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Alphaherpesvirus infection in a free-ranging narwhal *Monodon monoceros* from Arctic Canada

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ABSTRACT: We report the detection of an alphaherpesvirus infecting an adult female narwhal *Monodon monoceros* captured live during a tagging project in Tremblay Sound, Nunavut, Canada, in August 2018. The individual had 2 open wounds on the dorsum but appeared in good overall health. A blowhole swab was collected, and subsequent virus isolation was performed using a beluga whale primary cell line. Non-syncytial cytopathic effects were seen, in contrast to syncytial cytopathic effects described for monodontid alphaherpesvirus 1 (MoAHV1) isolates previously recovered from beluga whales *Delphinapterus leucas* from Alaska, USA, and the Northwest Territories, Canada. Next-generation sequencing was performed on a sequencing library generated from the DNA of the viral isolate and the analysis of the assembled contigs permitted the recovery of 6 genes, conserved in all members of the family *Orthoherpesviridae*, for downstream genetic and phylogenetic analyses. BLASTN (basic local alignment search tool, searching nucleotide databases using a nucleotide query) analyses of the narwhal herpesvirus conserved genes showed the highest nucleotide identities to MoAHV1, ranging between 88.5 and 96.8%. A maximum likelihood phylogenetic analysis based on concatenation of the 6 conserved herpesviruses amino acid alignments revealed the narwhal herpesvirus (NHV) to be the closest relative to MoAHV1, forming a clade within the subfamily *Alphaherpesvirinae*, genus *Varicellovirus*. NHV is the first alphaherpesvirus characterized from a narwhal and represents a new viral species, which we propose to be known as Varicellovirus monodontidalpha2. Further research is needed to determine the prevalence and potential clinical impacts of this alphaherpesvirus infection in narwhals.

KEY WORDS: *Alphaherpesvirinae* · *Herpesviridae* · Monodontid alphaherpesvirus 1· MoAHV1 · Narwhal herpesvirus · Next-generation sequencing · Virus isolation

1. INTRODUCTION

The narwhal *Monodon monoceros* is a medium-sized toothed cetacean reaching approximately 4–4.5 m in body length and is 1 of 2 species in the family Monodontidae, the other being the beluga whale *Delphinapterus leucas* (Heide-Jørgensen 2018). Both species have overlapping geographic ranges, with narwhals limited to the eastern Canadian Arctic, Greenland, and Russia. Narwhals are hunted throughout their range for subsistence use by indigenous groups, which has occurred for thousands of years

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(COSEWIC 2004). Fisheries and Oceans Canada (DFO) is a co-management partner in the sustainable hunt of narwhals, together with the Nunavut Wildlife Management Board, Regional Wildlife Organizations, and Hunters and Trappers Organizations (HTOs). As part of this management process, aerial surveys are undertaken to estimate population size. In addition, electronic tagging studies are conducted to determine population structure, define large-scale movements, and provide correction factors for the aerial surveys (Watt et al. 2015, Doniol-Valcroze et al. 2020).

The Arctic is undergoing rapid climate change, with loss of sea ice and increasing sea surface temperatures, which is further exacerbated by ongoing anthropogenic activities, including commercial fishing, mining development, shipping traffic, and noise pollution from increased vessel traffic which can negatively impact narwhals (Wassmann et al. 2011, Halliday et al. 2022). Consequently, Arctic marine mammals are exposed to increasing threats such as loss of habitat due to melting sea ice, changes in the prey base and potential predators, and increased competition for food (Moore & Huntington 2008, Reeves et al. 2012, 2014, Laidre et al. 2015). In addition, the health status of individual narwhals is likely to be affected by climate change through impacts on immune status, body condition, and transfer of pathogens (Burek et al. 2008). While little is currently known about infectious diseases affecting narwhals, they are known to be serologically reactive to *Brucella* spp. (Nielsen et al. 2001), a bacterium that is the causative agent of brucellosis. Brucellosis can cause severe reproductive disease in wildlife and livestock (Davis 1990), but it is unknown whether infection results in negative health impacts in narwhals.

The family *Orthoherpesviridae* encompasses a number of double-stranded DNA viruses that infect mammals, birds, and reptiles (Gatherer et al. 2021, ictv.global/taxonomy). The family is further subdivided into 3 subfamilies: *Alphaherpesvirinae*, *Betaherpesvirinae*, and *Gammaherpesvirinae*, comprising viruses with the ability to establish latency in the host whereby infection persists for life, ensuring long-term survival of the virus. Latent viruses can reactivate and cause lytic infection at any time. Varicella zoster virus (genus *Varicellovirus*, species *Varicellovirus humanalpha3*) causes human chickenpox, which is characterized by self-resolving skin or mucosal lesions upon primary infection. The virus then establishes a persistent latent infection of sensory neuronal nuclei and may reactivate later in life, causing shingles (Kinchington et al. 2012).

