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Abstract: In the years 2011–2012, a consensus nested polymerase chain reaction was used for the detection of adenovirus (AdV) infection in reptiles. During this screening, three new AdVs were detected. One of these viruses was detected in three lizards from a group of green striped tree dragons (*Japalura splendida*). Another was detected in a green anole (*Anolis carolinensis*). A third virus was detected in a Jackson's chameleon (*Chamaeleo jacksonii*). Analysis of a portion of the DNA-dependent DNA polymerase genes of each of these viruses revealed that they all were different from one another and from all previously described reptilian AdVs. Phylogenetic analysis of the partial DNA polymerase gene sequence showed that all newly detected viruses clustered within the genus *Atadenovirus*. This is the first description of AdVs in these lizard species.

Key words: *Anolis, Atadenovirus*, green anole, green striped tree dragon, Jackson's chameleon, *Japalura*.

INTRODUCTION

Adenoviruses (AdVs) are nonenveloped, icosahedral, double-stranded DNA viruses, with a diameter of 80–100 nm. AdVs are classified into five accepted genera within the family *Adenoviridae*, with the genus *Mastadenovirus* in mammalian hosts; the genus *Aviadenovirus* in avian hosts; the genus *Siadenovirus* in chelonians, amphibians, and birds; the genus *Atadenovirus*, primarily in squamates but also in birds and several mammals; and the genus *Ichtadenovirus* from a white sturgeon (*Acipenser tranmontanus*).[7] A sixth genus, *Testadenovirus*, has been proposed to encompass AdVs found in testudinid hosts.[11] A proposed explanation for the similarity of atadenoviruses from such varied hosts is that these viruses coevolved within squamate hosts and AdVs of other hosts represent later host switches.[18]

AdVs are commonly detected in different species of lizards and snakes (Table 1). All of the AdVs detected in squamates so far appear to belong in the genus *Atadenovirus*, and in many cases, it has been hypothesized that specific viruses may represent host-specific viruses that have coevolved with their lizard hosts.[39,52] Snake atadenoviruses, on the other hand, have appeared to be less species specific.[35] It is interesting to note that snakes are not basally divergent within the squamates and are closely related to the Iguanidae, Agamidae, and Chamaeleonidae families,[41,50] so that the differences in species specificity between lizard and snake atadenoviruses cannot be explained based on host evolution.

Clinical signs described in lizards and snakes with AdV infection include dehydration, lethargy, inappetence, regurgitation, vomiting, limb paresis, anorexia, and weakness, as well as central nervous system (CNS) signs including head tilt and circling, opisthotonus, and spasms. Gastrointestinal problems and pneumonia have also been reported.[12,15,16,27,29,31,39] There are several reported cases where AdV infection was described without any clinical signs.[20,24,38] Therefore, the primary pathogenic role of AdVs is not always clear. Nevertheless, the pathogenicity of these viruses has been demonstrated by an experimental transmission study only in snakes, where an AdV isolated from a boa constrictor (*Boa constrictor ortonii*) with hepatic necrosis was inoculated into a neonatal boa constrictor, which died 14 days after inoculation with hepatic necrosis.[21] Pathologic lesions found in lizards and snakes frequently involve the liver, which may be diffusely pale and swollen.[15] The liver can also be enlarged[24] and diffusely mottled. The intestine is also commonly affected, e.g., hemorrhage in the intestinal lumen.[20,21,32] Histologically, basophilic and eosino-

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Table 1. Squamate species in which AdV infection have been reported.

