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Research Article

Serum Virome of Southern Beaufort Sea polar bears (*Ursus maritimus*) during a period of rapid climate change

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Climate change affects the behavior, physiology and life history of many Arctic wildlife species. It can also influence the distribution and ecology of infectious agents. The southern Beaufort Sea (SB) subpopulation of polar bears (*Ursus maritimus*) has experienced dramatic behavioral changes due to retreating sea ice and other climate-related factors, but the effects of these changes on physiology and infection remain poorly understood. Using serum from polar bears sampled between 2004 and 2015 and metagenomic DNA sequencing, we identified 48 viruses, all of the family *Anelloviridae*. Anelloviruses are small, ubiquitous infectious agents with circular single-stranded DNA genomes that are not known to cause disease but, in humans, covary in diversity and load with immunological compromise. We therefore examined the usefulness of anelloviruses as biomarkers of polar bear physiological stress related to climate and habitat use. Polar bear anelloviruses sorted into two distinct clades on a phylogenetic tree, both of which also contained anelloviruses of giant pandas (*Ailuropoda melanoleuca*), another ursid. Neither anellovirus diversity nor load were associated with any demographic variables, behavioral factors or direct physiological measures. However, pairwise genetic distances between anelloviruses were positively correlated with pairwise differences in sampling date, suggesting that the polar bear "anellome" is evolving over time. These findings suggest that anelloviruses are not a sensitive indicator of polar physiological stress, but they do provide a baseline for evaluating future changes to polar bear viromes.

Key words: Anellovirus, climate change, Polar bear, virus, wildlife health

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Introduction

Host-associated microbiota, including bacteria, fungi, protists and viruses, play an important role in health by influencing physiological processes. In some cases, microbiota composition can alter susceptibility to infectious disease through the presence or absence of specific microorganisms (Hernández-Gómez, 2020), yet baseline characterization of wildlife microbiomes is often lacking (Smith *et al.,* 2009; Stephen *et al.,* 2019; Watson *et al.,* 2019). Like other microbiota, viruses may be mutualistic, commensal or parasitic (Trevelline *et al.,* 2019; Plyusnin *et al.,* 2020; Zhu *et al.,* 2021); however, viruses are more likely than other classes of microbes to emerge and cause epidemics in wildlife populations (Dobson and Foufopoulos, 2001). Climate change is affecting viral disease emergence risk through the behavior and physiology of wildlife hosts and vectors (Caminade *et al.,* 2019; Baker *et al.,* 2022; Carlson *et al.,* 2022). These effects are particularly important for threatened or endangered wildlife populations (Le Roux and McGeoch, 2008; Moore and Huntington, 2008; Thomas, 2010).

The Arctic is experiencing the effects of global warming at a significantly faster rate than other regions of the world (Cohen *et al.,* 2014; IPCC, 2018; DeRepentigny *et al.,* 2020). The warming climate has led to changes in the behavior and life history for many Arctic wildlife, including polar bears (*Ursus maritimus*). For the southern Beaufort Sea (SB) subpopulation of polar bears, loss of sea ice habitat has been associated with changes in abundance (Bromaghin *et al.,* 2015, 2021), recruitment (Rode *et al.,* 2010), habitat use (Atwood *et al.,* 2016b; Rode *et al.,* 2022), physiology (Pagano *et al.*, 2020; Fry *et al.,* 2019, 2023), diet and toxicant load (Atwood *et al.,* 2016a; McKinney *et al.,* 2017; Bourque *et al.,* 2018; Watson *et al.,* 2021) and bacterial microbiome diversity (Watson *et al.,* 2019). Yet, little is known about the pathogens of polar bears. A review of infectious agents in polar bears reported exposure to viral pathogens but did not look for active infection (Fagre *et al.,* 2015). Serological studies have revealed exposure of wild polar bears to canine morbillivirus (Philippa *et al.,* 2004), phocine morbillivirus and dolphin morbillivirus (Cattet *et al.,* 2004; Philippa *et al.,* 2004; Kirk *et al.,* 2010), calicivirus (Tryland *et al.,* 2005), dolphin rhabdovirus (Philippa *et al.*, 2004), canine adenovirus (Philippa *et al.,* 2004) and a single report of rabies virus (Taylor *et al.,* 1991), but no viral DNA was identified from wild polar bears. Viruses have been identified in captive polar bears either serologically or through health effects and pathology, including West Nile Virus (Dutton *et al.*, 2009) and herpesviruses (e.g. equine herpesvirus-1, equine herpesvirus-9, suid herpes virus-1) (Greenwood *et al.,* 2012).

A 14-year epizootic of alopecia syndrome in SB polar bears described by Atwood *et al.* (2015), led to a broad investigation into its etiology that included the analysis of skin, feces, nasal, oral and rectal swabs, as well as pathogens in blood (USGS unpublished data.). Next-generation sequencing to look for viruses in affected polar bears revealed no causative viruses for the alopecia syndrome but did reveal the presence of viruses of the family *Anelloviridae* in the serum of polar bears. Anelloviruses are small (1.6–3.9 kb), single-stranded circular DNA viruses with two main open reading frames (ORFs), with the largest, ORF 1, encoding the capsid protein (Varsani *et al.,* 2021). These small, highly genetically diverse viruses appear to be commensal and omnipresent in humans (Kaczorowska and van der Hoek, 2020; Arze *et al.,* 2021). Human anelloviruses (also known as "torque teno viruses," or TTVs) infect healthy individuals, occur at high prevalence and may be the most abundant eukaryotic virus in the human virome (Virgin *et al.,* 2009). Anelloviruses have also been identified in wildlife species, including non-human primates (Romeo *et al.,* 2000), a number of felids (Kraberger *et al.,* 2021), palm civets (*Paguma larvata;* Nishizawa *et al.,* 2018), bats, rodents, marsupials (de Souza *et al.,* 2018) and marine mammals, such as Pacific harbor seals (*Phoca vitulina richardsii*; Ng *et al.,* 2011), fur seals (*Arctocephalus gazella*; Crane *et al.,* 2018), California sea lions *(Zalophus californianus;* Ng *et al.,* 2009), Weddell seals (*Leptonychotes weddellii)* and Risso's dolphins (*Grampus griseus;* Fahsbender *et al.,* 2017). In nearly every described instance, individuals appear to be infected with multiple anelloviruses. See Varsani *et al.* (2021) for a complete list of known mammalian hosts. Anelloviruses have not been shown to cause disease. In humans, anelloviruses appear to be an infectious biomarker of immunological function, with diversity and load increasing with immune system suppression (Thom and Petrik, 2007; Spandole *et al.,* 2015), although the mechanisms for infection and replication remain unknown because no cell culture system nor animal model has been identified (Nasser *et al.,* 2009; Kaczorowska and van der Hoek, 2020).