Alphaherpesviruses cause a range of diseases, from inconsequential to severe, in both humans and wildlife. Given that these viruses are widespread, it is not surprising that infections are reported in both phocids and cetaceans (Smolarek Benson et al. 2006). The monodontid alphaherpesvirus 1 (MoAHV1), species *Varicellovirus monodontidalpha1*, was recently isolated and characterized from beluga whales from the Northwest Territories, Canada, and Alaska, USA (Nielsen et al. 2018, Gatherer et al. 2021). MoAHV1 was first isolated from the blowholes of healthy belugas and skin lesions of dead stranded belugas. For the latter, it was not possible to ascertain whether MoAHV1 contributed to the stranding (Nielsen et al. 2018). MoAHV1 grows in primary beluga cells, and the full genome has been determined (Davison et al. 2017). In the present study, we describe the isolation and genetic characterization of a related alphaherpesvirus from a free-ranging narwhal from Nunavut, Canada, in 2018.

2. MATERIALS AND METHODS

2.1. Narwhal tagging and health assessment

On August 18, 2018, an adult female narwhal (body length of 382 cm) accompanied by a calf was caught by net in Tremblay Sound, Nunavut, Canada (Fig. 1) as part of an ongoing DFO tagging program (DFO License to Fish for Scientific Purposes S-18/19-1029-NU and Animal Use Protocol FWI-ACC-2018-22). Upon capture, the narwhal was actively moving in the net and continued to show strong expected movements during handling. On close inspection following restraint of the animal, 2 large open wounds connected by a subcutaneous tracking wound were observed on the dorsum, cranial to the dorsal ridge (30 × 20 and 15 × 10 cm diameter). At the wounds, skin and 50–60% of the blubber were absent, and the underlying muscles were exposed and moderately necrotic. Pus and fibrin were observed coating the muscle tissue, and a malodorous odor was present. From the appearance of the wounds, it was suspected that both were at least a few days to a few weeks old and most likely caused by a single rifle bullet (Fig. 2). In addition to the physical examination, blood was collected from the ventral fluke vein for blood gases analysis, a complete blood count (CBC), and blood biochemistry analysis including serum cortisol. A swab of the blowhole was submitted for bacteriology and 2 pieces of skin (3 cm in diameter) that sloughed off from the margins of the

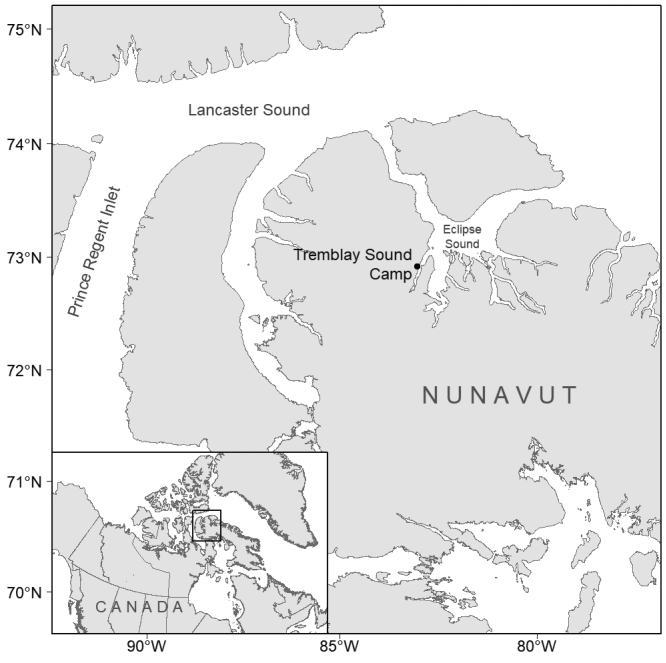


Fig. 1. The study area, Tremblay Sound, Nunavut, Canada, where the narwhals were sampled during electronic tagging activities

wounds during handling were collected and subsequently submitted for bacteriology and histopathology. An Acousonde™ tag (Model B003B, Greeneridge Sciences) attached with 2 suction cups was applied behind the dorsal ridge of the narwhal. The tag provided data on movement, dive, and acoustic behavior for up to 5 d following release (Shuert et al. 2021). Three additional healthy narwhals were also captured, tagged, and released in the same area without incident.

