**Szent István University**

**Postgraduate School of Veterinary Science**

# Diversity and Phylogeny of Adeno- and Parvoviruses Infecting Reptiles and Amphibians

Brief Summary of Doctoral Thesis

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# Introduction and Objectives

Adenoviruses (AdVs) are double stranded DNA viruses, which are present in all major vertebrate groups. The individual viruses usually possess a rather narrow host spectrum. With PCR-based methods becoming more wide-spread as well as DNA sequencing, investigation of these viruses started to involve lower vertebrate hosts besides mammals and birds. Moreover, genome characterization became available without the actual isolation of viruses providing the chance for novel phylogenetic as well as taxonomic conclusions. At the very start of this work, a couple of isolate of reptilian AdVs already existed whereas there had been only one AdV isolated from amphibians assigned to genus *Siadenovirus*. Despite of the numerous AdV detections in reptiles, there had been only one complete genome characterization performed. The full genome sequence of snake adenovirus 1 (SnAdV-1) clearly indicated the virus to be a member of the *Atadenovirus* genus, similarly to all other squamate AdVs to date.

Out of the two subfamilies of family *Parvoviridae* that unites all parvoviruses (PVs) to date, the members of *Parvovirinae* have been found to infect vertebrates; reptiles, birds and mammals. All reptilian PVs thus far have been observed in concurrent infection with AdVs hence they are assumed to be dependoparvoviruses. However, based on methods of molecular virology, this has been confirmed in case of two snake PVs only. Members of genus *Dependoparvovirus* require the presence of helper viruses (mostly AdVs or other large DNA viruses) to replicate efficiently. The only exceptions to date are anseriform dependoparvoviruses that are capable of autonomous replication. Members of genus *Dependoparvovirus* are considered to have coevolved with diapsids (tuataras, squamates, crocodiles and birds) and their presence in mammalian hosts is assessed to be a recent host switch. This is based on the autonomous replication ability of anseriform dependoparvoviruses and the basal phylogenetic position of snake adeno-associated virus (SAAV). There are no data on the replication abilities of reptilian dependoparvoviruses. There have been no PVs ever detected in amphibian hosts.

The main objective of this work was to assess the diversity and evolution of AdVs and PVs in reptilian and amphibian hosts, mostly because of the limited information available on the viruses of such lower vertebrates. Furthermore, investigating the genome organisation of novel reptilian dependoparvoviruses was assigned as a further aim, as well increasing the rather low number of such viruses in reptiles. To this end, PCR screening and partial genome characterizations of viruses from both families were involved. Moreover, we attempted the complete genome characterization of novel PVs as well as of two lizard atadenoviruses (LAdV-1 and 2), isolated in Germany prior to this work. We expected new information to be concluded on the evolution of the *Atadenovirus* and *Dependoparvovirus* genera, based on the phylogeny reconstructions of the further genome fragments acquired by PCRs designed by us. The

simultaneous screening for PVs and large DNA viruses was expected to provide information on the eventual autonomous replication abilities of reptilian dependoparvoviruses.

# Materials and Methods

## Origin of the examined samples

The bulk of the samples originated from a pet store at Budapest specialized in reptiles. Samples provided by private owners as well as samples, especially road kills, collected in the wild were also screened. Ten Mississippi alligator (*Aligator mississippiensis*) liver samples originated from the United States. Altogether 314 reptilian and 207 amphibian samples were screened for the presence of AdVs, whereas out of these 165 reptilian and 60 amphibian samples for PVs. All orders of reptiles were included except Rhyncocephalia. As for amphibians, two (Anura, Caudata) out of the three orders were represented. DNA was extracted from various internal organs, such as liver, lungs, kidney, intestine and gonads.

The two lizard AdVs examined at full genome level were isolated at the University of Hohenheim, cultured on iguana heart cells (IgH-2,118 ATCC: CCL-108) and Russell viper heart cell line (VH-2, ATCC: CCL-140). LAdV-1 had been obtained from a gila monster (*Heloderma suspectum*) as early as 2004, while LAdV-2 was isolated from Mexican beaded lizards (*Heloderma horridum*) only in 2008.

