Dadáková, E, Brožová, K, Piel, AK, Stewart, FA, Modrý, D, Celer, V and Hrazdilová, K

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Adenovirus infection in savanna chimpanzees (*Pan troglodytes schweinfurthii*) in the Issa Valley, Tanzania

Dadáková Eva[1], Brožová Kristýna[1], Piel Alex K.[2,3], Stewart Fiona A.[2,3], Modrý David[4,5,6], Celer Vladimír[1,6], Hrazdilová Kristýna[6,7]

[1] Department of Infectious Diseases and Microbiology, University of Veterinary and Pharmaceutical Sciences Brno, Palackého tř. 1946/1, 612 42 Brno, Czech Republic

[2] School of Natural Sciences and Psychology, Byrum Street, Liverpool John Moores University, Liverpool, UK L33AF

[3] Ugalla Primate Project

[4] Department of Pathological Morphology and Parasitology, University of Veterinary and Pharmaceutical Sciences Brno, Palackého tř. 1946/1, 612 42 Brno, Czech Republic

[5] Biology Centre, Institute of Parasitology, Czech Academy of Sciences, Branišovská 31, 370 05, České Budějovice, Czech Republic

[6] CEITEC-VFU, University of Veterinary and Pharmaceutical Sciences Brno, Palackého tř. 1946/1, 612 42 Brno, Czech Republic

[7] Department of Virology, Veterinary Research Institute, Hudcova 296/70, 621 00 Brno, Czech Republic

Corresponding author: Kristýna Hrazdilová, kristyna@hrazdilova.cz, +420 541 562 271, +420 604 214 439

Abstract

Adenoviruses are a widespread cause of diverse human infections with recently confirmed zoonotic roots in African great apes. We focused on savanna-dwelling chimpanzees in the Issa Valley (Tanzania), which differ from those from forested sites in many aspects of behavior and ecology. DNA polymerase gene targeting PCR detected AdV in 36,7% (69/188) of fecal samples. We detected five groups of strains within the HAdV-E and two distinct groups within the HAdV-C species based on partial hexon sequence. All detected AdVs from the Issa Valley are related to those from nearby Mahale and Gombe National Parks suggesting chimpanzee movements and pathogen transmission.

More than 50 years ago Jane Goodall started her chimpanzee research in Gombe, Tanzania. Her behavioral study triggered an interest in the Eastern chimpanzee (*Pan troglodytes schweinfurthii*) [1], later expanding to include investigation into zoonotic diseases. More recently, much work has been conducted on pathogen prevalence and transmission in free-ranging great apes, stimulated also by the range of important zoonotic viral infections, like simian immunodeficiency virus [2, 3], simian foamy virus [4], simian T-lymphotropic virus type I [5], and ebola [6]. The human adenoviruses (AdV) have zoonotic roots as well, originating from at least four independent transmission events from African great apes [7] .

Adenoviruses are non-enveloped icosahedral dsDNA viruses; all simian and human AdVs belong to the genus *Mastadenovirus* (family *Adenoviridae*), including seven species of Human mastadenovirus (HAdV-A to -G), and in the 2016 release of the International Committee on Taxonomy of Viruses (ICTV) taxonomy, also eight species of Simian mastadenovirus (SAdV-A to -H), and many unassigned species. Recently, several other new AdV species from primate hosts were proposed [8–10], however, these have not yet been adopted by the ICTV. In wild chimpanzees HAdV-A to -F and SAdV-A have been identified [11], but all available whole genome sequences originate from cell cultures or captive animals [12, 13]. Frequent shedding of AdV in feces [12] allows non-invasive studies on AdVs in wild animals in their natural habitat, allowing scientists to assess the diversity of circulating strains, their phylogenetic relationships and evolution [7, 11].

Adenoviral infections are reported from three out of four chimpanzee subspecies (Figure 1); no data from the Nigeria-Cameroon chimpanzee *P. t. ellioti* are available. Chimpanzees were recognized as the ancestral host of the HAdV-E species and of one clade within HAdV-C species [7], whilst HAdV-A to -G and SAdV-A were sporadically detected as a result of rare cross-transmission events (Figure 1). Savanna-mosaic dwelling chimpanzees differ from those from forested sites in many aspects of behavior, diet and ranging, and hence in the pattern, rate, and level of social interactions [14]. Such differences may consequently affect the spectrum and diversity of pathogens circulating in the community [15]. Adaptation of chimpanzees to arid environments may reveal adaptations of early hominids, which evolved in a similar type of habitat [16]. All the AdV studies to date have been conducted in forest-dwelling chimpanzees. Today, research on savanna-mosaic chimpanzees continues at three sites: Semliki (Uganda), Fongoli (Senegal) and Issa Valley (Tanzania) [16–18], however, none of these populations has been investigated for the diversity of AdVs.

