

**Part 1:** **TITLE, AUTHORS, APPROVALS, etc**

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| **Code assigned:** | **2020.008D** |  |
| **Short title:** Create one new genus (*Testadenovirus*) and six new species (*Rowavirales*: *Adenoviridae*) | | |
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**Author(s) and email address(es)**

|  |  |
| --- | --- |
| Tarján ZL, Pénzes JJ, Doszpoly A, Harrach B, Benkő M | [lztarjan@gmail.com](mailto:lztarjan@gmail.com); [judit.penzes@ufl.edu](mailto:judit.penzes@ufl.edu); [andor.doszpoly@gmail.com](mailto:andor.doszpoly@gmail.com); [balazs.harrach@gmai.com](mailto:balazs.harrach@gmai.com); [maribenko@gmail.com](mailto:maribenko@gmail.com) |

**Author(s) institutional address(es) (optional)**

|  |
| --- |
| Research Centre for Agricultural Research, Institute for Veterinary Medical Research, Hungary [TZL, DA, HB, BM], University of Florida, USA [PJJ] |

**Corresponding author**

|  |
| --- |
| Benkő, Mária (maribenko@gmail.com) |

**List the ICTV Study Group(s) that have seen this proposal**

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| *Adenoviridae* Study Group |

**ICTV study group comments and response of proposer**

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| The SG found the proposals acceptable. Two species names on Fig. 1 are not correct. Clarification of the text is proposed.  Response: Species names and other mistakes were corrected. Unclear parts were removed. |

**Authority to use the name of a living person**

|  |  |  |
| --- | --- | --- |
| **Taxon name** | **Person from whom the name is derived** | **Permission attached (Y/N)** |
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**Submission dates**

|  |  |
| --- | --- |
| Date first submitted to SC Chair | July 31, 2020 |
| Date of this revision (if different to above) | August 19, 2020 |

**ICTV-EC comments and response of the proposer**

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**Part 3:** **TAXONOMIC PROPOSAL**

**Name of accompanying Excel module**

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| --- |
| 2020.008D.R.Adenoviridae\_1ngen\_6nsp |

**Abstract**

|  |
| --- |
| Here, we propose the establishment of a new, sixth genus in the family *Adenoviridae* for novel adenoviruses (AdVs) that have recently been discovered in several hosts belonging to superfamily Testudinoidea of the order Testudines. These AdVs form a lineage that is phylogenetically distant from the existing AdV genera. We propose the genus name *Testadenovirus*, which refers to the host origin. This genus would be represented by the exemplar red-eared slider AdV-1 classified into the type species *Pond slider testadenovirus A*. We also propose the creation of five new species in existing genera: *Bovine atadenovirus E, Lizard atadenovirus B, Guinea pig mastadenovirus A, Psittacine siadenovirus D* and *Psittacine siadenovirus E.* The new species are justified by multiple species demarcation criteria, including phylogenetic distance, genome organization, host and serology. |