## Polymerase chain reaction

Various heat-resistant DNA polymerase enzymes were utilized during the PCR screenings. Fragments smaller than the estimated length of 1000 bp were obtained by Taq polymerase enzymes. To acquire longer fragments, more processive and accurate recombinant enzymes were utilized.

AdV infections were detected using a consensus nested PCR method designed by Wellehan et al. (2004). These primers were designed to amplify a 300-bp-long fragment of the DNA polymerase gene that is highly conserved throughout the whole *Adenoviridae* family.

For PV screening, we designed PCR primers aiming at a 600-bp-long fragment of the *cap* gene. This region was supposed to be conserved in all members of the *Dependoparvovirus* genus.

Samples positive for PVs yet negative for AdVs were further examined for the presence of other DNA viruses that harbor their own DNA-dependent DNA polymerase enzyme genes in their rather large genome.

To obtain further fragments from the AdV-positive samples, several PCR systems were designed throughout the conserved genome region flanked by the IVa2 and fiber genes. The length of such fragments varied between 200 and 1000 bp. We attempted the detection of

the genus-specific p32K gene as well. Two primer pairs were designed to obtain a 250- or 400-bp-long fragment from the other major ORF of PVs, the *rep*.

## Complete genome characterization

We performed the sequencing of the two LAdVs in collaboration with the German research team. All the other viruses were sequenced exclusively by us. Initially, short fragments of the genomes were obtained using the PCR systems described above, whereas the short fragments of the *rep* were amplified initially in case of PVs. Unidirectional PCRs using one primer were also performed in several cases, especially to gain sequences of the genome ends. The ITR sequences of the AdV genomes were acquired by using the 5'/3' RACE kit. Parvoviral ITR sequences were enhanced using PCR systems based on single-stranded adapters linked to the genome ends. The amplified fragments were then cloned and sequenced.

## *In silico* analysis of sequences

Nucleotide sequences (nt) were assembled and corrected if necessary using the Gap4 program of the Staden package. Sequences were identified using the BLAST algorithms online.

Longer genome fragments were annotated either by JavaScript DNA Translator 1.1 or by the freeware Artemis Genome Browser. Splice acceptor and donor sites were predicted manually and their validity was checked using the Splice Site Prediction algorithm at Neural Network online. The validity of suspected polyadenilation signals was verified by the Soft Berry POLYAH online software. Conserved protein motifs were identified using the SMART program and database online.

Various softwares were involved in multiple alignment preparation. Phylogeny reconstructions were based on amino acid (aa) sequences that are suitable for examining long-term evolution. Model selection was performed by ProtTest and guide trees were constructed using the Protdist, then the Fitch algorithms of PHYLIP v3.696. Maximum likelihood calculations and topology testing by bootstrap analysis were carried out on the ATGC-Montpellier online platform of phyML3.

# Results

## Adenoviruses

Prevalence of AdVs corresponded to 13.1% as 41 out of the 314 samples were positive. These included six different AdVs out of which three were novel. Previously unknown AdVs were obtained from a white-throated monitor (*Varanus albigularis*), two long-tailed grass lizards (*Takydromus sexlineatus*) and 23 short-tailed pygmy chameleons (*Rampholeon brevicaudatus*). This is the first time that an AdV in lacertids has been demonstrated. The prevalence of AdVs in the short-tailed pygmy chameleons was strikingly high (88.5%) and five variants of the virus could be differentiated at nucleotide (nt) level. The AdV infection of bearded dragons (*Pogona vitticeps*) is widely recognized. With small variations in the aa sequences, we detected this virus with a very high prevalence (88.9%). In snakes, AdV could be detected in seven cases. All these viruses have been described multiple times before. Three novel variants of SnAdV-1, along with the one deposited to GenBank, were demonstrated from the sample of a young red-tailed boa (*Boa constrictor*). Signs of chronic liver damage were observed in this young individual. We expanded the host spectrum of SnAdV-1 with two species, out of which the grass snake (*Natrix natrix*) is the first we found to harbor AdV in wild-living reptiles in Hungary. SnAdV-2 was detected in a novel host species as well, namely in the Sinaloean milk snake (*Lampropeltis triangulum sinaloe*).

