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Research Article

Discovery of novel DNA viruses in small mammals from Kenya

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ABSTRACT

Emergence and re-emergence of infectious diseases of wildlife origin have led pre-emptive pathogen surveillances in animals to be a public health priority. Rodents and shrews are among the most numerically abundant vertebrate taxa and are known as natural hosts of important zoonotic viruses. Many surveillance programs focused more on RNA viruses. In comparison, much less is known about DNA viruses harbored by these small mammals. To fill this knowledge gap, tissue specimens of 232 animals including 226 rodents, five shrews and one hedgehog were collected from 5 counties in Kenya and tested for the presence of DNA viruses belonging to 7 viral families by PCR. Diverse DNA sequences of adenoviruses, adeno-associated viruses, herpesviruses and polyomaviruses were detected. Phylogenetic analyses revealed that most of these viruses showed distinction from previously described viruses and formed new clusters. Furthermore, this is the first report of the discovery and full-length genome characterization of a polyomavirus in *Lemniscomys* species. This novel polyomavirus, named LsPyV KY187, has less than 60% amino acid sequence identity to the most related *Glis glis* polyomavirus 1 and *Sciurus carolinensis* polyomavirus 1 in both large and small T-antigen proteins and thus can be putatively allocated to a novel species within *Betapolyomavirus*. Our findings help us better understand the genetic diversity of DNA viruses in rodent and shrew populations in Kenya and provide new insights into the evolution of those DNA viruses in their small mammal reservoirs. It demonstrates the necessity of ongoing pathogen discovery studies targeting rodent-borne viruses in East Africa.

1. Introduction

More than 60% of the human pathogens documented to date are of zoonotic origin, causing over 70% of emerging infectious diseases in humans (Jones et al., 2008; Woolhouse and Gowtage-Sequeria, 2005). Emerging viral pathogens of animal origin have been noted to present growing challenges to global public health in the past few decades (Wang and Crameri, 2014). With the increasing human-animal interactions, incidences of interspecies viral transmission between animals and humans are expected to rise. In the absence of effective vaccines and treatment against these emerging zoonotic viruses, threats to public health security and economic growth are also expected to heighten (Allen et al., 2017; Heymann et al., 2015). It is therefore crucial to conduct virus surveillance among potential wildlife hosts in order to identify potential pathogens before they spill over to humans. Early discovery and characterization of novel viruses is also necessary as a critical step towards development of diagnostic, preventive and control approaches in case of disease emergence (Morse et al., 2012).

Rodents (Rodentia) and shrews (Soricomorpha) represent two large mammalian orders that are comprised by diverse species with widespread distribution and ecology. These small mammals have been shown to be reservoirs of a variety of viruses and are sources of many human diseases (Han et al., 2015; Luis et al., 2013). For instance, hantaviruses and arenaviruses are two major groups of zoonotic viruses harbored by rodents, causing severe viral hemorrhagic fever diseases in humans (Milholland et al., 2018; Gryseels et al., 2017; Golden et al., 2015).

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White-toothed shrews are the only known reservoirs of Borna disease virus 1, the spillover infection of which has caused fatal human encephalitis in Europe (Niller et al., 2020). Furthermore, rodents host a wide range of other viruses, including both RNA viruses such as astroviruses, hepaciviruses, paramyxoviruses, picornaviruses, etc. and DNA viruses such as adenoviruses, herpesviruses, poxviruses, etc. (Zheng et al., 2016; Donato and Vijaykrishna, 2017; Drexler et al., 2013; Sasaki et al., 2014; Lu et al., 2018; Hazel et al., 2000). Globalization, urbanization and land use changes have increased the risk of pathogen spillover from rodents and shrews to humans. Thus, understanding the virome diversity of these small mammals is of utmost importance for public health preparedness.

Kenya, an equatorial East African country with rich fauna, is home to about 106 species of rodents, 36 species of shrews and one species of hedgehog (Musila et al., 2019). Anthropogenic disturbances like expanded agricultural activities in the country may increase the chance of human contacts with rodents and rodent-borne pathogens (Young et al., 2017). Unfortunately, the viruses hosted by rodents and shrews in Kenya have been rarely investigated, and information about rodent-or shrew-borne viruses in the country is insufficient to advance disease prevention policies. Recently, we reported discovery of novel rodent viruses of various RNA viral families in Kenya, suggesting that Kenyan rodents harbor viruses with potential public health risk and raising the need for further researches that cover other virus families (Onyuok et al., 2019). Hence, in this study, a follow-up research that expanded sampling sites into more geographic zones and screened samples for multiple families of DNA viruses was conducted in Kenya.

2. Materials and methods

2.1. Study area

Field work was conducted in August/September 2016 and March 2019 at specific localities in five counties of Kenya (Fig. 1). 1) Kilifi County: we sampled in the rural villages adjacent to Mtwapa township in the coastal area at altitude range of 0–450 m above sea level (m a.s.l); 2) Nakuru County: the sampling area was within Rongai rural farms which lies within the rift valley at an altitude of 1,500 m a.s.l; 3) Trans-Nzoia County: animals were sampled at Kitale town within the rural and peri-urban areas at 2,000 m a.s.l; 4) Nairobi County: sampling was at Ngara Grocery Market and UhuruGardens both within the capital city. These locations are about 1,600 m a.s.l; 5) Kajiado County: at Ngong hills which has altitude of 1,840 m a.s.l. The areas were selected based on human activity, altitude and the climate so as to obtain samples representative of different geographic zones in the country (Coast, Rift Valley and Western).

2.2. Sample collection

From all the sampling sites, we designed a stratified, cross-sectional sampling method which enabled the data to be collected from a population as a representative subset at specific point in time of study period. At each site line transect were set around homesteads, farms and nearby natural habitats. Handling of the captured animals adhered to the guidelines provided by American Society for Mammalogist and National Museums of Kenya (biodiversity survey) (Sikes et al., 2011).

Animals were systematically captured alive using baited Sherman and Tomahawk live traps. Oat meal mixed with peanut butter was used as the bait. In market areas, fish pre-soaked in water or oil was preferentially used. Morphological taxonomic identifications of the trapped animals were conducted in the field by experienced mammalogists. Intravenous injection of ketamine was used to sedate the animals before cervical dislocation was applied to euthanize them. After morphometric measurements were taken, each animal was carefully dissected on a clean disinfected aluminum foil to enable the aseptic collection of the organs. For each animal, lung, liver and kidney samples were collected in sterile vials, labelled and preserved immediately in liquid nitrogen. The tissue samples were shipped to Wuhan Institute of Virology, China, where they