Species	Citation
Eastern bearded dragons (*Pogona barbata*)	27
Black-soil bearded dragons (also known as Rankin's dragon lizard) (*Pogona henrylawsoni*)	15
Central netted dragon (*Ctenophorus nuchalis*)	20
Western bearded dragon (*Pogona minor minor*)	20
Common agama (*Agama agama*)	5
Inland bearded dragons (*Pogona vitticeps*).	24, 29, 37, 52
Mexican beaded lizards (*Heloderma horridum*)	39
Gila monsters (*Heloderma suspectum*)	39, 52
Emerald monitor (*Varanus prasinus*)	39
Savanna monitor (*Varanus exanthematicus*)	22
Jackson's chameleon (*Chamaeleo jacksonii*)	23
Mountain chameleon (*Chameleo montinum*)	30, 52
Fat-tail geckos (*Hemitheconyx caudicinctus*)	52
Leopard geckos (*Eublepharis macularius*)	52
Tokay gecko (*Gekko gecko*)	52
Blue tongued skink (*Tiliqua scincoides intermedia*).	52
Hispaniolan gracile anole (*Anolis distichus ignigularis*)	4
Hispaniolan gracile anole (*A. distichus ravitergum*)	4
Boa constrictors (Common boa constrictor) (*Boa constrictor ortonii*)	19, 21, 33, 40, 42
Corn snakes (*Pantherophis guttatus*, formerly *Elaphe guttata guttata*)	1, 13, 16, 26, 31
Royal pythons (*Python regius*)	1, 38
Rosy boas (*Lichanura trivirgata*)	44
Mountain king snakes (*Lampropeltis zonata*)	43, 53
Mojave rattlesnake (*Crotalus scutulutus scutulatus*)	40
Palm viper (*Bothriechis marchi*)	43
Asp viper (*Vipera aspis*)	39
Four-lined rat snake (*Elaphe quatuorlineata*)	19
Aesculapian snake (*Elaphe longissima*)	19
California king snake (*Lampropeltis getulus californiae*)	16
Milk snake (*Lampropeltis triangulum*)	16
Gaboon viper (*Bitis gabonika*)	19
Indonesian pit viper (*Parias hageni*)	13
Death adder (*Acantrophis antarcticus*)	20
Bull snake (*Pituophis catenifer sayi*)	16

philic intranuclear inclusions in enterocytes, hepatocytes,[25,29] epithelial cells of the bile ducts, lung, renal tubules, pancreatic acini, and oral mucous membranes, as well as endothelial cells in the brain, have been described.[37,43,44,52]

AdVs have been detected in lizards by electron microscopy[21] and by polymerase chain reaction (PCR),[52] which represents an effective tool for the detection of AdVs in reptiles.[9,39] Screening of lizard and snake samples for AdVs using this PCR and sequencing of the PCR products led to the detection of three new squamate AdVs in this study.

MATERIALS AND METHODS

Case histories

Green anoles (Anolis carolinensis): These animals were imported in multiple shipments from Florida to Germany in the years 2011–2012 by a commercial dealer. A total of 2,400 animals were imported in five deliveries. The green anoles were housed in gauze cages in a greenhouse. The animals were separated into male and female groups of 25–30 animals each. Several animals demonstrated clinical signs such as weakness, cachexia, and greyish discoloration of the skin, with an increase in the mortality rate from October 2011 (0.8%) to January 2012 (15%). Skin, liver, and intestinal samples of one of the affected animals were submitted for virologic testing (Table 2).

Jackson's chameleon (Chamaeleo jacksonii): This animal was kept by a private owner. Clinical signs observed in the chameleon included poor body condition and cachexia. The liver was submitted for virologic testing (Table 2).

Table 2. Case histories, results of virologic screening, parasitologic, bacteriologic, cytologic, and coprologic examinations.[a]

Species	Case history	Sample	AdV PCR	Rana PCR[51]	IIV PCR[33]	PMV PCR[2]	Virus isolation results (IgH2)	Other findings
Green anole *(Anolis carolinensis)*	Imported from Florida to Germany, obtained from a group of animals that demonstrated weakness, dermatitis, and increased mortality rate.	Skin	−	+	+		Ranavirus + IIV isolated	Parasitological examination demonstrated the presence of Flagellates, *Coccidia*, Oxyurids, *Oochoristica* ssp.
		Liver	−	+	−		Ranavirus isolated	
		Intestine	+	+	−		Ranavirus isolated	
Jackson's Chameleon *(Chamaeleo jacksonii)*	Cachexia. Gross pathology: diffusely black-colored liver. Pathohistologically: melano-phagocyte hyperplasia of liver, lungs, and spleen;mild diffuse lymphocytic infiltrations in the small intestine; few to moderate numbers of multifocal eosinophilic and basophilic large intranuclear inclusion bodies within enterocytes. (Fig. 1)	Liver	+	−	+		IIV isolated	Bacteriological testing: *Proteus vulgaris* in liver, lungs, heart, and intestine; *Salmonella* Blijdorp in the intestine. Cytological examination (DiffQuik): liver, lung, kidney: Melano-macrophages, heterophilic granulocytes, lymphocytes, macrophages, uniform small bacillary bacteria.
Green striped tree dragon *(Japalura splendida)*								
Animal 1	Group of animals imported from China via Florida to Germany. Demonstrated dehydration, lethargy, central nervous system signs, dermatitis. Gross pathology: hemorrhagic, edematous gastrointestinal tract; ecchymotic hemorrhages in the fatty tissue and the liver.	Skin	−	−	+	−	—	Coprologic examination demonstrated the presence of flagellates, *Heterakis* spp., *Spirurida* spp., *Choleoeimeria* spp., and larval ascarids (probably *Hexametra* spp.).
		Lung	−	−	+	−		
		Liver and kidney	−	−	−	−		
		Intestine	+	−	−	−		
Animal 2		Skin	−	−	+	−	IIV isolated	
		Lung	−	−	+	−		
		Liver and kidney	−	−	+	−		
		Intestine	+	−	+	−		
Animal 3		Skin	−	−	+	−	IIV isolated	
		Lung	−	−	+	−		
		Liver and kidney	−	−	−	−		
		Intestine	+	−	+	−		