2.2. Virus isolation

Blowhole swabs were collected in ampoules containing virus transport media (VTM) from the wounded narwhal and the 3 healthy narwhals and immediately frozen and held at −20°C before shipping to DFO (Freshwater Institute, Winnipeg, Manitoba, Canada) for attempted virus isolation as previously described (Nielsen et al. 2018). Briefly, the frozen ampoules were rapidly thawed and 350 µl aliquot samples were aseptically inoculated onto 25 cm^2 tissue culture flasks (Corning) containing 80% confluent cultures of a primary beluga kidney cell line (BWK) (Nielsen et al. 1989) grown in Dulbecco's modified Eagle medium/Ham's F-12 (1:1) supplemented with 10% fetal bovine serum (HyClone) containing penicillin 200 units ml^{-1}, streptomycin

Fig. 2. The 2 infected wounds on the dorsum of the captured female narwhal from which the narwhal herpesvirus was isolated

200 µg ml^{-1}, and gentamycin 50 µg ml^{-1} as previously described (Tuomi et al. 2014). After adsorption for 1 h at 37°C, the inoculum was removed, and 5 ml of fresh media was added to each flask. A mock-infected flask inoculated with sterile media served as the negative control. Flasks were returned to the 37°C incubator and examined daily using an inverted microscope for signs of virus infection, evidenced by visual changes in the appearance of the cells or cytopathic effect (CPE). Cells were passaged (1:2) at weekly intervals into fresh media and observed for up to 1 mo.

2.3. Next-generation sequencing

Supernatant media from BWK cells showing CPE was passaged, expanded, frozen, and submitted to the Wildlife and Aquatic Veterinary Disease Laboratory (Gainesville, Florida, USA) for next-generation sequencing. After thawing, media was spun down at 9000 rpm (9075 × *g*) at 4°C for 20 min in a Beckman J2-MI centrifuge, and then the supernatant was centrifuged at 29 000 rpm (94 222 × *g*) at 4°C for 1 h 15 min in a Beckman fixed-angle rotor type 55.2 Ti. DNA was extracted from the resulting pellet using a QIAamp Viral DNA Mini Kit (Qiagen), following the manufacturer's instructions. A DNA sequencing library was generated using a NEBNext Ultra II Library Prep Kit (New England Biolabs) and sequenced on an Illumina MiSeq sequencer using a v3 chemistry 600 cycle kit. CLC Genomics Workbench (Qiagen) was used to de novo assemble the paired-end reads and to perform BLASTX (basic local alignment search tool [BLAST], searching protein databases using a translated nucleotide query) searches of the resulting contigs against the National Center for Biotechnology Information (NCBI) non-redundant protein database.

2.4. Phylogenetic and genetic analyses

Phylogenetic analyses were conducted by first separately aligning the amino acid (aa) sequences of 6 conserved herpesvirus genes (uracil-DNA glycosylase, helicase-primase helicase subunit, DNA packaging terminase subunit 1, major capsid protein, envelope glycoprotein B, and DNA polymerase catalytic subunit) recovered in this study to the homologous proteins encoded by 96 herpesviruses (42 alphaherpesviruses, 24 betaherpesviruses, and 30 gammaherpesviruses) downloaded from the NCBI GenBank database. Geneious Prime v2019.2.1 (https://www.geneious.com) was used to align the aa sequences of the individual genes with the multiple alignment using fast Fourier transform (MAFFT) option and then to concatenate the 6 alignments. IQ-TREE software (Trifinopoulos et al. 2016; http://iqtree.cibiv.univie.ac.at/) was used to determine best-fit models and generate maximum likelihood phylogenetic trees with 1000 non-parametric bootstraps for both the individual protein and the concatenated alignments.

