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ANNOTATED SEQUENCE RECORD

Novel smacoviruses identified in the faeces of two wild felids: North American bobcat and African lion

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Abstract

Smacoviruses are small circular single-stranded DNA viruses that appear to be prevalent in faeces of a range of animals and have also been found in a few insect species. In this study, we report the first viral genomes from faeces of free-roaming wild felids on two continents. Two smacoviruses were recovered from the faeces of two North American bobcats (*Lynx rufus*), and one was recovered from an African lion (*Panthera leo*). All three genomes are genetically different, sharing 59-69% genome-wide sequence identity to other smacoviruses. These are the first full smacovirus genome sequences associated with a large top-end feline predator, and their presence in these samples suggests that feline faeces are a natural niche for the organisms that these viruses infect.

The *Smacoviridae* are a family of circular single-stranded DNA viruses that have been identified in the faecal excrement of many mammals and birds, and a few arthropods [19]. The genomes of smacoviruses range from 2.3 to 3 kb in size and contain at least two bidirectionally transcribed open reading frames (ORFs), which encode a replication-associated protein (Rep) and a capsid protein (CP). These ORFs are separated by two intergenic regions, one of which contains an origin of replication. At present, more than 170 smacoviruses have been documented, with six established genera; *Bovismacovirus, Cosmacovirus, Dragsmacovirus, Drosmacovirus, Huchismacovirus* and *Porprismacovirus*. Recently, it was shown that DNA matching a smacovirus was found in the archaeon "*Candidatus* Methanomassiliicoccus intestinalis", suggesting that smacoviruses may infect faeces-dwelling archaea [7]. Despite the broad range of animals with which smacoviruses have been associated, none have been documented in a member of the family *Felidae*. Felids, both domestic and wild, are known to harbour a multitude of viruses, including; feline immunodeficiency virus [13, 20], feline foamy virus [4, 11], feline leukaemia virus [5, 16], feline anellovirus [9, 21], feline gammaherpesvirus [2, 18], feline coronavirus [15], and several more.

In two separate studies, faecal samples were collected from bobcats (*Lynx rufus*) in California, USA, and African lions (*Panthera leo*) in the Serengeti National Park in Tanzania. Faecal samples described in this study were collected from three wild cats. One bobcat sample was collected during necropsy, the other from a live trap on the day of capture, and the African lion sample was collected shortly after defecation was observed. Viral DNA was extracted, and circular molecules were enriched from the faecal samples according to a previously described protocol [12]. Enriched DNA was sequenced on an Illumina 2500 platform, and contigs were generated through *de novo* assembly of paired-end reads using metaSPAdes v. 3.12.0 [1]. Three contigs with similarities to smacoviruses were identified in BLASTx searches and amplified from the three individual samples using abutting primers (Table 1) by PCR. The amplicons were then cloned and sequenced using the Sanger method [12].

We have tentatively named the three novel smacoviruses reported in this study "Lynx rufus associated smacovirus 1" (LruSmV 1, MK796234), "Lynx rufus associated smacovirus 2" (LruSmV 2, MK796235) and "Panthera leo associated smacovirus" (PlSmV, MK796236). Their genomes are 2,435

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Table 1 Sample information and primers used for recovery of full genomes by PCR

