Evaluating the diversity of the Feline Immunodeficiency Virus (FIV): a leopard perspective

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To obtain more insights into the prevalence and diversity of species-specific Feline Immunodeficiency Virus (FIV) strains in naturally occurring felid species, 26 leopards (*Panthera pardus*) from the Kruger National Park (KNP), South Africa, were sampled. Prevalence was determined using a PCR protocol designed to target a 577 bp fragment in the *pol*-RT gene. Overall prevalence of FIV_{Ppa} was estimated at 73%, with no difference in prevalence between male and female leopards. Consistent with previous FIV studies on other felid species, prevalence appears to increase with age (adult = 84%; subadult = 43%). Phylogenetic analyses of these novel sequences were conducted against a revised FIV *pol*-RT species-specific reference dataset using both Bayesian and maximum likelihood methods. Within FIV_{Ppa} two distinct evolutionary groupings are present, which suggests the possibility of geographic variation within FIV_{Ppa} and the possibility of distinct subtypes, similar to what has been found in lions (*Panthera leo*) and domestic cats (*Felis catus*). The larger FIV_{Ppa} dataset provides new insights into the epidemiology of this under-studied FIV strain and with such high prevalence rates, further studies should focus on immunological and clinical consequences of FIV in wild felids.

Key words: FIV diversity, FIV reference dataset, geographic variation, Kruger National Park, *Panthera pardus*, FIV *pol*-RT.

INTRODUCTION

Lentiviruses (*Retroviridae*) such as the well-known Human Immunodeficiency Virus (HIV) are not unique to humans and have been isolated from a number of mammalian taxa (Miller, Cairns, Bridges & Sarver, 2000). For example, two well-studied lentiviruses in animals include the primate associated Simian Immunodeficiency Virus (SIV) and the feline associated Feline Immunodeficiency Virus (FIV). HIV in humans represents a recent cross-species transmission of SIV from non-human primates, and apart from SIV, FIV represents the only other well-studied natural animal model to explain the evolution of Immunodeficiency Viruses in mammals. In fact, HIV, SIV and FIV are structurally, genomically and seemingly pathogenically similar (Pedersen, Yamamoto, Ishida & Hansen, 1989; Bendinelli *et al*., 1995; Miller *et al*., 2000; O'Brien, Troyer, Roelke, Marker & Pecon-Slattery, 2006).

FIV (previously feline T-lymphotropic lentivirus) was first described in the domestic cat (*Felis catus*) nearly 30 years ago (Pedersen *et al*., 1989) and has since been detected in several non-domestic felids (Olmsted *et al*., 1992; Brown, Miththapala & O'Brien, 1993; Troyer *et al*., 2005). Within the family Felidae (comprising 38 species), 19 species have been identified as sero-reactive to