[a] AdV indicates adenovirus; PCR, polymerase chain reaction; IIV, invertebrate iridovirus; IgH2, iguana heart cells; PMV, paramyxovirus (ferlavirus); −, negative; +, positive.

Green striped tree dragons (Japalura splendida): A group of 207 animals was imported from China via Florida to Germany by the same importer as the green anoles. The green striped tree dragons were housed in groups of 50 animals. The following clinical signs were seen in this group of animals: 21 lizards demonstrated CNS signs such as incoordination, circling, and inability to climb, as well as dehydration and lethargy. Animals died within 24–36 hr after the first clinical signs were seen. Dermatitis was also observed in several animals in this group. Five formalin-fixed animals were submitted for pathologic examination. Internal organs from five other animals were submitted fresh for virologic testing (Table 2).

Virus isolation

Virus isolation was attempted from all AdV-positive samples on iguana heart cells (IgH2, ATCC: CCL-108) as described previously.[1] Briefly, samples were added to 3 ml Dulbecco's modified Eagles medium (DMEM) (Biochrom AG, 12247 Berlin, Germany) supplemented with antibiotics and then sonicated and centrifuged at $2{,}000 \times g$ for 10 min. Two hundred microliters of the supernatant was inoculated onto IgH2 in 30-mm-diameter Cellstar® tissue culture dishes (Greiner Bio-One GmbH, 72636 Frickenhausen, Germany). After incubation for 2 hr at 28°C, 2 ml DMEM supplemented with 2% fetal calf serum (Biochrom AG), 1% nonessential amino acids (Biochrom AG), and antibiotics was added to each dish. Samples were incubated for 14 days at 28°C and observed regularly for cytopathic effects.

PCR and sequencing

DNA was extracted from the supernatant of previously sonicated samples using the DNAeasy® kit (Qiagen GmbH, 40724 Hilden, Germany) following the instructions of the manufacturer. A consensus nested PCR targeting a portion of the DNA-dependent DNA polymerase gene was used for screening.[52] PCR products were separated on 1.5% agarose gels (Biozym, 31840 Oldendorf, Germany) in TAE buffer containing 0.5 μg ethidium bromide and visualized under 320-nm ultraviolet light. Positive PCR amplicons were purified with a peqGOLD Extraction Kit (PEQLAB Biotechnology GmbH, 91052 Erlangen, Germany) and sequenced by a commercial company (Eurofins MWG GmbH, 85560 Ebersberg, Germany).

Analysis of sequences

Obtained sequences were analyzed with the STADEN Package version 2003.0 Pregap4 and Gap4 programs.[10] All sequences, after editing out primers, were compared to the data in GenBank (National Center for Biotechnology Information, Bethesda, Maryland 20894, USA) online (http://www.ncbi.nih.gov) using BLASTX. For analysis of the sequences, multiple alignments were performed with the ClustalW algorithm of the BioEdit Sequence Alignment Editor program[17] using default settings. For this, homologous nucleotide or amino acid sequences of DNA-dependent DNA polymerase genes of AdVs were retrieved from GenBank through the nonredundant AdV database of the Molecular Virology Group at the Veterinary Medical Research Institute, Budapest, Hungary (http://www.vmri.hu/~harrach). Amino acid sequences were also used for phylogenetic calculation. Calculation of phylogenies using multiple methods is helpful for understanding the possible true topology of the resulting trees. Phylogenetic calculation was performed using MrBayes analysis with the TOPAli v2 platform,[36] as well as maximum likelihood method based on the Dayhoff matrix model[45] using the Mega 5.05 program[48] and Fitch-Margoliash method with global rearrangements, using PHYLIP program package version 3.6.[14]

A Shimodaira-Hasegawa test[46] to obtain support values (SHL) was carried out using the PHYml-aLTR program v2.4.5[3] applying the JTT amino acid substitution model, with fixed portion of invariable sites (p-ivar. = 0.00). This was performed to test phylogenetic accordance between the calculated maximum-likelihood tree and alternative trees.