Our goal was to characterize the serum virome, including anellovirus diversity and load, of SB polar bears, using samples collected over 11 years with measurable climate driven changes in the Arctic. We were especially interested in evaluating whether infection and viral load covaried with demographic and physiologic factors, including blood-based measures of immune function and physiological stress, as well as habitat use driven by climate change. We hypothesized that viral richness and viral load would increase in polar bears using on-shore summer habitats. If viruses, specifically anelloviruses, covaried with demographic, physiological or behavioral factors, they could represent a novel ecoimmunological tool for monitoring polar bear populations for immunological "health."

Methods

We examined serum samples from 24 unique polar bears collected as part of ongoing population monitoring studies by the US Geological Survey (Table 1). Polar bears were captured on land and on the sea ice of Alaska's southern Beaufort Sea (USA) from 2004 to 2015 (Figure 1). Briefly, helicopters were used to locate polar bears, which were chemically

Table 1: Summary of demography, anellovirus richness and total anellovirus load for 24 polar bears from the Southern Beaufort Sea Subpopulation

ID	Polar Bear	Capture Year	Sex	Age (years)	Summer Habitat Use	Anellovirus Richness	Total Anellovirus load (log_{10} vRPM/kb)
1	20 725	2004	M	19	On-shore	25	0.98
2	21 214	2013	F	3	On-shore	28	0.54
3	20 562	2009	F	22	On-shore	34	0.87
4	21 256	2013	M	6	On-shore	35	1.18
5	21 253	2014	F	4	Off-shore	19	0.17
6	21 335	2014	M	3	On-shore	5	0.06
7	22 302	2015	F	17	Off-shore	3	0.02
8	22 308	2015	F	2	Off-shore	27	0.54
9	20 300	2013	M	23	On-shore	22	0.39
10	20 733	2006	M	4	On-shore	14	0.12
11	20 772	2005	M	8	On-shore	19	0.43
12	20 859	2006	F	3	On-shore	13	0.45
13	20 988	2009	M	11	Off-shore	20	0.44
14	21 231	2011	M	10	Off-shore	12	0.08
15	21 249	2011	F	1	On-shore	0	0.00
16	21 347	2013	F	5	Off-shore	22	0.92
17	6336	2005	F	23	On-shore	34	1.49
18	6836	2005	F	16	Off-shore	7	0.10
19	20 125	2010	M	10	On-shore	37	1.21
20	20 710	2004	M	4	Off-shore	23	0.99
21	20 730	2004	F	5	On-shore	6	0.13
22	21 023	2009	M	1	On-shore	38	2.01
23	21 153	2011	M	2	On-shore	25	0.56
24	21 219	2010	F	14	On-shore	25	1.02

immobilized. Physical examination found the bears to be free of obvious signs of injury or disease. They were then weighed (kg), marked and age was determined using visual measures and dental analyses (see Atwood *et al.*, 2016b). Blood samples were collected by venipuncture of the femoral vein evacuated into plain tubes (Vacutainer; BD Biosciences, Franklin Lanes, NJ) and stored to prevent freezing. Serum was separated by centrifugation at 1500×*g* for 5 min (TRIAC, Clay Adams, Parsippany, NJ), frozen at −20°C immediately, then transferred to −80°C for long term storage. All animal research was conducted under appropriate permits, including animal care and use approvals (Marine Mammal Research Permit MA690038-17 and USGS multi-year IACUC approvals up to 2014–17).

We identified viruses in the serum of polar bears following previously described methods (Sibley *et al.*, 2016; Bennett *et al.*, 2020; Campbell *et al.*, 2022). Briefly, we centrifuged polar bear serum for 10 minutes at 10,000×*g* to pellet cellular debris, and total nucleic acids were extracted from 200 μl of supernatant using the QIAmp MinElute Virus Spin Kit (Qiagen, Hilden, Germany). We used the Superscript IV system (Thermo Fisher, Waltham, MA) with random hexamers to reverse transcribe RNA to cDNA, and prepared cDNA libraries using the Nextera XT DNA sample preparation kit (Illumina, San Diego, CA, USA). We sequenced libraries on a MiSeq instrument using 150 × 150 cycle V2 paired-end sequencing chemistry (Illumina), and sequencing adapters were removed from the resulting reads by on-board Illumina processing software.