**Text of proposal**

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| |  | | --- | | **1.** We propose the establishment of **a new genus** for AdVs that infect (and have supposedly coevolved with) testudine hosts. This is supported by the following findings.  **Phylogeny**  The existence of a distinct lineage of AdVs infecting turtles has been described in different countries and turtle hosts (1, 2, 4, 6, 7, 10, 19). In the NCBI database, partial sequences from the DNA polymerase and hexon genes are currently available from viruses found in 5 and 3 different turtle species, respectively (<https://sites.google.com/site/adenoseq/>). In phylogenic reconstructions based on short partial sequences, these novel AdVs appear very close to each other but well-separated from all other known AdVs (4). A targeted PCR survey resulted in the recognition of seven genetic variants of the novel virus in yellow-bellied and red-eared sliders (*Trachemys scripta scripta* and *T. s. elegans*), with some animals harbouring more than one AdV. The distribution of the different variants was not related to the subspecies affiliation of their hosts. From one AdV variant, derived from a red-eared slider, an approximately 15-kbp PCR fragment encompassing the central part of the genome was sequenced. Phylogenic reconstructions based on the full length amino acid (aa) sequences of the DNA polymerase and penton base proteins, as well as on the partial hexon protein sequences, further confirmed the phylogenetic distinctness and genus-level separation of the novel AdV clade (Figs. 1, 2 and 3).  **Hosts and coevolution**  Turtles (Testudines) represent a very (if not the most) ancient lineage of vertebrates, appearing 220-260 million years ago. Thus, it is not surprising that their AdVs are markedly different from the AdVs of other vertebrate orders and classes. The ancient host origin alone could merit the classification of these AdVs into a separate genus.  We hypothesize that these AdVs have coevolved with members of the Testudines. One proof for this hypothesis is the remarkable similarity between the phylogenetic tree topologies of AdVs from red-eared slider, yellow-bellied slider, ornate box turtle (*Terrapene ornata ornata*), eastern box turtle (*Terrapene carolina carolina*), pancake tortoise (*Malacochersus tornieri*) and red-footed turtle (*Chelonoidis carbonarius*) and that of their hosts (Fig. 4). The separation of two major AdV clades corresponds to that of the hosts’ families (Testudinidae and Emydidae).  **Genome differences**  The G+C content of the sequenced genome portion of red-eared slider AdV-1 (NCBI GenBank Acc. No.: JN632576) is balanced (55.2%), and therefore the possibility of a recent host switch has not been considered.  The absence of the protein V gene, along with the phylogenetic data and the hosts, clearly separates this lineage from the other AdV genera.  Red-eared slider AdV-1 has the longest pVI protein (286 aa) among the known AdVs except porcine AdV-3 (which is a mastadenovirus). At the same time, it has the shortest pVII protein (57 aa) after white sturgeon AdV-1 (Fig. 5). The protease cleavage signals on pVI and pVII are usually characteristic of a given genus (Fig. 5 and Fig. 6). The second cleavage signal on pVII is of type I in siadenoviruses and the proposed testadenoviruses, as well as in mastadenoviruses. However, all the known siadenovirus cleavage signals start with ‘L’, while in red-eared slider AdV-1 it starts with ‘M’ (Fig. 5). White sturgeon AdV-1 even lacks this second cleavage signal.  The second cleavage signal in pVI is even more different (Fig. 6). All known AdVs have a type II signal in this position, whereas red-eared slider AdV-1 alone has an exclusive type I signal. Furthermore, the third fragment (pVIc, which, by attaching to it, enhances the enzymatic capability of the viral protease) of the processed pVI contains 11 aa in every AdV but red-eared slider AdV-1, where its length is only 10 aa. These differences underscore the uniqueness of the novel AdV lineage compared to members of the other genera.  To reflect the characteristic host range, we propose the name ***Testadenovirus*** for the new genus.  **2.** We propose the creation of a new AdV species in the above proposed genus for the classification of the exemplar virus from red-eared slider. As red-eared and yellow-bellied sliders were found to harbour identical variants, and are of subspecies of the same slider species, which have the common name of pond sliders, we propose to name the new species ***Pond slider testadenovirus A***.  **3.