RESULTS

AdVs were detected in the intestine sample of the green anole, in the liver sample of the Jackson's chameleon, and in intestine samples of three out of five green striped tree dragons tested. The AdV detected in the green anole was named Anolis AdV-3; the AdV detected in the Jackson's chameleon was named Chameleon AdV-2; and the AdV detected in green striped tree dragons was named Green striped tree dragons AdV-1. No AdVs were isolated in cell culture from any of the tested AdV-positive samples.

Pathologic examination in the case of green anoles demonstrated catarrhal enteritis in individual animals. In the case of the Jackson's chameleon, a diffusely black-colored liver was

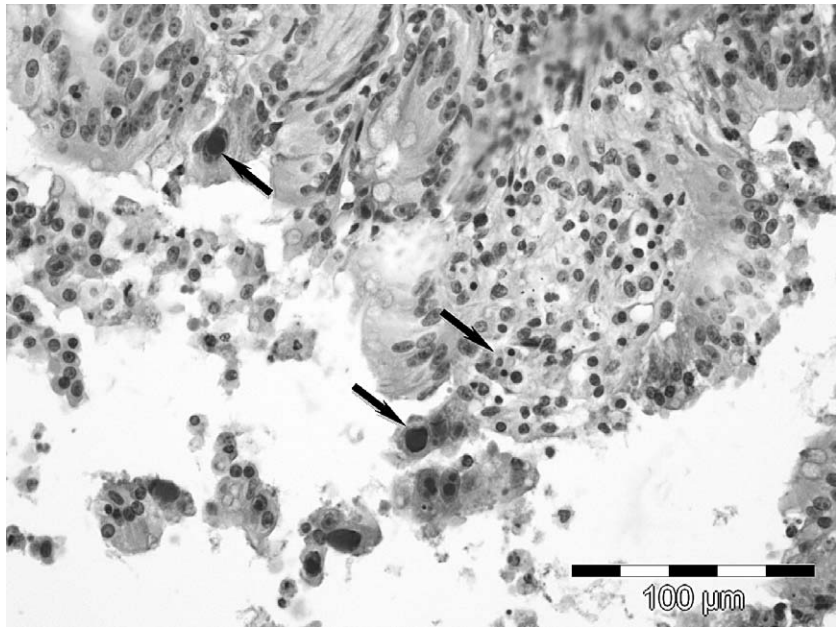


Figure 1. Intestine of the Jackson's chameleon (*Chamaeleo jacksonii*). Eosinophilic and basophilic intranuclear inclusion bodies (arrows). Hematoxylin and eosin, ×1,000.