3. RESULTS

3.1. Narwhal release and health assessment

Minor cuts and bleeding from capture in the net were noted on the left pectoral flipper, the fluke stalk, and on the edges of the fluke. Overall, the animal exhibited minimal blood loss and swam away strongly after release. The 2 large open wounds were treated by the onsite veterinarian with gentle manual debridement of necrotic tissue and local long-acting cephapirin infusion spread over the wound. Since narwhals are hunted for subsistence, parenteral antibiotics were not used for human safety reasons. Because reference ranges are unknown for narwhals, blood work results were compared to those of its closest relative, free-ranging beluga (St. Aubin et al. 2001), and indicated a mild non-regenerative anemia likely secondary to a chronic inflammatory response (wound infection), which was also suggested by hypoalbuminemia as shown in the biochemistry results (Table 1). A concomitant loss of albumin through the large cutaneous wounds may have also contributed to the hypoalbuminemia. Additional assessment of blood gases and biochemistry parameters indicated increased lactate levels and decreased bicarbonates and blood pH levels (Table 1). These results were similar to observed changes in the other 3 non-wounded tagged narwhals that recovered uneventfully (K. Béland unpubl. data) and likely indicate the physiological response to capture and handling. All other blood parameters, including white blood cell count and cortisol levels (Table 1), were similar to values obtained in other narwhals tagged and sampled in 2017 and 2018 (K. Béland unpubl. data). Bacteriology testing of the wound resulted in 1+ growth of β-hemolytic *Streptococcus* spp. No significant tissue alterations were observed during histological examination of the wound-associated epidermis. The bacterial culture of the blowhole sample was negative.

Dive data from the Acousonde™ tag revealed that the narwhal dove regularly over the 5 d after capture

Table 1. Blood parameters of the adult female narwhal from which the narwhal herpesvirus was isolated. ALT: alanine transaminase; AST: aspartate transaminase; BUN: blood urea nitrogen; CBC: complete blood count; CK: creatine kinase; GGT: gamma-glutamyltransferase; LDH: lactate dehydrogenase; WBC: white blood cell

Blood biochemistry				Blood gases and CBC	
Analyte	Concentration	Analyte	Concentration	Analyte	Concentration
Glucose (mmol l^{-1})	7.1	Total protein (g l^{-1})	71.0	pH	6.924
BUN (mmol l^{-1})	14.8	Albumin (g l^{-1})	29.5	pCO_2 (mmHg)	37.1
Phosphorus (mmol l^{-1})	2.67	Globulin (g l^{-1})	41.5	pO_2 (mmHg)	295
Creatinine (µmol l^{-1})	138	Albumin/globulin ratio	0.71	Base excess (mmol l^{-1})	−25
Cholesterol (mmol l^{-1})	4.02	Calcium (mmol l^{-1})	2.56	TCO_2 (mmol l^{-1})	9
Triglycerides (mmol l^{-1})	1.87	Potassium (mmol l^{-1})	4.43	SO_2 (%)	100
AST (U l^{-1})	82	Sodium (mmol l^{-1})	162.5	Lactates (mmol l^{-1})	>20
ALT (U l^{-1})	10	Chloride (mmol l^{-1})	116.4		
Alkaline phosphatase (U l^{-1})	48	Anion gap (mmol l^{-1})	45.4	Hematocrit (%)	47
GGT (U l^{-1})	21	Magnesium (mmol l^{-1})	0.95	Estimated WBCs (cells µl^{-1})	5400
LDH (U l^{-1})	279	Cortisol (mmol l^{-1})	96.8		
CK (U l^{-1})	58				

and release. The narwhal made several dives >600 m and traveled a total distance of 135 km between the time the tag was applied and the time it was released. This pattern of behavior was similar to that of the other tagged and healthy narwhals.

3.2. Virus isolation

Small foci of swollen, rounded, refractile cells developed in the cell monolayer 14 d post-infection (DPI) from swab material from the wounded narwhal. CPE progressed until the entire monolayer detached from the flask and displayed CPE development similar to that described for MoAHV1 (Nielsen et al. 2018), though no syncytia (multinucleated giant cells) were observed. This effect could be replicated upon dilution and passage onto fresh cells (Fig. 3). Upon passage, it was noted that the time until the first sign of CPE was shortened to 6 or 7 DPI, probably due to either the low amount of virus present in the inoculum or the extra time the virus needed to adapt to growth in BWK cells. No CPE was detected in the control and flasks inoculated with material from the other 3 healthy narwhals.

