

REVIEW ARTICLE

Adenoviruses across the animal kingdom: a walk in the zoo

 Balázs Harrach , Zoltán L. Tarján and Mária Benkő

Institute for Veterinary Medical Research, Centre for Agricultural Research, Budapest, Hungary

Correspondence

B. Harrach, Institute for Veterinary Medical Research, Centre for Agricultural Research, H-1143 Budapest, Hungária krt. 21, Hungary
 Tel: +36 1 467-4081
 E-mail: harrach.balazs@agrar.mta.hu

(Received 6 October 2019, revised 11 November 2019, accepted 12 November 2019, available online 9 December 2019)

doi:10.1002/1873-3468.13687

Edited by Urs Greber

Adenoviruses (AdVs) infect representatives of numerous species from almost every major vertebrate class, albeit their incidence shows great variability. AdVs infecting birds, reptiles, and bats are the most common and diverse, whereas only one AdV has been so far isolated both from fish and amphibians. The family *Adenoviridae* is divided into five genera, each corresponding to an independent evolutionary lineage that supposedly coevolved with its respective vertebrate hosts. Members of genera *Mastadenovirus* and *Aviadenovirus* seem to infect exclusively mammals and birds, respectively. The genus *Ichtadenovirus* includes the single known AdV from fish. The majority of AdVs in the genus *Atadenovirus* originated from squamate reptiles (lizards and snakes), but also certain mammalian and avian AdVs are classified within this genus. The genus *Siadenovirus* contains the only AdV isolated from frog, along with numerous avian AdVs. In turtles, members of a sixth AdV lineage have been discovered, pending official recognition as an independent genus. The most likely scenario for AdV evolution includes long-term cospeciation with the hosts, as well as occasional switches between closely or, rarely, more distantly related hosts.

Keywords: *Adenoviridae*; adenovirus; animal viruses; *Atadenovirus*; *Aviadenovirus*; coevolution; *Ichtadenovirus*; *Mastadenovirus*; *Siadenovirus*; *Testadenovirus*

Adenoviruses (AdVs) have been isolated from, or detected in, representatives of numerous vertebrate species, but never in invertebrates. We are interested in the exploration of the prevalence and diversity of AdVs in the possible highest number of animals, including domestic or wild, captive or free-living individuals. A snapshot of the present status of this quest will be presented by overviewing the actual and proposed virus taxonomy according to the host animals' taxa.

The viruses, in the family *Adenoviridae*, are currently grouped into five genera [1]. The genus *Mastadenovirus* contains AdVs of mammalian host origin. Similarly, only avian AdVs are contained in the genus

Aviadenovirus. The genus *Ichtadenovirus* was established to include the only known AdV from a fish host [2]. Members of the remaining two genera originate from a wider spectrum of host animal species. *Atadenovirus* includes AdVs found in various ruminant and avian hosts, in two marsupial-associated samples, in addition to viruses detected in animals from the order Squamata, that is, lizards, snakes, and worm lizards. These reptiles are hypothesized to be the host lineage with which atadenoviruses have coevolved. Members of the genus *Siadenovirus* have been found to infect birds, a single frog, and a few tortoise species. Earlier, siadenoviruses had been thought to represent the

Abbreviations

AdV, adenovirus; BtAdV, bat adenovirus; CAdV, canine adenovirus; EDSV, egg drop syndrome virus; FAdV, fowl adenovirus; HAdV-1, human adenovirus 1; HAdV-A, *Human mastadenovirus A*; PAdV, porcine adenovirus; PsAdV, psittacine adenovirus; SnAdV, snake adenovirus.

amphibian lineage of AdVs [3]. A sixth AdV lineage has been discovered more recently in a number of testudinoid turtles, and was proposed, albeit not approved yet, to become an additional genus *Testadenovirus* [4]. The genera are further subdivided into AdV species, each of which may contain several virus types, designated by the host name with ascending numbers. Originally, the AdV types had been demarcated based on their serological distinctness; however, in the past decade, the majority of novel types, for example, all human AdVs (HAdVs) over type number 52, are assigned according to the full genomic sequence.

In spite of the over 65-year history of AdV research, the overwhelming majority of our knowledge concerning the replication cycle and the exact function of the early viral genes still comes from the study of HAdVs. Only a handful of animal AdVs, mainly belonging to the genera *Mastadenovirus* and *Aviadenovirus*, have been examined in detail. A striking divergence from the well-known HAdVs has been discovered even in other members of the genus *Mastadenovirus*, both in genome length and in genetic content. The length of the fully sequenced AdV genomes ranges between 25 and 48 kbp. The central part of the genome is occupied by a cassette of 16 genes that are well-conserved across the genera, whereas the genome ends contain early regions (E1 and E4) contributing to efficient replication, at least for the well-studied mastadenoviruses. The number of these ORFs is also very variable, from as few as five in siadenoviruses to as many as 28 ORFs in the fish AdV. Each AdV lineage, acknowledged as genus, also shares a specific genome organization; nonetheless, smaller or larger differences can be present among AdVs of the same genus. These traits are used in the demarcation of the AdV species (<https://sites.google.com/site/adenoseq/>). The emerging picture of diversity foreshadows the existence of slightly different AdV replication strategies.