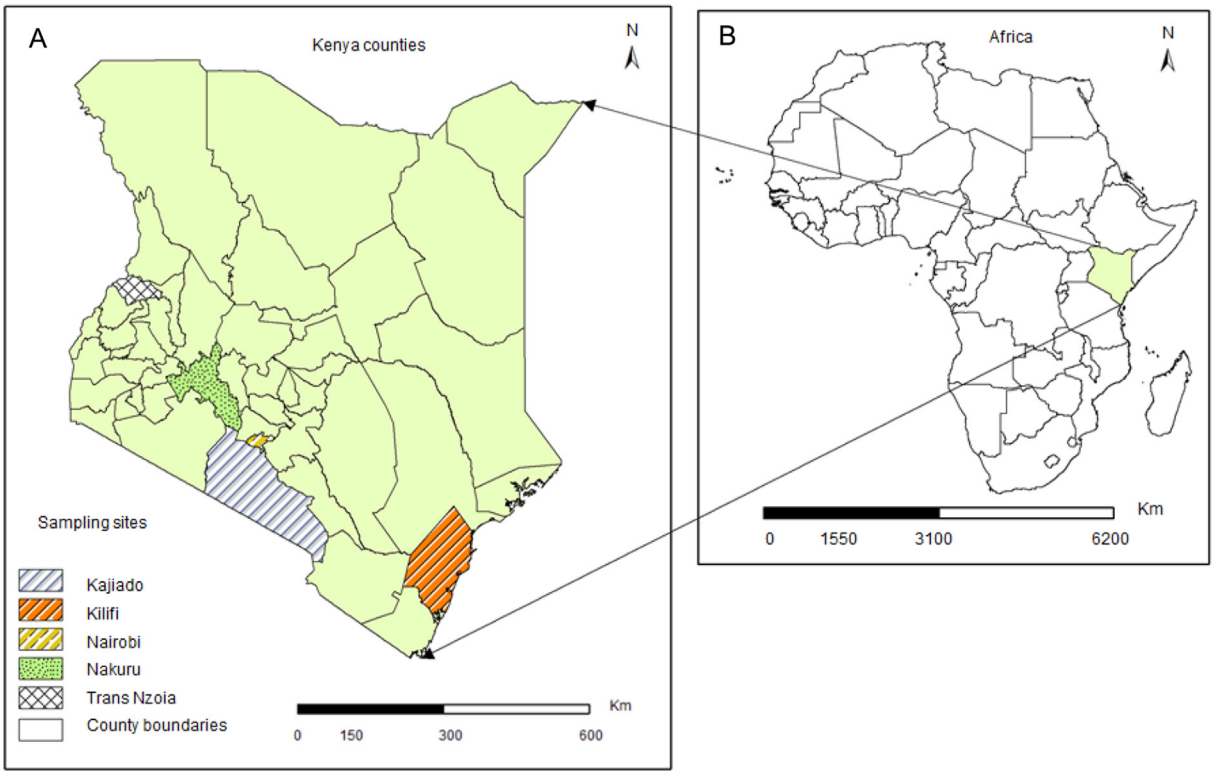

Fig. 1. Map of Kenya showing the location of five counties where sampling of small mammals was carried out in August/September 2016 and March 2019. **A** The location of Kilifi, Nakuru, Trans Nzoia, Nairobi and Kajiado counties in Kenya. **B** The location of Kenya in Africa.

were cryopreserved at −80 °C before virus screening. The remaining animal carcasses were deposited at the National Museums of Kenya as voucher specimens.

2.3. Molecular detection of DNA viruses and phylogenetic analysis

All the tissue samples were lysed by the Qiagen Tissue Lyser II as per the manufacturer's instruction (Qiagen, Germany). Viral nucleic acids, which contained both RNA and DNA, were extracted using high pure viral RNA kit (Roche, Germany) according to the manufacturer instructions. First, the viral nucleic acid from the three different tissues of the same individual animal was pooled. PCR assays utilizing Platinum Taq DNA Polymerase kit (Invitrogen, CA, USA) were applied to screen for the following DNA viruses: adenoviruses (AdVs) (Li et al., 2010b), adeno-associated viruses (AAVs) (Li et al., 2010a), bocaviruses (Shan et al., 2011), hepadnaviruses (Wang et al., 2017), herpesviruses (Chmielewicz et al., 2001), polyomaviruses (PyVs) (Johne et al., 2005), and poxvirus (Nitsche et al., 2004). Once the pooled tissues of an animal tested positive for a virus, the nucleic acids from three individual tissues of the same animal were re-tested singly to identify the positive organ of the animal. Host species identification of positive samples was confirmed by sequencing the mitochondrial cytochrome *b* (*cyt b*) gene as described by Irwin et al. (1991). The primers used in the study are listed in Supplementary Table S1. PCR amplification products with expected size were purified using the E.Z.N.A ® Gel Extraction Kit (Omega Bio-tek) following the manufacturer's instructions.

Purified DNA was directly sequenced or cloned using pGEM T-Easy vector system (Promega). Sequences of the detected virus gene fragments were determined by Sanger sequencing. Contamination was ruled out by repeating the experiments for the positive organs with newly extracted nucleic acid. Sequencing results were assembled and edited by SeqMan program and were preliminarily identified using BLAST. Sequences were aligned with representative strains (including previously published strains from rodents and shrews, strains of recognized species, and strains with high sequence similarity) of the relevant viral family or genus using Clustal W. Phylogenetic analysis was performed in MEGA version 7.0 using the maximum-likelihood (ML) approach with bootstraps of 1000 replicates (Hall, 2013).

2.4. Full-length genome sequencing of a novel polyomavirus

The kidney sample from a *Lemniscomys striatus* was selected for full-length genome sequencing of a novel polyomavirus using genome walking approach. Pairs of specific primers were designed from the initial partial VP1-coding sequence. Nested PCR assays were performed by two sets of primers. PLY187F1/R1 (5′-3′: AGGTTAAGGAAGAGGCTTGTG, CCTCATTAAGCAGGGGTAGCTG) and PLY187F2/R2 (5′-3′: ATCAAGGTCAGGAACAGCTACC, TCAATGGTAGCATCAGGACCTG) were used to amplify a 0.8-kb fragment. The second set of primers, PLY187AF/AR (5′-3′: GATGGACTGTTCATAAGTAGTGCTG, CCTCAGTCTTCACAGACACAGC) and PLY187BF/BR (5′-3′: GCCAAGGTACTTCAATGTTAGG, GCACAGGACCATGTAGGTATTTC) was designed from the above 0.8-kb fragment to amplify the remaining 4.5-kb genome. PCR was conducted in a 50-μL reaction system containing 10 μL of 5 × Prime STAR® Buffer (Mg^{2+} plus), 4 μL of dNTP Mixture (2.5 mmol/L each), 1.5 μL of both forward and reverse primer (0.3 μmol/L), 3 μL of nucleic acid (for first round) or 3 μL of the first round PCR product (for the second round PCR) as DNA template, 0.5 μL Prime STAR®HS DNA polymerase (2.5 U/μL) and DNase free water topping up to 50 μL. PCR was performed at following condition: 98 °C for 30 s, followed by 30 cycles (for the first round PCR) and 40 cycles (the second round PCR) of 98 °C for 10 s, 53 °C for 30 s, 72 °C for 50 s/kb (extension time depends on the expected size of amplicon), and a final extension at 72 °C for 5 min. The PCR products were taken for Sanger sequencing and sequencing results were assembled by SeqMan program to obtain the full-length virus genome sequence. The viral genome sequence was analyzed using BioAider (Zhou et al., 2020). The deduced amino acid (aa) sequences were then compared to sequences of related polyomaviruses available in the GenBank.

3. Results

3.1. Samples and virus detection

From 2016 to 2019, a total of 226 rodents belonging to 16 genera, five shrews and a hedgehog were trapped and their tissues were sampled from different localities in 5 counties in Kenya, which representatively covered the Coastal, Western, and Rift Valley regions of the country (Table 1 and Fig. 1). Liver, lung and kidney tissues of each animal were screened for the presence of DNA viruses of seven families. AdV, AAV, herpesvirus and PyV, were detected in 25 out of the 232 animals (Table 2). Overall positive rates of these viruses ranged from 0.86% (AdV and AAV) to 4.74% (PyV). The positive samples were derived from small mammals of 10 rodent species and one shrew species (*Crocidura olivieri*) (Table 2). At least one of the four viral families was found in the animals sampled from Nakuru, Trans Nzoia, Nairobi or Kajiado county, but no positive results were detected in the samples from Kilifi (Table 2). All samples tested negative for bocavirus, hepadnavirus and poxvirus. Co-infection of two or more of these virus strains in one animal was not observed.

3.2. Adenovirus

Small mammals' tissue samples were screened for AdV by nested PCR targeting a 261-bp fragment of the DNA polymerase (*pol*) gene. Of the 232 animals, only the lung samples of two multimammate rats (*Mastomys natalensis*) collected from Trans Nzoia County tested positive (Table 2). The virus sequences detected from the two rodents were 100% identical, suggesting they were from the same AdV strain. Phylogenetic analysis of the partial *pol* gene identified the Kenyan rodent AdV as the member of the genus *Mastadenovirus* (Fig. 2). This novel AdV was divergent from most existing species in the genus including the AdVs of primates, carnivores, bats, squirrels and murine AdVs 1 and 3. However, it was clustered with the clade of murine AdV-2 and a group of rat AdVs derived from diverse *Rattus* species in southern China, with which it shares up to 71.0% nucleotide (nt) identity. To our knowledge, this is the first time that AdVs have been identified in rodents from East Africa.

