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Full genome sequence analysis of a novel adenovirus from a captive polar bear (*Ursus maritimus*)

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ABSTRACT

The presence of a novel adenovirus (AdV) was detected by PCR and sequencing, in the internal organs of a captive polar bear that had died in the Budapest zoo. The virus content of the samples proved to be high enough to allow for conventional Sanger sequencing on PCR-amplified genomic fragments. With this approach, the sequence of the entire genome of the putative polar bear adenovirus 1 (PBAdV-1) was obtained. Although the genome was found to be short, consisting of 27,952 base pairs merely, with a relatively balanced G + C content of 46.3 %, its organisation corresponded largely to that of a typical mastadenovirus. Every genus-common gene could be identified except that of protein IX. The short E3 region of the PBAdV-1 consisted of two novel, supposedly type-specific ORFs only, whereas no homologue of any of the E3 genes, usually conserved in mastadenoviruses, such as for example that of the 12.5 K protein, were present. In the E4 region, only the highly conserved gene of the 34 K protein was found besides two novel ORFs showing no homology to any known E4 ORFs. *In silico* sequence analysis revealed putative splicing donor and acceptor sites in the genes of the E1A, IVa2, DNA-dependent DNA polymerase, pTP, 33 K proteins, and also of U exon protein, all being characteristic for mastadenoviruses. Phylogenetic calculations, based on various proteins, further supported that the newly-detected PBAdV is the representative of a new species within the genus *Mastadenovirus,* and may represent the evolutionary lineage of adenoviruses that coevolved with carnivorans.

1. Introduction

Adenoviruses (AdVs) are medium-sized icosahedral, non-enveloped viruses with double stranded, linear DNA genome. Presently, the family *Adenoviridae* is divided into five approved and one pending genera (Harrach et al., 2011) (https://talk.ictvonline.org; https://sites.google.com/site/adenoseq). *Mastadenovirus* and *Aviadenovirus* contain viruses infecting exclusively mammals or birds, respectively. The most recently accepted genus *Ichtadenovirus* was established for the only AdV found in fish to date, sturgeon adenovirus 1 (Benkő et al., 2002). The remaining two genera (*Atadenovirus* and *Siadenovirus*) have more diverse host spectra. Atadenoviruses have been found in various snakes, lizards, birds, as well as in a tortoise, several ruminants and even in a marsupial (Wellehan et al., 2004; Papp et al., 2009; Pénzes et al., 2014; Garcia-Morante et al., 2016; Szirovicza et al., 2016). Siadenoviruses are known to occur commonly in birds, but have also been detected in tortoises and in a frog (Rivera et al., 2009; Kovács and Benkő, 2011; Ballmann and Vidovszky, 2013; Ballmann and Harrach, 2016; Lee et al., 2016). Recently, a sixth genus, *Testadenovirus* has been proposed for the AdVs discovered in testudinoid turtles (Doszpoly et al., 2013). Adenoviruses usually have a narrow host range restricted to a single, or several closely related, vertebrate species. Several evidences imply that AdVs have been co-evolving with their hosts (Harrach et al., 2011; Podgorski et al., 2018). However, multiple assumed host switches of AdVs have been described, e.g. from Squamate reptiles to birds and ruminants, from monkeys and apes to humans, from bats to dogs (Benkő and Harrach, 2003; Wellehan et al., 2004; Kohl et al., 2012).

Thanks to the increased sensitivity of broad-range PCRs, numerous novel AdVs have been discovered and described in the past couple of decades (Benkő et al., 2002; Wellehan et al., 2004; Papp et al., 2009; Rivera et al., 2009; Ballmann and Vidovszky, 2013; Doszpoly et al., 2013; Ballmann and Harrach, 2016; Garcia-Morante et al., 2016; Lee et al., 2016; Szirovicza et al., 2016; Podgorski et al., 2018) (https://sites.google.com/site/adenoseq). In the majority of these cases

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however, actual isolation of the detected viruses has been hampered by the lack of appropriate cell lines.

Mastadenoviruses occur in a wide range of wild and domestic mammals including humans, as well as numerous ape and monkey species (Kohl et al., 2012; Vidovszky et al., 2015; Podgorski et al., 2016, 2018). Primary infection with AdVs usually results in mild, transient and self-limiting respiratory or enteric disease. In healthy individuals, most AdVs are harmless, yet some of them have been incriminated as the causative agent of specific severe, sometimes — though very rarely — fatal diseases. Such an exception is canine adenovirus 1 (CAdV-1) that causes the infectious canine hepatitis (ICH, or Rubarth's disease). While ICH might be life-threatening, the infection by CAdV-2, a genetic variant of CAdV-1 causes only mild respiratory disease, the so-called kennel cough. The involvement of CAdV-1 in fox encephalitis cases has also been described (Benkő, 2008). Serological positivity to both CAdV types has been detected in additional members of the order Carnivora such as various bears, coyote, jackal, raccoon, wolf, etc. PCR detection and isolation were accomplished from samples of red foxes (*Vulpes vulpes*) in the UK (Thompson et al., 2010) and in Italy (Balboni et al., 2013). More recently, new AdVs, genetically most closely related to CAdVs, have been discovered and, based on their clustering on the phylogenetic trees, a close common ancestry of CAdV-1, -2 and the AdV of certain vespertilionid bats have been hypothesized (Kohl et al., 2012; Vidovszky et al., 2015). A novel AdV was isolated from a wild living skunk (*Mephitis mephitis*) in Canada. The full genomic sequence analysis of this skunk adenovirus (SkAdV-1) revealed common characteristics with the CAdV genomes (Kozak et al., 2015). The phylogeny inference also suggested that SkAdV-1 and CAdVs and certain bat AdVs share close common ancestry.