We analysed viral sequences using CLC Genomics Workbench v. 20.0.4 (QIAGEN, Aarhus, Denmark) trimming low-quality bases (Phred quality score < 30), discarding short reads (<75 bp) and subjecting the remaining reads to *de novo* assembly using the CLC assembler with automatic word and

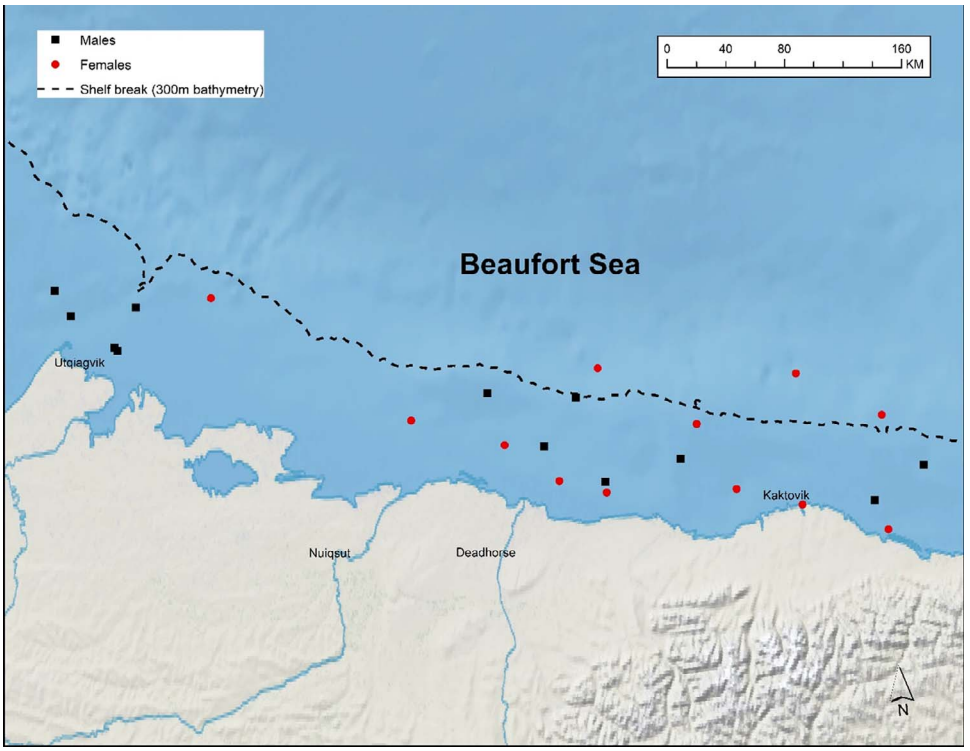

Figure 1: Study area and capture location of the 24 polar bears included in this study from the Southern Beaufort Sea subpopulation in northern Alaska, 2004–2015.

bubble size selection and a minimum contiguous sequence (contig) length of 500. We analysed contigs for nucleotide- (blastn) and protein-level (blastx) similarity to known viruses in GenBank. For blastx, we applied the BLASTX algorithm with the BLOSUM62 matrix to sequences translated into all 6 frames. We analysed all sequence data at the individual read level by mapping reads to viruses in the GenBank database using the CLC mapping tool at low stringency (length fraction of 0.5, similarity fraction of 0.6). We disregarded contigs matching viruses of known non-mammalian hosts (e.g. bacteria, invertebrates, plants, fungi), then mapped reads back to viral contigs to calculate the proportion of reads mapping to each virus (for virus-specific load) or the proportion of reads mapping to any virus (for total viral load). We normalized these measures for sequencing depth and target sequence length, expressing viral loads as $\log_{10}$ viral reads per million per kilobase of target ($\log_{10}$ vRPM/kb), which has been shown to correlate with quantitative real-time polymerase chain reaction data (Toohey-Kurth *et al.*, 2017).

Phylogenetic relationships among viruses were inferred from ORF 1 nucleotide sequences. We first aligned sequences of newly identified viruses with published sequences of related viruses in the GenBank database using the Prank algorithm (Löytynoja, 2014) in TranslatorX (Abascal *et al.*, 2010), with the Gblocks algorithm (Castresana, 2000) to remove poorly aligned regions. We inferred maximum-likelihood phylogenetic trees from the alignments using PhyML 3.0 with smart model selection (Lefort *et al.*, 2017) and 1000 bootstrap replicates to assess statistical confidence in clades. We used Figtree v. 1.4.4 to display final phylogenetic tree (Rambaut, 2018).

We assessed whether viral richness (number of viruses in each bear) and total viral load ($\log_{10}$vRPM/kb) were related to demographic characteristics (sex, age (years), age∗sex and capture year) and physiologic biomarkers of immune function. We included two markers of immune function: globulin, a protein that bridges the adaptive and innate immune response and leukocyte count, a measure of the innate immune response. Globulin levels were generated from the comprehensive diagnostic panel for the VetScan VS2 biochemistry analyser (Abaxis, Union City, California) and leukocytes were counted using an HM5 bioanalyser (Abaxis, Union City, CA). see Fry *et al.*, 2019 for complete methods). We included hair cortisol levels as a measure of chronic stress (Meyer and Novak, 2012; Manenschijn *et al.*, 2013; Karlén *et al.*, 2015) (see Van der Walt *et al.*, 2021 for cortisol methods).