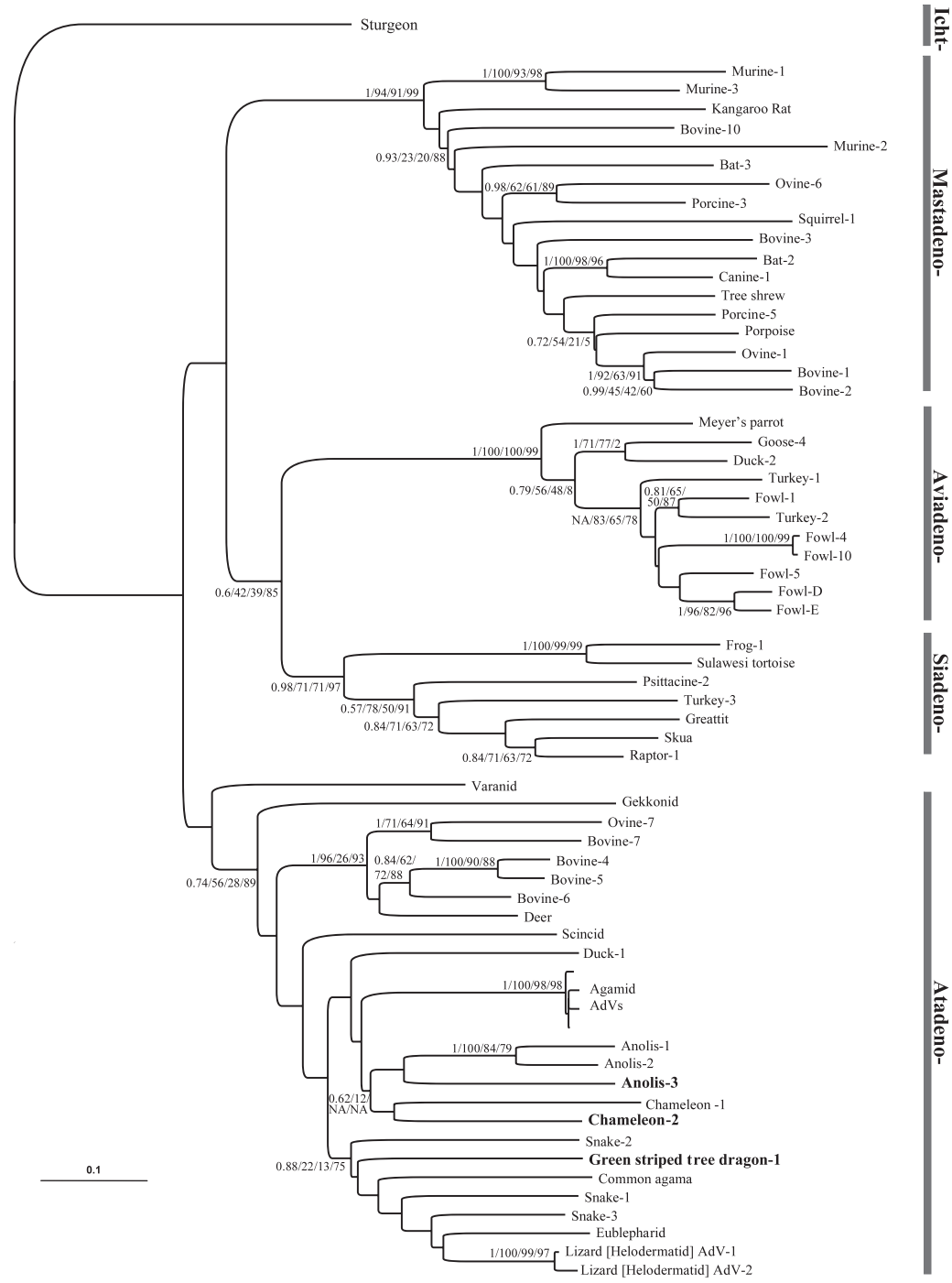
noted, the small intestine and rectum were moderately filled with yellow liquid content. Histologic examination demonstrated melanophagocytic hyperplasia of the liver, lungs, and spleen. In the small intestine, mild diffuse lymphocytic infiltration was observed and few to moderate numbers of multifocal eosinophilic and basophilic large intranuclear inclusion bodies were seen within enterocytes (Fig. 1). Pathologic examination of green striped tree dragons revealed hemorrhagic, edematous gastrointestinal tracts and ecchymotic hemorrhages in the fatty tissue and the liver. Histologic examination showed basophilic and eosinophilic intracytoplasmic inclusion bodies within hepatocytes.[6] Other viruses as well as different parasites and bacteria were detected in all cases (Table 2).

AdV sequences were 272 nucleotides long after the primers were edited out. All newly detected lizard AdVs demonstrated less than 67% nucleotide homology and less than 72% amino acid homology to other known AdVs over the region examined.

Sixty-four amino acid sequences (91 amino acids long) were used for the calculation of a phylogenetic tree (Fig. 2). Phylogenetic analysis clustered all newly detected viruses within the genus *Atadenovirus*. All newly detected AdVs found in lizards were distinctly different from previously detected lizard AdVs and from one another. Phylogenetic analysis of newly detected Anolis AdV-3 demonstrated that this virus belongs to a clade that includes the sequences from previously reported Anolis AdVs 1 and 2 and Chameleon AdV-1. The sequence of the new Chameleon AdV-2 belongs to the same clade, although the sequence demonstrated the highest identity to Agamid AdVs by BLAST analysis. This clade is, however, not well supported. The newly detected Green striped tree dragon AdV forms a well-supported clade with snake, gecko, helodermatid, and other agamid AdVs. The relationships between newly detected Anolis AdV-3 and two previously detected Anolis AdVs, along with both Chameleon AdVs within one clade, and kinship between newly described Green striped tree dragon AdV and other agamid, helodermatid, snake, and gecko AdVs in another clade are both well supported by the Bayesian posterior probability values, and latter kinship also by the SHL test support values (Fig. 2). All sequences were submitted to GenBank and received the accession numbers KF886532, KF886533, and KF886534.