We also wanted to test our former hypothesis on the continuous coevolution of AdVs with their vertebrate hosts [3]. To this end, a plethora of randomly collected samples from live and dead animals have been screened. A broad-range nested PCR with consensus primers, targeting the viral DNA polymerase gene, seemed to be capable of detecting any unknown AdV [5]. Sequencing of the amplicon usually gives enough information for the preliminary classification of any newly detected AdV at least at the genus level. The samples originated both from live and dead animals. When excretes or intestinal samples are used, it is important to differentiate between ingested or infecting viruses, that is, those actually replicating in the host organism. For example, if a murine AdV

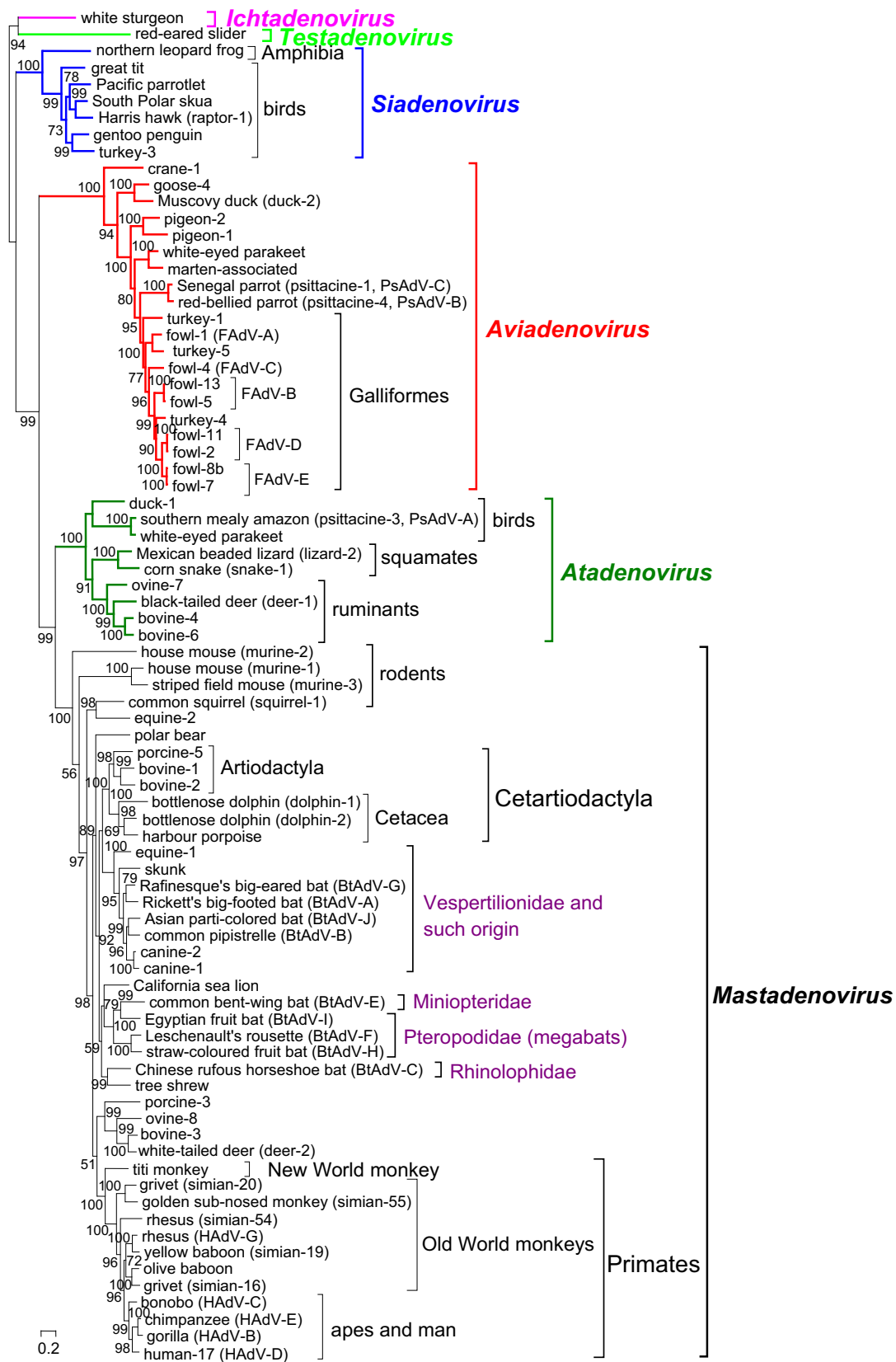
sequence is obtained from the PCR testing of intestinal content of buzzards or other birds of prey, the obvious interpretation is that the prey and not the predator had been infected. Apart from such exceptional cases, no birds have been proved to harbor mastadenoviruses. For a strong hypothesis of infection, the AdVs should be detected in the internal organs. Full genome sequences revealing genome organization and G+C content of newly identified AdVs are useful and required tools for the definitive classification of any novel virus type into a given officially recognized species. Next-generation sequencing technologies make these operations easy and fast, especially in case of successful virus isolation. Unfortunately, in most of the cases, *in vitro* propagation of new AdVs is hampered by the lack of appropriate cell cultures. In such cases, one has to rely on partial genomic characterizations, which provide useful hints about the incredible natural variety of AdVs.