** We propose the establishment of a novel species, ***Bovine atadenovirus E*** for the classification of bovine AdV-6 (BAdV-6). This virus was isolated and described as a new serotype long ago (17). Subsequently, it has been detected repeatedly (8). Its full hexon gene (12) then finally its entire genome have been sequenced (5). The genomic DNA has a low (35.1%) G+C content. BAdV-6 was classified into genus *Atadenovirus* (Fig. 2) without any species assignment. One of the AdV species demarcation criteria is the 10-15% difference in the aa sequence of the DNA polymerase. In phylogeny inference as well as in pairwise aa sequence identity, the BAdV-6 DNA polymerase was found to be adequately different from the most similar atadenoviruses, namely BAdV-4 and deer AdV-1 (Fig. 1, Fig. 7). The different host species, compared to deer AdV-1 (syn. Odocoileus AdV) and the lack of cross-neutralization with BAdV-4 or BAdV-7 justify the novel species.  **4.** Bearded dragon AdV-1 (its earlier syn. name being agamid AdV-1) was first characterised by a consensus-primer-based PCR (21). Later, it was found to be common in captive-bred bearded dragons (*Pogona vitticeps*) in numerous countries. It was also detected in central netted dragons (*Ctenophorus nuchalis*) in Australia. Recently, the whole genome of bearded dragon AdV-1 has been published (14). The virus belongs to genus *Atadenovirus*, like all AdVs from squamate reptiles. Its genome encodes proteins of the C-type lectin-like domain superfamily, which are non-homologous to ORF19 of aviadenoviruses, the only hitherto adenoviral protein of the same superfamily. Based on species demarcation criteria (phylogeny and individual pairwise distance of the DNA polymerase, host, genome organisation), it merits the establishment of a new species to include it (Fig. 1, Fig. 7). Since this virus has been detected in lizards of different species, it seems to be logical to follow the earlier naming policy and propose the species name of ***Lizard atadenovirus B***.  **5.** Short sequences of AdVs from guinea pig (*Cavia porcellus*) have been described previously (16). By now, the full genomic sequences of two strains, one AdV from Australia (MN986925) and another one from Germany (MN986926) have been reported (11). Based on phylogeny, pairwise distance (Fig. 1, Fig. 8) and the unique host, these AdVs merit the establishment of a new species with a proposed name of ***Guinea pig mastadenovirus A***.  **6.** To date, full sequences of several psittacine AdVs have been described. Some AdVs found in birds of various psittacine species turned out to be of identical types. It is essential that such viruses have a uniform, valid naming. We adopted the approach of using a collective name (i.e. psittacine) with ascending type numbers, as opposed to the current practice, according to which the numbering is re-started each time when the virus is detected in a new host. Following this logic, we propose the name psittacine AdV-5 for the siadenoviruses, detected in four different hosts. These hosts include Pacific parrotlet (*Forpus coelestis*; MK695679) (9) and sun parakeet (syn. sun conure; *Aratinga solstitialis;* MN450070). Both of these viruses are now fully sequenced. Additionally, the AdVs reported from a cockatiel (*Nymphicus hollandicus*; MH507070) (3) and a budgerigar (*Melopsittacus undulatus;* AB485763) should also be named as psittacine AdV-5. Another psittacine siadenovirus shows adequate divergence for being named with the next available type number, psittacine AdV-6. It comes also from a budgerigar and is fully sequenced (MN687905) (15). In spite of representing two different types, psittacine AdV-5 and psittacine AdV-6 should be classified into a common species, based on the species demarcation criteria (Fig. 1, Fig. 7). According to the earlier naming policy, we propose the species name ***Psittacine siadenovirus D***. *Psittacine siadenovirus C* has just been accepted (20). The appropriateness of the general name (Psittacine) is justified by the occurrence of these viruses in members of multiple psittacine species.  **7.** We propose the establishment of a further species, ***Psittacine siadenovirus E***, to include the siadenovirus found recently in the little corella (*Cacatua sanguinea*; MK227353) (18). This virus fulfils the species demarcation criteria (Fig. 1, Fig. 7).  The introduction of the above-proposed type and species naming would likely prevent the mixing up of these viruses in future since the emergence of additional similar viruses is expectable.  This work was supported partly by a grant provided by the Hungarian Scientific Research Fund (OTKA NN128309). | |

