Identification of a novel parvovirus in the Arctic wolf (Canis lupus)

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Abstract

Through the utilization of a viral metagenomic approach, a novel virus has been found in a pharyngeal metagenomic library derived from an Arctic wolf (*Canis lupus*). This virus has been temporarily designated as AWPV and assigned a GenBank accession number BK063423. The genome of AWPV is comprised of 4,920 base pairs, and its nucleotide composition is composed of 36.4% A, 23.4% T, 18.2% G, and 22.0% C, with a GC content of 40.2%. The viral genome demonstrates a typical pattern of parvovirus organization, with two predicted ORFs: ORF1, which encodes non-structural proteins NS1 and NS2, and ORF2, which encodes VP1 and VP2. By performing a pairwise sequence comparison and a phylogenetic analysis based on the NS1 and VP1 protein sequences, it has been suggested that AWPV may represent a novel species within the genus *Protoparvovirus*. This discovery of a novel parvovirus has enhanced our comprehension of the mammalian virus ecology and has facilitated an improved understanding of potential future infectious diseases.

Introduction

The family *Parvoviridae* comprises round, non-enveloped viruses that have linear, single-stranded DNA genomes ranging from 4–6 kb(1), and they have been detected in nearly all major groups of vertebrates, as well as in both proto- and deuterostome invertebrates(2). In 1975, the family *Parvoviridae* was established, and in 1993 it was divided into two subfamilies, *Parvovirinae* and *Densovirinae*, to classify viruses that infect either vertebrate or invertebrate hosts(1). As of now, according to the classification principles of the International Committee for the Taxonomy of Viruses (ICTV), the family *Parvoviridae* comprises 3 subfamilies, 28 genera, and 175 species. Reports of parvoviruses have surfaced in numerous countries and have affected a wide range of hosts, including mammals such as humans(3), mice(4), canines(5), and chimpanzees(6), as well as arthropods such as crickets(7), and birds such as ducks(8), red-crowned cranes(9), and pigeons(10).

Parvoviral genomes are characterized by long inverted terminal repeats (LTRs) located at both the 5' and 3' ends, which can adopt hairpin-like structures and play a role in the viruses' expression and transcription strategies(11). They encode two open reading frames (ORFs), where ORF1 encodes non-structural proteins NS1 and NS2, and ORF2 encodes structural proteins VP1 and VP2(12). Parvoviruses can be classified as belonging to the same species if their NS1 proteins have a shared amino acid sequence identity of over 85%. 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 a coverage of over 80% between any two members(2).

At present, there are 18 species within the genus *Protoparvovirus*, consisting of 15 officially recognized species and three that are currently proposed(13). Protoparvovirus carnivoran2 (CPV-2), belonging to the genus *Protoparvovirus* of the family *Parvoviridae*, is a highly contagious viral pathogen that primarily affects canids, particularly dogs. It is characterized by causing severe gastroente ric disease in its hosts, including symptoms such as vomiting, diarrhea, loss of appetite, and dehydration(5, 14). CPV-2 has a

wide range of hosts, including dogs, foxes, and wolves, and is mainly transmitted through the fecal-oral route(15). The CPV-2 first invades the pharynx and then enters the bloodstream within a few days of infection, reaching the intestines and bone marrow, causing severe leukopenia and may also cause viremia, which may subsequently lead to myocarditis(15, 16).

Here, we present the genome characterization of a novel Arctic wolf parvovirus (named AWPV) identified from an Arctic wolf pharyngeal metagenomic library. 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*.

Materials and Methods

Metagenome assembly

While studying potential pathogenic viruses in mammals, an available library - SRR12366691 - was downloaded from the SRA database. This library was uploaded by Du et al. from Hainan Medical University and corresponds to the host Arctic wolf, collected from an aquarium in Xi'an, China. The method for processing the samples has been described in the previous(17). 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(18, 19). 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). Afterwards, the resultant files were subjected to quality control using the options '–phred33 –length 35 –stringency 3 –fastqc'. PRINSEQ-lite v0.20.4 (-derep 1)(20) was employed to mark duplicated reads. An in-house pipeline was utilized to assemble this library. The assembly of single-end reads was carried out using MEGAHIT v1.2.9(21) with default parameters. Contigs with a sequence length greater than 1,500 bp were kept after the assembly process. Following the aforementioned steps, the outcomes were imported into Geneious Prime v2022.0.1(22) to be sorted and confirmed manually.