We present the great diversity of the animal AdVs through an imaginary walk in the zoo, by grouping them according to the host taxonomy. The phylogeny reconstruction, based on the full amino acid sequence of the adenoviral DNA polymerase, reflects the virus–host coevolution with earlier important interclass host changes (Fig. 1). The divergence values of the DNA polymerase, which is one of the most conserved proteins, were chosen by the International Committee on Taxonomy of Viruses as the most important species demarcation criterion [1].

AdV in fish (*Ichta*denovirus)

White sturgeon adenovirus

Although several early reports have been published on the EM observation of AdV-like particles in different organs of diseased fishes; to date, only a single piscine isolate has been confirmed as an AdV based on molecular evidence [6]. The full-genome sequence analysis of this virus, obtained from the white sturgeon in the USA, held multiple surprises [2]. As coming from one of the most ancient vertebrate hosts, it was expected to be the most primitive AdV with short and simply organized genome. However, exactly to the contrary, white sturgeon AdV-1 was found to possess the longest hitherto sequenced AdV genome with 28 novel ORFs, only five of which could be assigned to a probable function. Another significant difference was the unusual location of the fiber-like genes at the left end of the genome. No other fish AdVs could be recovered in spite of targeted efforts with nested consensus PCR to screen more than 430 samples from 44 fish species.



This came as a surprise especially because of the outstandingly large number of fish species. Metagenomics studies on marine and freshwater samples or fish genome sequencing have not produced any similar sequences as yet. Accordingly, we will need to consider the modification of our hypothesis concerning the presence of this virus in the white sturgeon. Other fish viruses thought to be AdV, based on EM morphology, turned out to be putative members of a candidate new virus family *Adomaviridae*. Adomaviruses seem to be intermediates or ‘mosaics’ of polyomaviruses, papillomaviruses, parvoviruses, and very ancient AdVs, thus giving hints of the evolutionary origin of AdVs [7].

AdV in amphibians (*Siadenovirus*)

Frog adenovirus

The single amphibian isolate (frog AdV-1) has been obtained from the northern leopard frog, interestingly enough on a cell line of reptilian origin. It has the shortest hitherto sequenced AdV genome with the simplest organization with merely five novel ORFs in place of the mastadenoviral early regions (E1, E3 and E4) [8]. It is intriguing to imagine how these genes, without hardly known functions, can perform all the steps necessary for efficient replication. Other viruses, reported as novel AdV in frogs, have been identified later as members of the family *Iridoviridae*. Since no additional siadenovirus (or any other AdV) could be detected in frogs or salamanders during our intensive screenings covering dozens of samples, we tend to discard the hypothesis on the amphibian origin of this virus lineage. Based on currently available data, it seems to be more likely that the presence of a siadenovirus in the frog was the result of a larger-scale switch from a yet unknown host. It has to be added that the number of different bird species, individuals of which were found to harbor siadenovirus, has been increasing fast in the past decade (see later).

Nonetheless, the host origin of siadenoviruses needs to be considered still unclear, since every single siadenovirus, examined to date, possesses genomic DNA of biased base composition toward A+T, a deemed indicative feature of host-switching events [3,5].

AdVs in turtles (*Testadenovirus*, *Siadenovirus*, *Atadenovirus*)

Testadenoviruses

These viruses are supposed to have coevolved with turtles (i.e., members of the order Testudines).

Although originally, the adenoviral lineage coevolving with reptiles was hypothesized to be *Atadenovirus*, it turned out soon that atadenoviruses, specifically those with genomic DNA of nonbiased base composition, are confined to lizards, worm lizards, and snakes, classified into the largest reptilian order Squamata (scaled reptiles). More recently, new virus sequences, representing multiple virus species of a putative novel AdV lineage, have been discovered in red-eared and yellow-bellied sliders, box turtles, and pancake tortoises [4]. A similar virus was also detected in red-footed tortoises [9]. It seems that these viruses might be the specific AdVs coevolving with animals belonging to the order Testudines. Unfortunately, neither *in vitro* isolation nor full-genome sequencing attempts have been successful as yet. Although a large part of the genome, including the entire DNA polymerase gene, from the red-eared slider AdV was PCR-amplified and sequenced (GenBank accession No. JN632576.2; Fig. 1), the sequences of the genome ends, characteristic for the genera, are still unknown. Thus, the proposal for the establishment of a novel genus (*Testadenovirus*) has not been approved officially. No pathology was connected to the presence of these viruses, a phenomenon suggesting long-term adaptation of the virus to its environmental niche, thus providing indication for the species of origin.