Out of the 207 amphibian samples, only seven proved to be positive corresponding to a prevalence of 3.4%. All positive samples originated from poison dart frogs (*Dendrobates auratus*, *Phyllobates vittatus*) that deceased during mass mortality events in a pet store in Budapest. Two variants, differing in three aa of a novel amphibian AdV (frog AdV 2, FrAdV-2), were detected in both samples. This is the first time since 1973 that AdV in an amphibian is found.

According to the BLAST homology searches, all AdVs involved in this work could be assigned to genus *Atadenovirus*.

Out of the conserved genes of family *Adenoviridae,* we could amplify the complete pVII and pX genes using primers designed for the penton base and pVI genes. We detected and examined the protease cleavage sites in both proteins. Almost the whole sequence of the penton base gene was obtained in the case of the pygmy chameleon (PCAdV) and the white-throated monitor (VAdV-2) AdVs. From the p32K gene, specific for the genus *Atadenovirus*, a 261-bp-long fragment from the PCAdV, together with a 217-bp-long one from the grass lizard AdV (GLAdV) were amplified and sequenced.

A 8161-bp-long contig was sequenced and assembled from the genome of the first variant of FrAdV-2, whereas basically the half of the expected genome size of the second

variant (12033 bp) was also determined. The two variants were 99.4% identical, hence we assembled them. The chimera contig with a G+C content of 36.6% was eventually 14114 bp in length, stretching from IVa2 to the protease gene and containing nine complete and two partial genes. Typically for genus *Atadenovirus*, the intergenic regions were short, with genes overlapping on a regular basis. Splicing was detected in case of the IVa2 and pTP genes. Splicing of the IVa2 gene has not been reported in atadenoviruses before.

The complete genome sequence of the two LAdVs was determined, including the ITRs (Fig. 1). There was a significant difference in their length, which was 36 628 bp for LAdV-1, and 32 965 bp for LAdV-2. The length difference was due to two more genes in LAdV-1. The G+C content was 44% (LAdV-1) and 44.2% (LAdV-2) of the complete genomes. The lengths of the ITRs were found to be the longest among atadenoviruses, 125 and 126 bp, respectively. In non-reptilian atadenoviruses, the ITRs never exceed 70 bp.

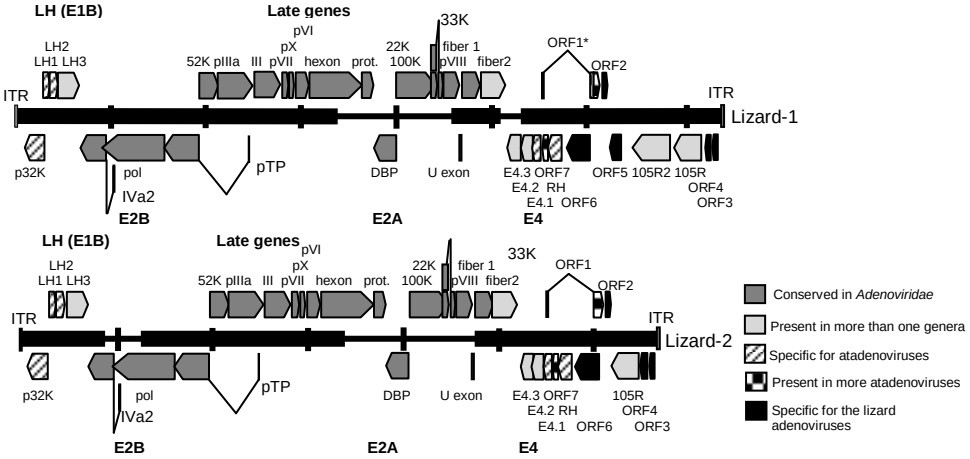


**Figure 1** Genetic map of the lizard adenoviruses. LAdV-1 of the gila monsters (above) is 36 628 bp long with 125-bp-long ITRs. The genome of LAdV-2, originating from Mexican beaded lizards, is significantly shorter, 32 965 bp with ITRs of 126 bp. Genes are presented by arrows, indicating the direction of their transcription. ORF1 of LAdV-1 is only present as a pseudogene (*). Vertical bars mark every 5000 bp. Thick black lines mark the regions sequenced by us.