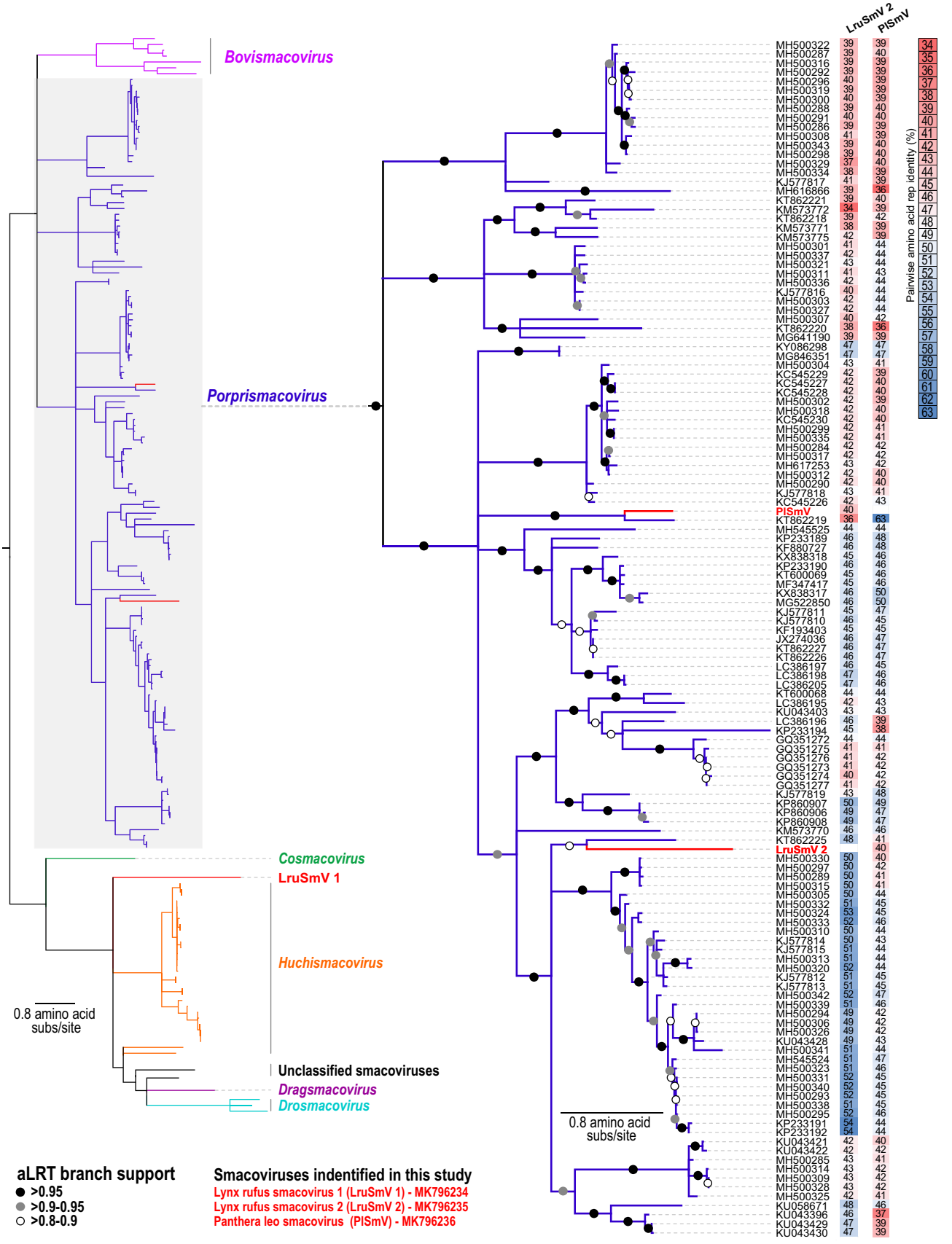
Sample source	Isolate name	Genome size (nt)	Forward primer	Reverse primer
Bobcat	Lynx rufus smacovirus 1	2,435	TTTCAGCAAGCTAGTAAGAAG GTGTTCATCGATGG	TGTATTCATCCTTCCATTACCTTC TCTATATCCGC
Bobcat	Lynx rufus smacovirus 2	2,595	CAACTTACTGGTGATTCGTCCTCT AATTATCCG	AGATACATTCTCATACACACCCTG ACCATAATACG
African lion	Panthera leo smacovirus	2,650	GTCCTTCTGTAGTATGCCATTTTA TACACC	CTATAGAAGGTCAAGGTACAGTTA CAGAAG