Fig. 1. Phylogenetic tree shows the diversity of animal AdVs and suggests several possible coevolutionary AdV lineages and host switches. Maximum likelihood analysis of amino acid sequences of the DNA-dependent DNA polymerase from selected AdVs. Multiple alignment: MultAlin (<http://multalin.toulouse.inra.fr/multalin/>); model selection: ProtTest 2.4 (<http://darwin.uvigo.es/>); maximum likelihood calculation: PhyML 3.1 with model LG+I+G and Shimodaira-Hasegawa-like branch test for statistical test for branch support on the platform of Galaxy/Pasteur (<https://galaxy.pasteur.fr>). Unrooted calculation; white sturgeon AdV-1 and slider AdV-1 are selected as outgroup for visualization. The AdVs are shown by type name (the word ‘adenovirus/AdV’ was removed to avoid crowding) or by the name of the host, or both. The informal abbreviation of the accepted or tentative species is shown for the psittacine and fowl aviadenovirus types and the bat and human mastadenovirus types. The species classification of the other viruses is shown in Table S1. Large square brackets mark the AdV genera, whereas the smaller ones mark the AdV lineages of different vertebrate groups with which their coevolution is assumed. The families of bat hosts are shown in lilac. The CAdVs, the equine AdV-1, and the skunk AdV were found earlier to have BtAdV origin and host switch is assumed. The five official and the proposed sixth genera are shown in different colors. The bar indicates a 20% difference between two neighboring sequences. SH branch support values are shown at the nodes if more than 50. Further details about the analyzed sequences are shown in Table S1.

Siadenoviruses and atadenoviruses switching to turtles

Previously, a siadenovirus, namely Sulawesi tortoise AdV-1, was detected in members of a large group of smuggled turtles. The virus caused mortality not only among the confiscated individuals but also in other turtles of other testudinoid species in a couple of zoos that gave shelter to the rescued animals. This clearly indicates the capacity of Sulawesi tortoise AdV-1 to easily cross certain species boundaries [10].

Besides, an atadenovirus was detected in a Greek tortoise (spur-thighed tortoise) in dead animals of a collection in Spain [11]. This case is also considered as a host-switching event.

AdVs in squamate reptiles (*Atadenovirus*)

The first representatives of this genus had been discovered in cattle and poultry. The exceptionally high (> 60%) A+T content, observed in the genomic DNA, was used to name the genus. Because of their striking phylogenetic distance from the genera *Mastadenovirus* and *Aviadenovirus*, a targeted screening was initiated by scrutinizing lower vertebrates. Indeed, all snake and lizard AdVs were found to belong to the genus *Atadenovirus*. This was supported by the common genome organization as well as by their clustering in phylogenetic analyses. However, their balanced base composition specifically distinguished these atadenoviruses. This nonbiased G+C content seems to be the proof for the constant coevolution. The atadenovirus genomes do not contain obvious homologues of the E1 and E3 regions, and genes of protein V and IX (known from mastadenoviruses only). Nonetheless, in the overall genomic organization, the squamate-originated atadenoviruses show closer relation to mastadenoviruses than to members of any other genera [12].

Lizard adenoviruses

A large number of lizard AdV genomes has been detected by PCR, and partially or fully characterized. The majority of the hosts were captive or even captive-bred specimen, as reptiles are becoming more and more popular pets. The newly discovered hosts include, among others, Mexican beaded lizard and gila, from each of which an isolated strain was obtained as well. From free-living individuals, bearded dragons, anole lizards, and worm lizards, more reliable data can be deduced, which usually confirm a link between a certain host species and AdV type [13–16].

The full genome of lizard AdV-2, isolated from Mexican beaded lizard, was determined and the structure of the virion was also studied. This is the first AdV recognized to have three, identical (long) fiber projections on certain vertices, and a single shorter fiber in the other pentons. This morphological variation with alternating numbers of fibers on the vertices has not been found yet in any other AdV [17].

Snake adenoviruses

Three types of snake AdVs (SnAdVs) are known along with several genomic variants. SnAdV-1 was described from individuals of different species including corn snake, boa, and ball python, all captive bred. SnAdV-2 was detected in eastern corn snake, California kingsnake, and asp viper, and SnAdV-3 in gopher snake [18,19]. Full genome sequence of SnAdV-1 has been published [20] and its structure was also studied [21]. One should keep in mind that all these AdVs have been found in captive-bred snakes.

The presence of AdVs has been reported from Nile crocodiles, but their molecular confirmation remained fruitless. No AdVs have been found, if looked at all, in members of the smallest reptilian order Rynchocephalia, that is, tuataras.

AdVs in birds (*Aviadenovirus*, *Atadenovirus*, *Siadenovirus*)

The AdVs found in birds represent three different genera and, accordingly, show great variety both in genetic characteristics and pathogenesis.