Isolation of an AdV from California sea lions (*Zalophus californianus*) diseased with clinical signs similar to those caused by CAdV-1 has also been reported, however serology and PCR indicated the presence of a virus distinct from CAdV and was named California sea lion AdV-1 (CSLAdV-1) (Goldstein et al., 2011). Seemingly the same virus was recovered from South American sea lion (*Otaria flavescens*) and named as otarine AdV-1 (Inoshima et al., 2013) and from Hawaiian monk seal (*Neomonachus schauinslandi*) (Cortes-Hinojosa et al., 2016). Finally, the complete genome of CSLAdV-1 was sequenced and this proved its difference from CAdVs (Cortes-Hinojosa et al., 2015). A rapidly increasing number of novel AdVs are being detected in different carnivoran samples by the nested PCR targeting the most conserved part of the gene of the adenoviral DNA-dependent DNA polymerase (*pol*) (Wellehan et al., 2004), thus from northern elephant seals (*Mirounga angustirostris,* phocine AdV-1), Pacific harbor seals (*Phoca vitulina richardii,* phocine AdV-2), and again from another California sea lion with ocular lesions (this virus named otarine AdV-2) (Wright et al., 2015). More recently, *pol* sequences became available from a cat-associated AdV (Lakatos et al., 2017), as well as from pine martens (*Martes martes*) and Eurasian otters (*Lutra lutra*) (Walker et al., 2017).

In this report, we describe the detection, full genome sequencing and phylogenetic analysis of an AdV found in a captive polar bear (*Ursus maritimus*) which had died in the Budapest Zoo. Routine PCR screening of the internal organs, namely the liver and the lungs revealed the likely presence of a hitherto unknown AdV. The amount of viral genomic DNA in the samples was found high enough to allow for conventional Sanger sequencing by direct primer walking on PCR-amplified genome fragments. Based on our results we propose this novel polar bear AdV to be a founding member of a new species within the genus *Mastadenovirus*.

2. Materials and methods

2.1. Samples

A 23-year-old female polar bear, born in captivity in Italy, died suddenly at the Budapest Zoo in 2011. Gross pathology revealed a tumour of about 10 cm in diameter in the liver. Upon histopathology, multifocal glomerulonephritis, chronic fibrosis and amyloidosis were found in the kidney. The tumour was classified as a biliary cystadenoma. Bacteriological examination indicated beta-2 toxin-producing *Clostridium perfringens* enterotoxaemia as the immediate cause of the death. For routine PCR screening, DNA extraction from liver and lung samples was performed as described earlier (Ballmann and Harrach, 2016).

2.2. PCR

For the initial diagnostic PCR or whenever the expected size of the PCR product did not exceed 1000 bp, Dream Taq Green (ThermoScientific) Master Mix (2×) and for the amplification of larger DNA fragments the PrimeSTAR Max or GXL DNA Polymerase (Takara Bio USA, Inc.) kits were applied. The initial detection of AdV DNA was performed by general nested PCRs with degenerate, consensus primers targeting conserved regions of the *pol* (Wellehan et al., 2004), IVa2 (Pantó et al., 2015) and hexon genes (the latter performed by single round PCR) (Kiss et al., 1996).

The whole genome was sequenced using a primer walking approach. Specific primers were designed based on the sequences gained first with the degenerate primers and later based on the sequences gained by the custom-made primers (specific PCR primers as listed in supplementary Table S1). PCR reaction volume was 25 µl, consisting of 12.5 µl DreamTaq Master Mix (2×) or PrimeSTAR Max or GXL DNA Polymerase (Takara), 0.5 µl (50 pmol/µl) of each primer (outer and inner for the first and second round, respectively) and 1 µl of target DNA. Milli-Q water was added up to 25 µl final volume. In the second cycle of nested PCR, 2 µl of the reaction mixture of the first cycle was used as a template. For single round PCR, the same amounts were used, as in the first round of nested PCR.

The nested PCR profile comprised an initial denaturation step at 94 °C for 5 min, followed by 45 cycles of denaturation at 94 °C for 0.5 min, annealing at 46 °C for 1 min and elongation at 72 °C for 1 min. The final elongation step lasted 3 min at 72 °C. For the amplification of the hexon gene, the following thermocycling conditions were used: initial denaturation at 95 °C for 5 min; 35 cycles of denaturation, annealing and elongation, 1 cycle consisting of 95 °C for 0.5 min, 55 °C for 0.5 min and 72 °C for 0.5 min, respectively, and a final elongation step at 72 °C for 5 min. Using specific primers, the PCR conditions were set according to manufacturer's instruction (Takara Bio USA, Inc.). The PCR products were analysed by electrophoresis in 1 % agarose gels containing GelRed™ (Biotium USA, Inc.).

2.3. DNA sequencing

DNA fragments of expected size were cut from the agarose gel and cleaned using NucleoSpin® Gel and PCR Clean-up kit (Macherey-Nagel, Germany). Sequencing was performed on both strands with the BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems/Life Technologies Corporation®, Carlsbad, CA, USA) using the consensus primers. The capillary electrophoresis was performed on the ABI Prism® 3100 Genetic Analyzer (Applied Biosystems, USA) by a commercial supplier.

2.4. Bioinformatics and phylogenetic analysis

The identity of the newly-gained sequences was checked by using different BLAST algorithms on the NCBI website. Sequence assembly and editing were performed with the Staden package (Staden et al., 1998). Genome annotation was carried out with Artemis Genome Browser (Berriman and Rutherford, 2003) and CLC Main Workbench. The determination of the splicing donor and acceptor sites, as well as the cleavage signals, was accomplished manually by comparison of conserved patterns (Farkas et al., 2002; Ruzindana-Umunyana et al.,

Table 1
Predicted gene products of PBAdV-1 compared in size and aa sequence identity to Carnivora AdVs and HAdV-5. Virus abbreviations are in the text.