In the present study, 188 fecal samples from nonhabituated eastern chimpanzees (*P. troglodytes schweinfurthii*) inhabiting the Issa Valley, western Tanzania were collected during 2012 and 2013. As chimpanzees were unhabituated at the time, researchers collected fecal samples from opportunistic encounters with chimpanzees, and from under fresh chimpanzee nests built the previous night. The entire region is one of the driest and most open chimpanzee habitats, with an altitudinal range of 900– 1800 m above sea level. The habitat is dominated by savanna (Miombo) woodland, characterized by *Brachystegia* and *Julbernardia* trees [18]. The population density of Issa chimpanzees is estimated to be ~0.25 individuals/km^2 [19]. All fecal samples (10 – 20 g) were preserved in equal volume of RNAlater (Sigma-Aldrich, USA), stored at -20 °C on site and subsequently shipped to the Czech Republic, where they were kept at -20/-80 °C until DNA extraction by PSP Spin Stool DNA Kit (Stratec, Germany). Adenovirus-positive samples were detected by nested PCR targeting conserved DNA polymerase (DPOL) gene. Consequently, 1800 nt long fragments of hexon gene (spanning all 7 hypervariable regions) were amplified from selected samples, primers used are listed in Online Resource [9]. All adenoviral sequences were deposited to GenBank (accession numbers MF176075-MF176106 for DPOL and MF176107-MF176134 for hexon gene). Herein used names for the candidate AdVs follows Wevers et al. [11] using abbreviations derived from the host species and continuous numbering. The nucleotide dataset including all available respective sequences from chimpanzees and representatives from other hosts was aligned and guided by CLUSTALW amino acid alignment [20]; poorly aligned regions were eliminated by Gblocks [21]. We compared the hexon gene nucleotide sequences to those from 213 isolates available in GenBank (see Online Resource) by Maximum Likelihood method (ML) using PhyML [22] and the best evolution model (GTR+G+I) was chosen based on likelihood ratio test computed in R [23].

Based on DPOL targeting PCR, AdV DNA was detected in 36,7 % (69/188) of the screened samples. As the Issa chimpanzees were not habituated at the time of sample collection, it was not possible to attribute the fecal samples to specific individuals. Detection rate of AdVs in our sample set is generally lower than the published prevalence in chimpanzees from Democratic Republic of Congo (42 %), Cameroon (38 %), and Republic of Congo (69,6 %) in *P.t.troglodytes* or 51 % in *P. troglodytes* spp. sampled across Africa [7, 12, 24].

To survey circulating AdV strains among the Issa chimpanzees, we sequenced 33 random samples resulting in at least one DPOL and/or hexon partial sequence. Preliminary BLAST analysis revealed the presence of HAdV-C and HAdV-E. Co-infection with different HAdV species or strains was observed in six samples based on both DPOL and hexon sequences, but DPOL sequences were used for AdV interspecies co-infection identification only. In five samples we detected co-infection of HAdV-C and HAdV-E and one sample was infected by two distinct HAdV-E strains (Figure 2). AdV coinfection rate in wild chimpanzees has been described in detail only for central chimpanzees from Odzala-Kokoua National Park (Republic of Congo) on a very limited number of individuals (16 AdV positive from 23 tested), where six of 16 infected individuals (37,5 %) carried more than one AdV species [24]. In our dataset, we detected six coinfections in 33 sequenced samples (18,2 %). However, presented rates of AdV co-infections are probably underestimated due to the restraints of PCR product cloning approach. The AdV diversity and common multiple infections are reflected also in frequent recombination events reported in AdVs [25].

The highly conserved sequence of a DPOL gene ensured the detection of AdVs, but did not allow the proper differentiation of AdVs allocated to the same species. Thus, a hexon based phylogenetic analysis was performed (Figure 2). The overall topology of a DPOL-derived tree (data not shown) was in accordance with the hexon tree.

