. 2012 Oct;86 (20):11024–11030. <https://doi.org/10.1128/jvi.01427-12>

- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst Biol.* 2012 May;61 (3):539–542.  
<https://doi.org/10.1093/sysbio/sys029>
- Ros C, Bayat N, Wolfisberg R, Almendral JM. Protoparvovirus cell entry. *Viruses.* 2017 Oct;9(11):313.  
<https://doi.org/10.3390/v9110313>
- Schmieder R, Edwards R. Quality control and preprocessing of metagenomic datasets. *Bioinformatics.* 2011 Mar;27 (6):863–864.  
<https://doi.org/10.1093/bioinformatics/btr026>
- Stilwell JM, Anis E, Wilkes RP, Rissi DR. Dual infection with an emergent strain of canine distemper virus and canine parvovirus in an Arctic wolf under managed care. *J Vet Diagn Invest.* 2019 Jul; 31 (4):594–597. <https://doi.org/10.1177/1040638719851832>
- Tuteja D, Banu K, Mondal B. Canine parvovirology – A brief updated review on structural biology, occurrence, pathogenesis, clinical diagnosis, treatment and prevention. *Comp Immunol Microbiol Infect Dis.* 2022 Feb;82:101765.  
<https://doi.org/10.1016/j.cimid.2022.101765>
- Vargas-Bermudez DS, Mogollon JD, Franco-Rodriguez C, Jaime J. The novel porcine parvoviruses: Current state of knowledge and their possible implications in clinical syndromes in pigs. *Viruses.* 2023 Dec;15 (12):2398. <https://doi.org/10.3390/v15122398>
- Wang Y, Yang S, Liu D, Zhou C, Li W, Lin Y, Wang X, Shen Q, Wang H, Li C, et al. The fecal virome of red-crowned cranes. *Arch Virol.* 2019 Jan;164 (1):3–16.  
<https://doi.org/10.1007/s00705-018-4037-x>
- Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R, Heer FT, de Beer TAP, Rempfer C, Bordoli L, et al. SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic Acids Res.* 2018 Jul 2;46(W1):W296-W303.  
<https://doi.org/10.1093/nar/gky427>
- Yeo YG, Kim HR, Park J, Kim JM, Shin YK, Lee KK, Kwon OK, Jeoung HY, Kang HE, Ku BK, et al. Epidemiological and molecular approaches for a fatal feline panleukopenia virus infection of captive Siberian tigers (*Panthera tigris altaica*) in the Republic of Korea. *Animals.* 2023 Sep;13 (18):2991.  
<https://doi.org/10.3390/ani13182991>
- Yeşil Y, Çelik M, Yılmaz B. Wild edible plants in Yeşilli (Mardin-Turkey), a multicultural area. *J Ethnobiol Ethnomed.* 2019 Nov;15 (1):52. <https://doi.org/10.1186/s13002-019-0327-y>
- Zhang P, Su H, Peng R, Chan JF, Bai S, Wang G, Huang Y, Hu X, Luo J, Liu S, et al. Identification of a novel astrovirus in pinnipeds. *Front Microbiol.* 2022 May;13:845601.  
<https://doi.org/10.3389/fmicb.2022.845601>

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