protein name	Size (aa) PBAdV	Size (aa) CAdV-1	aa sequence identity	Size (aa) CSLAdV	aa sequence identity	Size (aa) SkAdV	aa sequence identity	Size (aa) bat-WIV12 AdV	aa sequence identity	Size (aa) HAdV-5	aa sequence identity
E1A	246	239	18.1 %	245	20.1 %	267	18.9 %	198	24.5 %	289	23.4 %
19K	166	169	20.0 %	147	25.9 %	182	20.7 %	138	21.3 %	176	27.5 %
55 K	446	444	21.7 %	418	25.4 %	453	22.4 %	432	54%%	496	21.5 %
IX	–	103	–	156	–	99	–	84	–	140	–
IVa2	447	446	57.0 %	447	56.8 %	436	56.2 %	330	49.9 %	449	57.2 %
pol	1137	1149	56.2 %	1148	58.0 %	1143	56.1 %	1146	56.2 %	1198	51.5 %
pTP	603	608	59.5 %	605	52.9 %	610	57.8 %	600	56.4 %	671	51.2 %
52 K	334	389	47.8 %	390	45.5 %	396	44.9 %	350	54%%	415	45.3 %
pIIIa	481	548	42.9 %	570	41.0 %	581	39.0 %	577	40.7 %	585	41.2 %
III	480	477	60.2 %	537	53.0 %	478	61.1 %	483	60.3 %	571	51.9 %
pVII	181	132	28.2 %	188	25.2 %	144	27.1 %	101	35.6 %	198	26.1 %
V	227	421	17.0 %	431	17.4 %	434	16.9 %	381	20.6 %	368	20.9 %
pX	74	68	44.3 %	65	50.7 %	70	40.0 %	65	52.6 %	80	31.9 %
pVI	218	238	43.5 %	238	43.9 %	273	38.1 %	199	46.6 %	250	37.6 %
hexon	913	905	70.8 %	991	65.3 %	907	71.2 %	916	69.0 %	952	65.4 %
protease	207	206	57.8 %	204	56.9 %	206	58.7 %	201	59.6 %	204	57.4 %
DBP	452	454	40.9 %	495	40.8 %	437	41.5 %	440	52.5 %	529	31.5 %
100K	696	689	47.5 %	686	49.6 %	697	47.2 %	710	50.4 %	807	43.2 %
22K	118	128	31.9 %	188	21.0 %	168	25.7 %	129	35.4 %	196	21.3 %
33 K	154	149	39.1 %	170	29.0 %	153	38.1 %	156	38.5 %	229	29.2 %
pVIII	194	224	32.9 %	217	33.2 %	222	32.3 %	205	33.3 %	227	30.4 %
E3 ORFA	206	117	not homologue	320	not homologue	115	not homologue	129	not homologue	107	not homologue
E3 ORFB	105	194	not homologue	88	not homologue	401	not homologue	–	–	63	not homologue
U exon (whole/ first exon)	98/55	54	19.0 %	55	24.5 %	55	20.0 %	55	39.3 %	217	16.4 %
fiber	552	543	18.5 %	610	11.5 %	606	21.4 %	656	23.4 %	581	24.6 %
E4 ORF6/7	–	86	–	78	–	89	–	–	–	150	–
E4 34 K	245	259	21.7 %	247	24.9 %	257	20.5 %	247	28.2 %	294	23.8 %
E4 ORFB	161	124	not homologue	132	not homologue	129	not homologue	108	not homologue	114	not homologue
E4 ORFA	67	122	not homologue	142	not homologue	124	not homologue	130	not homologue	116	not homologue

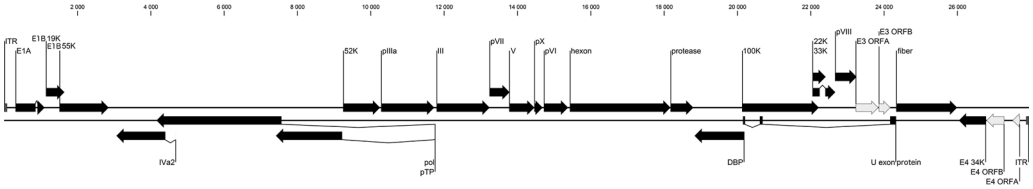

Fig. 1. Genome map of the polar bear adenovirus. The genome is represented by two black lines (each for one strand) marked at 2 kbp intervals. The ORFs supposed to encode proteins are shown as arrows. The exons of spliced genes are connected by lines. Conserved ORFs for mastadenoviruses are shown with black arrows, new ORFs with white arrows. The ITRs are marked with grey squares. Note the missing protein IX gene.

2002; Mangel and San Martin, 2014; Podgorski et al., 2016). Multiple alignments of the amino acid sequences were prepared with the Muscle program of the MEGA6 package. The selection of the best substitution model selection for the phylogenetic tree was performed using ProtTest (Darriba et al., 2011) and model LG + I + G was chosen for the aa sequences of *pol* and *hexon* genes. The final tree was calculated with PhyML on the ATGC bioinformatics platform of the French National Institute of Bioinformatics (Guindon et al., 2010). Approximate Likelihood-Ratio Test (aLRT SH-like) was applied for statistical tests for the branches (Anisimova and Gascuel, 2006). The phylogenetic trees were displayed in FigTree 1.4.4 program.

3. Results

3.1. Genome characteristics

The genome of the PBAdV-1 strain BK35 (GenBank accession no. MF773580) was found to be 27,952 bp, with inverted terminal repeats of 80 bp on both ends. The viral DNA has an average G + C content of 46.33 %, which can be considered as non-biased. The genome was predicted to contain 27 genes as presented in Table 1 in comparison to other carnivoran AdVs (CAdV-1, CSLAdV-1 and SkAdV-1), bat-WIV12 AdV and human adenovirus 5 (HAdV-5). The number and arrangement of the predicted genes were similar to those of other mastadenoviruses; every genus-specific gene except that of protein IX could be identified. One fiber gene could be determined. The E3 region contains two novel ORFs (E3 ORFA, E3 ORFB) but lacks the 12.5 K gene. In the E4 region, two novel ORFs (E4 ORFA, E4 ORFB) were found besides the conserved 34 K gene (called ORF6 in HAdVs) but gene ORF6/7 was missing. The three exons of the U exon protein gene could be predicted (Podgorski et al., 2016). The splicing donor and acceptor sites, characteristic for the genus, were predicted in *pol*, and in the E1A, pTP, IVa2 and 33 K genes, too. No evidence of gene duplication was found. A schematic genetic map of the PBAdV-1 is presented in Fig. 1. The protease cleavage sites of the precursor proteins could be determined by the alignment of consensus sites (M/L/I)XGG'X (type I), (M/L/I)XGX'G (type II)

Table 2
The protease cleavage sites of the precursor proteins of PBAdV-1. The numbers show the first aa of the cleavage signal.