DISCUSSION

AdV infection has repeatedly been described in association with CNS signs in lizards.[29,39] Interestingly, additional viral infections with ranavirus and/or invertebrate iridoviruses (IIVs) were de-



NA – not apllicable

Figure 2. Phylogenetic distance tree of partial adenovirus (AdV) DNA polymerase amino acid sequences (91 amino acids) is shown. Posterior probabilities generated by the Bayesian method/Fitch–Margoliash bootstrap values/maximum likelihood bootstrap values/ Shimodaira-Hasegawa–like support values are shown on branches, separated by slashes. NA, not applicable. New viruses are shown in bold lettering. Abbreviations and GenBank accession numbers (in parentheses): Agamid AdVs (DQ077706, EU914205, FJ196812, ACH86251), Anolis AdV-1 (KC544015), Anolis AdV-2 (KC544016), Anolis AdV-3 (KF886534), Bat AdV-2 (JN252129), Bat AdV –3

tected in all but one of the lizards tested (Table 2). Ranavirus infections in lizards have been described in association with skin lesions and hepatitis.[6,34,47] Also, skin lesions have been detected in lizards tested positive for the presence of IIV.[28] Infection without any clinical signs also has been reported for each virus.[35]

In the case of the group of green striped tree dragons, CNS signs as well as dermatitis were observed. In this case it is hypothesized that the causative agent for the disease was not a bacterial infection, because an initial antibiotic treatment did not reduce the mortality rate of the diseased animals.[6] It is, however, not possible to identify a primary pathogen in this case. Clinical signs observed in the green anoles included weakness and skin lesions. In this case it is hypothesized that the skin lesions could be the result of ranavirus or/and IIV infection, because these two viruses were detected in a skin sample from a diseased animal. The only clinical sign observed in the Jackson's chameleon was cachexia. In this case, coinfections with different viruses (AdV and IIV) and bacteria (*Proteus* spp.) may have led to the fatal course of disease.

Coinfection of AdVs and other infectious agents in lizards have been described previously.[5,29] Infections with AdVs and other viruses have also been reported.[1,39] Viruses that have coevolved with their hosts, as is hypothesized for atadenoviruses in reptiles,[8,18] may also be nonpathogenic for their natural hosts, or lead to development of clinical disease only in weakened hosts. It is therefore possible that in the presented cases in which the hosts were infected by multiple viruses, the AdVs contributed to the development of clinical disease.

In the cases of the green striped tree dragons and green anoles, it is possible that stress from recent importation and adapting to a new environment and new diet could clinically predispose these reptiles to develop disease. Overcrowding may also have influenced the epidemiology of disease, increasing both the spread of virus and the susceptibility of individuals to infection and disease development.

The comparison of partial DNA polymerase gene sequences of our three newly detected lizard AdVs demonstrated less than 80% sequence identity. The detected viruses therefore seem to represent distinct AdV species. An AdV described in a common agama recently also demonstrated less than 80% sequence identity to all previously detected atadenoviruses.[5] AdVs that have been described in snakes (SnAdV-1, SnAdV-2, and SnAdV-3) have also demonstrated less than 80% identity to one another and with all known atadenoviruses in this portion of the genome.[16,26] Earlier studies of AdVs of lizards using the same portion of the genome showed that six AdVs detected in seven different lizard species demonstrated less than 90% sequence identity.[52] The finding of these three new distinct AdVs also demonstrates the increasing frequency of the detection of genetically distinct atadenoviruses. These findings are rapidly broadening our understanding of the diversity of AdVs of reptiles.

In the case of the green striped tree dragons, AdV was found in three of five animals submitted for virologic testing. There are several reported cases in which single animals within a group were infected with AdV.[15,37] Although the route of transmission of AdVs in reptiles has not been studied, it is believed to be transmitted via the fecal-oral route or by direct contact via oronasal secretion.