In Issa chimpanzees, we detected five new groups of strains clustering within the HAdV-E. These AdV species have been described in chimpanzees, bonobos (P. paniscus) and humans [7, 11, 24, 26] and chimpanzees are considered be the ancestral host [7]. Three of our HAdV-E groups, namely PtroAdV 17-19, evince 93-96% pairwise hexon nucleotide sequence identity to different sequences acquired from captive chimpanzees (Table). Relatedness of sequences from wild and captive chimpanzees confirms the persistence of chimpazee AdV strains in captivity. The only identified HAdV-E sequence to date was contracted via horizontal transmission - from chimpanzee to human, not the opposite [7]. The last two groups of HAdV-E isolates (PtroAdV-21 and -22) clusters closely to previously described AdV strains from *P. t. schweinfurthii* from Uganda (Table, Figure 2).

Two distinct groups of our strains were identified within the HAdV-C species. The first one, formed by our sequences PtroAdV-15.1 and -15.2, is closely related to PtroAdV-9 from *P. t. schweinfurthii*. The second group of our strains, PtroAdV-16.1 to -16.4, clusters with PtroAdv-6 (*P. t. schweinfurthii*), and with two sequences from captive chimpanzees (SAdV-31.1 and -31.2). As the closest relatives of Issa AdV HAdV-C strains have already been detected in eastern chimpanzees (PtroAdV-6 and PtroAdV-9, both from Ngamba Island, Uganda), we can confirm wide distribution of this subclade among the *P. t. schweinfurthii* in East Africa. HAdV-C of human, gorilla, chimpanzee and bonobo cluster in clearly distinct clades (Figure 2) with topology reflecting the co-evolutionary processes during hominine evolution [7], which suggests strict host specificity of these viruses.

The third most abundant AdV species detected in chimpanzees (Figure 1), HAdV-B, was not detected at Issa. We also did not detect any HAdV-A, -D, -F or SAdV-A strains in the Issa population. HAdV-D seems to be exclusively limited to human hosts. The only HAdV-D sequence from *P. t. schweinfurthii* (Uganda) is likely the result of an isolated human to chimpanzee cross-species transmission event [11]. HAdV-A and -F were reported from *P. t. verus* and *P. t. schweinfurthii* only in few sporadic cases [7, 11].

Figure 1 shows all sequences of AdV strains reported from wild chimpanzees and bonobos. In most of the research sites HAdV-B, -C and -E have been identified. Issa chimpanzees host several strains of HAdV-C and HAdV-E, which is in accordance with report from Mahale Mountains National Park, Tanzania [26]. Unfortunately, sequences of Mahale isolates are not available in any public database, but based on published phylogenetic trees and corresponding relationships, we can assume that the Mahale isolate clone_327 is closely related to the PtroAdV-21 isolate. Strain PtroAdV-1 identified in another Tanzania locality, Gombe [11], clusters

closely to our PtroAdV-22 strains. These findings suggest the circulation of related strains not only in the Greater Mahale Ecosystem (including Issa and Mahale), but even in the Greater Gombe Ecosystem. Despite geographical isolation of Tanzania chimpanzees confirmed by their lower genetic diversity [27], relatedness of AdV strains from Issa to those from Gombe and Mahale localities confirms that the Malagarasi river is not an absolute barrier to chimpanzee movements and pathogen transmission [28].

The absence of strains of presumptive human origin, together with the fact that human AdV strains are not reported even from habituated chimpanzee communities with much higher contact with humans, suggests high host specificity of these viruses even in phylogenetically closely related hosts.

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Compliance with Ethical Standards