**Supporting evidence**

***Ichtadenovirus***

white sturgeon-1, *Sturgeon ichtadenovirus A*

***Testadenovirus***

red-eared slider-1, *Pond slider testadenovirus A*

frog-1, *Frog siadenovirus A*

great tit-1, *Great tit siadenovirus A*

chinstrap penguin-2, *Penguin siadenovirus A*

turkey-3, *Turkey siadenovirus A*

South Polar skua-1, *Skua siadenovirus A*

raptor-1, *Raptor siadenovirus A*

psittacine-7 (little corella), *Psittacine siadenovirus E*

psittacine-6 (budgerigar, strain BrdKdnyDNA)

psittacine-5 (Pacific parrotlet)

psittacine-5 (sun parakeet)

*Psittacine siadenovirus D*

***Siadenovirus***

crane-1

goose-4, *Goose aviadenovirus A*

duck-2, *Duck aviadenovirus B*

pigeon-2, *Pigeon aviadenovirus B*

pigeon-1, *Pigeon aviadenovirus A*

white-eyed parakeet-2

marten-associated-1

psittacine-1 (Senegal parrot), *Psittacine aviadenovirus C*

psittacine-4 (red-bellied parrot), *Psittacine aviadenovirus B*

turkey-1, *Turkey aviadenovirus B*

fowl-1, *Fowl aviadenovirus A*

turkey-5, *Turkey aviadenovirus D*

fowl-4, *Fowl aviadenovirus C*

fowl-5, *Fowl aviadenovirus B*

turkey-4, *Turkey aviadenovirus C*

fowl-9, *Fowl aviadenovirus D*

fowl-6*, Fowl aviadenovirus E*

***Aviadenovirus***

duck-1, *Duck atadenovirus A*

white-eyed parakeet-1

psittacine-3 (Southern mealy amazon), *Psittacine atadenovirus A*

bearded dragon-1, *Lizard atadenovirus B*

lizard-2, *Lizard atadenovirus A*

snake-1, *Snake atadenovirus A*

ovine-7, *Ovine atadenovirus D*

deer-1, *Deer atadenovirus A*

bovine-4, *Bovine atadenovirus D*

bovine-6, *Bovine atadenovirus E*

***Atadenovirus***

murine-2, *Murine mastadenovirus B*

murine-1, *Murine mastadenovirus A*

murine-3, *Murine mastadenovirus C*

squirrel-1, *Squirrel mastadenovirus A*

equine-2, *Equine mastadenovirus B*

polar bear-1, *Polar bear mastadenovirus A*

bottlenose dolphin-1, *Dolphin mastadenovirus B*

bottlenose dolphin-2, *Dolphin mastadenovirus A*

harbor porpoise-1

porcine-5, *Porcine mastadenovirus C*

bovine-1, *Bovine mastadenovirus A*

bovine-2, *Ovine mastadenovirus A*

equine-1, *Equine mastadenovirus A*

skunk-1, *Skunk mastadenovirus A*

canine-1, *Canine mastadenovirus A*

bat-2, *Bat mastadenovirus B*

Asian parti-colored bat, *Bat mastadenovirus J*

bat-3, *Bat mastadenovirus A*

bat-250-A, *Bat mastadenovirus G*

tree shrew-1, *Tree shrew mastadenovirus A*

bat-WIV9, *Bat mastadenovirus C*

porcine-4, *Porcine mastadenovirus B*

California sea lion-1, *Sea lion mastadenovirus A*

bat-WIV17, *Bat mastadenovirus F*

straw-colored fruit bat, *Bat mastadenovirus H*

Egyptian fruit bat, *Bat mastadenovirus I*

bat-WIV12, *Bat mastadenovirus D*

bat-WIV13, *Bat mastadenovirus E*

porcine-3, *Porcine mastadenovirus A*

guinea pig-1, *Guinea pig mastadenovirus A*

ovine-8, *Ovine mastadenovirus C*

Odocoileus-2, *Deer mastadenovirus B*

bovine-3, *Bovine mastadenovirus B*

**primate adenoviruses**

***Mastadenovirus***

97

99

99

84

100

100

100

100

100

100

81

99

84

79

48

96

75

100

92

100

100

98

100

100

93

29

100

43

100

100

98

98

100

99

97

100

51

95

82

99

99

100

100

100

100

100

100

81

44

94

15

91

99

100

100

93

91

97

61

95

92

88

100

98

99

100

99

75

57

100

100

0.2

**Fig 1.** Phylogenetic tree of AdVs based on maximum likelihood analysis of DNA-dependent DNA polymerase (*pol*) amino acid sequences from one AdV from each species or candidate species, or distinct strain with the full *pol* sequence known. Only the proposed species *Psittacine siadenovirus D* is represented by three virus strains. Multiple alignment: MultAlin; model selection: ProtTest 2.4; 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 Galaxy/Pasteur platform. Unrooted calculation; white sturgeon AdV-1 and red-eared slider AdV-1 are selected as outgroups for visualization. Species names (if any) are shown after the virus names. Genus names are noted and the branches belonging to special genera are shown by different colours. Red letters designate the proposed novel species and genus. From the name of the AdV types the word ′adenovirus/AdV′ was removed for clarity. The branches of primate AdVs are collapsed. The bar indicates 20% difference between two neighbouring sequences. SH (Shimodaira-Hasegawa) branch support values are shown at the nodes.

***Ichtadenovirus***

white sturgeon-1, *Sturgeon ichtadenovirus A*

***Testadenovirus***

red-eared slider-1, *Pond slider testadenovirus A*

frog-1, *Frog siadenovirus A*

great-tit-1, *Great tit siadenovirus A*

chinstrap penguin-2, *Penguin siadenovirus A*

turkey-3, *Turkey siadenovirus A*

South Polar skua-1, *Skua siadenovirus A*

raptor-1, *Raptor siadenovirus A*

*Psittacine siadenovirus E*

psittacine-7 (little corella),

psittacine-6 (budgerigar, strain BrdKdnyDNA)

psittacine-5 (Pacific parrotlet, sun parakeet)