The first genes in both genomes was the genus-specific p32K, located on the *l* strand, followed by three more genus-specific genes, the LHs on the *r* strand. The organization of the central region of both genomes was typical for the *Adenoviridae* family. Similarly to FrAdV-2, the sequence of the IVa2 and pTP genes of the E2B region included introns. Two fiber genes were identified, for the first time in genus *Atadenovirus,* in both genomes. Structural analysis of the LAdV-2 virions was carried out in Spain, revealing fiber-1 to be positioned solitarily and fiber-2 as triplets on one penton base each. This is the first time that a penton with three fibers is observed in the *Adenoviridae* family. The aa sequence of fiber-1 was 95% identical in both

viruses and scored the highest homology valeue with the fiber of SnAdV-1. Fiber-2 appeared to display the lowest identity (87%) out of the homologous genes of the two LAdVs.

The region following the E4 was found to be the most variable one in both cases. Only five out of eleven (LAdV-1) and four out of the nine (LAdV-2) genes in total possessed homologues in other adenoviral genomes. There was only one RH gene identified, similarly to SnAdV-1 but contrary to non-reptilian atadenoviruses. The length of the LAdV RH genes was approx. double the size of the one in SnAdV-1. Similarly to the ones in non-reptilian AdVs, an F-box motif could be identified in their aa sequence. Such motifs of eukaryotes are responsible for the regulation of the cell cycle. Their presence is assumed to be due to host switch in non-reptilian atadenoviruses, where the motif is located in proximity to the N-terminal, unlike in the LAdVs, where it was observed in the middle of the extraordinary long RH. We carried out phylogeny reconstruction, handling the two halves of the protein independently as separated at the metionin preceding the F-box. This supported the different evolution origin of the two moieties. The merge of these proto-RH genes appears to be a secondary trait.

The only homologue of ORF7 is ORF1 in SnAdV-1, believed to be a type-specific ORF for this virus. The same applies to ORF1, which is a homologue of ORF1 in duck AdV-1 (DAdV-1). Even though the complete ORF appears to be well-conserved, two stop codons transform this gene into a pseudogene in case of LAdV-1. The 105R gene has originally been described in the genome of a mastadenovirus, the tree shrew AdV. A homologue of it has been found in the genome of SnAdV-1. It was also present in both LAdV genomes. Interestingly, in LAdV-1, two copies of the 105R homologue were found. We detected the presence of various IG domains in these proteins, including those similar to the ones in immune cell receptors, Coxackie-adenovirus receptors, adhesion proteins and NK cells.

Out of the ORFs with no recognizable homologues in the *Adenoviridae* family, ORF2 was to encode a protein that is a member of the C-type lectin family. Possible lectin-like genes have been demonstrated in pox- and asfarviruses previously as well as in surface receptors of NK cells. In the protein sequence of ORF4, a coiled-coil motif, similarly to protein IX of mastadenoviruses, could be identified. We assume this protein to enhance the activity of the major late promoter, correspondingly to and instead of IX, as it has no homologue in atadenoviruses.

Phylogeny reconstructions were performed in case of four proteins, indicating GLAdV to be the most basal out of genus *Atadenovirus*. This corresponds with the presumed Squamata origin of atadenoviruses, as well as with the hypothesized evolution of the host species. Based on the short polymerase sequences, FrAdV-2 clustered with non-reptilian, i.e. ruminant, atadenoviruses. The monophily of squamatid and non-squamatid atadenoviruses could only be observed on the p32K tree. The RH-based phylogenetic tree displays the RH genes of atadenoviruses as the descendants of three lineages, placing the first and second proto-RHs of

the LAdVs in different clusters. All viruses in this study displayed a balanced or high G+C content, except FrAdV-2, similarly to non-reptilian atadenoviruses.