Aviadenoviruses

This virus lineage is supposed to have coevolved continuously with avian hosts. Different AdV types, infecting poultry and waterfowl, were recognized soon after the discovery of the first AdVs in humans and domestic mammals. Nowadays, a complex picture on the widespread occurrence of numerous specific AdVs in representatives of an ever-growing number of bird species is emerging, thanks to the increased efficiency of detection methods as well as to the intensifying attention toward wild animals. The incredible diversity of avian AdVs can be explained by the outstandingly high number of avian species. The presence of over 10 000 species makes the birds the second vertebrate group in terms of richness of species right after the fish. The class Aves is divided into two main groups. The infraclass Palaeognathae includes the flightless ratites and the flying tinamous species. The other

infraclass Neognathae contains all the remaining birds in two larger clades termed Galloanserae and Neoaves.

Ratites

From this group, AdVs have been reported only from ostriches albeit not from their native distribution area but from commercial farms in Europe and China. Therefore, these results should be handled with precaution all the more so since the isolation was performed on primary chicken cells, and the recovered AdVs were identical with earlier described fowl AdV (FAdV) types [22]. No direct PCR detection of AdVs has been reported ever from rhea or cassowary, thus we still cannot be sure if any such ancient avian AdVs exist at all, or how much they would differ from the known members of the genus *Aviadenovirus*.

Galloanserae (Galliformes, Anseriformes)

Besides the twelve traditionally recognized FAdV types, well-known from chickens, additional aviadenoviruses have been reported from poultry and waterfowl, including four types from turkey, three from goose, and two from Muscovy duck [23–25]. FAdVs are assigned to five virus species, *Fowl aviadenovirus A* to *Fowl aviadenovirus E*, and specific pathologies seem to be connected to some of them [26]. For example, FAdV-1 from species *Fowl aviadenovirus A* causes gizzard erosion, and FAdV-4 from *Fowl aviadenovirus C* is responsible for severe, often fatal cases of the so-called hepatitis-hydropericardium syndrome in many Asian countries. Another clinical manifestation of AdV infection in chickens is the inclusion body hepatitis, caused by particular serotypes from *Fowl aviadenovirus D* and *Fowl aviadenovirus E*. FAdV-1 has been reported several times from birds other than chickens, including quail and Alagoas curassow, a pheasant-like bird, the wild populations of which became extinct. Nowadays, novel FAdV types are reported exceptionally rarely; however, several strains showing significant divergence from FAdV-5 were just described in Central Europe as candidate type FAdV-13 [27]. During metagenomics studies on fecal samples from Australian wild birds, partial sequences of an aviadenovirus were identified in Pacific black duck [28]. In phylogeny reconstruction, this virus appeared to be a close relative of duck AdV-2 and -3 originating from Muscovy ducks. Aviadenoviruses, occurring in birds belonging to the orders Galliformes and Anseriformes, appear as monophyletic clades, respectively, reflecting their presumably long cospeciation with these hosts (Fig. 1).

Neoaves

PCR-amplification and partial characterization of novel AdVs, detected in racing and fancy pigeons, have been reported recently [29,30]. The full genome sequence of two psittacine aviadenoviruses (PsAdVs) has been published. Type 1 was found to occur in Senegal parrot, red-fronted parrot, and red-breasted parakeet, whereas PsAdV-4 originated from red-bellied parrot [31,32]. Additional aviadenovirus sequences, obtained from Meyer's parrot, white-eyed parakeet [33], four different falcon species (Aplomado falcon, American kestrel, orange-breasted falcon, Teita falcon), gull, and Humboldt penguin [34], as well as neotropic cormorant [35] were also published. Metagenomics study of crane fecal samples revealed almost the complete genome sequence of a new aviadenovirus with a surprisingly low (34%) G+C content [36]. Aviadenoviruses, invading members of the order Columbiformes, are monophyletic. Similarly, the AdVs, found in birds belonging to the order Psittaciformes, are also very close to each other (Fig. 1).

We hypothesize that eventually every bird species might possess at least one, but likely several, aviadenovirus types. However, discovery of these viruses seems to be more unlikely than that of the *Atadenovirus* and *Siadenovirus* members infecting birds. These latter AdVs are usually more pathogenic in birds and cause disease or death of the infected individuals, thus rendering them as a more obvious source of positive samples.

Atadenoviruses

The so-called egg drop syndrome virus (EDSV) was isolated from chicken more than 40 years ago [37] when it caused severe losses in the layer flocks all over the world by eventuating a sharp drop in the egg production. Later by serological investigation of archived serum samples from wild birds, it was revealed that EDSV has existed in ducks previously, hence its official name became duck AdV-1. Its transmission to chickens and worldwide spreading was supposed to have happened through widely used contaminated poultry vaccines. Vaccination against EDSV is still in practice in certain regions. PsAdV-3 was isolated from southern mealy parrot [38]. Atadenovirus sequences were obtained from the droppings of long-tailed finches [39], as well as from chimney swift fledglings with necrotizing ventriculitis [40].

Phylogenetic calculations show three separate lineages of atadenoviruses, evolving presently with the squamate reptiles, birds, and ruminants, respectively (Fig. 1). In the case of the two latter hosts, we assume precedent host-switching events (see later).