←

(AB303301), Bovine AdV-1 (YP_094032), Bovine AdV-2 (AP_000006), Bovine AdV-3 (AP_000026), Bovine AdV-4 (AAK13183), Bovine AdV-5 (not released yet), Bovine AdV-6 (YP_007346998), Bovine AdV-7 (U57335), Bovine AdV-10 (AF238882), Canine AdV-1 (AAB05434), Chameleon AdV-1 (AY576679), Chameleon AdV-2 (KF886533), Common agama AdV (KC155825), Duck AdV-1 (AP_000539), Eublepharid AdV-1 (AY576677), Fowl AdV-1 (AP_000410), Fowl AdV-2 (HM853995), Fowl AdV-3 (HM853996), Fowl AdV-4 (GU188428), Fowl AdV-5 (DQ 159938), Fowl AdV-6 (HM853999), Fowl AdV-7 (HM854000), Fowl AdV-8 (GU734104), Fowl AdV-9 (AC_000013), Fowl AdV-10 (HM854003), Fowl AdV-11 (HM854004), Frog AdV (AAF86924), Gekkonid AdV (AY576681), Goose AdV (JF510462), Greattit AdV (ACW84422), Green striped tree dragon AdV-1 (KF886532), Helodermatid AdV-1 (AAS89696), Helodermatid AdV-2 (EU914207), Kangaroo rat AdV (not released yet), Mascovy duck AdV (not released yet), Parrot AdV (AY644731), Murine AdV-1 (AC_000012), Murine AdV-2 (HM049560), Murine AdV-3 (EU835513), Odocoileus AdV (AF361168), Ovine AdV-1 (not released yet), Ovine AdV-6 (not released yet), Ovine AdV-7 (AAD45950), Psittacine AdV-2 (EU056825), Porpoise AdV (JN377908), Porcine AdV-3 (AB026117), Porcine AdV-5 (AAK26504), Raptor AdV (NC_015455), Scincid AdV (AY576682), Squirrel AdV (GU735083), Skua AdV (YP_004935931), Snake AdV-1 (AAL89790), Snake AdV-2 (ACH91014), Snake AdV-3 (FJ012164), Sturgeon AdV (AY082701), Tortoise AdV (EU056826), Tree shrew AdV (AF258784), Turkey AdV-1 (GU936707), Turkey AdV-2 (not released yet), Turkey AdV-3 (AC_000016), Varanid AdV (ACH86253).

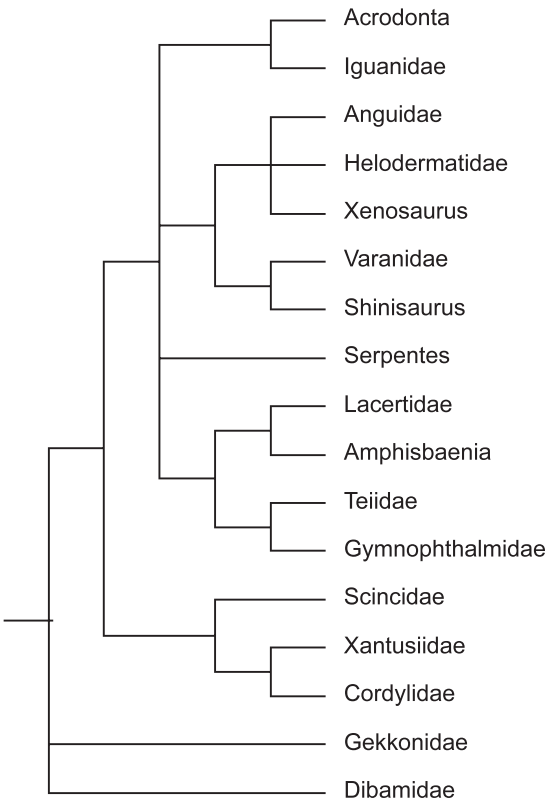


Figure 3. Phylogenetic tree for squamate reptiles.[49]

All of the lizard species in this report belong to the infraorder Iguania, which includes, among others, the families Iguanidae, Agamidae, and Chamaeleonidae. The phylogenetic analysis of squamate reptiles has demonstrated that iguanians are most closely related to the snakes and Anguimorpha (e.g., Helodermatidae, Anguidae, Varanidae), all of which have been included in the Toxicofera. They are clearly distinct from the gekkonid lizards, which belong to the Bifurcata. The Scincidae also fall outside the Toxicofera and have been included in the Scinciformata.[49,50] The topology of a phylogenetic tree of squamate reptiles (Fig. 3) is not similar to the phylogenetic tree of squamate AdVs (Fig. 2) in which AdVs detected previously in lizards belonging to the infraorder Gekkota (Eublepharid AdV-1 and Gekkonid AdV-1) cluster distinctly from each other. This contradicts the theory that the majority of squamate AdVs described have coevolved with their hosts and indicates that host switches, at least between different squamate species, are possible and perhaps even common. In that case, Eublepharid AdV-1 might have switched hosts from a member of the Toxicofera to a gekkonid host.