## Parvoviruses

Using our newly designed PCR system for the detection of PVs, seven of the 165 reptilian samples turned out be positive (4.2%), whereas all the amphibian samples remained negative. Out of the positive samples, four proved to contained novel PVs. One of the novel ones was detected in four bearded dragon samples, out of which one proved to be negative for the presence of potential helper viruses. PV infection was also detected in one pygmy chameleon sample positive for AdV. This was the first time when the presence of PVs was observed in any members of the family Chamaeleonidae. The only amphisbaenian sample, originated from *Trogonophis wiegmanni*, proved to be positive for PV, whereas no concurrent AdV, herpesvirus, or other potential helper DNA viruses could be demonstrated. Only one out of the snake samples proved to harbor PV, namely the sample of an adult corn snake, showing signs of persistent anorexia. SnAdV-1 was also present in the same sample. The PV detected is a novel one, being the hitherto third snake PV. All four novel PVs could be assigned to genus *Dependoparvovirus* according to the phylogenetic calculations based on the aa sequences of the VP fragments.

We managed to determine the sequence of approx. one half of the genome size in case of the pygmy chameleon PV (PCPV) and the corn snake PV (CSPV). The length of these sequences was 1487 and 1821 nt, respectively. The complete genome sequence of the bearded dragon PV (BDPV) was successfully obtained, encompassing a length of 4590 nt. The ITRs proved to be long, folding up to a regular hairpin-shaped secondary structure. Even though the palindromic region that is responsible for the hairpin structure resembled that of the SAAV, the single-stranded region proved to be significantly longer. We presume this region to be important for site specific integration of the viral genome into the host cell.

In all three PVs, two primary ORFs, the *rep* and *cap*, could be identified, as well as an alternative ORF, encoding the assembly-activating protein (AAP), completely embedded in the *cap* gene. All three viruses included one splice donor and two acceptor sites in their genome. A cryptic poly A signal could be observed within the intron and between the ORFs in all cases, however, the significance of this site in polyadenilation of mRNAs transcribed from the two proximal promoters remains unknown. The intron length was observed to be shorter than 190 nt in all reptilian PVs, including SAAV. We consider these short introns as ancestral traits, as the intron length in all other dependoparvoviruses is significantly longer. A well conserved, supposedly strong poly A signal was observed, following the *cap* gene, in the BDPV genome.

According to its complete genome sequence, BDPV can be proposed as a new species, named *Squamate dependoparvovirus 2*.

According to the phylogenetic tree based on the complete Rep protein sequences, it is confirmed that BDPV is not only a dependoparvovirus, but clusters as the most basal member of the genus together with SAAV. Reptilian dependoparvoviruses appeared as a monophyletic cluster on the phylogenetic tree based on the complete AAP protein sequence (Fig. 2).