3.3. Next-generation sequencing and phylogenetic analyses

The BLASTX searches identified herpesvirus-related contigs of a novel herpesvirus, hereinafter referred to as narwhal herpesvirus (NHV). The complete coding sequences of the 6 conserved herpesvirus genes were identified: uracil-DNA glycosylase (1266 bp), helicase-primase helicase subunit (2463 bp), DNA packaging terminase subunit 1 (2184 bp), major capsid protein (4122 bp), envelope glycoprotein B (2727 bp), and DNA polymerase catalytic subunit (3570 bp), and were deposited in the NCBI GenBank database (Table 2). BLASTN (BLAST search of nucleotide databases using a nucleotide query) searches of the NHV conserved genes against the NCBI nucleotide database showed the highest nucleotide identities to MoAHV1 (GenBank accession no. MF678601), ranging between 88.5 and 96.8% (Table 2). The best-fit models for the aa alignments of individual genes are presented in Table 2. The maximum likelihood tree based on the concatenated aa sequence alignment of the 6 conserved herpesvirus genes was built using the LG + F + I + G4 model (see Table 2 for definitions of abbreviations). All 7 maximum likelihood phylograms supported NHV as the sister group to the MoAHV1. The NHV/MoAHV1 clade was supported as a member of the genus *Varicellovirus*, subfamily *Alphaherpesvirinae*, infecting mammals of the order *Cetartiodactyla* (Fig. 4; Fig. S1 in the Supplement at www.int-res.com/articles/suppl/d154p131_supp.pdf).

4. DISCUSSION

We report the first isolation in cell culture, genetic and phylogenetic characterization of a herpesvirus from a narwhal. Isolation was made from a single blowhole sample collected from a live-captured, wounded, and presumably stressed adult female

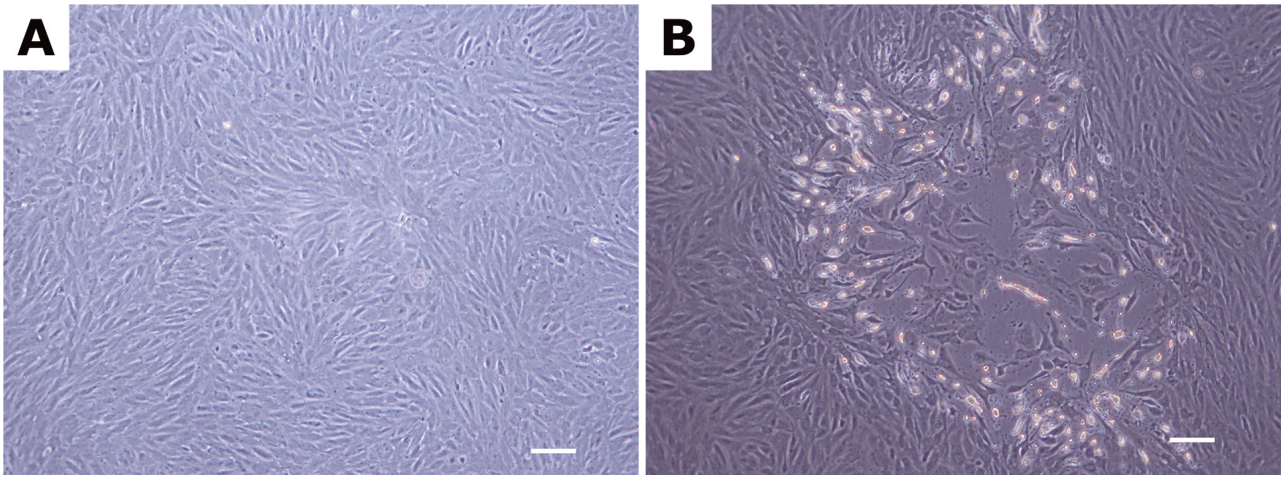


Fig. 3. *In vitro* growth characteristics of the narwhal herpesvirus in primary beluga kidney cells, passage 2. (A) Uninfected and (B) infected cells showing focal cytopathic effect including cell rounding, increased refractility, and death without the formation of syncytia 8 d post-inoculation at second passage. Scale bars = 100 µm

Table 2. The 6 conserved herpesvirus genes from the narwhal herpesvirus (NHV) deposited in GenBank, nucleotide sequence identities of NHV to monodontid alphaherpesvirus 1 (GenBank accession no. MF678601), and the best-fit models determined in IQ-TREE with the Bayesian information criterion option implemented to infer the maximum likelihood tree. BLASTN: basic local alignment search tool, searching nucleotide databases using a nucleotide query; LG (Le & Gascuel 2008) and JTT (Jones et al. 1992): substitution models; F: empirical equilibrium frequencies; G: gamma-distributed site rates with gamma shape parameter estimated from the alignment; I: fraction of invariable sites estimated from the alignment