Search for novel vertebrate-associated viruses

The contigs were aligned with the non-redundant protein (nr) database (downloaded in February 2023) utilizing the BLASTx program built in DIAMOND v2.0.15(23), with a cut-off E-value of $< 10^{-5}$. The taxonomic identification was carried out using the built-in rma2info program in MEGAN6(24), and the viruses of interest were filtered out from the results. Geneious Prime was used to predict putative open reading frames (ORFs) using built-in parameters (Minimum size: 400) (22). These predictions were subsequently validated by comparing them to ORFs found in related viruses. Comparisons to the Conserved Domain Database (CDD) were used to annotate these ORFs. Finally, we obtained a putative novel parvovirus with complete genome organization structure.

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(25) and MAFFT v7.3.1, which employed the E-INS-I algorithm(26). MrBayes v3.2(27) 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(25) software to verify all Bayesian inference trees. The Sequence Demarcation Tool v1.2(28) 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(28).

Prediction of potential genome recombination events

The Recombination Detection Program v4.39 (RDP4) software was used to analyze genomic alignments of both reference strains and the AWPV strain. This was done through various algorithms including RDP, GENECONV, Chimaera, MaxChi, BootScan, and SiScan, in order to detect possible recombination events(29).

Prediction of spatial structure

The three-dimensional structure of the viral structural protein identified in this study was predicted using ColabFold(30), and SWISS-MODEL(30) was employed to compare and screen models that possess comparable spatial structures from the PDB database. To visualize the results, PyMOL v2.0 (www.pymol.org) was utilized.

Data availability

The novel parvovirus sequence obtained in this study have been deposited in GenBank database under accession number BK063423.

Results

Overview of the pharyngeal metagenomic library of the Arctic Wolf

This library was sequenced using the Illumina HiSeq 2500 platform, generating 28,946,890 raw reads. Following quality control, 28,945,811 clean reads were obtained, which were then 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 (Supplementary Table 1). 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%), and *Herelleviridae* (3.08%) (Supplementary Table 1). However, the vast majority of them are already known. 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. In order to determine whether the host of this library is the Arctic wolf, we performed a BLASTx search by comparing the contigs assembled from the NGS data of this library (specifically those containing potential mammalian sequence reads) against the complete mitochondrial proteome database downloaded from GenBank. The search results revealed that the majority of sequences among the mammalian matches corresponded to the Arctic wolf (Supplementary Table 2).

Identification of a novel parvovirus

In this research, a virus named AWPV, belonging to the family *Parvoviridae* and possessing a complete genome organization structure, was obtained using the sequence assembly tool of Geneious Prime. AWPV has a genome size of 4,920 bp, characterized by a GC content of 40.2% and a nucleotide composition consisting of 36.4% A, 23.4% T, 18.2% G, and 22.0% 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}GPASTGKS^{415} [GXXXXGK(T/S)], a Walker B loop at ^{448}IIWVEE^{453} (xxxxEE), and a Walker B' loop at ^{465}KAICSGQSIRIDQK^{478} (KxxxxGxxxxxxxK). In addition, we identified a conserved replication initiator motif ^{132}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_2 (PLA2) motif was identified, which contains the expected calcium-binding (YLGPG) site and catalytic residues (Fig. 1A).

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(31) and foxes(13, 32), 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 Supplementary Table 3). 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 its survival in the natural environment and entry into host cells(33). 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(34), 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 (Supplementary Fig. 1).

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(35). 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 of them are related to CPV-2. For example, Justin M. 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(36). 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 that was found stranded and deceased in 2010(31). The Newlaviruses were detected and identified in the carcasses of Italian foxes(32). Due to the possibility of this Arctic wolf being captive, we cautiously speculate that AWPV may also have originated from interactions with other captive animals. Further research was deemed necessary to investigate the epidemiology and transmission dynamics of AWPV in both captive and wild Arctic wolf populations, in order to gain a comprehensive understanding of this virus and its potential impact on the health of these animals. The vast temporal and geographic range of both wild and captive hosts raises questions as to whether other, as-yet-undiscovered intermediate hosts may be carrying these viruses. While the role of these newly identified viruses in host morbidity and mortality remains uncertain, it is clear that members of the family *Parvoviridae* have achieved a worldwide distribution.