*Psittacine siadenovirus D*

***Siadenovirus***

goose-4, *Goose aviadenovirus A*

duck-2, *Duck aviadenovirus B*

pigeon-2, *Pigeon aviadenovirus B*

pigeon-1, *Pigeon aviadenovirus A*

fowl-1, *Fowl aviadenovirus A*

turkey-1, *Turkey aviadenovirus B*

turkey-4, *Turkey aviadenovirus C*

fowl-5, *Fowl aviadenovirus B*

fowl-6, *Fowl aviadenovirus E*

***Aviadenovirus***

duck-1, *Duck atadenovirus A*

psittacine-3 (Southern mealy amazon), *Psittacine atadenovirus A*

*Lizard atadenovirus B*

bearded dragon-1,

lizard-2, *Lizard atadenovirus A*

snake-1, *Snake atadenovirus A*

ovine-7, *Ovine atadenovirus D*

deer-1, *Deer atadenovirus A*

*Bovine atadenovirus E*

bovine-6,

***Atadenovirus***

*Guinea pig mastadenovirus A*

Guinea pig-1,

murine-1, *Murine mastadenovirus A*

California sea lion-1, *Sea lion mastadenovirus A*

tree shrew-1, *Tree shrew mastadenovirus A*

bottlenose dolphin-1, *Dolphin mastadenovirus B*

bovine-3, *Bovine mastadenovirus B*

canine-1, *Canine mastadenovirus A*

bat-2, *Bat mastadenovirus B*

equine-1, *Equine mastadenovirus A*

simian-1, *Human mastadenovirus G*

human-2, *Human mastadenovirus C*

***Mastadenovirus***

100

94

54

97

83

97

100

89

99

96

74

86

99

98

97

92

99

100

56

92

100

86

83

86

84

100

100

90

100

76

84

98

97

98

0.2

54

**Fig 2.** Midpoint rooted phylogenetic tree of AdVs based on maximum likelihood analysis of full length penton base amino acid sequences from AdVs one from each species and candidate species, or distinct strain with full *pol* sequence known (except three strains belonging to proposed species *Psittacine siadenovirus D*). Multiple alignment: T-Coffee Server; model selection: ProtTest 3.4.2; maximum likelihood calculation and statistical test: PhyML 3.0 with model LG+I+G+F and Shimodaira-Hasegawa-like approximate likelihood-ratio test for branches on the ATGC bioinformatics platform. Species names (if any) are shown after the virus names. Genus names are noted and the branches belonging to special genera are shown by different colours. Red letters sign the proposed novel species and genus. The word ′adenovirus/AdV′ was removed from the names of the AdV types for clarity. The bar indicates 20% difference between two neighbouring sequences. SH (Shimodaira-Hasegawa) branch support values are shown at the nodes.

***Ichtadenovirus***

white sturgeon-1, *Sturgeon ichtadenovirus A*

eastern box turtle

red-footed tortoise-1

red-eared slider-1, *Pond slider testadenovirus A* white sturgeon-1, *Sturgeon ichtadenovirus A*

***Testadenovirus*** red-eared slider-1, *Pond slider testadenovirus A*

frog-1, *Frog siadenovirus A* frog-1, *Frog siadenovirus A*

great-tit-1, *Great tit siadenovirus A* great-tit-1, *Great tit siadenovirus A*

chinstrap penguin-2, *Penguin siadenovirus A* chinstrap penguin-2, *Penguin siadenovirus A*

turkey-3, *Turkey siadenovirus A* turkey-3, *Turkey siadenovirus A*

raptor-1, *Raptor siadenovirus A*

South Polar skua-1, *Skua siadenovirus A*

***Siadenovirus*** psittacine-6 (budgerigar, strain BrdKdnyDNA)

lizard-2, *Lizard atadenovirus A* psittacine-5 (Pacific parrotlet, sun parakeet)