Cleavage site type Precursor protein	type I	type II	type IIb
pTP	302	172	
pIIIa		461	
pVII	27		17
pX	41		
pVI	30	204	
pVIII	128		

and NTGW'G (type IIb) of selected adenoviruses (Farkas et al., 2002) (Table 2 and Fig. 2).

3.2. Phylogenetic analyses

Phylogenetic trees, obtained by maximum likelihood analysis based on the full aa sequence of the DNA-dependent DNA polymerase (a) and the hexon (b) proteins are presented in Fig. 3. The topology of both trees as well as the genome analysis confirmed that PBAdV-1 belongs to the genus *Mastadenovirus* but forms a independent branch corresponding to a species-level separation.

4. Discussion

Adenoviruses have been found in many vertebrates, but their appearance in exotic mammals has not been studied in much detail. Human AdVs are definitely the best described AdVs, but non-human AdVs are getting more attention too, because of their widespread occurrence in nature and the possibilities to use them as vaccines or as vectors in gene therapy (Alonso-Padilla et al., 2016). We have extensive knowledge about non-human primate, ruminant and bird AdVs, but those from other vertebrate species, especially the exotic ones, are left behind. The main reason might be the problematic sampling of these animals, as well as the lack of specific tissue and cell cultures to isolate and propagate these viruses. However, molecular biology provides methods to identify and even fully sequence non-isolated viruses, and the International Committee on Taxonomy of Viruses decided that complete genomic sequences can be accepted as representatives of official virus species (Simmonds et al., 2017).

Here we present the complete coding sequence of a polar bear adenovirus, PBAdV-1. The death of the specimen was caused by beta-2 toxin producing *Clostridium perfringens* enterotoxaemia. Consequently, the pathogenicity of the novel virus is unclear.

Our investigation showed that the PBAdV-1 has a similar genome arrangement to other mastadenoviruses, but it has a non-typical short genome of 27,952 bp. Since seroprevalence of canine AdVs has been described in wild carnivorans, *inter alia* bears and polar bears (Zarnke and Evans, 1989; Knowles et al., 2018), we expected to find a canine AdV in the sample. However, phylogenetic analysis of the virus indicates that the PBAdV-1 is a member of a new species within the genus *Mastadenovirus*. Although the PBAdV-1 is very different from all other AdVs found so far, the primers we use in routine PCR detection of AdVs (targeting genes IVa2, *pol*, hexon) amplified this AdV successfully. We could not perform next generation sequencing in this situation since we do not have the appropriate cell line to isolate the virus and produce larger amounts of DNA. However, primer walking and traditional (Sanger) sequencing was fully adequate, most probably mainly because of the high amount of virus in the tissue samples.

Genome analysis showed the presence of all genus specific genes except the one coding protein IX. The lack is surprising since this gene has been detected in all other wild type mastadenoviruses so far. Protein IX functions as a cement protein for the AdV capsid and stabilizes the structure, furthermore functions as transcriptional activator and seems to be involved in virus-induced nuclear reorganization, too (Rosa-Calatrava et al., 2001). Studies show, that in the absence of protein IX, HAdV virions are heat-labile, yet more easily growing on certain, especially on Coxsackie and Adenovirus Receptor (CAR)-negative cell lines (Colby and Shenk, 1981; Sargent et al., 2004; de Vrij et al., 2011). Further studies are needed to clarify whether protein IX-deleted AdVs have an increased pathogenicity.

The predicted putative splicing sites in the E1A, pTP, *pol*, IVa2, 33 K and U exon protein genes are typical for the genus. Compared to other mastadenoviruses some genes seem to be shorter (52 K, pIIIa, V and pVIII) and the similarity is very low; max. 71.2 % in hexon gene (Table 1). The protease cleavage signals in the precursor proteins pTP, pIIIa, pVII, pX, pVI and pVIII could also be determined (Table 2) and are well comparable to those in other mastadenoviruses. The novel ORFs in the regions E3 and E4 are not similar to any know AdV ORF and neither to any protein deposited in the GenBank.

It is surprising how well the 34 K homologue is preserved during the evolution. It is present in all mast- and aviadenoviruses, as well as in atadenoviruses, where even in duplicated (multiplicated). Only members of the genus Siadenovirus are devoid of this gene (Gilson et al., 2016). The 34 K protein of mastadenoviruses forms Cullin-based ubiquitin ligase complexes that, in association with E1B 55 K (present also in PBAdV-1), target cellular proteins for degradation. Thus it may be understandable why this gene is the only gene preserved in the E4 region of the PBAdV-1.

An interesting question is, whether the AdVs found in the members of order Carnivora co-evolved with their hosts. Canine and skunk AdVs had been hypothesized to originate from bat AdVs by host switch (Kohl et al., 2012; Kozak et al., 2015; Vidovszky et al., 2015). Surprisingly the skunk AdV found originally in Canada has been found also in a New World monkey from a European (Hungarian) zoo (Gál et al., 2013) and in African pigmy hedgehogs kept as pets in Japan and the USA (Madarame et al., 2016; Needle et al., 2019). This almost unprecedented ability among AdVs to switch species barrier among evolutionarily so different host suggests that skunk is not the real host the virus co-evolved with. Similarly, CAdVs have been described from a

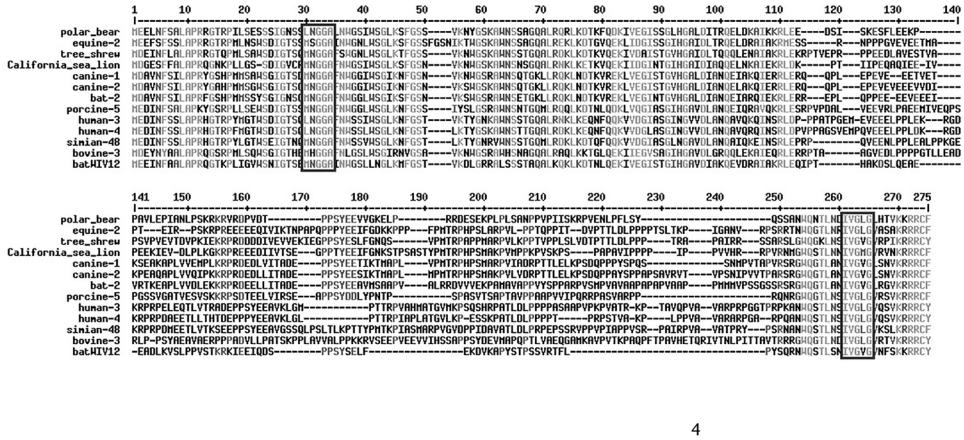

Fig. 2. Multiple alignments of precursor protein pVI sequences of selected mammalian adenoviruses. The conserved protease cleavage signals are marked with grey frames. The sequence alignment was performed using the MultAlin 5.4.1 program (http://multalin.toulouse.inra.fr/multalin). Precursor protein pVI was selected as representative of all cleaved precursor proteins, as it is one of the shortest ones and cleavage signals are best visible.

