Study of the diversity of adenoviruses and some other viruses in vertebrate animals (K 100163)

(2012-2017)

Project Closing Final Report

Adenoviridae

In a quest for the exploration of the abundance and diversity of adenoviruses (AdVs) occurring in the vertebrate hosts, we continued the PCR screenings on samples from a wide range of animals. Surprisingly, all our piscine samples remained negative. Thus the white sturgeon AdV is still the sole representative of fish AdVs. Nonetheless, the genomic and phylogenetic analyses further confirmed the existence of a distinct virus lineage and justified the establishment of the genus *Ichtadenovirus*. The large number of negative PCR results obtained from the amphibian samples was also somewhat unexpected. It seems that the prevalence of AdVs in fish and amphibians is either extremely low, or more special AdVs exist that cannot be detected easily by our PCR system. Interestingly however, several genomic variants of a novel atadenovirus were demonstrated in captive-bred dart poison frogs commercialized in Hungary. The samples originated from a stock in which morbidity and mortality were experienced. In the internal organs of individuals belonging to two common species (*Dendrobates auratus* and *Phyllobates vittatus*), previously unknown partial DNA polymerase gene sequences were amplified by PCR. Phylogeny reconstruction indicated the presence of two hitherto unknown AdVs, both being new member of the genus *Atadenovirus*. Additional genomic fragments were obtained by consensus (atadenovirus-specific) PCR primers. Thus the existence of two novel atadenoviruses able to infect amphibian hosts was confirmed unequivocally. This finding rises new perspectives in the theory on the co-evolution of adenoviruses and their vertebrate hosts. However, the improved hypothesis needs further shaping. The central part of the genome of the slider AdVs, i.e. members of the proposed genus *Testadenovirus* was determined but the sequence of the genus-specific end regions are still unresolved.

We detected novel genomic variants of an atadenovirus, described earlier from anolis lizards. A large part, encompassing approximately half of the entire genome estimated to be around 30 kilo base pairs (kb) was acquired by PCR and sequenced. By screening over 300 specimens by PCR, novel atadenoviruses were discovered in cloacal samples of different squamate reptiles, wild-caught in different Spanish national parks and nature reserves. Three hitherto unknown atadenovirus sequences were obtained from 11 Carpetan rock lizards (*Iberolacerta cyreni*), 2 Iberian green lizards (*Lacerta schreiberi*) and 9 Iberian worm lizards (*Blanus cinereus*), respectively. In the Carpetan rock lizards, two slightly divergent genomic variants of the same virus type seemed to be present. These Advs are the very first ones described from representatives of the two interesting families (Lacertidae and Ambphisbaenidae) of the order Squamata. In another Spanish collaborative work, the presence of an AdV belonging to the genus *Atadenovirus* was confirmed in a tortoise (*Testudo graeca*) presenting hyperplastic stomatitis and esophagitis. This is the third AdV genus the representatives of which have been found in turtles. The full genomic sequence of the atadenovirus described from bearded dragons (*Pogona vitticeps*) and incriminated as a

possible pathogen all over the world, was also finished by Sanger method using consecutive PCRs with consensus and specific primers. The project took a long time as the size of the full genome (over 35 kb) turned out to be considerably longer than initially thought (to be under or at most 30 kb). The annotation of the genome also resulted in the recognition of several surprising traits, such as the presence of multiple genes seemingly coding for C-lectin domains (marked by red arrows on Figure 1). Further study of these genes is of interest. Unfortunately, we did not succeed with the isolation of the virus.