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Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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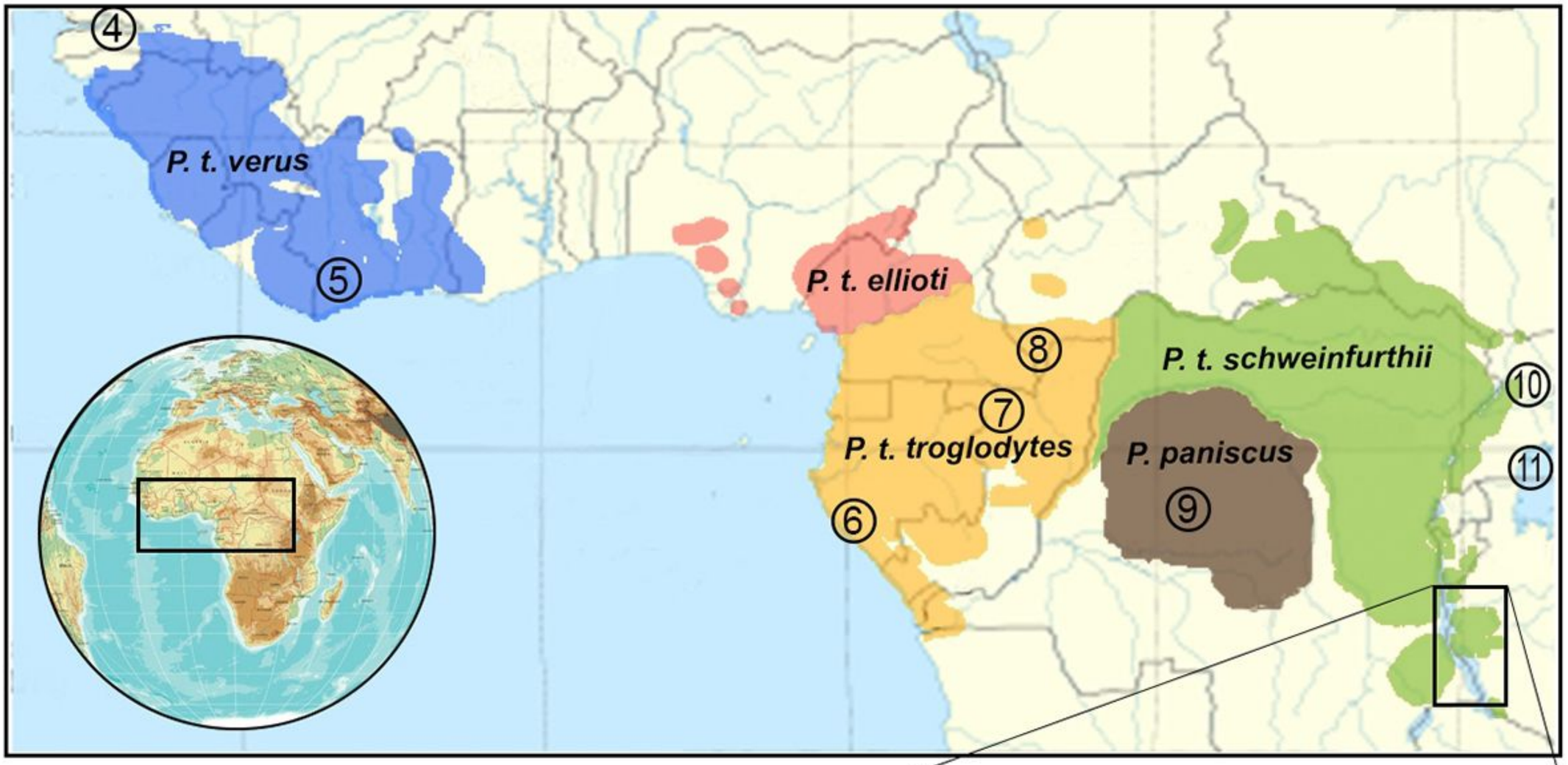
Fig.1 Distribution map of wild chimpanzee AdV species. Localities of all so far published AdV species are numbered. Herein described study site, Ugalla (Tanzania), is highlighted in red. Host range is displayed in colour: bonobos (*P. paniscus*) in brown, and chimpanzee subspecies (*P. t. verus, ellioti, troglodytes, schweinfurthii*) in blue, red, orange and green.