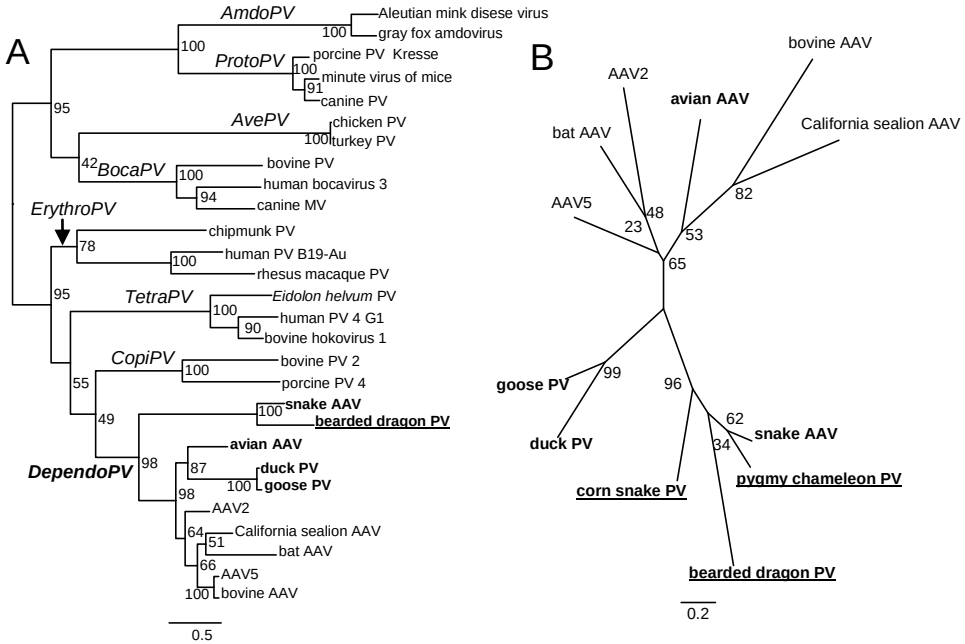


**Figure 2** Results of phylogeny reconstructions based on the full Rep (570 aa, maximum likelihood, RtREV+I+G+F, α=1.29, pinv=0.03) (A), and the full assembly-activating protein sequence (212 aa, maximum likelihood, HIVb+G+F, α=0.95) (B). Diapsid dependoparvoviruses are presented in bold, novel reptilian dependoparvoviruses are underlined. The monophily of squamate dependoparvoviruses is supported by both trees, contrary to all diapsid dependoparvoviruses. (AAV – adeno-associated virus, PV – parvovirus)

# Discussion and Conclusions

The diversity of reptilian viruses is well reflected by our results; from the 314 samples of deceased specimens, seven novel viruses could be derived, as well as the host spectra of two, already known viruses expanded with three novel host species.

This is the first time that parvoviral infections in lizards are indisputably described. In amphibians, a cardinal vertebrate group of conservation programs, we detected a novel AdV, which may be of significance as a serious pathogen.

## Adenoviruses

According to our results, the adenoviral infection in neither bearded dragons nor pygmy chameleons is new-fangled. This can be concluded from the fact that both viruses possess several genotypic variants. In bearded dragons this even manifests at aa level, suggesting an even longer co-evolutional period suitable for genotype segregation. The more extensive trade of bearded dragons compared to that of pygmy chameleons might have been an accelerating factor for this. Mixing of animals, but not wary handling of individuals of different origin harboring various pathogens, can provide an excellent background for novel pathogenic types to emerge. AdVs of serpents, an evolutionally younger reptilian group, are capable of bestriding the species barrier, unlike AdVs of most animals. The extremely rapid adaptive radiation, which principally affected snake morphology, but not the immune system, could provide a plausible explanation to this.

Besides the complete genome characterization of the two lizard AdVs, we managed to gain more genome fragments of previously non-isolated amphibian and reptilian AdVs for the first time. The primer pair aiming at the amplification of the compete pVII and pX genes, proved to be the most successful. The genus-specific p32K gene has been detected and analyzed in two, non-isolated lizard AdVs for the first time. This protein appears to have a different evolutional background in squamate and non-squamate atadenoviruses, which is supported by the phylogeny tree reconstruction. We are certain that this gene is present in all atadenoviruses involved in this project, however, its highly variable sequence makes it difficult to detect.

At the beginning of this work, it was already accepted that genus *Atadenovirus* is of squamate origin, even though some of its members infect birds, ruminants and a marsupial. Our results corroborated this hypothesis, as all squamate AdVs to date proved to be atadenoviruses. The G+C content could be considered balanced, not only in the longer, but even in the short fragments of squamate AdVs, as well as in all other squamate AdV sequences derived to date. The biased G+C content of the non-squamate atadenoviruses is believed to be the result of adaptation mechanisms to the new host. Phylogenetic calculations indicate GLAdV to be the

most basal atadenovirus, which is also in concordance with not only the supposed squamate origin of the *Atadenovirus* genus but with the evolution of the hosts as well. Certain features of lizard and snake AdVs appear to be ancestral traits of the genus *Atadenovirus*. The ITRs, for instance, appear to decrease in length after host switches. Contrary to this, the low number of RH genes seems to be an ancestral feature, as its number is always one in squamate AdVs and varies between two and six in non-squamate AdVs. This suggests that the duplication and multiplication of these genes might also be also related to adaptation mechanisms to the new hosts.