In summary, the identification of AWPV increases our understanding of the diversity of canine parvoviruses and provides limited assistance in improving virus taxonomy. While there is no direct evidence to suggest whether AWPV will have significant negative effects on its host, further dynamic monitoring of the virus is needed in the future.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Written informed consent for publication was obtained from all participants.

Availability of data and materials

The novel parvovirus sequence obtained in this study have been deposited in GenBank database under accession number BK063423.

Competing interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Authors' contributions

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

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Not applicable.

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Figures

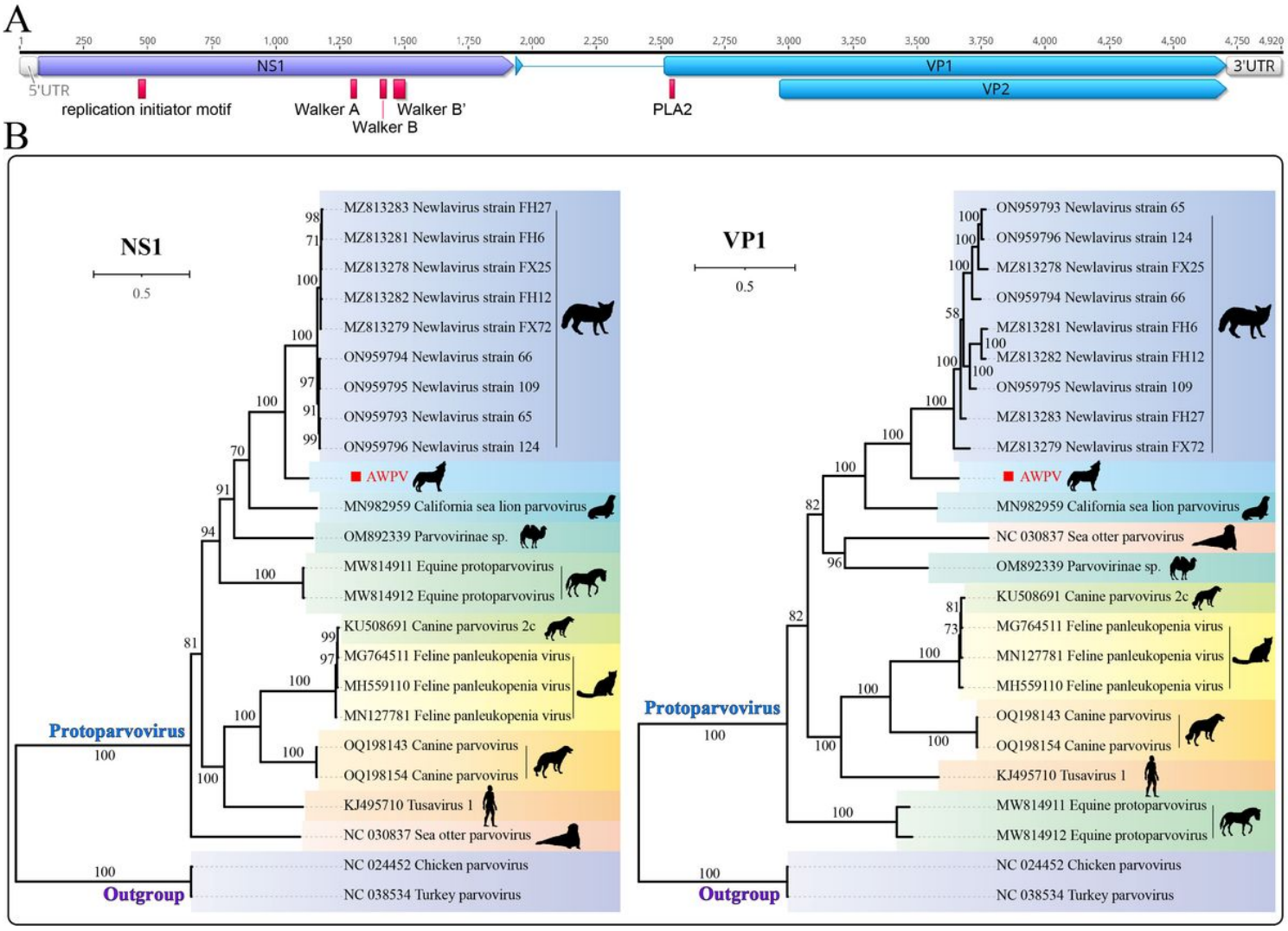

Figure 1

Genome and Phylogenetic Analysis of a novel parvovirus. **(A)** 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.