We assessed the effect of climate driven changes in summer habitat use by polar bears on virus richness and total viral load. Some SB polar bears opportunistically scavenge bowhead whale (*Balaena mysticetus*) carcasses left by subsistence hunters in summer and fall (Herreman and Peacock, 2013; Rogers *et al.*, 2015). Bears with $>5\%$ bowhead whale in

Table 2: ORF1 characteristic of viruses identified in serum samples from 24 Southern Beaufort Sea polar bears

Virus ID	Year Detected	Size (nt)[a]	Closest nt match accession[b]	Closest nt match taxon(year)[c]	% nt ID to closest match[e]	Clade	GenBank accession number
PbV-1	2004	1653	ASH99133	Gpan20684 (2017)	50.28	B	OP970915
PbV-2	2004	2064	ASH99133	Gpan20684(2017)	53.64	B	OP970916
PbV-3	2004	1872	ASH99133	Gpan20684(2017)	57.98	B	OP970917
PbV-4	2004	2031	ASH99133	Gpan20684(2017)	60.00	B	OP970918
PbV-5	2006	1431	ASH99109	Gpan20681(2017)	41.63	A	OP970919
PbV-6	2005	1464	ASH99109	Gpan20681(2017)	44.04	A	OP970920
PbV-7	2004	1404	ASH99085	Gpan20859(2017)	38.42	A	OP970921
PbV-8	2005	1398	ASH99079	Gpan21094(2017)	43.36	A	OP970922
PbV-9	2004	1626	ASH99133	Gpan20684(2017)	42.56	B	OP970923
PbV-10	2009	1269	ASH99085	Gpan20859(2017)	36.46	A	OP970924
PbV-11	2004	1413	ASH99079	Gpan21094(2017)	39.95	A	OP970925
PbV-12	2004	2298	ASH99133	Gpan20684(2017)	56.11	B	OP970926
PbV-13	2006	930	ASH99085	Gpan20859(2017)	46.05	A	OP970927
PbV-14	2005	1509	ASH99079	Gpan21094(2017)	39.82	A	OP970928
PbV-15	2004	2061	ASH99106	Gpan21094(2017)	59.96	B	OP970929
PbV-16	2004	1485	ASH99133	Gpan20684(2017)	44.03	A	OP970930
PbV-17	2004	1485	ASH99109	Gpan20681(2017)	54.30	B	OP970931
PbV-18	2004	1494	ASH99109	Gpan20681(2017)	36.04	A	OP970932
PbV-19	2004	1416	ASH99109	Gpan20681(2017)	41.33	A	OP970933
PbV-20	2005	1416	ASH99109	Gpan20681(2017)	38.20	A	OP970934
PbV-21	2005	1407	ASH99085	Gpan20859(2017)	39.25	A	OP970935
PbV-22	2004	1416	ASH99109	Gpan20681(2017)	40.15	A	OP970936
PbV-23	2004	2133	ASH99133	Gpan20684(2017)	54.54	B	OP970937
PbV-24	2004	2091	ASH99133	Gpan20684(2017)	56.32	B	OP970938
PbV-25	2004	1455	ASH99079	Gpan21094(2017)	38.24	A	OP970939
PbV-26	2004	2133	ASH99133	Gpan20684(2017)	54.44	B	OP970940
PbV-27	2004	1413	ASH99133	Gpan20684(2017)	38.18	A	OP970941
PbV-28	2004	1416	ASH99109	Gpan20681(2017)	41.61	A	OP970942
PbV-29	2004	1416	ASH99079	Gpan21094(2017)	41.40	A	OP970943
PbV-30	2004	1425	ASH99085	Gpan20859(2017)	38.78	A	OP970944
PbV-31	2004	1374	ASH99079	Gpan21094(2017)	53.76	B	OP970945
PbV-32	2004	771	ASH99109	Gpan20681(2017)	44.91	A	OP970946
PbV-33	2004	1566	ASH99133	Gpan20684(2017)	58.79	B	OP970947
PbV-34	2004	2067	ASH99133	Gpan20684(2017)	55.68	B	OP970948
PbV-35	2004	1569	QZE11967	Gpb08AV03–5(2022)	45.38	B	OP970949
PbV-36	2004	1443	ASH99106	Gpan21066(2017)	37.33	A	OP970950
PbV-37	2004	2064	ASH99133	Gpan20684(2017)	55.09	B	OP970951
PbV-38	2004	2133	ASH99133	Gpan20684(2017)	53.74	B	OP970952

Continued

Table 2: Continued

Virus ID	Year Detected	Size (nt)[a]	Closest nt match accession[b]	Closest nt match taxon(year)[c]	% nt ID to closest match[e]	Clade	GenBank accession number
PbV-39	2004	1410	ASH99085	Gpan20859(2017)	36.29	A	OP970953
PbV-40	2004	2094	ASH99133	Gpan20684(2017)	55.65	B	OP970954
PbV-41	2009	1323	YP_009505746	Tbc-TTV14(2001) [d]	29.21	B	OP970955
PbV-42	2004	1467	ASH99079	Gpan21094(2017)	45.89	A	OP970956
PbV-43	2005	1476	ASH99106	Gpan21066(2017)	41.82	A	OP970957
PbV-44	2004	1410	ASH99133	Gpan20684(2017)	57.79	B	OP970958
PbV-45	2006	1413	ASH99079	Gpan21094(2017)	41.50	A	OP970959
PbV-46	2004	2007	ASH99133	Gpan20684(2017)	59.58	B	OP970960
PbV-47	2009	1545	QZE11973	Gpb08AV05–5(2022)	39.90	B	OP970961
PbV-48	2004	1005	ASH99106	Gpan21066(2017)	39.62	A	OP970962

[a]Length refers to the length of the nucleotide (nt) sequence for ORF1, used for phylogenetic and viral load analyses; [b]GenBank accession number of closest match using BLASTx is shown [c]all Gp reference viruses are giant panda from China in (year), except [d]Tupasis, Japan, 2001, [e] % identity refers to percent nucleotide identity of ORF1 to the closest match in GenBank.

their diet are considered to be using on-shore summer habitat (Atwood *et al.*, 2017; McKinney *et al.*, 2017). Using on-shore habitat versus sea ice habitat leads to increased risks associated with contact with humans, other polar bears and other wildlife. These behaviors have been shown to affect exposure to toxicant load and bacterial pathogens; and therefore, may influence viral richness and load (Atwood *et al.*, 2016b, 2017; McKinney *et al.*, 2017; Bourque *et al.*, 2018).