3.3. Adeno-associated virus

A PCR assay was applied to detect a *cap* gene fragment of AAV around 440-bp. Novel AAV sequences were detected in specimens of the only two African woodland dormice (*Graphiurus murinus*) collected from Trans-Nzoia. Samples from other species were negative for AAV. Strains from the two individual dormice were similar, sharing 91.7% nt identity. The AAVs were found to be widely present in different tissues of the positive animals showing no organ tropism (Table 2). These *Graphiurus murinus* AAVs were phylogenetically more closely related to the clades of diverse variants of bat AAVs than to the AAVs previously reported from mice and rats (Fig. 3). Nevertheless, the nt identities of the partial *cap* gene between the Kenyan rodent AAVs and known rodent, bat or primate AAVs were not higher than 74%, indicating huge genetic distance between them and the bat AAVs.

3.4. Herpesvirus

The presence of herpesvirus DNA in tissues was tested by a nested pan-herpesvirus PCR targeting the partial terminase gene. Ten animals tested positive, including seven rodents from five different species, and three shrews belonging to the species *Crocidura olivieri* (Table 2). A prevalence rate of about 20% was found in both *Aethomys kaiseri* (2/10) and *Arvicanthis niloticus* (2/11) (Table 2). In four of the ten positive

Table 1
Numbers of animals from different small mammal species collected in different counties of Kenya.

Order	Species	No. of animals					
		2016			2019		Total
		Kilifi	Nakuru	Trans Nzoia	Nairobi	Kajiado	
Rodentia	***Gliridae***						
	Graphiurus murinus	–	–	2	–	–	2
	Muridae: Gerbillinae						
	Gerbilliscus robustus	5	–	–	–	–	5
	Muridae: Deomyinae						
	Lophuromys acquilus	–	–	6	–	–	6
	Lophuromys sp.	–	–	–	–	5	5
	Muridae: Murinae						
	Aethomys kaiseri	–	10	–	–	–	10
	Arvicanthis niloticus	–	7	4	–	–	11
	Grammomys macmillani	–	–	2	–	–	2
	Lemniscomys striatus	–	5	2	12	1	20
	Mastomys natalensis	–	37	14	33	–	84
	Mus minutoides	–	10	2	–	1	13
	Mus triton	–	5	4	–	–	9
	Mus sp.	–	–	–	1	–	1
	Oenomys hypoxanthus	–	–	1	–	–	1
	Otomys tropicalis	–	1	–	–	–	1
	Otomys sp.	–	–	–	4	–	4
	Rattus rattus	2	3	10	–	–	15
	Rattus norvegicus	–	–	–	31	–	31
	Nesomyidae						
	Cricetomys gambianus	–	1	–	–	–	1
	Dendromus sp.	–	–	–	–	1	1
	Sciuridae						
	Paraxerus ochraceus	2	–	–	–	–	2
	Spalacidae						
	Tachyoryctes splendens	–	2	–	–	–	2
Soricomorpha	***Soricidae***						
	Crocidura olivieri	–	3	2	–	–	5
Erinaceomorpha	***Erinaceidae***						
	Aterelix albiventrix	1	–	–	–	–	1
	Total	10	84	49	81	8	232

Table 2
Individual animals and tissues positive for four DNA viral families or genera.

Virus	Positive host species	No. of positives/No. tested individuals						
		Kilifi	Nakuru	Trans Nzoia	Nairobi	Kajiado	Total	Positive tissues
Adenovirus	*Mastomys natalensis*		0/37	2/14	0/33		2/84	lung (2)
Adeno-associated virus	*Graphiurus murinus*			2/2			2/2	lung (2), kidney (2), liver (1)
Herpesvirus	*Aethomys kaiseri*		2/10				2/10	kidney (1), lung (1)
	Arvicanthis niloticus		2/7	0/4			2/11	kidney (2), liver (1)
	Grammomys macmillani			1/2			1/2	lung (1), kidney (1), liver (1)
	Lemniscomys striatus		0/5	1/2	0/12	0/1	1/20	kidney (1)
	Mus minutoides		1/10	0/2		0/1	1/13	lung (1), kidney (1), liver (1)
	Crocidura olivieri		2/3	1/2			3/5	lung (3), kidney (3), liver (2)
Polyomavirus	*Arvicanthis niloticus*		0/7	1/4			1/11	lung (1), kidney (1), liver (1)
	Lemniscomys striatus		2/5	0/2	3/12	1/1	6/20	kidney (6), liver (4)
	Lophuromys acquilus			1/6		0/5	1/11	lung (1), kidney (1), liver (1)
	Mastomys natalensis		0/37	1/14	1/33		2/84	lung (2), kidney (2), liver (1)
	Rattus norvegicus				1/31		1/31	kidney (1)

animals, herpesviruses were discovered in all three tested tissues, while only the lung or kidney was positive in other four rodents.

Phylogenetic analysis classified the newly identified herpesviruses into the subfamily *Betaherpesvirinae* (Fig. 4). The phylogeny of this subfamily exhibited clear host restriction, in which viruses from primates, rodents, bats and shrews fell into four separate clades (Fig. 4). All of the seven Kenyan rodent herpesviruses clustered into one clade of the genus *Muromegalovirus* which refers to murine or rat cytomegaloviruses. These rodent herpesviruses were genetically diverse, forming two clusters. Herpesviruses detected in four rodent species, *Aethomys kaiseri*, *Arvicanthis niloticus*, *Grammomys macmillani* and *Lemniscomys striatus*, represented four distinct strains. They were clustered with murid betaherpesvirus 1, showing 85.0%–92.5% nt sequence identities to the known virus isolates of this species. The other herpesvirus from *Mus*