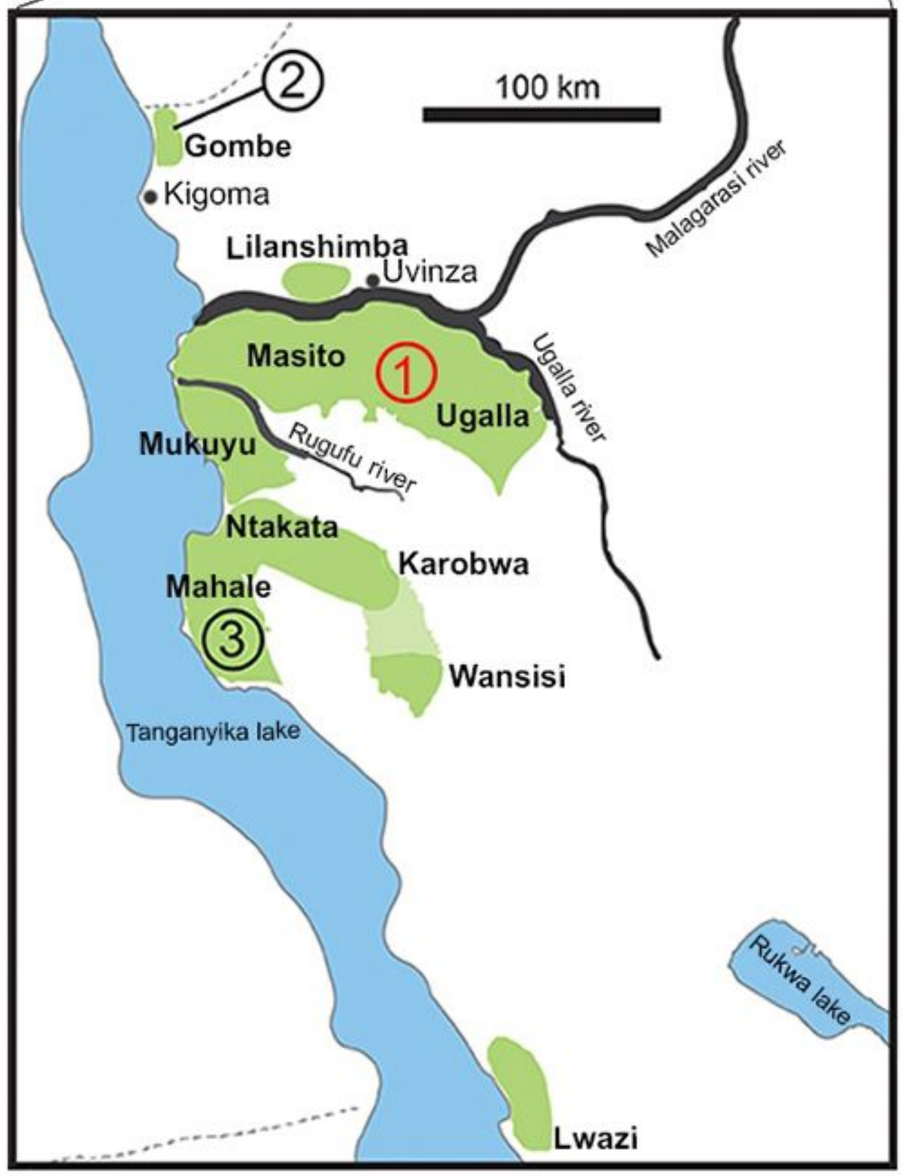
Fig.2 A maximum likelihood phylogenetic tree based on partial nucleotide sequences of hexon gene. (a) Overall topology of the tree of all AdV species (b) detailed view of HAdV-E species clade (c) detailed view of HAdV-C species clade. Node-supports refer to bootstrap values (figures above 50% from 1000 displayed only). AdVs described in this study are marked with red branches and their names and abbreviations refer to the host species and strain. Five pairs of symbols indicate co-infection detected from one sample. The origin of already published AdVs addresses to the localities marked in the Figure 1 or to the samples from Democratic Republic of Congo (DRC), captive animals (ZOO), research centers (RC) or cell cultures (CC); three Atadenovirus sequences used as an outgroup and all non-primate Mastadenovirus sequences are not displayed (for details see Online Resource).