We examined these relationships individually with generalized linear models using the Wald method to test for significance at an alpha level of 0.05. For viral presence/absence we conducted logistic regression. Regression analyses were conducted using base R 4.2.1 (R Core Team, 2021). To examine possible genetic changes in anelloviruses over time, we computed pairwise patristic distances between ORF 1 nucleotide sequences and compared them to pairwise differences in the year of anellovirus detection using the Mantel tests of matrix correlation (Mantel, 1967) with 10 000 permutations using the APE package in R (Paradis *et al.*, 2004).

Results

In the serum of 24 polar bears (Table 1), we identified 48 distinct anelloviruses (Table 2, Supplement A) and no other viruses associated with eukaryotic hosts. All polar bear anelloviruses identified shared the typical genome architecture for this type of virus (Supplementary Figure 1A). Amino acid similarity of ORF 1 to known viruses was low, as expected from published results (Varsani *et al.*, 2021) and ranged from 29.21%—60.00%. (Table 2). A maximum likelihood phylogenetic tree based on ORF 1 nucleotide sequences (final alignment = 2163 positions) of newly discovered anelloviruses (n = 48) and the closest BLAST matches in Genbank (reference sequences, n = 8) consisted of two clades. Clade A is comprised of 26 polar bear anelloviruses and clade B included 22 polar bear anelloviruses (Figure 2). In all cases, sequences clustered most closely with viruses from the same host species indicating that the newly identified polar bear anelloviruses are more similar to each other than to previously described anelloviruses. Of the polar bears infected by anelloviruses (n = 23), all (100%) were infected with viruses from clade A. Polar bear 7 (PB#7) was the only individual that was not infected by a virus from clade B. Clade B contained three divergent groups in addition to sequences from giant pandas (*Ailuropoda melanoleuca*) and a tree shrew (*Tupaia belangeri*) (Figure 2). Pairwise genetic and temporal distances between polar bear anelloviruses were positively correlated, indicating that anelloviruses from samples collected closer together in time were also more genetically similar (Figure 3; $r = 0.16$; $P = 0.028$; slope = 0.007% change per year of detection).

Average anellovirus richness in SB polar bears was 20 (Figure 4a, range, 0–38; median, 22; SD, 11.1). One individual (PB#15), a year-old cub, did not have any detectable anelloviruses, while the only other year-old cub in our sample (PB#22) had the highest anellovirus richness of 38 (Table 1, Supplementary Figure 1B). The mean total anellovirus load from the serum of our sample population was 0.61 $\log_{10}$ vRPM/kb (Figure 4b; range, 0–2.01; median, 0.49; SD, 0.52). The prevalence of each virus in the populations ranged from 8% to 83% (Figure 5).

Anellovirus richness and total viral load increased slightly with age for females and declined slightly with age for males, but these relationships were not statistically significant (Table 3). Similarly, year of capture did not significantly influence viral richness or load (Table 3). Physiological