nt, 2595 nt and 2,650 nt, respectively, in size (Table 1). All contain two large open reading frames oriented in opposite directions, encoding the Rep and CP proteins. 174 complete smacovirus genome sequences available in the GenBank database were compiled and used for comparison with the three sequences reported here. Full-genome, Rep and CP amino acid sequence datasets were assembled and used to determine pairwise identity values using SDT 1.2 [14]. Genome-wide pairwise comparison showed that the three genomes share 54-57% nucleotide sequence identity. With all other smacoviruses, the closest relative for each is as follows: LruSmV 1 shares 59% sequence identity with chimpanzee associated porprismacovirus 2 (GQ351273) [3], LruSmV 2 shares 61% sequence identity with Chlorocebus cynosuros associated smacovirus (LC386199), and PlSmV shares 67% sequence identity with sheep faeces associated smacovirus 3 (KT862219) [17] (Supplementary Data 1). Rep and CP amino acid comparison with all smacoviruses showed they share 39-63% sequence identity in Rep and 35-60% sequence identity in CP (Fig. 1 and Supplementary data 1).

Smacovirus Rep amino acid sequences were aligned using MAFFT [10], and the resulting alignment was used to construct a maximum-likelihood phylogenetic tree using PhyML 3.0 [8] with the best-fit amino acid model rtREV+G+I+F selected using ProtTest [6]. Branches with less than 0.8 aLRT branch support were collapsed. The phylogenetic tree was rooted with the Rep sequences of the nanoviruses. Based on the taxonomic guidelines put forward by the *Smacoviridae* subcommittee for the International Committee on Taxonomy of Viruses [19], the results of phylogenetic analysis and sequence comparisons support the assignment of each of these three smacoviruses to a distinct species.

Fig. 1 Maximum-likelihood phylogenetic tree based on Rep protein sequences (left), displaying the six described genera. The LruSmV 1 Rep is most similar to that of Papio cynocephalus associated smacovirus (LC386204), sharing 38% amino acid sequence identity. An expanded subtree of the Rep-based phylogeny of the porprismacoviruses is displayed on the right. Names in red letters indicate the feline-faeces-associated Reps of the genomes identified in this study. A pairwise amino acid sequence comparison of the two novel feline smacovirus Rep proteins in the genus *Porprismacovirus* to those of all other members of the genus is shown next to the phylogenetic tree

Phylogenetic analysis showed that the Rep of LruSmV 1 is divergent, forming a singleton clade that is closest to the huchismacoviruses. The Reps of LruSmV 2 and PlSmV group in a clade consisting of the members of the genus *Porprismacovirus* (Fig. 1). The Rep proteins of LruSmV 2 and PlSmV share a common ancestor with porcine associated porprismacovirus 10 (KT862225) and sheep faeces associated smacovirus 3 (KT862219), respectively (Fig. 1) [17]. It is worth noting that these two LruSmVs were recovered from the faeces of individual bobcats living in the Los Angeles area (California, USA), but they are genetically distinct from each other. If smacoviruses do in fact infect archaea [7], this may indicate that these two distinct viruses are hosted by different archaeal species. Here, we describe the first smacoviruses associated with felid faeces and show that they are highly diverse, representing three distinct species. Continued research in this area is needed to determine the true hosts of these viruses and to elucidate what relationship they have to other felid viruses and those infecting other animals.


Bovismacovirus
Porprismacovirus
Cosmacovirus
LruSmV 1
Huchismacovirus
Unclassified smacoviruses
Dragsmacovirus
Drosmacovirus
0.8 amino acid subs/site
LruSmV 2
PlSmV
Pairwise amino acid rep identity (%)
0.8 amino acid subs/site
aLRT branch support
>0.95
>0.9-0.95
>0.8-0.9
Smacoviruses indentified in this study
Lynx rufus smacovirus 1 (LruSmV 1) - MK796234
Lynx rufus smacovirus 2 (LruSmV 2) - MK796235
Panthera leo smacovirus (PlSmV) - MK796236

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