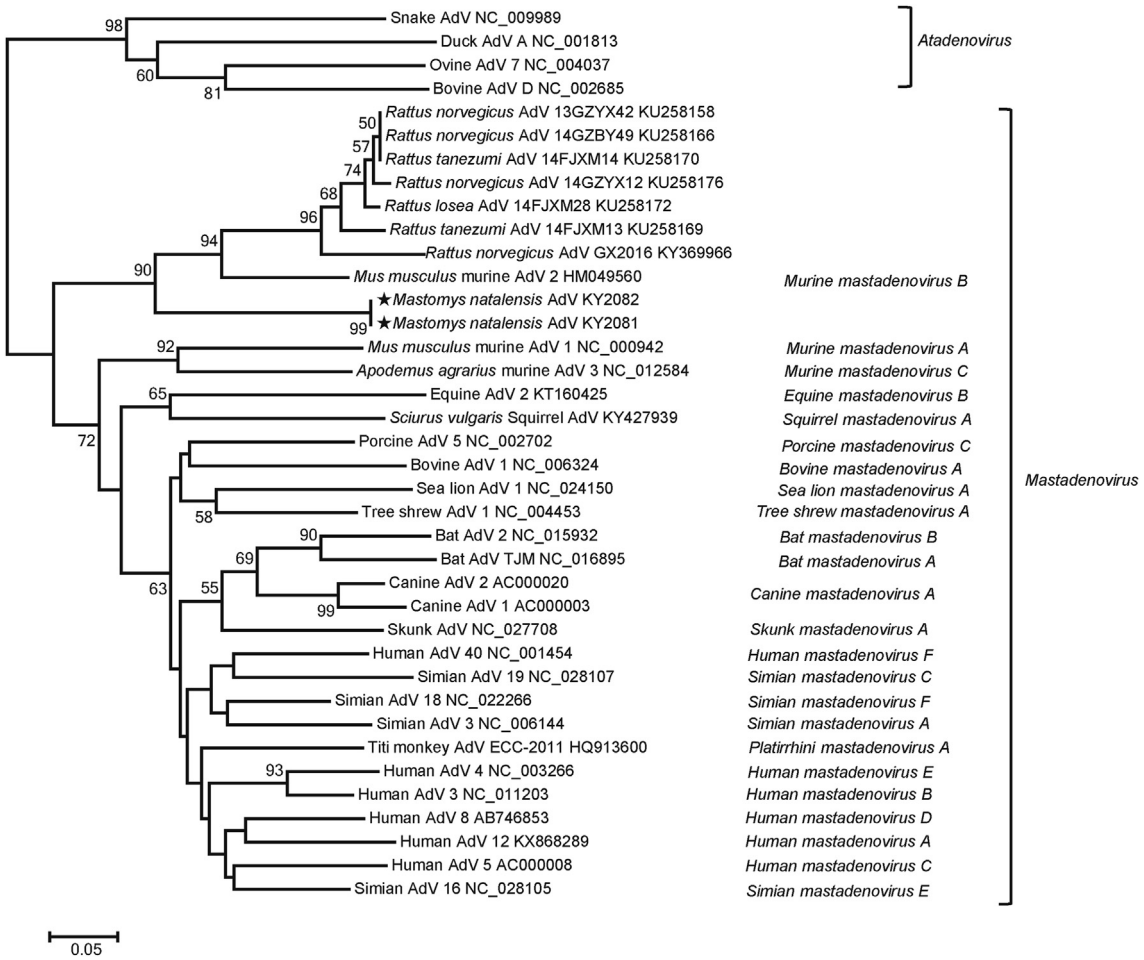

Fig. 2. Phylogenetic analysis based on partial amino acid sequences of the DNA polymerase of viruses belonging to the genera *Mastadenovirus* and *Atadenovirus*. Representative species of mastadenovirus are marked. Stars indicate AdVs detected in the present study. The tree was generated by using the neighbor-joining method with the *p*-distance model and a bootstrap of 1000 replicates. Bars indicate an evolutionary distance of 0.05 substitutions per site.

minutoides was phylogenetically related to murid betaherpesvirus 8, sharing 77.9% nt sequence identity (Fig. 4). The three herpesviruses discovered in shrews from Kenya were closely clustered with shrew herpesviruses previously reported in other African countries. *Crodicura olivieri* herpesviruses KY2083 and KY2230, which were found in the western and Rift Valley regions of Kenya, showed 99.7% and 99.5% identity in the partial *ter* gene to the shrew herpesviruses DRC2 from the Democratic Republic of Congo (DRC) and CMR1 from Cameroon, respectively. The remaining strain, *Crocidura olivieri* KY2275, was most closely related to shrew herpesvirus DRC3, with 86.5% sequence identity (Fig. 4).

3.5. Polyomavirus

Tissue samples of 232 small mammals were subjected to PCR targeting a partial VP1-coding sequence of PyVs. Twenty-two specimens from 11 animals (4.7%) were tested positive. The PyV positive samples were from five host species: *Arvicanthis niloticus*, *Lemniscomys striatus*, *Lophuromys acquilus*, *Mastomys natalensis* and *Rattus norvegicus* (Table 2). Strains detected in different rodent species were distinct from each other and represent novel PyVs as determined by sequencing and BLAST analysis. Of the five positive species, the highest prevalence rate (30%) of PyV was identified in *Lemniscomys striatus*. The PyV infecting this rodent species was discovered in Nairobi and Nakuru Counties in the Rift Valley region.

The phylogenetic tree of the partial *VP1* gene revealed that the newly detected rodent PyVs were divided into two large clades, which correspond to two genera of *Polyomaviridae* (Fig. 5). All PyVs from *Lemniscomys striatus* and one of the *Mastomys natalensis* PyVs named KY2007 were phylogenetically placed into the *Betapolyomavirus* genus. They formed a well-supported monophyletic group with some recognized rodent PyVs, including the squirrel (*Sciurus carolinensis*) PyV 1 and edible dormouse (*Glis glis*) PyV 1 (Fig. 5). The 240-bp partial *VP1* gene sequences of these novel Kenyan rodent PyVs exhibited less than 71% nucleotide sequence identities to known PyVs. The *Mastomys natalensis* PyV KY2007 was distinct from *Mastomys natalensis* PyV 1 identified in a previous study in Zambia, showing only 67% nucleotide sequence identity. The remaining four PyVs discovered in this study belonged to the clade formed all by alphapolyomaviruses. Within this clade, *Rattus norvegicus* PyV KY100 and *Mastomys natalensis* PyV KY127, together with already published PyVs, constituted a monophyletic group exclusively comprising PyVs from rodents (Fig. 5). *Rattus norvegicus* PyV KY100 shared 86%–88% nucleotide sequence identity to the *Rattus norvegicus* and *Apodemus flavicollis* PyVs reported in China and Germany. *Mastomys natalensis* PyV KY127 was more distinct to all known alphapolyomaviruses and therefore had higher novelty. Its partial VP1 sequence shared less than 76% nucleotide identity to previously described rodent PyVs, including the *Mastomys natalensis* PyV 2 found in West Africa. Two novel rodent PyVs from *Arvicanthis niloticus* and *Lophuromys acquilus* were separated from other rodent alphapolyomaviruses, and were more

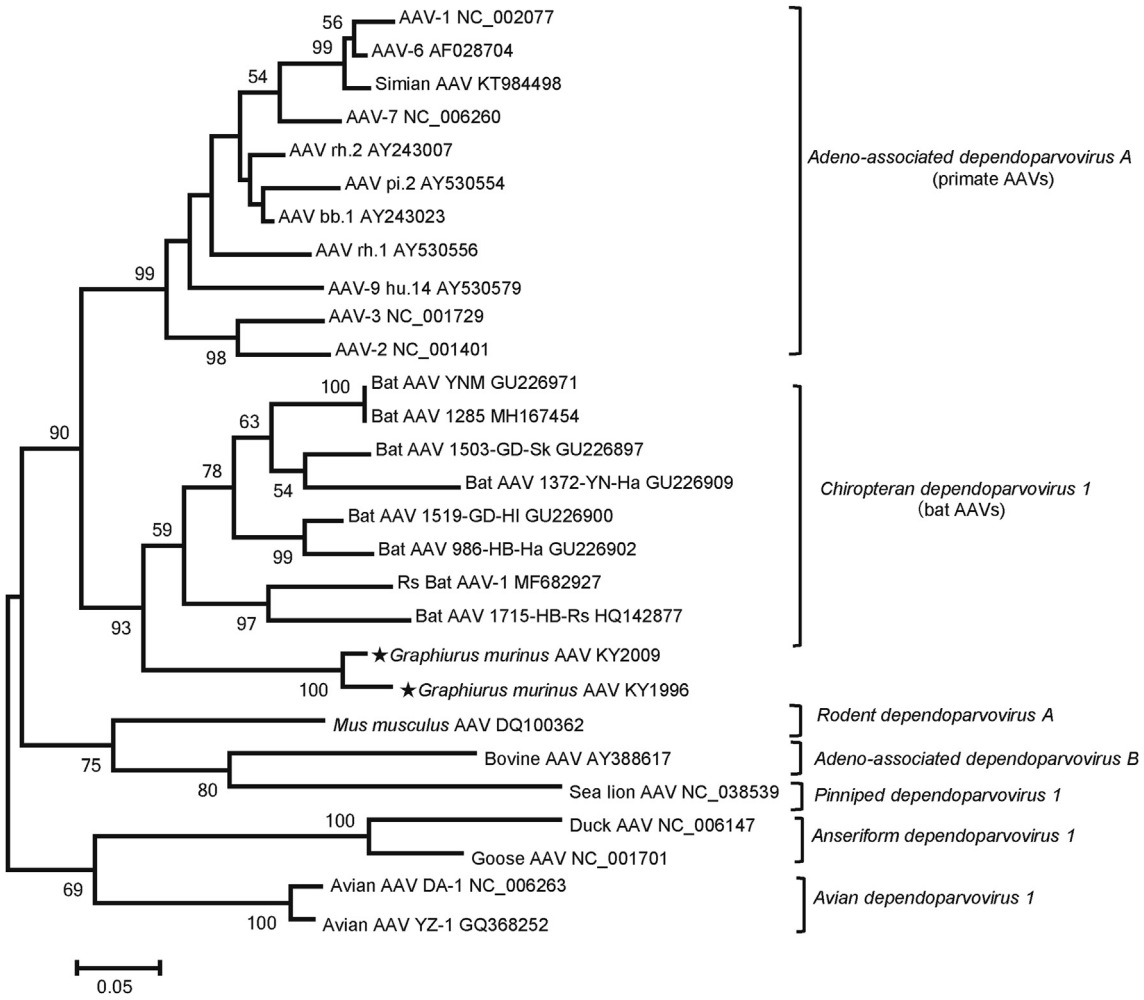

Fig. 3. The maximum-likelihood tree based on 426-bp partial *cap* gene sequences of AAVs. The novel rodent AAVs detected in this study are denoted by stars. Bootstrap values were calculated from 1,000 replicates. Bars indicate an evolutionary distance of 0.05 substitutions per site.

related to shrew PyVs and human PyV-12, but their VP1 sequence identities to these PyVs were less than 70%.