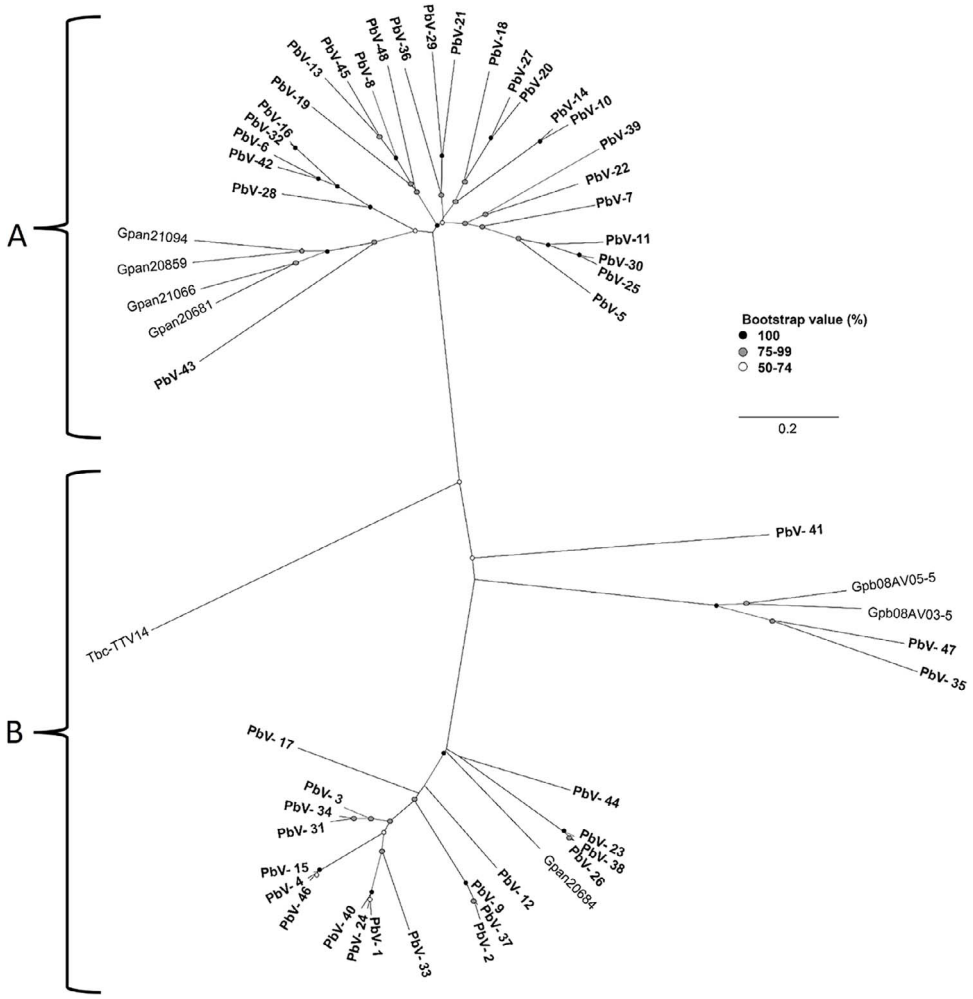

Figure 2: Maximum-likelihood phylogenetic tree of polar bear anelloviruses identified in this study(**bold**) and most closely-related viruses. Bootstrap values (%) are based on 1000 replicates, bootstrap values > 50% are labeled. All Giant panda (*Ailuropoda melanoleuca*) taxon (Gp) are from China in year referenced. All PbV sequences are from the USA, Southern Beaufort Sea polar bear subpopulation. Sequence Tbc-TTV14 is from a tree shrew (*Tupaia belangeri*) from Japan. Scale bar indicates nucleotide substitutions per site. Clades are indicated by A and B. See Table 2 for GenBank accession numbers for all viruses and year of detection.

biomarkers were also not significantly correlated with richness or load of anelloviruses (Table 3). Whether polar bears spent the summer using on-shore habitat or off-shore habitat did not significantly influence anellovirus richness or load (Table 3, Supplement C).

Discussion

We characterized the serum virome component of the microbiome of 24 polar bears from the SB subpopulation and identified 48 new anelloviruses. The anelloviruses we identified sort into two clades together with anelloviruses of another ursid, the giant panda (Varsani *et al.*, 2021). These results are similar to reports of host-associated anelloviruses in other wildlife species (Fahsbender *et al.*, 2017; Kraberger *et al.*, 2021; Varsani *et al.*, 2021). Similarly, our finding of a large number of anelloviruses in individual bears aligns with data from felids (Kraberger *et al.*, 2021), palm civets (Nishizawa *et al.*, 2018), primates (Hrazdilová *et al.*, 2016) and suids (Huang *et al.*, 2010). The diversity of anelloviruses and the species specificity suggests that within the host recombination of the viruses with a likely coevolutionary relationship between anelloviruses and their hosts (Fahsbender *et al.*, 2017;Arze *et al.*, 2021 ; Kraberger *et al.*, 2021). The large number of anelloviruses in individual polar bears was consistent over time, supporting the notion that anelloviruses persistently infect hosts and may, like in humans, be controlled when immune function is not suppressed (Arze *et al.*, 2021). Interestingly, we identified a weak but statistically significant trend of increasing genetic differentiation between viruses over time in SB polar bear anelloviruses. We caution that this trend

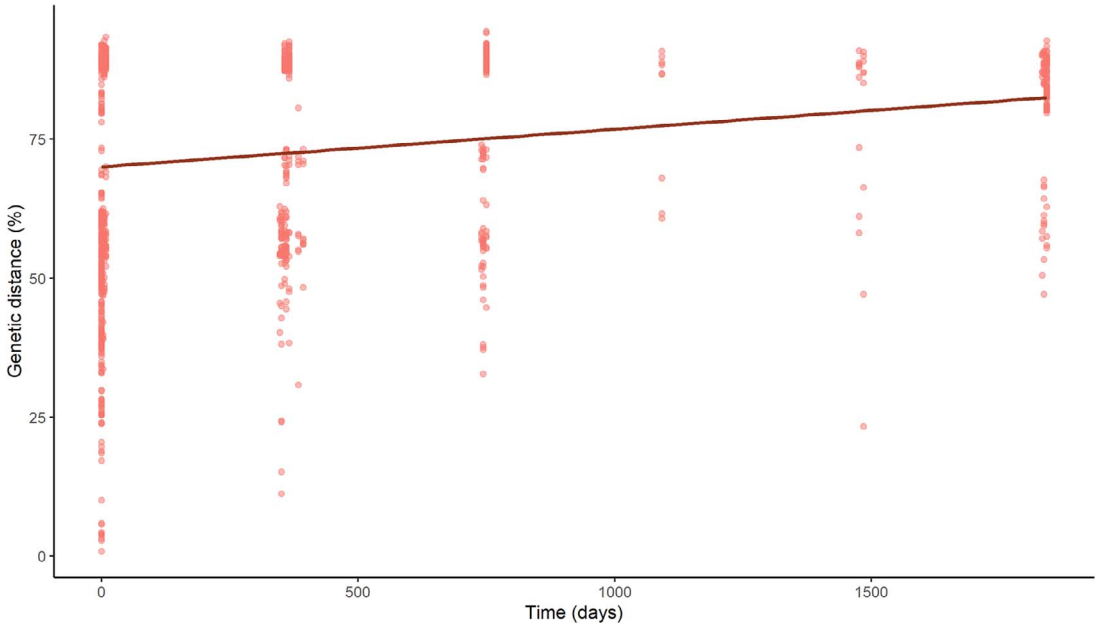

Figure 3: Pairwise genetic and temporal distance of 48 polar bear anelloviruses found in Southern Beaufort Sea polar bears between 2004 and 2015. Solid line is the least squares line (pairwise distance = 0.007x + 70.03, $r = 0.1596$).