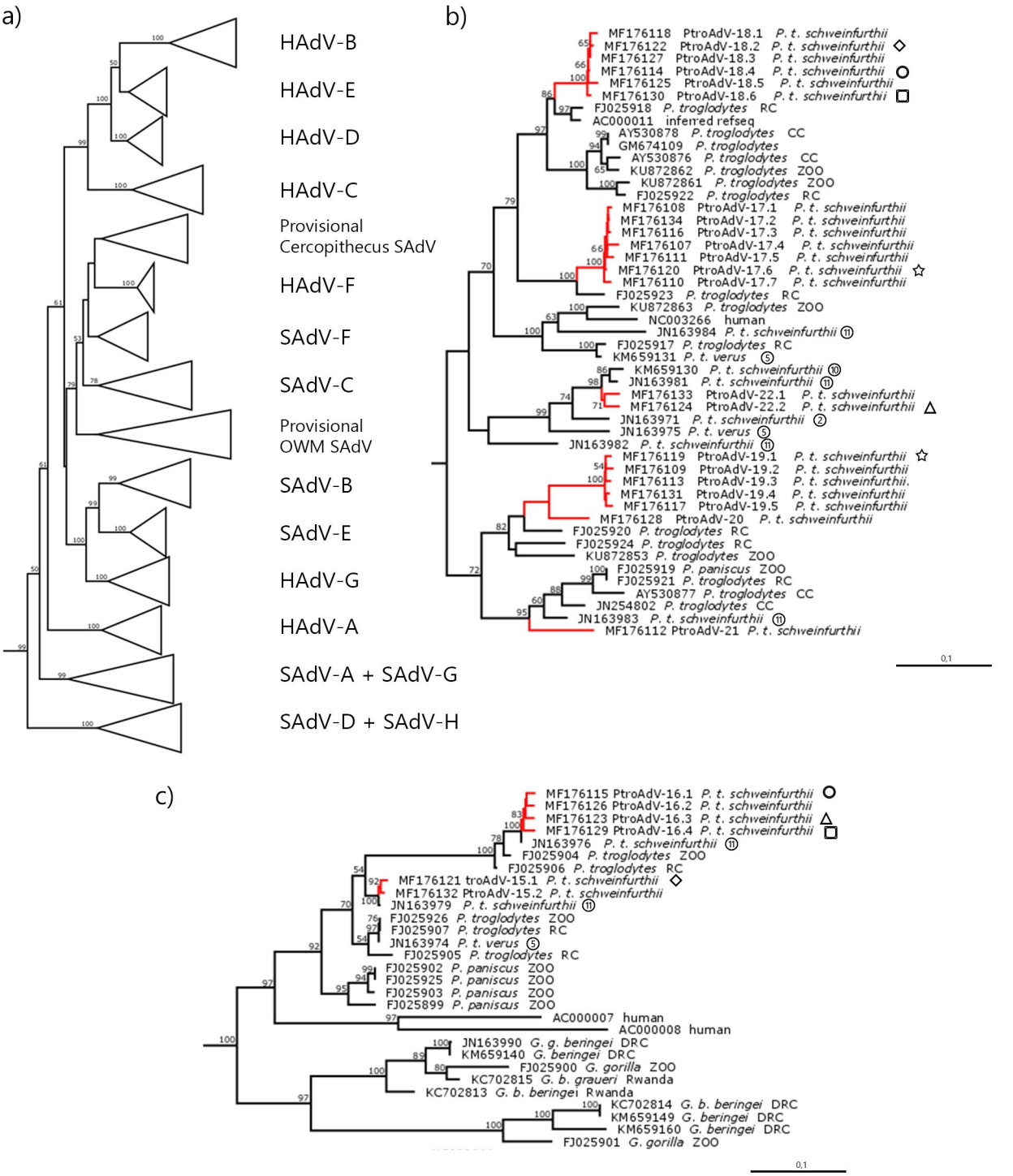
Table The closest related sequences (based on ML tree) for herein described AdV strains and their origin. P-dist refers to pairwise hexon nucleotide sequence identity

		p-dist	NCBI seq	Host
HAdV-E	PtroAdV-17.1 – 17.7	95%	FJ025923 Simian adenovirus 26	Captive *P.troglodytes*
	PtroAdV-18.1 – 18.6	96%	FJ025918 Simian adenovirus 25.2	Captive *P.troglodytes*
	PtroAdV-19.1 – 19.5 PtroAdV-20	93%	FJ025920 Simian adenovirus 30	Captive *P.troglodytes*
	PtroAdV-21	92%	JN163983 PtroAdV-13	Uganda *P.t.schweinfurthii*
	PtroAdV-22.1 - 22.2	98%	JN163981 PtroAdV-11	Uganda *P.t.schweinfurthii*
HAdV-C	PtroAdV-15.1 - 15.2	99%	JN163979 PtroAdV-9	Uganda *P.t.schweinfurthii*
	PtroAdV-16.1 - 16.4	99%	JN163976 PtroAdV-6	Uganda *P.t.schweinfurthii*