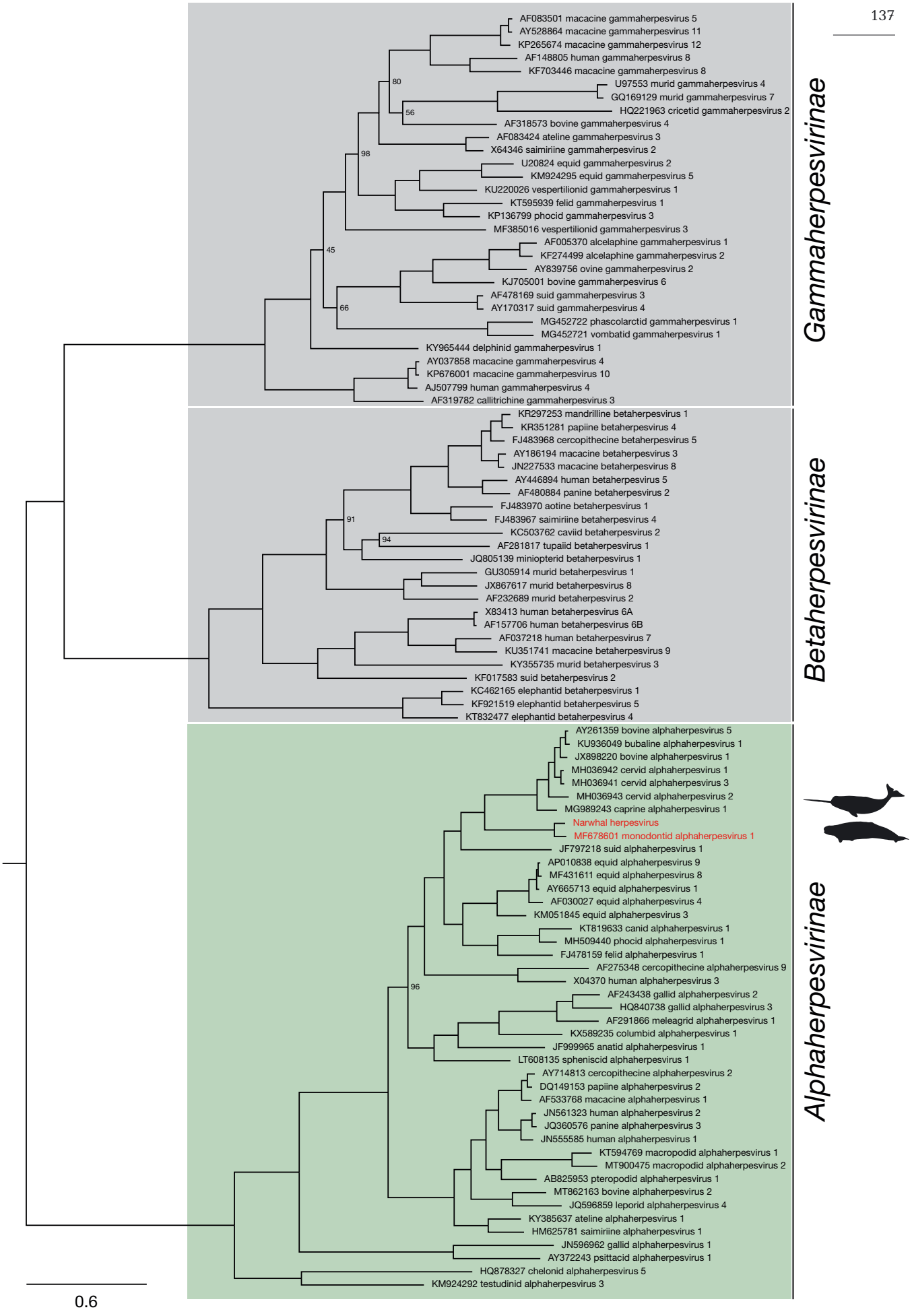
Conserved herpesvirus protein	Gene length (bp)	Protein length (aa)	BLASTN coverage (%)	BLASTN identity (%)	Best-fit model	GenBank accession no.
Uracil-DNA glycosylase	1266	421	65	88.5	LG + I + G4	OP852646
Helicase-primase helicase subunit	2463	820	100	93.5	JTT + I + G4	OP852647
DNA packaging terminase subunit 1	2184	727	100	96.8	LG + F + I + G4	OP852648
Major capsid protein	4122	1373	100	95.3	LG + F + I + G4	OP852649
Envelope glycoprotein B	2727	908	100	89.1	LG + F + I + G4	OP852650
DNA polymerase catalytic subunit	3570	1189	100	93.7	LG + F + I + G4	OP852651

with a calf. Stress brought on by events such as capture, tagging, caring for a calf, and a worsening infected wound is known to exacerbate herpesvirus infections that can lead to the reactivation of latent infections which may become systemic, resulting in serious health outcomes (Grinde 2013). In cetaceans, herpesvirus infection can be associated with a range of pathological findings, although in some cases, herpesvirus-related lesions may not be present in infected animals. Alphaherpesviruses have been related to fatal systemic infections (Blanchard et al. 2001), lymphoid necrosis (Arbelo et al. 2010), nephritis and other kidney lesions (Felipe-Jiménez et al. 2021), central nervous system lesions (van Elk et al. 2016), and skin lesions (Manire et al. 2006) in cetaceans.