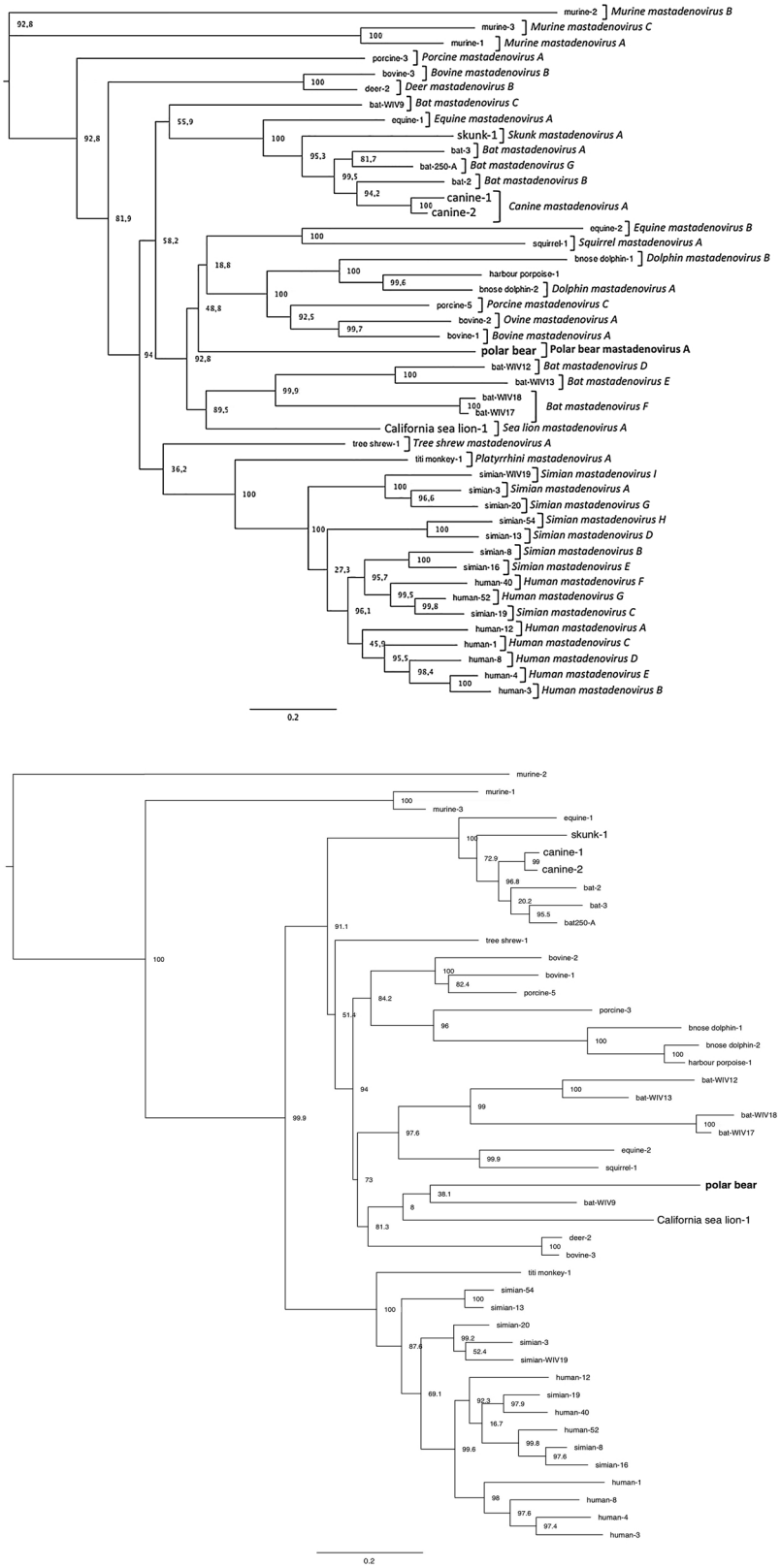

Fig. 3. Phylogenetic tree of full (a) DNA-dependent DNA polymerase and (b) hexon protein amino acid sequences of AdVs of selected species. Maximum likelihood calculation with LG + I+G model. From the names of the AdV types the word "AdV" was deleted for clarity. The AdVs isolated from carnivorans are shown with larger font, the AdV detected in polar bear with larger and bold font. The Approximate Likelihood-Ratio Test (aLRT) values are shown as percentages. On Fig. 3a virus species are shown, too.

large range of carnivorans (fox, coyote, jackal, raccoon, wolf, even from sea lion and various bears). This fact is indicative of a relatively recent host switch to dog, and is rather against a long co-evolutionary history with dogs. Thus, the remarkable pathogenicity of CAdVs might be explained by the lack of successful adaptation to a newly invaded host. On the other hand, PBAdV-1 shows large phylogenetic distance and a genome organization that differs from these bat originated viruses.