snake-1, *Snake atadenovirus A*

bearded dragon-1, *Lizard atadenovirus B*

duck-1, *Duck atadenovirus A*

psittacine-3 (Southern mealy amazon), *Psittacine atadenovirus A*

ovine-7, *Ovine atadenovirus D* goose-4, *Goose aviadenovirus A*

bovine-6, *Bovine atadenovirus E* duck-2, *Duck aviadenovirus B*

deer-1, *Deer atadenovirus A*

***Atadenovirus***

goose-4, *Goose aviadenovirus A*

duck-2, *Duck aviadenovirus B* turkey-4, *Turkey aviadenovirus C*

pigeon-2, *Pigeon aviadenovirus B* fowl-5, *Fowl aviadenovirus B*

pigeon-1, *Pigeon aviadenovirus A*

fowl-1, *Fowl aviadenovirus A*

turkey-1, *Turkey aviadenovirus B*

fowl-5, *Fowl aviadenovirus B*

turkey-4, *Turkey aviadenovirus C*

fowl-6, *Fowl aviadenovirus E*

***Aviadenovirus***

murine-1, *Murine mastadenovirus A*

bottlenose dolphin-1, *Dolphin mastadenovirus B*

simian-1, *Human mastadenovirus GBovine atadenovirus E*

human-2, *Human mastadenovirus C*

bovine-3, *Bovine mastadenovirus B*

tree shrew-1, *Tree shrew mastadenovirus A****Atadenovirus***

California sea lion-1, *Sea lion mastadenovirus A* Guinea pig-1,

equine-1, *Equine mastadenovirus A* murine-1, *Murine mastadenovirus A*

bat-2, *Bat mastadenovirus B* California sea lion-1, *Sea lion mastadenovirus A*

canine-1, *Canine mastadenovirus A*  tree shrew-1, *Tree shrew mastadenovirus A*

***Mastadenovirus*** canine-1, *Canine mastadenovirus A*

24 equine-1, *Equine mastadenovirus A*

99

48

94

45 simian-1, *Human mastadenovirus G*

70

97 human-2, *Human mastadenovirus C*

83

73

86

91

96

76***Mastadenovirus***

99

75

84

93

85100

8094

9354

7297

6883

9897

9189

8699

8896

9174

9486

9998

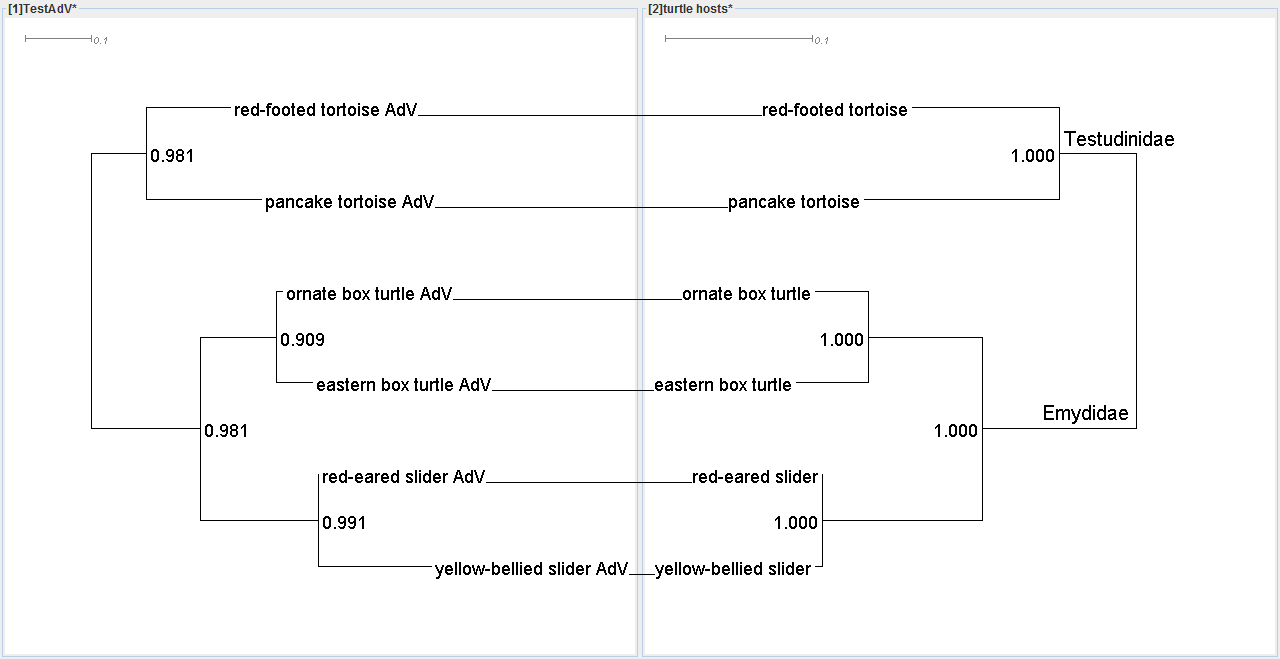
7997

9892

0.192

98

**Fig 3.** Midpoint rooted phylogenetic tree of AdVs based on maximum likelihood analysis of partial hexon amino acid sequences (137 aa). Multiple alignment: T-Coffee Server; model selection: ProtTest 3.4.2; maximum likelihood calculation and statistical test: PhyML 3.0 with model LG+I+G and Shimodaira-Hasegawa-like approximate likelihood-ratio test for branches on the ATGC bioinformatics platform. Species names (if any) are shown after the virus names. Genus names are noted and the branches belonging to specific genera are shown by different colours. Red letters designate the proposed testadenovirus species and genus. The word ′adenovirus/AdV′ was removed from the names of the AdV types for clarity. The bar indicates 10% difference between two neighbouring sequences. SH (Shimodaira-Hasegawa) branch support values are shown at the nodes.

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**Fig. 4.** Comparison of the phylogenetic trees of testadenoviruses (left) and turtle hosts (right) Midpoint-rooted phylogenetic tree reconstructions of testadenoviruses calculated on 92 amino acid-long alignment of DNA-dependent DNA polymerases (maximum likelihood method, JTT+I model) and turtle hosts calculated on 1086 nucleotide-long multiple alignment of the mitochondrial cytochrome b genes (maximum likelihood method, GTR+G model). The reliability of the nodes was tested using the Shimodaira–Hasegawa-like approximate likelihood-ratio test. The scale bars above the trees indicate the number of amino acid and nucleotide substitutions per site.