Siadenoviruses

Although turkey AdV-3, the causative agent of the so-called turkey hemorrhagic enteritis, a severe disease requiring prevention by vaccination, has been known since the 1970s [41], the number of different siadenoviruses detected in representatives of a plethora of bird species started to increase rapidly in the past two decades only. Raptor AdV-1, found in two diseased and dead owls and a Harris hawk from a private collection of birds of prey, could not be isolated, yet it was sequenced fully by using PCR-aided genome walking [42]. The capability of this virus to cross the species barrier is in line with its observed pathogenic characteristics. Further, full genome sequences were reported from South Polar skua [43], as well as from chinstrap and gentoo penguin AdVs [44]. From siadenoviruses found in Adélie penguin and Humboldt penguin [34], in budgerigar and great tit, partial sequences of different lengths are available. Two novel pigeon AdVs, types 4 and 5, were also classified as siadenoviruses [29]. Among the PsAdVs, PsAdV-2 is especially pathogenic and has been described already from more than half a dozen of different psittacine species and cockatoo [39,45]. Such a broad host range is a rare trait even among siadenoviruses. Gouldian finches, red-billed fire finches, and red-throated parrot finches were found shedding Gouldian finch AdV-1, a long-tailed finch that harbored a novel siadenovirus [39]. Recently, PsAdV-5 was reported from cockatiel with chronic liver disease [46] and a novel siadenovirus from Pacific parrotlets [47]. There are a large number of unpublished detections of siadenoviruses in additional wild-living avian species. On the phylogenetic tree, the siadenoviruses derived from avian species separate well from the single known amphibian siadenovirus (Fig. 1). Nonetheless, certain different siadenoviruses, detected in the same host, may appear phylogenetically distant from each other, leading us to presume that multiple host-switching events might have happened at different times. This is exemplified well by the two fairly divergent pigeon siadenovirus types (pigeon AdV-4 and -5), while the three pigeon types of the genus *Aviadenovirus* were monophyletic and close to each other in a phylogenetic tree based on partial DNA polymerase sequence (data not shown) [29].

AdVs in mammals (*Mastadenovirus*, *Atadenovirus*)

We have arrived at the last and highest-ranked class of the vertebrates, for which presentation of their AdVs seems to be more appropriate by taking the hosts in a reverse order to the evolutionary direction, since the

AdVs of man compose the largest and best-studied AdV group. So let us have a look at our fellow zoo visitors and animal caretakers.

Mastadenoviruses

AdVs in primates

The diversity of HAdVs has been recognized early, with newly isolated serotypes grouped according to their different biological and genomic properties. These former subgenera, designated from A to F, were later transformed into HAdV species. With time, the majority of these species was complemented by numerous viruses derived from nonhuman primates. By now, it is clear that species *Human mastadenovirus D* (HAdV-D), encompassing 32 serotypes and several recombinant types, is the only lineage that has continuously coevolved with man, which is supported by the fact that not a single similar AdV was found in apes or monkeys. To the contrary, many AdVs have been detected in, or isolated from, different great apes, such as chimpanzees, bonobos, or gorillas, that seemed to belong to species HAdV-B, HAdV-C, and HAdV-E. This suggests that apes also represent the host species of origin for the HAdVs classified into these species. An intriguing indication for the ape origin of HAdV-4, besides its remarkable pathogenicity, is the fact that this virus used to be the single constitutive member of species HAdV-E, long before the more recent recognition of numerous isolates from chimpanzees and gorillas in this species. On the other hand, the lineages represented by HAdV-A, F, and G clearly originate from Old World monkeys. Again, the unusual pathogenicity resulting mainly in diarrhea along with the slightly biased base composition of their DNA ($G+C < 50\%$) indicate that the HAdV types, classified into these species (HAdV-12, -18, -31, -40, -41 and -52), share a common ancestry with the simian AdVs derived from Old World monkeys. However, HAdVs are not the principal subject of this review.

Ape AdVs are known from chimpanzee, bonobo, and gorilla. In phylogeny inference, all these great ape AdVs cluster closely with HAdV-B, HAdV-C, and HAdV-E. The ancient gorilla AdVs seemingly belonging to HAdV-B might have switched to man in two independent occasions and continued to evolve and split into different HAdV types [48], which is reflected by the existence of two lineages, termed informally as 'subspecies' HAdV-B1 and -B2. This evolutionary scenario is also supported by overlaps between the topology of the phylogenetic tree of primate AdVs with that of the primate phylogeny.

There are a large number of Old World monkey AdVs, isolated from cynomolgus monkey (crab-eating macaque), rhesus macaque, grivet, golden snub-nosed monkey, yellow and olive baboon. Many further AdVs were only detected by PCR from Assam macaque, black-and-white colobus, red colobus, black-crested mangabey, Campbell's mona monkey, Diana monkey, hamadryas baboon, mandrill, and patas monkey. At least, 23 AdV serotypes (and numerous further genotypes), assigned to 10 species (*Human mastadenovirus G*, *Simian mastadenovirus A* to *I*), are described to date and most of them are even fully sequenced [49]. Similar to the scenario in HAdV-E, *Human mastadenovirus G* contains a single known HAdVs type among two dozen of macaque types as remaining members of the species. Not surprisingly, the corresponding type, HAdV-52, was found to use a similar cellular receptor as the macacine AdVs in this virus species [50].