3.6. Genomic characterization of a novel polyomavirus

As the partial PyV VP1-coding sequences detected in the six individuals of *Lemniscomys striatus* were highly similar, we selected the kidney sample from one positive animal for virus genome sequencing. The full-length genome sequence of a novel PyV designated *Lemniscomys striatus* polyomavirus KY187 (LsPyV KY187) was generated and annotated (Fig. 6A). The circular genome of LsPyV KY187 had a typical PyV genome organization. It consisted of a late region that encoded the viral structural proteins VP1, VP2 and VP3, and an early region that encoded the small and large T antigens (STAg, LTAg) on the opposite strand (Fig. 6A). The two regions were separated by a 597-bp non-coding control region (NCCR). Additionally, a potential extra ORF was found in the upstream of VP2 coding sequence. The LTAg and VP2 CDS of LsPyV KY187 were interrupted by an intron. By aligning the sequence of LsPyV KY187 with related PyVs, the splice donor and acceptor sites were identified for its LTAg and VP2 mRNA. The predicted positions of introns and exons of the two genes are presented in Fig. 6B.

To understand the phylogenetic placement of LsPyV KY187, we constructed a maximum likelihood (ML) tree using the sequences of conserved LTAg amino acid blocks of all currently recognized members of the genus *Betapolyomavirus*. The polyomaviruses infecting rodents were clustered in three different clades. LsPyV KY187 was found to belong to a well-supported clade consisting of polyomaviruses from various hosts of glires, including dormouse, hare and two species of squirrels (Fig. 7). In accordance with the partial VP1 phylogeny, LsPyV KY187 was most closely related to *Glis glis* polyomavirus 1 (GgliPyV1) and *Sciurus carolinensis* polyomavirus 1 (ScarPyV1). We further compared the aa sequences of the three structural proteins, LTAg and STAg of LsPyV KY187 to those of related polyomaviruses (Supplementary Table S2). Its VP1 protein showed 71.8% and 74.9% sequence identity to GgliPyV1 and ScarPyV1, respectively. In VP2, VP3, LTAg and STAg, the aa sequence identities of LsPyV KY187 to the two viruses were all less than 60%, ranging between 44.6% and 59.7%. For LTAg coding sequences, the genetic distance of LsPyV KY187 to the two most closely related species, *Glis glis polyomavirus 1* and *Sciurus carolinensis polyomavirus 1*, were calculated to be 49.2% and 54.9%, which were much higher than the threshold value (15%) for polyomavirus species demarcation. According to the taxonomic criteria of The International Committee on Taxonomy of Viruses (ICTV), LsPyV 187 can be tentatively assigned to a new species within the genus *Betapolyomavirus*.

4. Discussion

Owing to their relatively high rates of mutation and capability of adaption to new hosts, RNA viruses are considered the majority of the emerging and re-emerging viral pathogens (Jones et al., 2008). However, the etiological role of DNA viruses in the zoonotic diseases should not

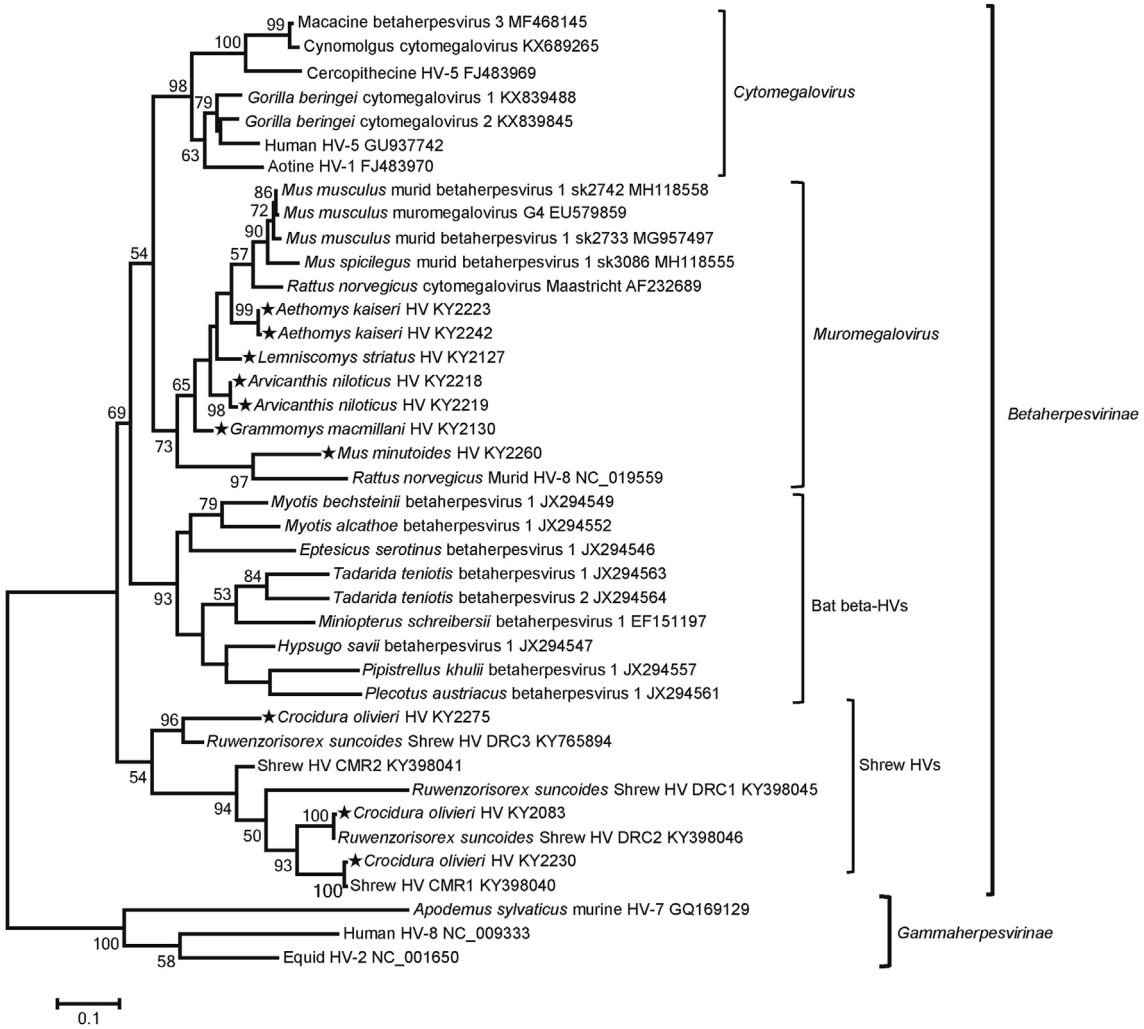

Fig. 4. The maximum-likelihood tree based on 417-bp partial terminase (*ter*) gene sequences of betaherpesviruses and gammaherpesviruses. Novel herpesviruses detected in this study are denoted by stars. Bootstrap values were calculated from 1000 replicates. Bars indicate an evolutionary distance of 0.1 substitutions per site.

escape the attention of public health, either. For instance, monkeypox virus infects humans as well as various species of rodents and non-human primates. In Africa, several monkeypox outbreaks in humans have been reported in the past decade (Silva et al., 2020). A few of animal herpesviruses also have the potential for cross-species transmission and may cause zoonotic infection (Tischer and Osterrieder, 2010). Rodents, the most abundant and diversified order of mammals which comprise about 40% of all mammalian species, are reservoir hosts of a great diversity of viruses. Due to the greater number of rodent species than bat species, the expected total number of zoonotic viruses in rodents are even more than in bats (Luis et al., 2013). According to a global database for rodent-associated viruses, members of at least 31 virus families have been reported from rodents worldwide, of which 10 are DNA viruses (Chen et al., 2017). The rich biodiversity of small mammals in Kenya equally supports the high diversity of viruses they harbor. We previously conducted RNA virus surveillance in wild and synanthropic rodents and shrews in Kenya, leaving DNA viruses unattended (Onyuok et al., 2019). To fill this knowledge gap, we tested tissues from over 200 small mammals sampled across different geographic zones in Kenya for infection by DNA viruses of 7 families.