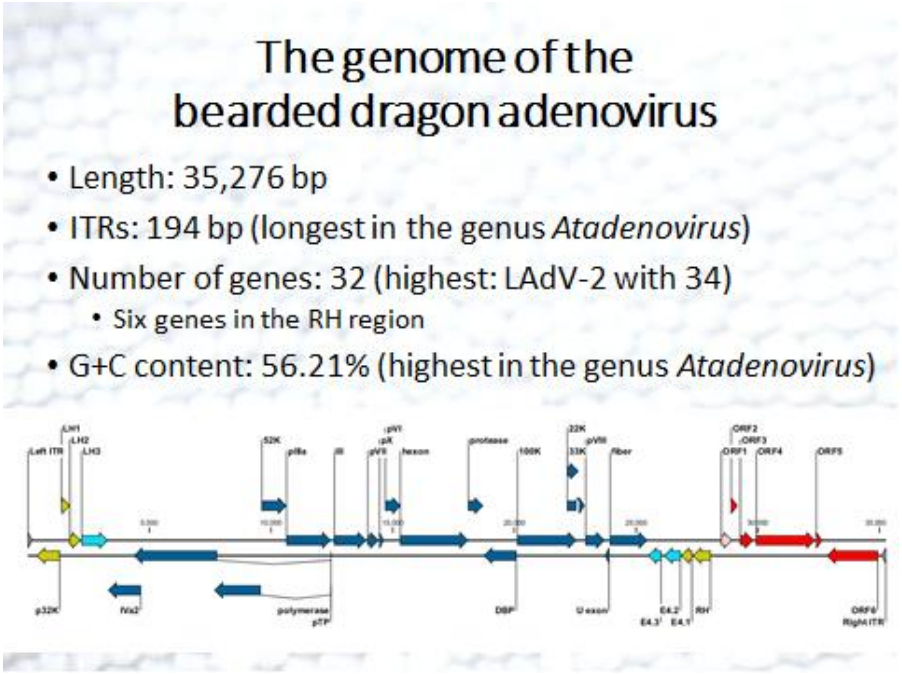

Figure 1. Schematic presentation of the bearded-dragon adenovirus genome

In an Austrian collaboration, we participated in the genomic annotation of the prototype strains of two important fowl adenovirus (FAdV) species, namely *Fowl aviadenovirus D* and *E* that had been sequenced at University of Veterinary Medicine Vienna. Full genomic sequencing of a Hungarian isolate of FAdV-5, representing species *Fowl aviadenovirus B* was started by NGS method. The virus, a member of the genus *Aviadenovirus* was obtained from Debrecen. The thorough genomic and phylogenetic study of this virus is interesting because it seems to be associated with morbidity among broiler chickens in the eastern part of Hungary. A great honour was that our young colleague, G. Kajan was asked to contribute the chapter on “Poultry Adenoviruses” in the book edited by Dongyou Liu on the “Molecular detection of animal viral pathogens”(CRC Press, 2016).

A number of new members of the genus *Mastadenovirus* as well as several previously isolated monkey AdVs were also detected and characterized by partial or full genome sequencing. By the largest number, the primate AdVs, particularly those of the small monkeys and prosimians were represented. One publication has appeared, another one is still in manuscript. A very interesting project included the full genome sequencing of a new mastadenovirus detected in the internal organs of a polar bear (*Ursus martimus*) that had died at the Budapest Zoo a couple of years ago. Attempts to isolate the virus remained unsuccessful, however, larger or smaller parts of its genome could be amplified from the sample material by a series of PCRs using initially consensus, later specific primers. By this hard way, the nucleotide sequence of the entire genome was determined, assembled and

annotated. The exact sequence of the inverted terminal repeats (ITRs) occupying the genome ends were also obtained by using the RACE kit. An interesting feature of the tentative polar bear adenovirus is the lack of the gene of protein IX. The phylogenetic place of the virus in a sister clade to the adenovirus described from a Californian sea lion (*Zalophus californianus*) seemed to support that these viruses represent the lineage co-evolving with members of the order Carnivores. The overall G+C content (46.33%) of the genome is close to the low scale yet it is still within the limits of unbiased range (45 to 55%).