According to our results, atadenoviruses appear to be more diverse than we had ever thought. The previously never observed presence of splicing in the IVa2 genes in the LAdVs and the FrAdV-2 are examples to this. The analysis of the LAdV genomes revealed five novel ORFs, with no homologues in any other AdVs. Their function can only be estimated. The two LAdVs belong to the same AdV species, although the right end of their genomes differ significantly. Even though there is no plausible explanation for the presence of two fiber genes in the LAdV genomes, it appears to be certain, that two fiber genes appeared multiple times in the various AdV genera. The fact that LAdV-2 has been demonstrated in dwarf bearded dragons (*Pogona minor minor*) supports the assumption that two different fibers enhance the host range of the virus. Mustering the amount of motifs and genes supposedly involved in interactions with the host's immune system (F-box motif of the RH, IG domains of the 105Rs, ORF2 homologous to C-lectins), we have to consider the possibility that LAdVs are in fact viruses of bearded dragons under current adaptation to the helodermatid hosts.

The novel AdV of poison-dart frogs is the first atadenovirus in an amphibian host. The possible host switch from squamates is supported by the phylogeny and the biased G+C content of its DNA. The occurrence of atadenoviruses in amphibians suggests that host switch events might have happened four times independently. We suspect the one to amphibians to be the most ancient and the one to ruminants as the most recent one.

## Parvoviruses

The number of PVs known in reptiles is now tripled. This is the first time that PVs are confirmed in lizards by molecular data. Furthermore, we described the first virus ever in a member of the suborder Amphisbaenia.

Our results suggest reptilian dependoparvoviruses to be capable of autonomous replication. At least, no concurrent infection with potential helper viruses could be detected in case of two samples. If dependoparvoviruses are indeed derived from autonomously replicating ancestors, these findings strongly support their diapsid origin.

The common reptilian-avian origin is disproved, however, by the results of phylogeny reconstructions. The monophily of diapsid dependoparvoviruses was not supported by any of the three phylogeny calculations. Squamate dependoparvoviruses, nevertheless, appear as a monophyletic cluster in all three cases. According to these results a single squamate origin of the *Dependoparvovirus* genus is more plausible.

# New scientific results

1. We detected seven novel reptilian viruses. We detected adenovirus infection in lacertids for the first time, and proved that the prevalence of adenoviruses in pygmy chameleons can be as high as that in bearded dragons. The four novel reptilian parvoviruses triple the number of reptilian parvoviruses characterized at molecular level. We described the first ever viral infection in worm lizards (suborder Amphisbaenia). We detected parvoviruses in lizards undisputedly for the first time.

2. We detected the presence of adenovirus in amphibians for the first time since 1973. The hitherto second frog adenovirus proved to be a member of genus Atadenovirus. This is the first proof of atadenoviruses in amphibians. The infection is supposed to be the result of a host switch.

3. We detected adenoviral infection in a free-living Hungarian reptile for the first time.

4. This is the first time when complete genome characterization of any lizard viruses is performed. At the same time, these are the first analyses of parvoviral and adenoviral genomes originating from lizards.

5. We identified two fiber genes for the first time in atadenoviruses and revealed a penton structure including three fibers per vertex, as a unique feature in the whole *Adenoviridae* family. We detected C-type lectines in adenoviruses for the first time as well as Ig domains in atadenoviruses.

6. We detected the presence of dependoparvoviruses in reptiles with no simultaneous infection by large DNA viruses. This suggests the capability of autonomous replication of squamate dependoparvoviruses.