From New World monkeys, only a single AdV isolate is available, namely titi monkey AdV-1, whose genome is sequenced fully [51]. More recently, a large number of AdVs have been detected by PCR and characterized by partial sequences from different New World monkeys, including cotton-top tamarin, red-handed tamarin, common squirrel monkey, red-bellied tamarin, golden-headed lion tamarin, tufted capuchin monkey, gray-bellied night monkey, red-faced spider monkey, and marmoset [52–54]. Apart from a single case report [51], no evidence implying human infection by New World monkey AdVs has ever been published.

The same might be true for the AdVs of prosimians, the most ancient lineage of primates. The first PCR screening on samples originating from Madagascar or European zoos has just been reported [54]. Novel AdVs were found in ring-tailed lemur, black-and-white ruffed lemur, eastern lesser bamboo lemur, red-fronted lemur, black lemur, and crowned lemur (<https://site.s.google.com/site/adenoseq/>). Prosimian AdVs form the basal branches of the phylogenetic tree of primate AdVs, followed in order by the New World monkey, Old World monkey AdVs, then great ape and HAdVs (Fig. 1). This topology is congruent with that of the known phylogeny of primates, thus underscoring the theory on the AdV–host coevolution [54].

AdVs in carnivorans

Infectious canine hepatitis, caused by canine AdV-1 (CAHV-1) is a well-known fatal disease of dogs. Its prevention is a constant component in the vaccination schedule of puppies worldwide. CAHV-1 causes encephalitis in foxes and was found to infect a number of other predators including wolf, coyote, jackal, otter,

raccoon, and bear [55,56]. A genomic variant, CADV-2, is associated with the so-called kennel cough of dogs. Although both CADVs have been studied intensively, including full-genome sequencing, their likely origin from bat AdV (BtAdV) was recognized more recently (see later) [57]. For a long time, no other AdVs from any members of the order Carnivora have been molecularly characterized. In the past decade, however, several such novel AdVs were recognized, some of which seemed also to have their origin in BtAdV. Among these is skunk AdV-1 found in striped skunk in Canada and sequenced entirely [58]. Interestingly, the isolation and partial genetic characterization of an almost identical virus was reported from a captive pygmy marmoset (a New World monkey) that had died in a Hungarian zoo. Moreover, apparently the same AdV type was also isolated from pet African pygmy hedgehogs (white-bellied or four-toed hedgehog) that had died in Japan and in the USA, respectively [59,60]. The ability of the skunk AdV-1 to cross the host species barrier in case of animals belonging to phylogenetically and geographically remote species is exceptional and needs further investigation (discussed below).

Besides the BtAdV-related viruses, partial DNA polymerase sequences have been obtained from a number of carnivorans including harbor seal, northern elephant seal, fur seal, cat, marten, least weasel, and Eurasian otter [61,62]. From California sea lion and polar bear AdVs, the full genome sequence was published [61,63].

AdVs in bats

The first BtAdV isolate was obtained accidentally during attempts to establish primary cell cultures from flying foxes for the study of certain zoonotic viruses. Soon after this, targeted PCR screening of bat samples resulted in an amazing wealth of novel partial DNA polymerase sequences [64–67]. This may be explained by the fact that the order Chiroptera is the second richest group of mammals right after the rodents, in terms of the number of species. Compared to the time elapsed since the start of their study, an enormously high number of putative BtAdVs has been detected in individuals of a huge palette of small bat species (microbats), as well as that of fruit bats and flying foxes (megabats). The number of actual isolates, close to a dozen at the moment, is also growing steadily. BtAdVs show a surprising diversity compared to the AdVs of any other mammalian animal group, reflected by the diverse phylogenetic relationships formed with AdVs from different host species (Fig. 1) [65,68]. Twelve full

genomes are available [57,69–71]. The known BtAdVs are classified into 10 species (*Bat mastadenovirus A* to *Bat mastadenovirus I*; <https://sites.google.com/site/adeseq/>). Strong signs of coevolution according to the bat families could be observed [65]. On the other hand, several older or recently found animal AdVs, such as the CAdVs and equine AdV-1, or skunk AdV-1, turned out to cluster so closely with certain BtAdVs that their close common ancestry cannot be denied (Fig. 1).

AdVs in rodents

For a long time, only two murine AdV types, both of them from house mouse, were known and studied in detail. However, the number of novel AdVs, detected in, or isolated from, different rodent hosts has increased lately. Full genome sequence is available also for MAdV-3 from striped field mouse. Partial sequences confirmed the existence of AdVs in rat, deer mouse, Chinese striped hamster, Daurian ground squirrel, Southwest China vole, and gray squirrel [72]. An AdV, found in common squirrel, has also been fully sequenced [73]. Novel AdVs were detected in different wild rodents in Cameroon [74]. Moreover, AdV isolates from captive guinea pigs kept in Australia and the Czech Republic have just been reported [75].