**Fig. 5.** Protease cleavage signals (type I, II, IIb and III) in pVII. The actual cleavage site is after the fourth aa. The cleavage signal of red-eared slider AdV-1 (type IIb and type I) are similar to those of the phylogenetically closely related siadenoviruses, but still distinct.



**Fig. 6.** The second protease cleavage signals (type I and II) in pVI (the exact cleavage site is indicated by arrows). Only the red-eared slider AdV-1 (slider-1) has a type I protease cleavage signal (M/I/L)XGG-X). The length of pVIc cofactors (designated by red or green capitals) are 10 aa (slider-1) or 11 aa (members of all other genera). The exemplars of the new candidate species in this proposal are designated by asterisks.

**TPnonmast_mat.emf**

**Fig. 7.** Pairwise identity of the DNA-dependent DNA polymerase amino acid sequences of the non-mastadenoviruses representing the official AdV species and further unclassified strains. Sequence Demarcation Tool ver. 1.2 (13), Muscle alignment. The first cut-off value is 90% identity, the second is 46%. Red-eared slider AdV-1 (slider\_A) shows less than 46% identity (42%) to the phylogenetically most closely related white sturgeon AdV-1 (sturgeon\_A; *Sturgeon ichtadenovirus A*) and all other known AdVs. Similarly, members of the established genera show less than 46% identity to any members of the other genera. The unclassified white-eyed parakeet AdV (whe\_parak) shows 90% identity with psittacine AdV-3 (psittaci3A; *Psittacine siadenovirus A*) and thus most probably should be classified into this species. Psittacine AdV-5 from Pacific parrotlet and also from sun parakeet, furthermore psittacine AdV-6 from budgerigar, show more than 95-99% identity and thus belong to the same novel proposed species *Psittacine siadenovirus D*.

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**Fig. 8.** Pairwise identity of the DNA-dependent DNA polymerase amino acid sequences of mastadenoviruses (except primate AdVs) representing different AdV species or unclassified strains. All of them, including guinea pig AdV-1, show more than 15% difference, which is the species demarcation criterion. Guinea pig AdV-1 shows only 54% identity with porcine AdV-3, the most closely related AdV.