It is interesting to note that the AdV detected in the group of green striped tree dragons (Green striped tree dragon AdV-1), which belong to the family Agamidae, demonstrated the highest identity with Helodermatid AdV-1 and Eublepharid AdV-1. Eublepharid AdV-1 has been described in a leopard gecko (*Eublepharis macularius*) and in fat-tail geckos (*Hemitheconyx caudicinctus*).[52] An AdV with 99% identity to Helodermatid AdV-2 in a partial DNA-dependent DNA polymerase gene sequence was also detected in a wild-captured western bearded dragon (*Pogona minor minor*) in Australia.[20] Thus, because of this apparent broader host spectrum, similar to that observed among Snake AdV (see later), it has been proposed to rename Helodermatid AdVs to Lizard AdVs (Harrach, pers. comm.). Helodermatid AdV-2 was first found in beaded lizards (*Heloderma horridum*),[39] and a closely related virus, Helodermatid AdV-1, has been found only in Gila monsters (*Heloderma suspectum*) so far.[39,52] Phylogenetic analysis also clustered this virus distinctly from other agamid lizard AdVs. The AdV detected in the Jackson's chameleon (Chameleonid AdV-2) demonstrated the highest identity to different agamid lizard AdVs rather than to Chameleon AdV-1, which was detected previously in a mountain chameleon (*Chamaeleo montium*, formerly *Triocerus montium*).[52] However, Chameleon AdV-2 belongs to the same clade as Chameleon AdV-1, although this clade is not well supported. An AdV infection has been described in a Jackson's chameleon before,[22] but unfortunately no sequence information was available from the virus in that case. The AdV detected in a green anole demonstrated the highest identity with Anolis AdV-2, which was detected in *Anolis distichus ravitergum*.[4] The phylogenetic analysis also demonstrated that the Anolis AdV-3 belongs to the same clade as the previously detected Anolis AdVs 1 and 2, but this clade is not well supported. A lack of species specificity has previously been postulated for AdVs from snakes. Snake AdV-1 has been described in both colubrid and boid snakes[12,35] and Snake AdV-2 has been found in viperid and colubrid snakes.[16,39] Snake AdV-3 has been detected in different species of colubrid snakes.[16] However, the sequences on which these findings are based are short (91 amino acids) and bootstrap values at this branching of the tree are low, so that further sequencing is necessary to find out the exact taxonomic position of these viruses.

In conclusion, although apparently the three newly detected AdVs from hosts belonging to

Iguania do not fully support the host-specificity coevolution–cospeciation theory of AdVs, as they do not form a monophyletic subgroup within the cluster of AdVs originating from toxicoferan hosts, yet we can find interpretations of these new data supporting the theory. There is a well-supported subgroup of AdVs from chameleons and anolis lizards in close connection with the Agamid AdV-1 isolates, which might represent the coevolved iguania AdV lineage. The green anole AdV and Jackson's chameleon AdV from this study belong to this subgroup, yet further AdV sequences from other lizards belonging to the infraorder Iguania are needed to test the assumption. A sister clade to this presumed lineage contains AdVs from related toxicoferan hosts (except for the Eublepharid AdV), of which two AdVs (from common agama and green striped dragon) also belong to Iguania (family Agamidae). At the same time, it is important to remember that many squamate AdVs have been detected in animals that are pet reptiles and are often captive bred. Also, wild-caught reptiles that are tested for AdV infections may be stationed in holding facilities for variable amounts of time prior to testing, where they may come into contact with large numbers of taxonomically distant animals and other reptiles, carrying a significantly different microbiome. In these cases the spillover of viruses from taxonomically and evolutionary very distant hosts is also possible. Therefore the AdVs detected in squamates cannot generally be assumed to exclusively reflect the AdVs found in natural hosts, and opportunities for host switches may be common. Further work is necessary to broaden our understanding of which AdVs can be found in different species of lizards and snakes. In addition, sequence data from a larger portion of the genome of the detected viruses are necessary to fully understand the origin of the members of the genus *Atadenovirus.* This would also help in developing risk analysis tools for AdV infections in reptiles and understanding how infections in individual species might affect other species within a collection.

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