7. Our results, in connection with the evolution of dependoparvoviruses suggest a more plausible single squamate origin instead of the earlier hypothesized common reptile-bird (Diapsida) origin.

# Publications and conference abstracts, this work was based on

## Publications

Pénzes J.J., Benkő M.: **Novel parvovirus from the worm lizard *Trogonophis wiegmanni* - First virus ever detected in amphisbaenian hosts**, Acta Vet. Hung., 62. 284-292, 2014.

Pénzes J.J., Menéndez-Conejero, R, Condezo, G.N., Ball, I., Papp, T., Doszpoly, A., Paradela, A., Pérez-Berná, A.J., López-Sanz, M., Nguyen, T.H., van Raaij, M.J., Marschang, R.E, Harrach B., Benkő, M., San Martín, C.: **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. 11304-11314, 2014.

Pénzes J., Doszpoly A.: **Adenovírusos fertőzöttség kimutatása szakállas agámákban (*Pogona vitticeps*) Magyarországon. [Detection of adenoviral infection in bearded dragons (*Pogona vitticeps*) in Hungary. In Hungarian]**, Magy. Állatorvosok, 133. 432-437, 2011.

Pénzes J.J., Pham, H.T., Benkő M., Tijssen, P.: **Novel parvoviruses in reptiles and genome sequence of a lizard parvovirus shed light on *Dependoparvovirus* genus evolution**, 2015. [submitted]

Pénzes J.J., Doszpoly A., Harrach B., Benkő M.: **PCR screening of carcasses of captive reptiles reveals a high prevalence of adenoviruses**, 2015. [prepared for submission]

## Conference abstracts

Pénzes J., Lopez, P., Martin, J., Harrach B., Benkő M.: **Novel adeno- and parvoviruses in reptiles; first virus detections ever in suborder Amphisbaenia**, Combined Exotics and Avian Conference – Tropical and Tropical, Cairns, Australia, 2014.

Pénzes J., Harrach B., Benkő M.: **Novel parvoviruses in reptiles: first results concerning autonomous replication of reptilian dependoviruses supports the Diapsida-origin of genus *Dependovirus***, 5th European Wildlife Disease Association Student Workshop, Veyrier-du-Lac, France, 2013.

Pénzes J., Romanova, I., Papp, T., Doszpoly A., Marschang, R.E., Harrach B.: **Genome sequencing and analyzis of two novel lizard adenoviruses**, 10th International Adenovirus Meeting, Umeå, Sweden, 2012.

Pénzes J., Benkő M**.: Prevalence and diversity of adenoviruses and parvoviruses detected in samples of reptiles and frogs kept in captivity**, International Conference on Reptile and Amphibian Medicine, Cremona, Italy, 2012.

Pénzes J., Doszpoly A., Benkő M., Harrach B.: **Novel amphibian and reptile adenoviruses provide further proofs for the reptilian origin of atadenoviruses**, 4th European Wildlife Disease Association Student Workshop, Veyrier-du-Lac, France, 2011.

Pénzes J., Romanova, I., Papp, T., Doszpoly A., Harrach B., Marschang, R.E.: **Genome sequencing and analysis of two novel lizard adenoviruses**, 21st Annual Meeting of the Gesellschaft fur Virologie (GfV) Freiburg, Germany, 2011.

Pénzes J., Doszpoly A., Benkő M.: **Examinations aiming at the verification of the reptilian origin of atadenoviruses**, 8th International Symposium on Viruses of Lower Vertebrates, Santiago de Compostella, Spain, 2010.

Pénzes J., Doszpoly A., Benkő M.: **Examinations aiming at the verification of the reptilian origin of atadenoviruses**, ESVV 8th International Congress of Veterinary Virology, Budapest, Hungary, 2009.

## Further publications

Tarján Z.L., Pénzes J.J., Tóth R.P., Benkő M.: **First detection of circovirus-like sequences in amphibians and novel putative circoviruses in fishes**, Acta Vet. Hung., 62. 134-144, 2013.

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