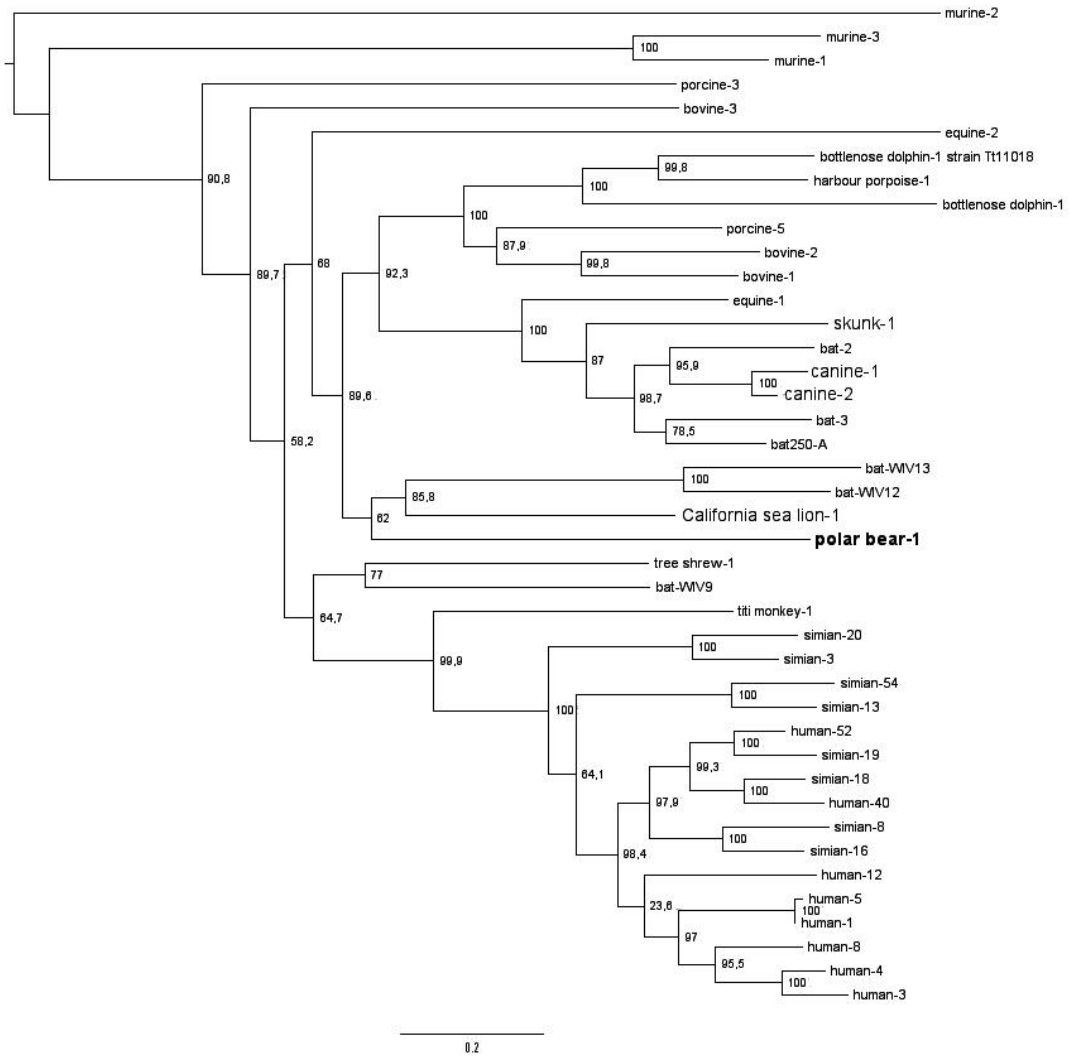

Figure 2. Phylogeny of selected mastadenoviruses based on complete amino acid sequences of the viral DNA polymerase (Maximum Likelihood calculation)

Herpesvirales: Alloherpesviridae

Another gene fragment of the new alloherpesvirus detected in Hungarian wels catfish (*Silurus glanis*) was obtained by consensus PCR. The sequence of the terminase gene fragment also proved to be novel. However, bridging of the two gene fragments by PCR has not been successful as yet. The genome organisation of alloherpesviruses is rather diverse, so that it is almost impossible to predict the relative orientation of, and the approximate distance between, the two known genes and gene fragments, respectively. We decided to

give a try to a metagenomics approach. To this end, we are testing different purification and concentration protocols in order to prepare a reliable template. Full characterisation of the virus is of interest because of the skin lesions that seem to be associated with the infection (Figure 3).