AdVs are a family of DNA virus commonly infect numerous species of almost all major vertebrate classes. *Mastadenovirus*, the largest genus of the family *Adenoviridae*, exclusively contains AdVs of mammalian host origin. In humans and many other mammals, AdV infections usually require predisposing factors for the development of severe diseases (Harrach et al., 2019). Currently, there are three recognized murine AdV (MAdV) species known as MAdV-1, MAdV-2 and MAdV-3. Among them, MAdV-2 is more divergent, occupying a phylogenetic branch independent from the cluster of the other two MAdV species. While MAdV-1 may cause fatal infection in neonate mice, it is not clear whether MAdV-2 may cause diseases (Hemmi et al., 2011). In other rodents, some cases of mortal enteritis in red squirrels potentially associated with squirrel AdV infection have previously been reported in Europe (Abendroth et al., 2017). In this study, we detected a novel AdV in *Mastomys natalensis* from Kenya. Previously, another *Mastomys natalensis* AdV (MnAdV) was reported from Cameroon in Central Africa. Apparently, these two strains are distinct. The Cameroonian strain clusters with MAdV-1/MAdV-3, and the Kenyan MnAdV is more closely related to MAdV-2 (Diffo et al., 2019). It suggests that there may be a wide diversity of AdVs in this African most widespread rodent species in peridomestic environments. In addition, DNA of AdVs closely related to MAdV-2 was detected in rodent species from DRC. Since that study used a PCR target gene different from ours, we cannot calculate the similarity between them and the Kenyan rodent AdVs (Kumakamba et al., 2020). Without the full sequence of polymerase gene, we are also unable to conclusively tell whether the novel MnAdV from Kenya represents a new species or a new subtype of MAdV-2 at this point.

Adeno-associated viruses (AAVs) are a group of small DNA viruses highly diverse among their mammalian and avian hosts. Because of their long-lasting expression and non-apparent pathogenicity, AAVs have been

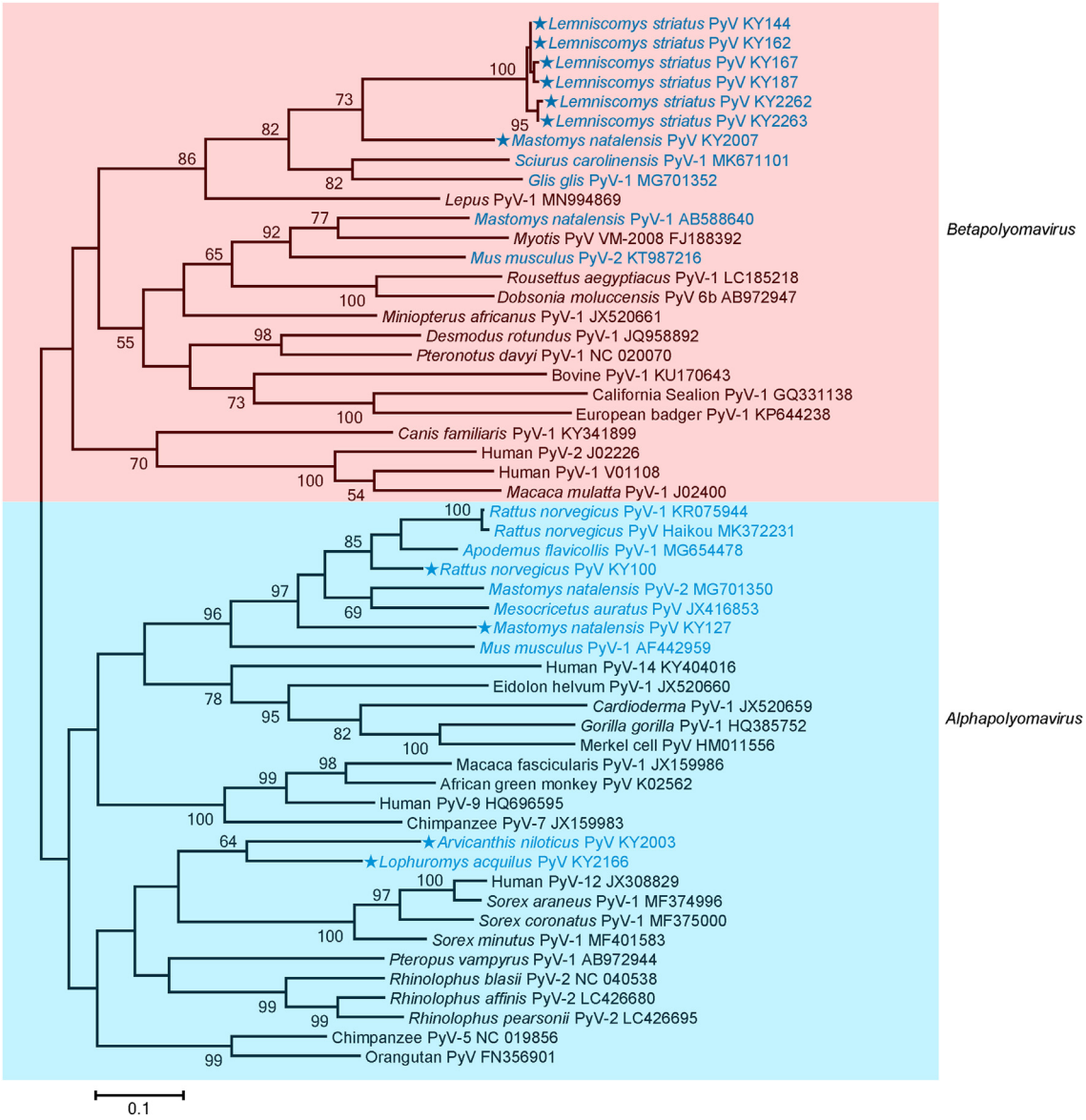

Fig. 5. Phylogenetic relationship of viruses belonging to the genera *Alphapolymavirus* and *Betapolyomavirus*. The maximum-likelihood tree was constructed using the partial *VP1* gene sequences. Novel polyomaviruses identified in this study are denoted by stars. Polyomaviruses detected in rodents are shown in blue fonts. Bootstrap values were calculated from 1000 replicates. Bars indicate an evolutionary distance of 0.1 substitutions per site.

frequently used as viral vectors in gene therapy researches (Mingozzi and High, 2011). Here we report the first detection of AAVs in African rodents. Phylogeny of known mammalian AAVs suggests that they generally coevolve with their specific hosts (Xiong et al., 2018). As very few rodent AAVs have been described to date, current knowledge of AAV diversity in rodents is largely incomplete. Global discovery of additional AAVs from rodents of different species and from different locations will help better understand the evolutionary patterns of mammalian AAVs. The productive replication of AAVs requires a co-infecting helper virus such as AdV or herpesvirus (Flotte and Berns, 2005). In our study, neither AdV nor herpesvirus was detected in the two samples from *Graphiurus murinus* that tested positive for AAV. A previous bat AAV surveillance study also revealed negative result of helper viruses in a majority of AAV positive bat samples (Li et al., 2010a). The lack of detection of helper virus could be partially attributed to the limitation of detection sensitivity of the pan-PCR assays. Without the presence of a helper virus, AAVs may integrate into the host chromosome and establish a latent infection (Xiong et al., 2018), but this cannot be distinguished by our *cap* gene targeting PCR assay.