**References**

1. Archer GA, Phillips CA, Adamovicz L, Band M, Byrd J, Allender MC (2017) Detection of copathogens in free-ranging Eastern box turtles (*Terrapene carolina carolina*) in Illinois and Tennessee. J Zoo Wildl Med 48: 1127-1134 PMID 29297797 doi 10.1638/2017-0148R.1
2. Brown LC, Krause KJ, Reavill DR, Childress AL, Wellehan JFX Jr (2016) Characterization of a novel testadenovirus in red-footed tortoises (*Chelonoidis carbonaria*). GenBank accession No. KU601299
3. Cassmann E, Zaffarano B, Chen Q, Li G, Haynes J (2019) Novel siadenovirus infection in a cockatiel with chronic liver disease. Virus Res 263: 164-168 PMID **30711577 doi** 10.1016/j.virusres.2019.01.018
4. Doszpoly A, Wellehan JFX, Childress AL, Tarján ZL, Kovács ER, Harrach B, Benkő M (2013) Partial characterization of a new adenovirus lineage discovered in testudinoid turtles. Infect Genet Evol 17: 106-112 PMID 23567817, doi 10.1016/j.meegid.2013.03.049
5. Erdei N, Szathmáry R, Harrach B, Benkő M (2012) Genome analysis of bovine adenovirus 6 reveals a need to establish a new species: *Bovine adenovirus E*. GenBank accession No. JQ345700
6. Farkas SL, Gál J (2009) Adenovirus and mycoplasma infection in an ornate box turtle (*Terrapene ornata ornata*) in Hungary. Vet Microbiol 138: 169-173 PMID 19375875 doi 10.1016/j.vetmic.2009.03.016
7. Franzen-Klein D, Adamovicz L, McRuer D, Carroll SA, Wellehan JFX, Allender MC (2020) Prevalence of box turtle adenovirus in eastern box turtles (*Terrapene carolina carolina*) presented to a wildlife rehabilitation center in Virginia, USA. J Zoo Wildl Med 50: 769-777 PMID 31926506 doi 10.1638/2018-0238
8. Graham DA, Calvert V, Benkő M, Curran W, Wylie M, Snodden DA, Moffet DA, Papp T, Adair BM, Smyth JA (2005) Isolation of bovine adenovirus serotype 6 from a calf in the United Kingdom. Vet Rec 156: 82-86 PMID 15689037 doi 10.1136/vr.156.3.82
9. Gregory CR, Nilsen RA, Linn SC, Hokamp JA, Cianciolo RE, Ritchie BW (2019) Histologic, ultrastructural, and complete genome sequence of a siadenovirus in a Pacific parrotlet (*Forpus coelestis*). GenBankaccession No. MK695679
10. Harrach B, Tarján ZL, Benkő M (2019) Adenoviruses across the animal kingdom: a walk in the zoo. FEBS Letters 593: 3660-3673 PMID **31747467 doi** 10.1002/1873-3468.13687
11. Hofmann-Sieber H, Gonzalez G, Spohn M, Dobner T, Kajon AE (2020) Genomic and phylogenetic analysis of two guinea pig adenovirus strains recovered from archival lung tissue. Virus Res 285: 197965 PMID 32311385 doi 10.1016/j.virusres.2020.197965
12. Lehmkuhl HD, Hobbs LA (2008) Serologic and hexon phylogenetic analysis of ruminant adenoviruses. Arch Virol 153: 891-897 PMID 18340400 doi 10.1007/s00705-008-0063-4
13. Muhire B, Martin DP, Brown JK, Navas-Castillo J, Moriones E, ZerbiniFM, Rivera-Bustamante R, Malathi VG, Briddon RW, Varsani A (2013) A genome-wide pairwise-identity-based proposal for the classification of viruses in the genus *Mastrevirus* (family *Geminiviridae*). Arch Virol 158: 1411–1424 PMID **23340592 doi** 10.1007/s00705-012-1601-7
14. Pénzes J, Szirovicza L, Harrach B (2020) The complete genome sequence of bearded dragon adenovirus 1 harbors three genes encoding proteins of the C-type lectin-like domain superfamily. Infect Genet Evol 83: 104321 PMID 32302697 doi 10.1016/j.meegid.2020.104321
15. Phalen DN, Agius J, Vaz FF, Eden JS, Setyo LC, Donahoe S (2019) A survey of a mixed species aviary provides new insights into the pathogenicity, diversity, evolution, host range, and distribution of psittacine and passerine adenoviruses. Avian Pathol 13: 1-25 31081348
16. Pring-Akerblom P, Blazek K, Schramlová J, Kunstýr I (1997) Polymerase chain reaction for detection of guinea pig adenovirus. J Vet Diagn Invest 9: 232-236 PMID 9249160 doi 10.1177/104063879700900302
17. Rondhuis PR (1968) A new bovine adenovirus. Arch Gesamte Virusforsch 25: 235-236 PMID 5709347 doi 10.1007/BF01258169
18. Sutherland M, Sarker S, Vaz PK, Legione AR, Devlin JM, Macwhirter PL, Whiteley PL, Raidal SR (2019) Disease surveillance in wild Victorian cacatuids reveals co-infection with multiple agents and detection of novel avian viruses. Vet Microbiol 235: 257-264 31383310 doi: 10.1016/j.vetmic.2019.07.012
19. [Tarján ZL](https://m2.mtmt.hu/gui2/?type=authors&mode=browse&sel=10039539), [Doszpoly A](https://m2.mtmt.hu/gui2/?type=authors&mode=browse&sel=10014087) (2019) Diversity of adenoviruses occurring in turtles. (in Hungarian) Magy Allatorvosok 141: 747-757
20. Walker PJ, Siddell SG, Lefkowitz EJ, Mushegian AR, Adriaenssens EM, Dempsey DM, Dutilh BE, Harrach B, Harrison RL, Hendrickson RC, Junglen S, Knowles NJ, Kropinski AM, Krupovic M, Kuhn JH, Nibert M, Rubino L, Sabanadzovic S, Simmonds P, Varsani A, ZerbiniFM, Davison AJ (2020) Changes to virus taxonomy and the Statutes ratified by the International Committee on Taxonomy of Viruses (2020). Arch Virol (online ahead of print) PMID 32816125 doi: [10.1007/s00705-020-04752-x](https://doi.org/10.1007/s00705-020-04752-x)
21. Wellehan JFX, Johnson AJ, Harrach B, Benkő M, Pessier AP, Johnson CM, Garner MM, Childress A, Jacobson ER (2004) Detection and analysis of six lizard adenoviruses by consensus primer PCR provides further evidence of a reptilian origin for the atadenoviruses. J Virol 78, 13366-13369 PMID 15542689 doi 10.1128/JVI.78.23.13366-13369.2004