Specifically in beluga whales, MoAHV1 infection has been associated with both healed and active skin lesions and was also found in a high proportion of belugas that did not present with skin lesions in Alaska and Arctic Canada populations (Nielsen et al. 2018). MoAHV1 was genetically identified in a focal papilloma-like penile lesion in a beluga from the St. Lawrence Estuary at Rivière-Ouelle, Quebec, Canada (Bellehumeur et al. 2015). Systemic MoAHV1 infection was assigned as the cause of death of a female calf with multisystemic herpesvirus vasculitis and multifocal necrosis in the adrenal gland reproductive tissue, spleen, lymph nodes, skin, and heart muscle (Burek-Huntington et al. 2015). Most recently, systemic MoAHV1 infection was described in a free-ranging beluga whale calf with congenital defects and suspected bacterial peritonitis. In that case, it was suggested that the alphaherpesvirus could have been acquired through uterine infection, during the birthing process, or nursing. Blowhole and genital swabs were positive for MoAHV-1 by conventional PCR (Burek-Huntington et al. 2022). No skin or other lesions were associated with the isolation of NHV in the present study, but a more comprehensive sampling of live and dead narwhals is needed to further investigate the pathogenicity of NHV.

Since a viable virus was recovered from the epithelial cells in the blowhole, the narwhal had an active infection at the time of sampling. It is known that active (isolation-positive) infections of MoAHV1 in belugas are relatively common and can approach >50% of sampled animals at some geographic locations, implying that MoAHV1 is likely enzootic in belugas (Nielsen et al. 2018). Further research is needed to determine whether this may be true for narwhals. Similar to that proposed for MoAHV1, NHV may be transmitted in aerosols during breathing, since the virus is present in the blowhole, in addition to direct sexual contact, as MoAHV1 has been isolated in the urogenital tracts of both female and male belugas (Nielsen et al. 2018). MoAHV1 and NHV were both isolated in BWK cells, but MoAHV1 produced syncytia, whereas NHV did not (Fig. 3B),

Fig. 4. Phylogram of the relationship of the narwhal herpesvirus (NHV) to other members of the family *Orthoherpesviridae* (grouped by subfamilies: *Alpha-*, *Beta-*, and *Gammaherpesvirinae*), based on the concatenated amino acid sequence alignment of 6 conserved herpesvirus proteins. The maximum likelihood tree was generated using 1000 bootstraps, and branch lengths are based on the number of inferred substitutions, as indicated by the scale. Virus GenBank accession numbers are listed, followed by virus names. Red font: NHV and monodontid alphaherpesvirus 1. All nodes are supported by 100% bootstrap values, except the nodes labeled with bootstrap values