Cytomegaloviruses (CMV) are the typical members of the subfamily *Betaherpesvirinae*. Human cytomegalovirus (HCMV) is a major cause of birth defects besides being highly pathogenic in immunocompromised patients (Boeckh and Geballe, 2011). Related herpesviruses have been widely reported from rodents (Ehlers et al., 2007). The murine cytomegalovirus (MCMV), also called murid betaherpesvirus 1, is highly prevalent in wild populations of house mice (Gouy De Bellocq et al., 2015). Distinct CMVs have also been isolated from brown rats in Europe, which represent two diverged species (Geyer et al., 2015). Both MCMV and rat CMVs are used as laboratory model for CMV infection of humans. In this study, we have found a variety of betaherpesviruses in wild rodent and shrew species in Kenya. The rodent herpesviruses were all from the subfamily *Murinae*, and they fell into the same clade with recognized murid betaherpesviruses, known as the genus *Muromegalovirus*. Sequences that we detected in five different murine species showed genetic uniqueness, and the novel viruses from each species phylogenetically appear in their own branch. However, the inclusion of these novel viruses does not affect the topology of the phylogeny among *Betaherpesvirinae*, in which viruses from primates,

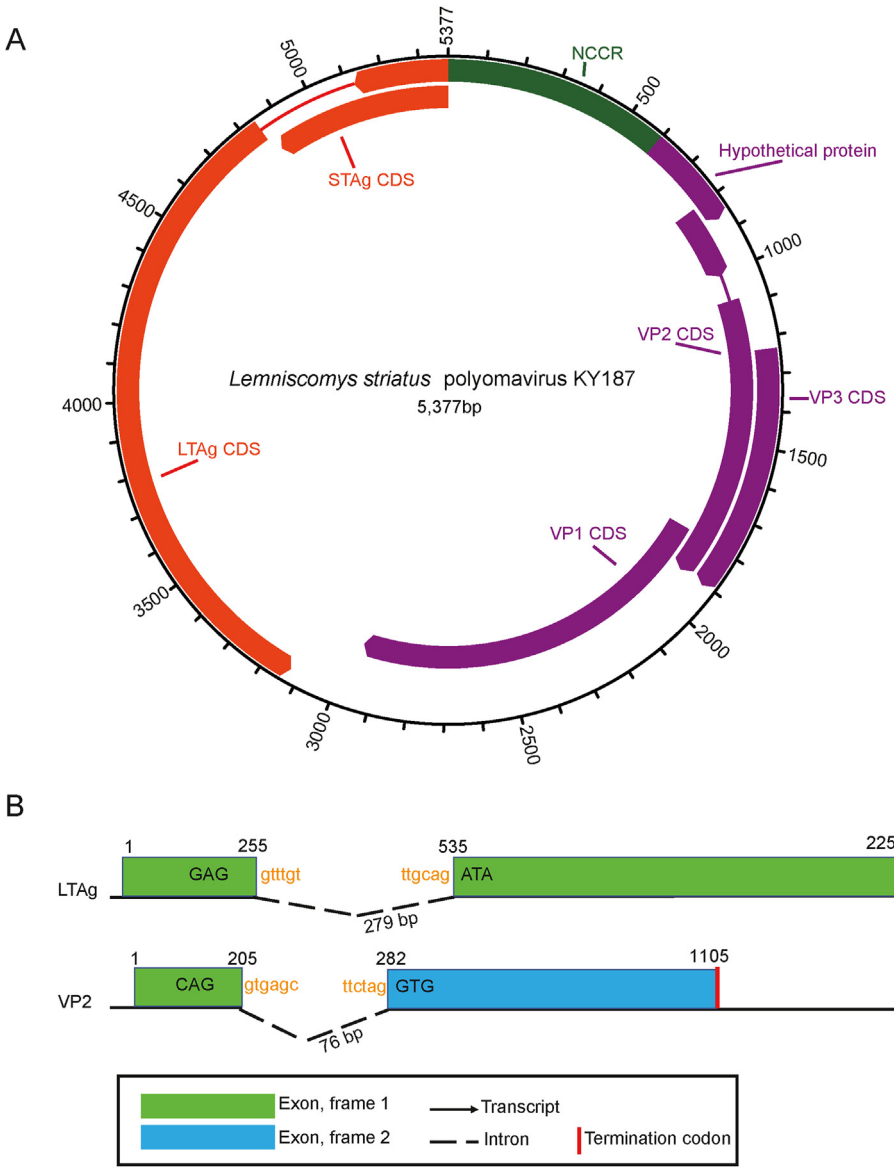

Fig. 6. Genome organization of *Lemniscomys striatus* polyomavirus KY187. **A** The scheme of the circular genome of LsPyV KY187. The *VP1*, *VP2*, *VP3* genes and CDS for the additional hypothetical protein are indicated by purple bars. *LTAg* and *STAg* antigen genes are indicated by orange bars. Thin lines within *LTAg* and *VP2* genes indicate introns. Non-coding control region (NCCR) is marked by dark green bars. **B** Exon sequences of the *LTAg* and *VP2* genes are depicted as colored bars. Predicted introns are depicted as dashed lines.

rodents, bats and shrews form separate clusters respectively. Previously, another study reported identification of diverse betaherpesviruses in a variety of rodent species from Germany and Thailand (Ehlers et al., 2007). However, as only the glycoprotein B and DNA polymerase (*dpol*) gene sequences are available for those viruses, we are unable to include them in our phylogenetic analysis. More gene sequencing of the novel herpesviruses will allow us to get a more complete picture of herpesvirus evolution and find out whether they have co-evolved with their hosts to a large part. Our findings indicate that diversity of rodent CMVs in nature may be greatly underappreciated. With only the partial fragment sequences of the terminase gene, we cannot determine whether the newly identified herpesviruses can be treated as novel species or not. Compared with those from rodents, novelty of the herpesvirus sequences obtained from Kenyan shrews is lower. Two strains differ by less than 1% from known shrew herpesviruses detected in central Africa, suggesting these viruses may circulate commonly in shrews across the Africa continent (Ntumvi et al., 2018).

Polyomaviruses (PyVs) are small viruses with circular dsDNA genome that infect mammals, birds and fishes. Some PyVs are associated with cancer in human or animal hosts. In the past decade, novel PyVs have been discovered at an increasingly high pace, with over 100 species of PyVs currently known according to the 10th ICTV report on PyV taxonomy (Moens et al., 2017a) (updated by 2020). Rodents and shrews are two natural hosts of mammalian PyVs. Within the genera *Alphapolyomavirus* and *Betapolyomavirus*, there are a total of 16 recognized species derived from rodents and 3 from shrews (Schulze et al., 2020). Little is known about the zoonotic potential of PyVs in these small mammals, except that the close similarity between the *Sorex araneus* PyV and human PyV-12 suggested possible cross-species transmission of a shrew PyV to humans (Gedvilaite et al., 2017). In this study, we further extended the knowledge about rodent PyV diversity by documenting three groups of distinct novel PyVs from Kenya. Our screening revealed a high prevalence of PyV among *Lemniscomys striatus* populations in Kenya. All PyVs identified from this rodent species belong to a single