AdVs in Cetartiodactyla

Even-toed ungulates (Artiodactyla)

Adenoviruses from domesticated ruminants, including cattle, sheep, and goat, have been long known. The presence of novel mastadenoviruses was detected also in alpaca, muntjac, and white-tailed deer. The full genome of this latter virus (deer AdV-2) has been sequenced [76]. After a long pause, a new ovine AdV, classified as type OAdV-8, was isolated from sheep in Hungary. Its full genome sequence is the first one published from an ovine mastadenovirus [77]. The number of porcine AdV (PAdV) types is also expected to grow in the near future with the detection of novel sequences in the USA (strain PAdV-WI from Wisconsin) and Slovenia (strain PAdV-SVN1) [78,79]. Two full genome sequences, from PAdV-3 and 5, have been determined.

Cetaceans

The full-genome sequence analysis of bottlenose dolphin AdV-1 and -2, as well as the partial sequence of harbor porpoise AdV, seems to support the

coevolution of these viruses with their hosts. Moreover, the phylogenetic tree of AdVs also justifies that the superorder Cetartiodactyla, represented by BAdV-1 and -2 along with PAdV-5, forms a sister clade of the cetacean AdVs (Fig. 1) [80].

Mastadenoviruses in other mammals

Several representatives of additional mammalian species were found to harbor AdV, including tree shrew, rabbit, and different shrew species [74,81]. One of the two AdV types known to infect horses, namely equine AdV-1, also appears to originate from micro BtAdVs (Fig. 1).

It is noteworthy that two large groups of mammals, the subclass Prototheria (e.g., platypus or echidna) and superorder Afrotheria (e.g., elephant, dugong, or hyrax), are not represented yet by mastadenoviruses at all. Similarly, the presence of AdVs in animals classified in the order Xenarthra (anteaters, tree sloths, and armadillos) has never been reported. No mastadenoviruses could be detected in marsupials (e.g., kangaroo, koala, or opossum) yet. To get a more complete picture about adenoviral evolution, these data would be indispensable.

Atadenoviruses

Atadenoviruses in marsupials

Atadenoviruses were found in the feces of wild-living brush-tail possum and captive kowari. However, since both samples were recruited from intestinal excretes, with the possibility that these AdVs might have originated from ingested feed, there is no direct proof for active infection.

Atadenoviruses in ruminants

Approximately half of the known bovine AdV types belong to the genus *Atadenovirus*. Other domestic and wild ruminants, such as sheep, goat, deer, moose, and elk, were also found to harbor atadenoviruses. The diversity of these AdV types implies that the presumed ancient host switch from the original reptilian host to a ruminant animal might have taken place before the divergence of the recent ruminant lineages. The full genomic sequences, for example, from ovine AdV-7, bovine AdV-4 to -8, or deer AdV-1 (from *Odocoileus*) [82] revealed characteristic differences, such as the duplication or even multiplication of the RH genes, compared to the reptilian AdV genomes.

Conclusions and perspectives

Adenoviruses seem to be common in vertebrates but the distribution of their frequency is rather uneven. According to our experiences, birds and bats are the richest source of AdVs exhibiting over 10% PCR-positivity if randomly collected samples are screened. The explanation for this phenomenon certainly includes the peculiar lifestyle that these two groups of animals share. Both of them have the capability of flying actively. The daily and seasonal migration allows them to cover large distances. Moreover, the outstandingly high number of species, the dense populations, and the frequently crowded nesting, roosting, and gathering places also contribute to the relatively easy transmission of different microorganisms, including viruses and AdVs as well.

Our theory on the coevolutionary history of AdVs with their hosts has to be modified slightly, inasmuch as no new fish or amphibian AdVs have been detected in a large number of fish amphibian samples. Now, it seems that AdVs invaded the vertebrates only after the separation of amniotes from the amphibians. The single fish and frog AdVs may be the results of later host switches. The absolute lack of sequences, similar to the known fish or frog AdVs, in both marine and freshwater metagenomics studies supports this scenario. Nonetheless, we plan to continue the intriguing quest for finding novel AdV types, especially in free-living animals in their native geographical areas. The results obtained from captive specimens have always to be handled with care. The breeding conditions of fancy birds and pet reptiles are often far from optimal, or at least they are non-natural. Mixing and handling of animals from geographically distant regions, or even from different continents, provide favorable circumstances for transmission and spreading of different microorganisms, including viruses, between the captive-bred individuals. Along with this, our chances to pick AdVs that are non-native to a given host are also increased. The practical benefit of such a descriptive and classification work could be forecasting infections with the more pathogenic AdVs that appear to have undergone a host switch. The discovery and availability of the genetically distant animal AdVs may provide new models and subjects for structural and functional studies on this diverse virus family.

Acknowledgements

This work was supported partly by a grant provided by the Nemzeti Kutatási, Fejlesztési és Innovációs Hivatal (OTKA NN128309). The funding source(s)

had no involvement in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. Special thanks are due to our distinguished colleagues who generously provided samples from zoos, pet shops, national parks, urban areas, private collections, and veterinary diagnostic facilities.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Origin and accession number of the adenoviral DNA polymerase sequences used in the phylogenetic calculation (shown in the same order as they appear on Fig. 1).