AF083501 macacine gammaherpesvirus 5
AY528864 macacine gammaherpesvirus 11
KP265674 macacine gammaherpesvirus 12
AF148805 human gammaherpesvirus 8
KF703446 macacine gammaherpesvirus 8
U97553 murid gammaherpesvirus 4
GQ169129 murid gammaherpesvirus 7
HQ221963 cricetid gammaherpesvirus 2
AF318573 bovine gammaherpesvirus 4
AF083424 ateline gammaherpesvirus 3
X64346 saimiriine gammaherpesvirus 2
U20824 equid gammaherpesvirus 2
KM924295 equid gammaherpesvirus 5
KU220026 vespertilionid gammaherpesvirus 1
KT595939 felid gammaherpesvirus 1
KP136799 phocid gammaherpesvirus 3
MF385016 vespertilionid gammaherpesvirus 3
AF005370 alcelaphine gammaherpesvirus 1
KF274499 alcelaphine gammaherpesvirus 2
AY839756 ovine gammaherpesvirus 2
KJ705001 bovine gammaherpesvirus 6
AF478169 suid gammaherpesvirus 3
AY170317 suid gammaherpesvirus 4
MG452722 phascolarctid gammaherpesvirus 1
MG452721 vombatid gammaherpesvirus 1
KY965444 delphinid gammaherpesvirus 1
AY037858 macacine gammaherpesvirus 4
KP676001 macacine gammaherpesvirus 10
AJ507799 human gammaherpesvirus 4
AF319782 callitrichine gammaherpesvirus 3
Gammaherpesvirinae
KR297253 mandrilline betaherpesvirus 1
KR351281 papiine betaherpesvirus 4
FJ483968 cercopithecine betaherpesvirus 5
AY186194 macacine betaherpesvirus 3
JN227533 macacine betaherpesvirus 8
AY446894 human betaherpesvirus 5
AF480884 panine betaherpesvirus 2
FJ483970 aotine betaherpesvirus 1
FJ483967 saimiriine betaherpesvirus 4
KC503762 caviid betaherpesvirus 2
AF281817 tupaiid betaherpesvirus 1
JQ805139 miniopterid betaherpesvirus 1
GU305914 murid betaherpesvirus 1
JX867617 murid betaherpesvirus 8
AF232689 murid betaherpesvirus 2
X83413 human betaherpesvirus 6A
AF157706 human betaherpesvirus 6B
AF037218 human betaherpesvirus 7
KU351741 macacine betaherpesvirus 9
KY355735 murid betaherpesvirus 3
KF017583 suid betaherpesvirus 2
KC462165 elephantid betaherpesvirus 1
KF921519 elephantid betaherpesvirus 5
KT832477 elephantid betaherpesvirus 4
Betaherpesvirinae
AY261359 bovine alphaherpesvirus 5
KU936049 bubaline alphaherpesvirus 1
JX898220 bovine alphaherpesvirus 1
MH036942 cervid alphaherpesvirus 1
MH036941 cervid alphaherpesvirus 3
MH036943 cervid alphaherpesvirus 2
MG989243 caprine alphaherpesvirus 1
Narwhal herpesvirus
MF678601 monodontid alphaherpesvirus 1
JF797218 suid alphaherpesvirus 1
AP010838 equid alphaherpesvirus 9
MF431611 equid alphaherpesvirus 8
AY665713 equid alphaherpesvirus 1
AF030027 equid alphaherpesvirus 4
KM051845 equid alphaherpesvirus 3
KT819633 canid alphaherpesvirus 1
MH509440 phocid alphaherpesvirus 1
FJ478159 felid alphaherpesvirus 1
AF275348 cercopithecine alphaherpesvirus 9
X04370 human alphaherpesvirus 3
AF243438 gallid alphaherpesvirus 2
HQ840738 gallid alphaherpesvirus 3
AF291866 meleagrid alphaherpesvirus 1
KX589235 columbid alphaherpesvirus 1
JF999965 anatid alphaherpesvirus 1
LT608135 spheniscid alphaherpesvirus 1
AY714813 cercopithecine alphaherpesvirus 2
DQ149153 papiine alphaherpesvirus 2
AF533768 macacine alphaherpesvirus 1
JN561323 human alphaherpesvirus 2
JQ360576 panine alphaherpesvirus 3
JN555585 human alphaherpesvirus 1
KT594769 macropodid alphaherpesvirus 1
MT900475 macropodid alphaherpesvirus 2
AB825953 pteropodid alphaherpesvirus 1
MT862163 bovine alphaherpesvirus 2
JQ596859 leporid alphaherpesvirus 4
KY385637 ateline alphaherpesvirus 1
HM625781 saimiriine alphaherpesvirus 1
JN596962 gallid alphaherpesvirus 1
AY372243 psittacid alphaherpesvirus 1
HQ878327 chelonid alphaherpesvirus 5
KM924292 testudinid alphaherpesvirus 3
Alphaherpesvirinae
80
56
98
45
66
91
94
96
0.6

indicating that NHV differs phenotypically from MoAHV1 in these cells. Syncytia versus non-syncytia production is a trait also seen in other related alphaherpesviruses (Okubo et al. 2016).

Phylogenetic analyses based on the individual and concatenated conserved herpesvirus protein alignments showed that NHV and MoAHV1 formed a well-supported clade most closely related to alphaherpesviruses within the genus *Varicellovirus* infecting *Cetartiodactyla* (Figs. 4 & S1). Species demarcation criteria for herpesviruses are flexible and are typically based on sequence data and epidemiological or biological characteristics of the virus to support taxonomic proposals (Gatherer et al. 2021). Based on the nucleotide divergence of the 6 conserved genes of NHV and MoAHV1 (88.5–96.8%; Table 2), we propose that NHV represents a new herpesvirus species. We suggest the viral species name of Varicellovirus monodontidalpha2, pending consideration by the International Committee on Taxonomy of Viruses.

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