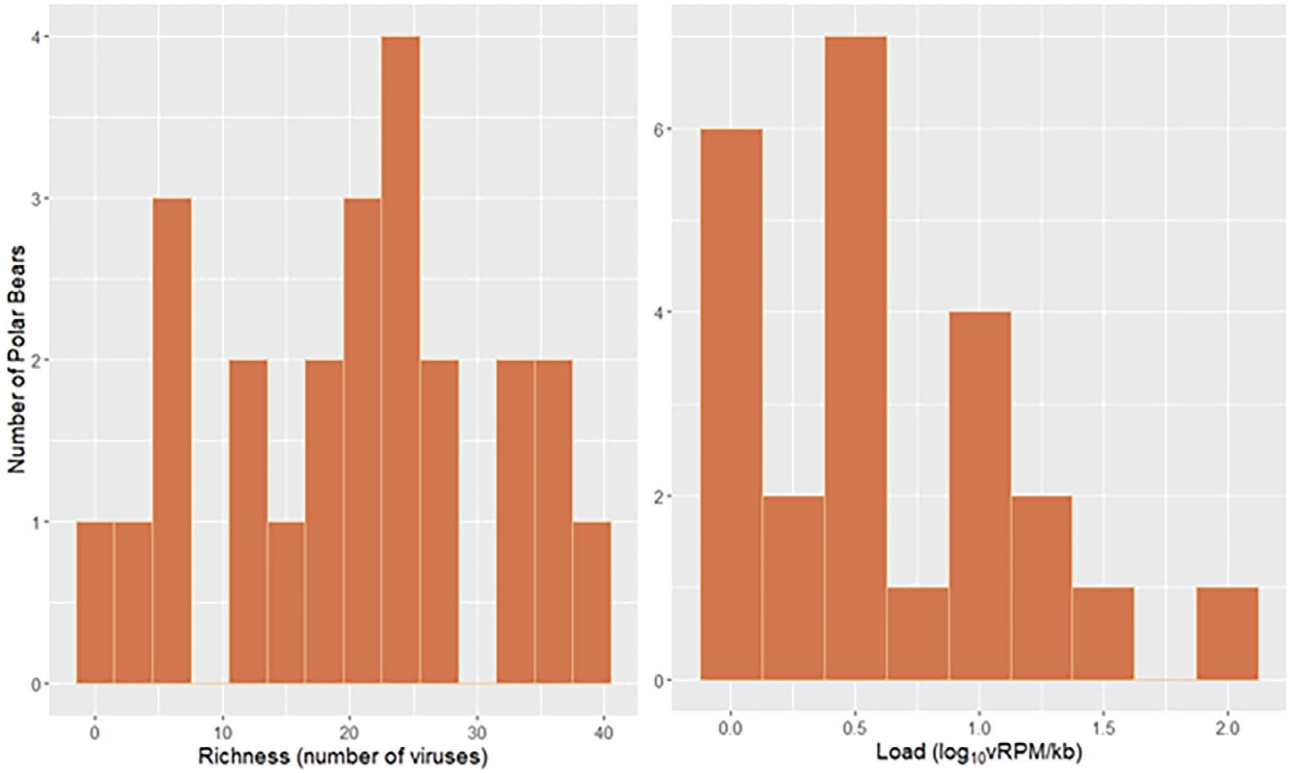

Figure 4: Histograms of (a) anellovirus richness and (b) total anellovirus loads in 24 Southern Beaufort Sea polar bears ($\log_{10}$ vRPM/kb = $\log_{10}$ viral reads per million per kilobase).

Virus ID	n*	Prevalence (%)	lower	upper	Virus ID	n	Prevalence (%)	lower	upper
1	16	66.7	0.48	0.86	25	7	29.2	0.11	0.47
2	14	58.3	0.39	0.78	26	13	54.2	0.34	0.74
3	16	66.7	0.48	0.86	27	2	8.3	-0.03	0.19
4	16	66.7	0.48	0.86	28	10	41.7	0.22	0.61
5	6	25.0	0.08	0.42	29	7	29.2	0.11	0.47
6	12	50.0	0.30	0.70	30	8	33.3	0.14	0.52
7	8	33.3	0.14	0.52	31	12	50.0	0.30	0.70
8	11	45.8	0.26	0.66	32	11	45.8	0.26	0.66
9	14	58.3	0.39	0.78	33	14	58.3	0.39	0.78
10	6	25.0	0.08	0.42	34	16	66.7	0.48	0.86
11	11	45.8	0.26	0.66	35	8	33.3	0.14	0.52
12	9	37.5	0.18	0.57	36	14	58.3	0.39	0.78
13	5	20.8	0.05	0.37	37	13	54.2	0.34	0.74
14	8	33.3	0.14	0.52	38	12	50.0	0.30	0.70
15	14	58.3	0.39	0.78	39	6	25.0	0.08	0.42
16	7	29.2	0.11	0.47	40	16	66.7	0.48	0.86
17	14	58.3	0.39	0.78	41	4	16.7	0.02	0.32
18	4	16.7	0.02	0.32	42	12	50.0	0.30	0.70
19	7	29.2	0.11	0.47	43	11	45.8	0.26	0.66
20	3	12.5	-0.01	0.26	44	9	37.5	0.18	0.57
21	3	12.5	-0.01	0.26	45	9	37.5	0.18	0.57
22	6	25.0	0.08	0.42	46	17	70.8	0.53	0.89
23	12	50.0	0.30	0.70	47	6	25.0	0.08	0.42
24	14	58.3	0.39	0.78	48	20	83.3	0.68	0.98