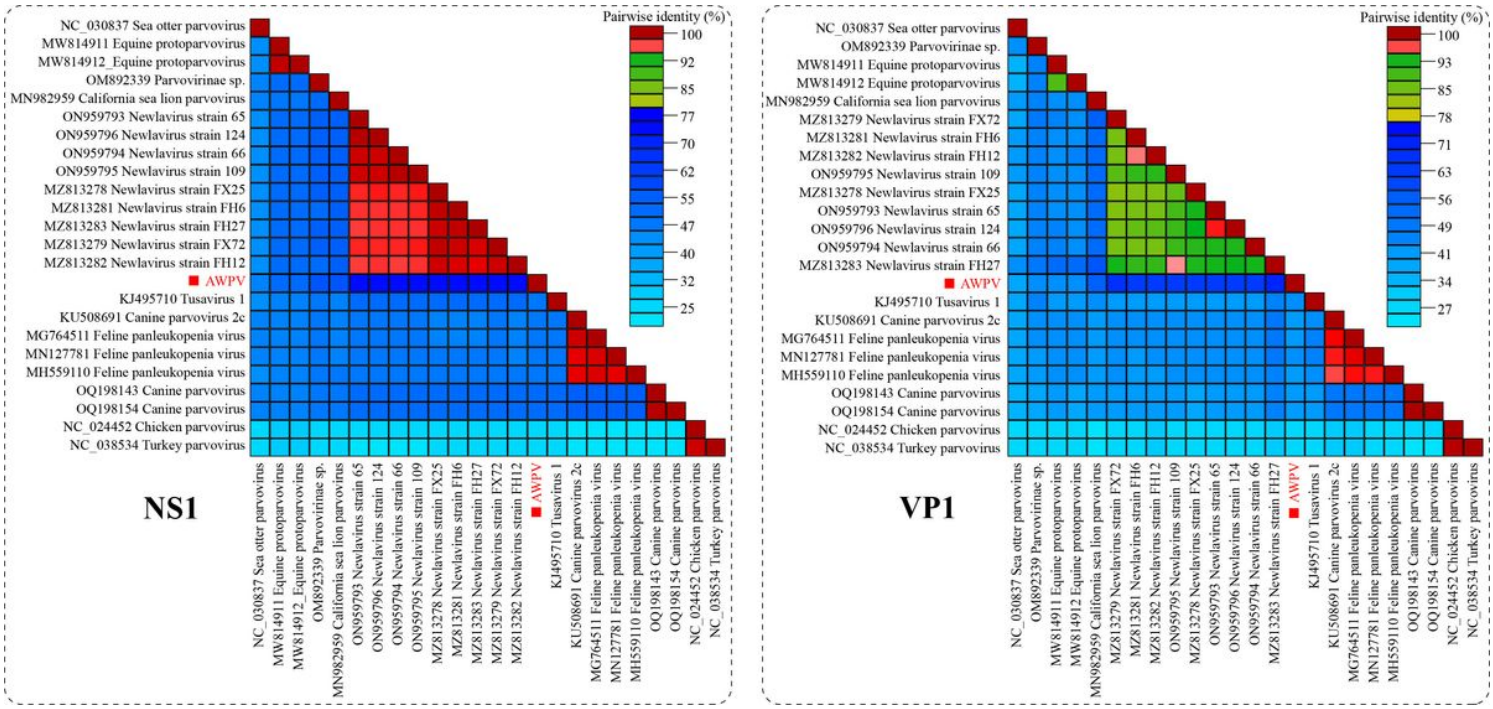

Figure 2

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

Supplementary Files

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- SupplementaryTable2.xlsx
- SupplementaryTable3.xlsx