	Localities	HAdV A	HAdV B	HAdV C	HAdV D	HAdV E	HAdV F	SAdV A	Ref.
1	Ugalla Tanzania			■		■			
2	Gombe NP Tanzania					■			[11]
3	Mahale NP Tanzania			■		■			[26]
4	River Gambia NP Gambia						■		[11]
5	Taï NP Ivory Coast	■	■	■		■			[11, 7]
6	Loango NP Gabon		■	■		■			[11, 7]
7	Odzala - Kokoua NP Republic of Congo		■	■		■			[24]
8	Dzanga - Sangha PA Central African Republic					■			[7]
9	Salonga NP Democratic republic of Congo			■		■			[11,7]
10	Budongo CFS Uganda		■	■		■	■		[7]
11	Ngamba Island ChS Uganda	■	■	■	■	■		■	[11]




a)
HAdV-B
HAdV-E
HAdV-D
HAdV-C
Provisional
Cercopithecus SAdV
HAdV-F
SAdV-F
SAdV-C
Provisional
OWM SAdV
SAdV-B
SAdV-E
HAdV-G
HAdV-A
SAdV-A + SAdV-G
SAdV-D + SAdV-H
b)
MF176118 PtroAdV-18.1 P. t. schweinfurthii
MF176122 PtroAdV-18.2 P. t. schweinfurthii
MF176127 PtroAdV-18.3 P. t. schweinfurthii
MF176114 PtroAdV-18.4 P. t. schweinfurthii
MF176125 PtroAdV-18.5 P. t. schweinfurthii
MF176130 PtroAdV-18.6 P. t. schweinfurthii
FJ025918 P. troglodytes RC
AC000011 infered refseq
AY530878 P. troglodytes CC
GM674109 P. troglodytes
AY530876 P. troglodytes CC
KU872862 P. troglodytes ZOO
KU872861 P. troglodytes ZOO
FJ025922 P. troglodytes RC
MF176108 PtroAdV-17.1 P. t. schweinfurthii
MF176134 PtroAdV-17.2 P. t. schweinfurthii
MF176116 PtroAdV-17.3 P. t. schweinfurthii
MF176107 PtroAdV-17.4 P. t. schweinfurthii
MF176111 PtroAdV-17.5 P. t. schweinfurthii
MF176120 PtroAdV-17.6 P. t. schweinfurthii
MF176110 PtroAdV-17.7 P. t. schweinfurthii
FJ025923 P. troglodytes RC
KU872863 P. troglodytes ZOO
NC003266 human
JN163984 P. t. schweinfurthii (11)
FJ025917 P. troglodytes RC
KM659131 P. t. verus (5)
KM659130 P. t. schweinfurthii (10)
JN163981 P. t. schweinfurthii (11)
MF176133 PtroAdV-22.1 P. t. schweinfurthii
MF176124 PtroAdV-22.2 P. t. schweinfurthii
JN163971 P. t. schweinfurthii (2)
JN163975 P. t. verus (5)
JN163982 P. t. schweinfurthii (11)
MF176119 PtroAdV-19.1 P. t. schweinfurthii
MF176109 PtroAdV-19.2 P. t. schweinfurthii
MF176113 PtroAdV-19.3 P. t. schweinfurthii.
MF176131 PtroAdV-19.4 P. t. schweinfurthii
MF176117 PtroAdV-19.5 P. t. schweinfurthii
MF176128 PtroAdV-20 P. t. schweinfurthii
FJ025920 P. troglodytes RC
FJ025924 P. troglodytes RC
KU872853 P. troglodytes ZOO
FJ025919 P. paniscus ZOO
FJ025921 P. troglodytes RC
AY530877 P. troglodytes CC
JN254802 P. troglodytes CC
JN163983 P. t. schweinfurthii (11)
MF176112 PtroAdV-21 P. t. schweinfurthii
0,1
c)
MF176115 PtroAdV-16.1 P. t. schweinfurthii
MF176126 PtroAdV-16.2 P. t. schweinfurthii
MF176123 PtroAdV-16.3 P. t. schweinfurthii
MF176129 PtroAdV-16.4 P. t. schweinfurthii
JN163976 P. t. schweinfurthii (11)
FJ025904 P. troglodytes ZOO
FJ025906 P. troglodytes RC
MF176121 troAdV-15.1 P. t. schweinfurthii
MF176132 PtroAdV-15.2 P. t. schweinfurthii
JN163979 P. t. schweinfurthii (11)
FJ025926 P. troglodytes ZOO
FJ025907 P. troglodytes RC
JN163974 P. t. verus (5)
FJ025905 P. troglodytes RC
FJ025902 P. paniscus ZOO
FJ025925 P. paniscus ZOO
FJ025903 P. paniscus ZOO
FJ025899 P. paniscus ZOO
AC000007 human
AC000008 human
JN163990 G. g. beringei DRC
KM659140 G. beringei DRC
FJ025900 G. gorilla ZOO
KC702815 G. b. graueri Rwanda
KC702813 G. b. beringei Rwanda
KC702814 G. b. beringei DRC
KM659149 G. beringei DRC
KM659160 G. beringei DRC
FJ025901 G. gorilla ZOO
0,1