Until a year ago, only one AdV has been identified from bears which could be characterized by sequencing; it was a CAdV-1 from a grizzly bear (*Ursus arctos horribilis*) cub found dead (Knowles et al., 2018). Besides this finding, only seropositivity has been reported (detected in brown bears, grizzly bears, and even polar bears), which was attributed to canine AdV infection (Chomel et al., 1998; Dunbar et al., 1998; Bronson et al., 2014; Di Francesco et al., 2015). Recently, the sequence of an almost identical AdV (sharing 99 % nt identity with our PBAdV-1) has been described from a captive-born polar bear cub that had died in the Berlin Zoo (Dayaram et al., 2018). Compared to that analysis, we determined the genome ends (ITRs), and predicted the splicing sites (for genes E1A, pTP, *pol*, IVa2, 33 K and U exon protein). This is especially important for the *pol* gene as it results a considerably longer protein, which is more suitable for phylogenetic analysis. Also important that both genome analyses show the lack of protein IX, which was such unique finding that first we attributed it to some possible technical problem. For this reason, we discussed this topic more in detail.

Our work provides a strong evidence for this virus being a genuine polar bear virus as opposed to the assumption of Dayaram et al. (2018) who have speculated a cross-species transmission of a novel pathogen. The geographical and temporal distances between the two cases practically exclude the existence of a common source. The two polar bears could obviously have no direct or indirect contact.

This novel virus shows adequate divergences from the other mastadenoviruses to merit the establishment of a novel species. Based on the above-mentioned facts, we propose confidently the establishment of a new virus species with the name of *Polar bear mastadenovirus A* (Harrach et al., 2011). The species demarcation criteria, such as like phylogenetic distance (*pol* amino acid sequence difference larger than 15 %), genome organization (missing protein IX, four novel E3 or E4 genes), G + C content and the novel host, all justify this proposal.

CRediT authorship contribution statement

Kinga P. Böszörményi: Conceptualization, Methodology, Investigation, Writing - original draft. **Iva I. Podgorski:** Methodology, Supervision. **Márton Z. Vidovszky:** Validation, Formal analysis. **Endre Sós:** Resources. **Mária Benkő:** Writing - review & editing, Project administration. **Balázs Harrach:** Supervision, Writing - review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.virusres.2019.197846.

References

Alonso-Padilla, J., Papp, T., Kaján, G.L., Benkő, M., Havenga, M., Lemckert, A., Harrach, B., Baker, A.H., 2016. Development of novel adenoviral vectors to overcome challenges observed with HAdV-5-based constructs. Mol. Ther. 24 (1), 6–16.

Anisimova, M., Gascuel, O., 2006. Approximate likelihood-ratio test for branches: a fast, accurate, and powerful alternative. Syst. Biol. 55 (4), 539–552.

Balboni, A., Verin, R., Morandi, F., Poli, A., Prosperi, S., Battilani, M., 2013. Molecular epidemiology of canine adenovirus type 1 and type 2 in free-ranging red foxes (*Vulpes vulpes*) in Italy. Vet. Microbiol. 162 (2-4), 551–557.

Ballmann, M.Z., Harrach, B., 2016. Detection and partial genetic characterisation of novel avi- and siadenoviruses in racing and fancy pigeons (*Columba livia domestica*). Acta Vet. Hung. 64 (4), 514–528.

Ballmann, M.Z., Vidovszky, M.Z., 2013. Detection of broad-host-range psittacine adenovirus (PsAdV-2) in representatives of different parrot species. [in Hungarian]. Magy Allatorvosok 135, 78–84.

Benkő, M., 2008. Adenoviruses: pathogenesis. In: Mahy, B.W.J., van Regenmortel, M.H.V. (Eds.), Encyclopedia of Virology (Third Edition). Elsevier, Oxford, pp. 24–29.

Benkő, M., Élő, P., Ursu, K., Ahne, W., LaPatra, S.E., Thomson, D., Harrach, B., 2002. First molecular evidence for the existence of distinct fish and snake adenoviruses. J. Virol. 76 (19), 10056–10059.

Benkő, M., Harrach, B., 2003. Molecular evolution of adenoviruses. Curr. Top. Microbiol. Immunol. 272, 3–35.

Berriman, M., Rutherford, K., 2003. Viewing and annotating sequence data with Artemis. Brief Bioinform 4 (2), 124–132.

Bronson, E., Spiker, H., Driscoll, C.P., 2014. Serosurvey for selected pathogens in free-ranging American black bears (*Ursus americanus*) in Maryland, USA. J. Wildl. Dis. 50 (4), 829–836.

Chomel, B.B., Kasten, R.W., Chappuis, G., Soulier, M., Kikuchi, Y., 1998. Serological survey of selected canine viral pathogens and zoonoses in grizzly bears (*Ursus arctos horribilis*) and black bears (*Ursus americanus*) from Alaska. Rev. Sci. Tech. 17 (3), 756–766.

Colby, W.W., Shenk, T., 1981. Adenovirus type 5 virions can be assembled *in vivo* in the absence of detectable polypeptide IX. J. Virol. 39 (3), 977–980.

Cortes-Hinojosa, G., Doescher, B., Kinsel, M., Lednicky, J., Loeb, J., Waltzek, T., Wellehan Jr., J.F., 2016. Coinfection of California sea lion adenovirus 1 and a novel polyomavirus in a Hawaiian monk seal (*Neomonachus schauinslandi*). J. Zoo Wildl. Med. 47 (2), 427–437.

Cortes-Hinojosa, G., Gulland, F.M., Goldstein, T., Venn-Watson, S., Rivera, R., Waltzek, T.B., Salemi, M., Wellehan Jr., J.F., 2015. Phylogenomic characterization of California sea lion adenovirus-1. Infect. Genet. Evol. 31, 270–276.

Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2011. ProtTest 3: fast selection of best-fit models of protein evolution. Bioinformatics 27 (8), 1164–1165.

Dayaram, A., Tsangaras, K., Pavulraj, S., Azab, W., Groenke, N., Wibbelt, G., Sicks, F., Osterrieder, N., Greenwood, A.D., 2018. Novel divergent polar bear-associated mastadenovirus recovered from a deceased juvenile polar bear. mSphere 3 (4), e00171–18.

de Vrij, J., van den Hengel, S.K., Uil, T.G., Koppers-Lalic, D., Dautzenberg, I.J., Stassen, O.M., Barcena, M., Yamamoto, M., de Ridder, C.M., Kraaij, R., Kwappenberg, K.M., Schilham, M.W., Hoeben, R.C., 2011. Enhanced transduction of CAR-negative cells by protein IX-gene deleted adenovirus 5 vectors. Virology 410 (1), 192–200.