Figure 3. Skin lesions in a wels catfish infected by the novel alloherpesvirus

Circoviridae

The parvovirus research was not continued much further yet some results are still waiting for publication. We are in a similar situation with the novel circoviruses derived from lower vertebrate hosts. The manuscript about the full genomic sequences of circoviruses derived from fish, frogs and a slider is still in preparation. Unfortunately, the pigeon circovirus genomes determined from the samples of Hungarian racing and fancy pigeons are no exception to this. By consensus PCR, we detected several novel circovirus and circovirus-like sequences in samples of different bats. The majority of these tentative viruses, present in alimentary tract of the bats, are certainly of insect host origin. We picked a putative genuine bat circovirus that had been detected twice independently in bats of the same species namely the lesser horseshoe bat (*Rhinolophus hipposideros*). The entire genome was successfully amplified by inverse PCR by using the diagnostic primers in outward orientation. Almost the full nucleotide sequence (except a small reiteration that proved to be impossible to resolve as yet) could be determined. In phylogeny reconstructions, based either on the individual (*rep* and *cap*) genes or on the entire genome, this virus always appeared among the circoviruses of mammalian animals. Therefore we hypothesize that this virus represents the circovirus lineage co-evolving with bats.

Miscellaneous results

After the end of this research project, our group was involved in the organization of two consecutive international conferences in Budapest. The venue was the beautifully renovated campus of the University of Veterinary Medicine. The first conference was a jubilee event in

a series that had started almost three decades ago. We took as a honour to be asked to host the 10th International Symposium on Viruses of Lower vertebrates. Indeed it was recognition of our achievements on this field. A second smaller meeting was with one day overlap the 4th International Symposium on Ranaviruses. On the joint day, more than 100 participants from more than 21 countries of five continents participated.

The two senior investigators, as active members of the ICTV (International Committee on Taxonomy of Viruses) are invited to the plenary session of ICTV at the IUMS 17th International Congress of Virology to be held in Singapore. B. H. is a member of the Executive Committee and was appointed to chair one of the six subcommittees (Animal DNA Viruses and Retroviruses Subcommittee). M. B. is the chair of the Adenoviridae Study Group and is the national Representative for Hungary. Her abstract submitted has been accepted for oral presentation at the International Congress of Virology. Unfortunately, the travel allowance that remained from the budget of this project was prohibited to be used for this congress but had to be returned to the NRDIO. Nonetheless, the oral presentation will acknowledge the support provided by this OTKA grant.

Our application to continue the research was rejected last year thus the future of a traditional Hungarian veterinary virology research topic that have been attracting collaboration from numerous foreign laboratories engaged with veterinary, human medical and basic virology for several decades is now seriously jeopardized.

With the final extension, this research project lasted for 5 years. During the last, extension year, two additional diploma work theses, by a graduate veterinary student and a biology MSc student were prepared. Both students were members of the Scientific Students' Associations, and participated at the XXXIIIrd National Conference in the Medicine and Human Health, and in the Biology Sections, at Pécs and Debrecen, respectively. Both students received a first class special prize for the presentation in their respective category.

In 2017, based on the above results, we have submitted a newer proposal to the ICTV for the establishment of 6 novel mastadenovirus and two aviadenovirus species. The cumulative impact factor of the papers published in the 5 years is 62.103, about half of which was produced during the very last year. Nonetheless, significant results are still waiting for publication.