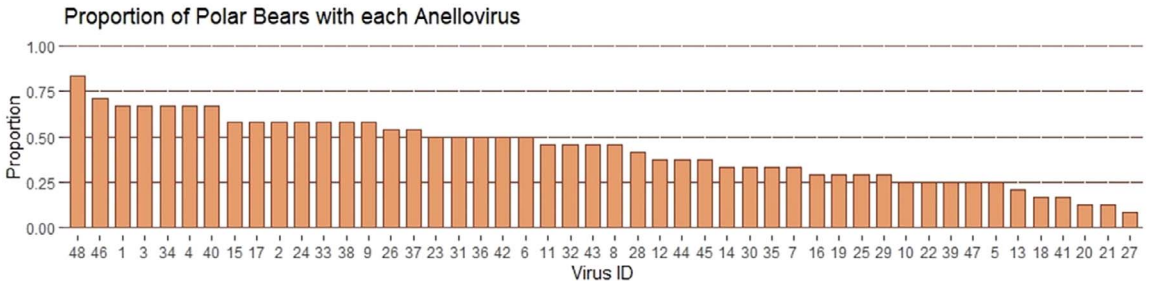

Figure 5: Prevalence (%) of each anellovirus in the sample population of 24 polar bears Southern Beaufort Sea polar bears with upper and lower 95% confidence intervals using the Wald Method and proportion of polar bears with each anellovirus. ∗number of polar bears infected with virus

should not be interpreted as an evolutionary rate, because our analysis was not lineage specific (due to very limited numbers of viruses from the same lineages over time). Rather, we speculate that this trend reflects a combination of anellovirus community turnover and molecular evolution of the ORF 1 gene.

Persistent infection is a hallmark of the anelloviruses (Arze *et al.*, 2021; Kraberger *et al.*, 2021). The polar bear "anellome" appears to be commensal and to vary by individual, consistent with results from other species (Crane *et al.*, 2018). Similar to anelloviruses of other species, polar bear anelloviruses appear to be diverse and host specific (Nishizawa *et al.*, 2018; Kraberger *et al.*, 2021). Contrary to our predictions, viral richness and load did not correlate with the demographic, physiologic or behavioral parameters we assessed. However, we do show that anelloviruses identified more closely in time have shorter genetic distances between them than those identified further apart; suggesting that the polar bear anellome is evolving, likely through a combination of point substitution and haplotype turnover (Arze *et al.*, 2021).

Anelloviruses have been found in blood, serum, feces, semen and the oral cavity and tissues of their hosts (Kaczorowska and van der Hoek, 2020). Mechanisms for virus

Table 3: Summary of model covariates and their relationship with anellovirus richness and load in 24 Southern Beaufort Sea polar bears (See Supplementary Figures 1C and 2C)

			Richness		Load (log_{10} vRPM/kb)	
Covariate	**Description**	**Sample size**	**t value**	**P -value**	**t value**	**P -value**
Sex	Male/Female	24	1.05	0.31	1.20	0.25
Age	Range: 0.5–23 years	24	0.76	0.46	0.57	0.58
Age*Sex	Interaction of age and sex	24	−0.75	0.46	−1.37	0.18
Year	Year of capture (Range: 2004–2015)	24	0.03	0.98	−0.83	0.41
Globulin	Measure of sustained immune response	16	2.00	0.07	1.11	0.29
Leukocyte Count	Measure of acute immune response	15	0.56	0.59	0.02	0.98
Hair Cortisol	Glucocorticoid hormone elevated during periods of stress	17	0.20	0.84	0.68	0.51
Summer Habitat Use	On-shore/off-shore*	24	1.23	0.23	1.37	0.19

Polar bears with > 5% bowhead in diet considered to use summer on-shore habitat.

transmission have been hypothesized to include diet, sexual, fecal-oral, respiratory and through blood transfusion and organ transplant (Arze *et al.*, 2021). A dietary route of infection for polar bear anelloviruses is possible but difficult to ascertain. The viromes of ringed seals (*Pusa hispida*), the primary prey of polar bears and bowhead whales (*Balaena mysticetus)*, the primary on-shore diet of SB polar bears, have yet to be investigated. Other modes of transmission, such as airborne or sexual transmission, are also possible but mechanisms have not been realized (Kaczorowska *et al.*, 2022b). Anelloviruses have been detected in human infants as young as 6 weeks old but were unrelated to maternal anelloviruses (Kaczorowska *et al.*, 2022a), providing no evidence to date of vertical transmission.

Our sample population was selected to maximize representation of bears across demographic characteristics, such as sex, age and summer habitat use, over 11 years during which polar bears underwent marked changes in habitat availability resulting from climate change (see Atwood *et al.*, 2016b, 2021, Bromaghin *et al.*, 2015, Johnson and Derocher, 2020, Rode *et al.*, 2014). Infection with viruses is difficult to detect. Infections can be short-lived or seasonal and can differ based on sample type. Our finding of a limited virome represent a baseline from which to evaluate virome changes. In terms of anelloviruses, longitudinal studies of individual bears over longer periods could reveal associations between anellovirus richness and load and physiological and ecological factors. Our findings are similar to Watson *et al.*'s (2019) investigation of the fecal microbiota of polar bears, which showed that neither sex nor age significantly influenced microbiota richness. Further, lack of a relationship between anellovirus load or richness and physiological biomarkers suggests that, unlike in humans, anelloviruses in polar bears do not appear to respond to physiological stress, at least within the range of physiological parameters we were able to examine, suggesting that immune function in these polar bears is competent in controlling anellovirus load (Arze *et al.*, 2021). Overall, anelloviruses are unlikely to be an effective ecoimmunological marker of immune function in polar bears; nevertheless, our findings of a relatively innocuous virome in polar bears provide a baseline against which to evaluate changes over time.

Author Contributions

T.LF., T.C.A. and T.L.G. conceived the project; L.A.O, E.D. and T.L.F. completed bioinformatics and T.L.F and A.C.K analysed the data; T.LF. led the writing of the manuscript. All authors contributed critically to the project and publication of this manuscript.

Conflicts of Interest

The authors have no conflicts of interest to declare.

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Data Availability

All viruses have been deposited into GENBANK https://www.ncbi.nlm.nih.gov/genbank/; virus accession numbers are reported in Table 2. The data that support the findings of this study are openly available in USGS Alaska Science Center

data repository at https://www.usgs.gov/centers/alaska-science-center/data; https://doi.org/10.5066/P9OXCRJ6.

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