Di Francesco, C.E., Gentile, L., Di Pirro, V., Ladiana, L., Tagliabue, S., Marsilio, F., 2015. Serologic evidence for selected infectious diseases in Marsican brown bears (*Ursus arctos marsicanus*) in Italy (2004-09). J. Wildl. Dis. 51 (1), 209–213.

Doszpoly, A., Wellehan Jr., J.F., Childress, A.L., Tarján, Z.L., Kovács, E.R., Harrach, B., Benkő, M., 2013. Partial characterization of a new adenovirus lineage discovered in testudinoid turtles. Infect. Genet. Evol. 17, 106–112.

Dunbar, M.R., Cunningham, M.W., Roof, J.C., 1998. Seroprevalence of selected disease agents from free-ranging black bears in Florida. J. Wildl. Dis. 34 (3), 612–619.

Farkas, S.L., Benkő, M., Élő, P., Ursu, K., Dan, A., Ahne, W., Harrach, B., 2002. Genomic and phylogenetic analyses of an adenovirus isolated from a corn snake (*Elaphe guttata*) imply a common origin with members of the proposed new genus *Atadenovirus*. J. Gen. Virol. 83, 2403–2410.

Gál, J., Hornyák, Á., Mándoki, M., Bakonyi, T., Balka, G., Szeredi, L., Marosán, M., Ludányi, T., Forgách, P., Sós, E., Demeter, Z., Farkas, S.L., 2013. Novel mastadenovirus infection and clinical disease in a pygmy marmoset (*Callithrix [Cebuella] pygmaea*). Vet. Microbiol. 167 (3-4), 695–699.

Garcia-Morante, B., Pénzes, J.J., Costa, T., Martorell, J., Martinez, J., 2016. Hyperplastic stomatitis and esophagitis in a tortoise (*Testudo graeca*) associated with an adenovirus infection. J. Vet. Diagn. Invest. 28 (5), 579–583.

Gilson, T., Blanchette, P., Ballmann, M.Z., Papp, T., Pénzes, J.J., Benkő, M., Harrach, B., Branton, P.E., 2016. Using the E4orf6-based E3 ubiquitin ligase as a tool to analyze the evolution of adenoviruses. J. Virol. 90 (16), 7350–7367.

Goldstein, T., Colegrove, K.M., Hanson, M., Gulland, F.M., 2011. Isolation of a novel adenovirus from California sea lions Zalophus californianus. Dis Aquat Organ 94 (3), 243–248.

Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W., Gascuel, O., 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst. Biol. (Stevenage) 59 (3), 307–321.

Harrach, B., Benkő, M., Both, G.W., Brown, M., Davison, A.J., Echavarría, M., Hess, M., Jones, M.S., Kajon, A., Lehmkuhl, H.D., Mautner, V., Mittal, S.K., Wadell, G., 2011. Adenoviridae. In: King, A.M.Q., Adams, M.J., Carstens, E.B., Lefkowitz, E.J. (Eds.), Virus Taxonomy. Ninth Report of the International Committee on Taxonomy of Viruses. Elsevier, Oxford, pp. 125–141.