Primers used for amplification of DPOL and hexon gene sequences

Targeted gene	Primer	Sequence 5'-3'	Product length
DPOL	SAdVpol-F1	AGGCTGTCBGTGTCNCCGTA	
	SAdVpol-R1	GTCTAYAAYATCTGTGGCATGTATGC	998 bp
	SAdVpol-F2	GGCYAGCACAAANGAGGC	
	SAdVpol-R2	TCGVCTCTGCTGGACCAA	649 bp
Hexon	SAdVhex-F1	TACATGCACATCGCCGGRCAGG	
	SAdVhex1-R1	GGGTAVAGCATGTTRGCWGC	cca 1900 bp
	SAdVhex-F2	CAGGAYGCYTCGGAGTACCTGAG	
	SAdVhex1-R2	AGGTAGTCRTTRAAYGACTG	cca 1800 bp

Accession numbers of sequences used in phylogenetic analysis and hidden in collapsed clades in tree in Figure 2:

HAdV-A: GU191019, AM749299, NC_001460, JN163978

HAdV-B: AC_000010, KM659156, JN163977, KM659129, FJ025910, FJ025912, JN163986, JN163988, KM659132, JN163989, KM659157, FJ025911, FJ025916, AC_000019, HQ292614, KM659172, KU872854, FJ025915, FJ025914, JN163987, KU872860, FJ025913, FJ025927, AC_000018, FJ025908, FJ025929, KM659171, FJ025909, FJ025928, FJ025930, KM659161

HAdV-D: JN935766, AB448778, JN226747, JN226760, JN226762, AP012285, JN226746, JN226764, EF153474, EF153473, JN226763, AY875648, JN226757, AB605240, FJ169625, FJ619037, JN226751, AB448767, GQ384080, JN226759, DQ393829, HQ883276, JN226758, JF799911, JN226752, JN162672, JN226749, AB448774, JQ326208, JN226765, AB562587, DQ149628, HM770721, JN163980, JN226748, JN226761, FJ824826, JN226753, AJ854486, AP012302, JN226756, AB333801, NC_012959, JN162671, JN226750

HAdV-F: NC_001454, AB728839, JN163973, JN163985

HAdV-G: DQ923122, DQ792570, JN163993, JN163992, NC_006879, KF053130

SAdV-A: JN163972, JQ776547, NC_006144, HQ241818

SAdV-B: EU293065, KP329561, NC_015225, HQ241820, JN880452, JN880451, JN880453, KC693021

SAdV-C: KC693022, KC693024, KU872851, KU872855, KF053124, KP329562, NC_025828, KC693023, KP329565

SAdV-D: KP329563

SAdV-E: KP329564, JN163991, JN163995

SAdV-F: NC_022266, KU872856, KU872857, KU872864

SAdV-G: NC_020485

SAdV-H: NC_025678

Provisional *Cercopithecus* SAdV: KP274048, KU872858, KU872859, KU872852

Provisional OWM SAdV: JN163996, JN163999, KU872865, JN163997, JN163998, JN163994

Non-primate Mastadenoviruses: AC_000012, M81889, NC_012584, HM049560, NC_014899, AC_000189, AF083132, AF030154, JN381195, AC_000190, AF258784, NC_027705, AC_000003, EF559262, CAU77082, JN252129, NC_015932, NC_016895, GU226970, JN418926, AC_000191, DQ630761, DQ630754, AF289262, NC_002702, AF252854, DQ630758, DQ630755, AF282774

Atadenovirus outgroup: AF036092, AC_000004, U40839