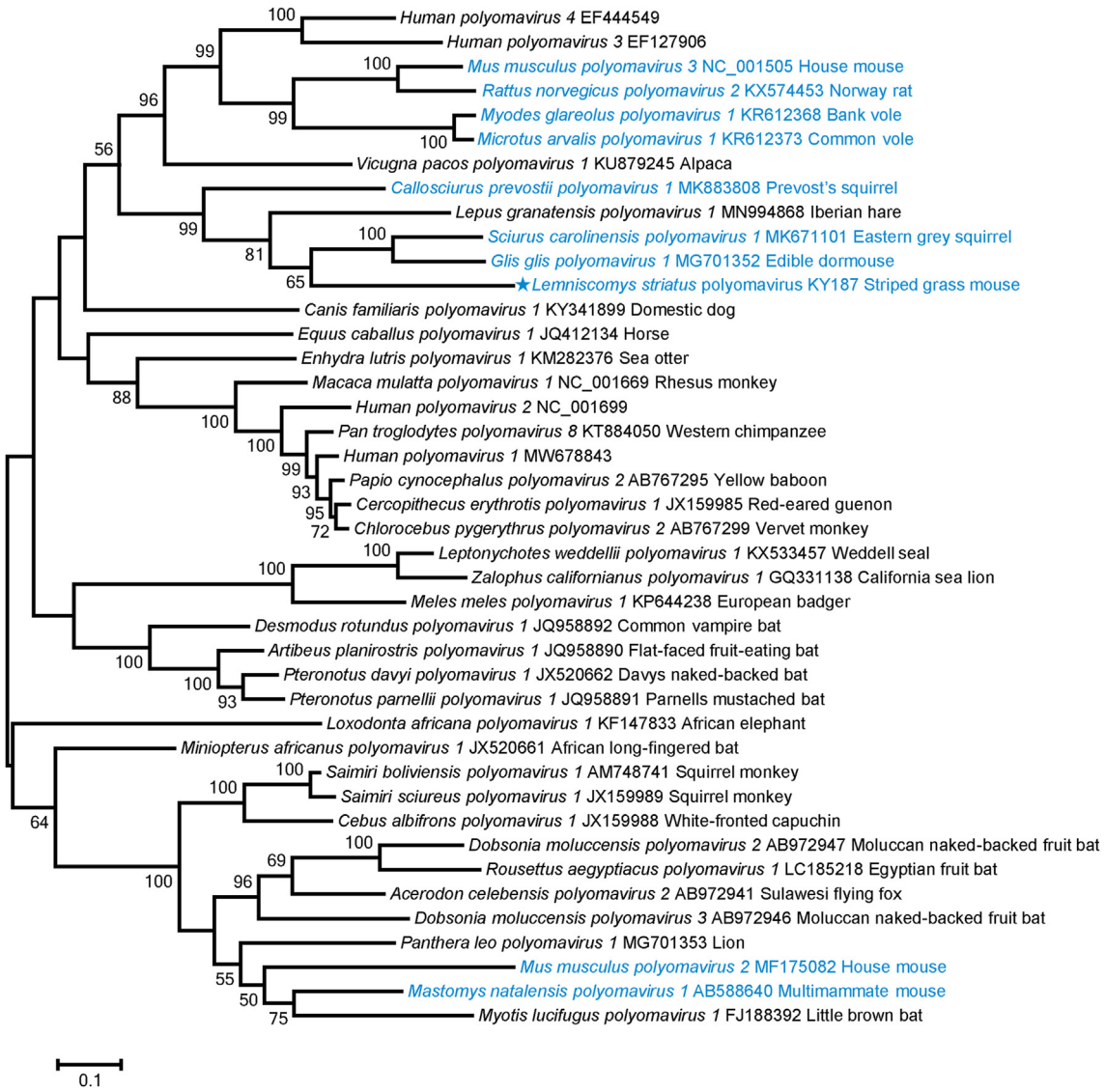

Fig. 7. Phylogenetic analysis of betapolyomaviruses based on conserved amino acid blocks of the LTAg sequences. The maximum-likelihood tree was constructed using WAG model. Bootstrap values were calculated from 1000 replicates. The tree includes all ICTV-recognized species within the genus *Betapolyomavirus*, which are named using the Latin binomials of their hosts and a serial number. The GenBank accession numbers and common names of the hosts of these polyomaviruses are also given. The novel polyomaviruses identified in this study is denoted by a star. Polyomaviruses detected in rodents are shown in blue fonts.

monophyletic clade, in which strains from two different sampling locations, Nairobi and Nakuru, appeared as two closely related subclades. Other PyVs that we found in some local African rodents such as in *Lophuromys* and *Arvicanthis* are also markedly different from those previously detected in other host species. The results further strengthen the assumption that PyVs are strongly host-specific (Moens et al., 2017b). Interestingly, we discovered two highly divergent *Mastomys natalensis* PyVs allocated to *Alphapolyomavirus* and *Betapolyomavirus* respectively. This is in line with previous observation that one single host species can be infected with multiple distantly related PyVs. The interspersion of PyVs in the phylogeny may have resulted from lineage duplications during PyV evolution which have driven their diversification (Ehlers et al., 2019). Previously, DNA of rodent PyVs were mainly found in spleen and lung (Ehlers et al., 2019; Schulze et al., 2020; Orba et al., 2011). But in our study, all of the novel PyVs can be detected in kidney samples, including three viruses exclusively in kidney. Three of the PyVs were widely distributed in lung, kidney and liver. In a previous study, PyV DNA was identified in kidney and chest-cavity fluid of bank voles and common voles (Nainys et al., 2015). These results suggest that rodent PyVs may have a broad tissue tropism, and kidney could be another preferential target tissue for monitoring PyVs in wild rodents.

This study reports the first full-length genomic characterization of PyV in rodents of the genus *Lemniscomys* (grass mouse). We found the DNA of this PyV abbreviated as LsPyV KY187 in both kidney and liver tissues. However, there was not any noticeable pathological alterations in the infected animal. Infection with this PyV is likely to be asymptomatic in immunocompetent adults of *Lemniscomys*, like many other PyVs in their natural hosts (Moens et al., 2017a). The genome arrangement of LsPyV KY187 is similar to that of ScarPyV1 and GgliPyV1, including the predicted splice sites in the *LTAg* and *VP2* genes. Congruently, phylogenetic analysis of LTAg, the marker employed by ICTV in the *Polyomaviridae* taxonomic analyses, also suggests their relatively close relationship. However, with the application of the ICTV criteria for PyV species demarcation, a new species of betapolyomavirus can be created for LsPyV KY187, as significant genetic distance exists between it and the other two known PyV species. In addition to KY187, given the novelty and diversity of rodent PyVs in Kenya revealed by this study, complete genome sequencing of other PyV strains may shed new light on taxonomy

of mammalian PyVs, and allow better understanding of the long-term dynamics of coevolution of PyVs with their African rodent hosts.

5. Conclusions

Taken together, in this study we discovered novel DNA viruses of four different families in wild and synanthropic rodents and shrews from Kenya, including a novel species of PyV whose complete genome has been characterized. For most of these viruses, our analysis was based on sequences of small-sized amplicons. To have a more robust phylogenetic conclusion, more sequences should be determined from these viruses in following studies. Moreover, as these viruses are not closely related to any virus known to cause disease in their small mammal hosts or in humans, their pathogenicity and potential risk of zoonotic transmission are unclear and need to be further assessed. Despite these issues to be addressed later, the findings of this study have enhanced our knowledge of the diversity and evolution of DNA viruses in these small mammal reservoirs. As there are increasing agricultural activities into the natural habitats of rodents in Africa, it highlights the necessity of continued surveillance over a wide-scale area, which could involve larger sample size and apply more high-throughput detection methods. Pathogenicity studies of novel viral pathogens are also required in future investigation. Such programs will contribute an important baseline for future prevention and control of emerging zoonotic diseases.

Data availability

Sequences generated by this study were deposited in the GenBank under accession numbers OL366010-OL366013, OL377720-OL377729 and OL439487-OL439497. These sequence data are also openly available in Science Data Bank at 10.11922/sciencedb.o00061.00002.

Ethics statement

The study was approved by the Research and Ethics and Committee of the Kenya Wildlife Service (KWS) with the permit KWS/BRM/5001. All institutional and national guidelines for care and handling use of animals were followed. The authors declare they have no conflicts of interest.

Author contributions

Griphin Ochieng Ochola: conceptualization, data curation, formal analysis, investigation, resources, visualization, writing-original draft; Bei Li: investigation, methodology; Vincent Obanda: resources, writing-review and editing; Sheila Ommeh: resources, writing-review and editing; Harold Ochieng: resources; Xing-Lou Yang: funding acquisition, methodology, supervision; Samson Omondi Onyuok: resources; Zheng-Li Shi: conceptualization, funding acquisition, project administration, supervision; Bernard Agwanda: conceptualization, resources, supervision, writing-review and editing; Ben Hu: conceptualization, data curation, formal analysis, visualization, writing-original draft; writing review and editing.

Conflict of interest

All authors declare that they have no competing interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.virs.2022.06.001.

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