Inoshima, Y., Murakami, T., Ishiguro, N., Hasegawa, K., Kasamatsu, M., 2013. An

outbreak of lethal adenovirus infection among different otariid species. Vet. Microbiol. 165 (3-4), 455–459.
Kiss, I., Matiz, K., Bajmóci, E., Rusvai, M., Harrach, B., 1996. Infectious canine hepatitis: detection of canine adenovirus type 1 by polymerase chain reaction. Acta Vet. Hung. 44 (2), 253–258.
Knowles, S., Bodenstein, B.L., Hamon, T., Saxton, M.W., Hall, J.S., 2018. Infectious canine hepatitis in a brown bear (*Ursus arctos horribilis*) from Alaska, USA. J. Wildl. Dis. 54 (3), 642–645.
Kohl, C., Vidovszky, M.Z., Muhldorfer, K., Dabrowski, P.W., Radonic, A., Nitsche, A., Wibbelt, G., Kurth, A., Harrach, B., 2012. Genome analysis of bat adenovirus 2: indications of interspecies transmission. J. Virol. 86 (3), 1888–1892.
Kovács, E.R., Benkő, M., 2011. Complete sequence of raptor adenovirus 1 confirms the characteristic genome organization of siadenoviruses. Infect. Genet. Evol. 11 (5), 1058–1065.
Kozak, R.A., Ackford, J.G., Slaine, P., Li, A., Carman, S., Campbell, D., Welch, M.K., Kropinski, A.M., Nagy, E., 2015. Characterization of a novel adenovirus isolated from a skunk. Virology 485, 16–24.
Lakatos, B., Hornyák, A., Demeter, Z., Forgách, P., Kennedy, F., Rusvai, M., 2017. Detection of a putative novel adenovirus by PCR amplification, sequencing and phylogenetic characterisation of two gene fragments from formalin-fixed paraffin-embedded tissues of a cat diagnosed with disseminated adenovirus disease. Acta Vet. Hung. 65 (4), 574–584.
Lee, S.Y., Kim, J.H., Seo, T.K., No, J.S., Kim, H., Kim, W.K., Choi, H.G., Kang, S.H., Song, J.W., 2016. Genetic and molecular epidemiological characterization of a novel adenovirus in Antarctic penguins collected between 2008 and 2013. PLoS One 11 (6), e0157032.
Madarame, H., Ogihara, K., Ochiai, H., Omatsu, T., Mizutani, T., 2016. Detection of skunk adenovirus 1 (SkAdV-1) in an African pigmy hedgehog (*Atelerix albiventris*). Vet. Rec. Case Rep. 4 (1), e000321.
Mangel, W.F., San Martin, C., 2014. Structure, function and dynamics in adenovirus maturation. Viruses 6 (11), 4536–4570.
Needle, D.B., Selig, M.K., Jackson, K.A., Delwart, E., Tighe, E., Leib, S.L., Seuberlich, T., Pesavento, P.A., 2019. Fatal bronchopneumonia caused by skunk adenovirus 1 in an African pygmy hedgehog. J. Vet. Diagn. Invest. 31 (1), 103–106.
Pantó, L., Podgorski, I.I., Jánoska, M., Markó, O., Harrach, B., 2015. Taxonomy proposal for Old World monkey adenoviruses: characterisation of several non-human, non-ape primate adenovirus lineages. Arch. Virol. 160 (12), 3165–3177.
Papp, T., Fledelius, B., Schmidt, V., Kaján, G.L., Marschang, R.E., 2009. PCR-sequence characterization of new adenoviruses found in reptiles and the first successful isolation of a lizard adenovirus. Vet. Microbiol. 134 (3-4), 233–240.
Pénzes, J.J., Menendez-Conejero, R., Condezo, G.N., Ball, I., Papp, T., Doszpoly, A., Paradela, A., Perez-Berna, A.J., Lopez-Sanz, M., Nguyen, T.H., van Raaij, M.J., Marschang, R.E., Harrach, B., Benkő, M., San Martin, C., 2014. Molecular characterization of a lizard adenovirus reveals the first atadenovirus with two fiber genes and the first adenovirus with either one short or three long fibers per penton. J. Virol. 88 (19), 11304–11314.
Podgorski, I.I., Pantó, L., Földes, K., de Winter, I., Jánoska, M., Sós, E., Chenet, B., Harrach, B., Benkő, M., 2018. Adenoviruses of the most ancient primate lineages support the theory on virus-host co-evolution. Acta Vet. Hung. 66 (3), 474–487.
Podgorski, I.I., Pantó, L., Papp, T., Harrach, B., Benkő, M., 2016. Genome analysis of four Old World monkey adenoviruses supports the proposed species classification of primate adenoviruses and reveals signs of possible homologous recombination. J. Gen. Virol. 97 (7), 1604–1614.
Rivera, S., Wellehan Jr., J.F., McManamon, R., Innis, C.J., Garner, M.M., Raphael, B.L., Gregory, C.R., Latimer, K.S., Rodriguez, C.E., Diaz-Figueroa, O., Marlar, A.B., Nyaoke, A., Gates, A.E., Gilbert, K., Childress, A.L., Risatti, G.R., Frasca Jr., S., 2009. Systemic adenovirus infection in Sulawesi tortoises (*Indotestudo forsteni*) caused by a novel siadenovirus. J. Vet. Diagn. Invest. 21 (4), 415–426.
Rosa-Calatrava, M., Grave, L., Puvion-Dutilleul, F., Chatton, B., Kedinger, C., 2001. Functional analysis of adenovirus protein IX identifies domains involved in capsid stability, transcriptional activity, and nuclear reorganization. J. Virol. 75 (15), 7131–7141.
Ruzindana-Umunyana, A., Imbeault, L., Weber, J.M., 2002. Substrate specificity of adenovirus protease. Virus Res. 89 (1), 41–52.
Sargent, K.L., Ng, P., Evelegh, C., Graham, F.L., Parks, R.J., 2004. Development of a size-restricted pIX-deleted helper virus for amplification of helper-dependent adenovirus vectors. Gene Ther. 11 (6), 504–511.
Simmonds, P., Adams, M.J., Benkő, M., Breitbart, M., Brister, J.R., Carstens, E.B., Davison, A.J., Delwart, E., Gorbalenya, A.E., Harrach, B., Hull, R., King, A.M., Koonin, E.V., Krupovic, M., Kuhn, J.H., Lefkowitz, E.J., Nibert, M.L., Orton, R., Roossinck, M.J., Sabanadzovic, S., Sullivan, M.B., Suttle, C.A., Tesh, R.B., van der Vlugt, R.A., Varsani, A., Zerbini, F.M., 2017. Consensus statement: virus taxonomy in the age of metagenomics. Nat. Rev. Microbiol. 15 (3), 161–168.
Staden, R., Beal, K.F., Bonfield, J.K., 1998. The Staden package. In: Misener, S., Krawetz, S. (Eds.), Computer Methods in Molecular Biology. Humana Press, Totowa, pp. 115–130.
Szirovicza, L., Lopez, P., Kopena, R., Benkő, M., Martin, J., Pénzes, J.J., 2016. Random sampling of squamate reptiles in Spanish natural reserves reveals the presence of novel adenoviruses in Lacertids (family Lacertidae) and worm lizards (Amphisbaenia). PLoS One 11 (7), e0159016.
Thompson, H., O'Keeffe, A.M., Lewis, J.C., Stocker, L.R., Laurenson, M.K., Philbey, A.W., 2010. Infectious canine hepatitis in red foxes (*Vulpes vulpes*) in the United Kingdom. Vet. Rec. 166 (4), 111–114.
Vidovszky, M., Kohl, C., Boldogh, S., Görföl, T., Wibbelt, G., Kurth, A., Harrach, B., 2015. Random sampling of the Central European bat fauna reveals the existence of numerous hitherto unknown adenoviruses. Acta Vet. Hung. 63 (4), 508–525.
Walker, D., Gregory, W.F., Turnbull, D., Rocchi, M., Meredith, A.L., Philbey, A.W., Sharp, C.P., 2017. Novel adenoviruses detected in British mustelids, including a unique *Aviadenovirus* in the tissues of pine martens (*Martes martes*). J. Med. Microbiol. 66 (8), 1177–1182.
Wellehan, J.F., Johnson, A.J., Harrach, B., Benkő, M., Pessier, A.P., Johnson, C.M., Garner, M.M., Childress, A., Jacobson, E.R., 2004. Detection and analysis of six lizard adenoviruses by consensus primer PCR provides further evidence of a reptilian origin for the atadenoviruses. J. Virol. 78 (23), 13366–13369.
Wright, E.P., Waugh, L.F., Goldstein, T., Freeman, K.S., Kelly, T.R., Wheeler, E.A., Smith, B.R., Gulland, F.M., 2015. Evaluation of viruses and their association with ocular lesions in pinnipeds in rehabilitation. Vet. Ophthalmol. 18 (Suppl 1), 148–159.
Zarnke, R.L., Evans, M.B., 1989. Serologic survey for infectious canine hepatitis virus in grizzly bears (*Ursus arctos*) from Alaska, 1973 to 1987. J. Wildl. Dis. 25 (4), 568–573.