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FIV, and infection has been confirmed in 11 species using PCR (Troyer *et al.*, 2005; Pecon-Slattery, Troyer, Johnson & O'Brien, 2008b). Lentiviruses infecting feline species are related (VandeWoude *et al.*, 1997; Troyer *et al.*, 2005) and in most instances FIV is species-specific (VandeWoude & Apetrei, 2006). Unique FIV lineages have been described in the domestic cat (FIV_{Fca}) (Olmsted *et al.*, 1989a; Olmsted, Hirsch, Purcell & Johnson, 1989b), African lion (*Panthera leo* – FIV_{Ple}) (Brown, Yuhki, Packer & O'Brien, 1994; Troyer *et al.*, 2005), puma (*Puma concolor* – FIV_{Pco}) (Olmsted *et al.*, 1992; Carpenter *et al.*, 1996), Pallas cat (*Otocolobus manul* – FIV_{Oma}) (Troyer *et al.*, 2005; Brown *et al.*, 2010), bobcat (*Lynx rufus* – FIV_{Lru}) (Franklin *et al.*, 2007; Lee *et al.*, 2012), cheetah (*Acinonyx jubatus* – FIV_{Aju}) (Troyer *et al.*, 2005), leopard (*Panthera pardus* – FIV_{Ppa}) (Carpenter *et al.*, 1996; Troyer *et al.*, 2005), ocelot (*Leopardus pardalis* – FIV_{Lpa}) (Troyer *et al.*, 2005), jaguarundi (*Puma yagouaroundi* – FIV_{Pya} formerly *Herpailurus yagouaroundi* – FIV_{Hya}) (Troyer *et al.*, 2005; Johnson *et al.*, 2006), snow leopard (*Panthera uncia* – FIV_{Pun}) (Troyer *et al.*, 2005) and spotted hyaena (*Crocuta crocuta* – FIV_{Ccr}) within the family Hyaenidae (Troyer *et al.*, 2005). The diversity among FIV strains within most of these host species is not that well documented, and more research is needed to answer questions regarding the presence and spatial distribution of FIV strains and subtypes. For those felids that have been more extensively studied, there is sufficient evidence to support large scale variation within species-specific strains of FIV. For example, five different monophyletic subtypes were detected in the domestic cat (FIV_{Fca} subtypes A–E), six in the lion (FIV_{Ple} subtypes A–F) and two in the puma (FIV_{Pco} subtypes A and B) as reviewed by VandeWoude and Apetrei (2006) and (O'Brien *et al.*, 2012). In some instances, these subtypes are also geographically confined (Troyer *et al.*, 2005; Antunes *et al.*, 2008).

Leopards are the largest of the spotted felids found in Africa (Skinner & Chimimba, 2005) and have a distribution that extends beyond that of many other felid species (Skinner & Chimimba, 2005; Henschel *et al.*, 2008). Like most members of the Felidae, leopards are solitary and are generally only seen together when males and females are mating or if a female is accompanied by subadults or cubs (Skinner & Chimimba, 2005; Macdonald & Loveridge, 2010). The large geographic but intermittent range of leopards across Africa leads to predictions that some geographic variation may be present in $FIV_{Ppa.}$ In addition, the solitary nature of leopards may limit contact between individuals, and the prevalence of FIV in leopards is therefore predicted to be lower than the prevalence of FIV in more social species such as lions.

Knowing the prevalence and diversity of FIV_{Ppa} in leopards is also important from a conservation perspective. The global leopard population is declining (Henschel *et al.*, 2008; Stein *et al.*, 2016) leading to the amendment of their conservation status from Near Threatened (Henschel *et al.*, 2008) to Vulnerable (Stein *et al.*, 2016) by the International Union for the Conservation of Nature (IUCN). Although the pathogenic effects of FIV in domestic cats have been more extensively studied, the pathogenic potential of the virus in wild felids is less well known (Brown *et al.*, 1994; Hofmann-Lehmann *et al.*, 1996; Pecon-Slattery *et al.*, 2008a; Adams *et al.*, 2009). In fact, baseline data for FIV in leopards is lagging behind as exemplified by the availability of only five FIV_{Ppa} *pol*-RT sequences in GenBank (Carpenter *et al.*, 1996; Troyer *et al.*, 2005).

This study aimed to extend the current knowledge on FIV diversity in wild felid species, and particularly in leopards. By screening free-ranging leopards occurring in the Kruger National Park (KNP), one of the largest protected areas in southern Africa, we aimed to provide new insights into the prevalence and diversity of FIV_{Ppa}. To place the newly documented findings in perspective with what is known for FIV in felids, we compare the prevalence of FIV to other felid species and provide a phylogenetic analysis of FIV using all previously published FIV sequences available from GenBank. Since no current predefined reference dataset exists for FIV, such as the Los Alamos HIV Database (http://www.hiv.lanl.gov), we proposed an updated FIV *pol*-RT reference dataset to be used in future studies (also see Troyer *et al.* (2005)). The FIV database represents a backbone where all duplicated sequences with different accession numbers were removed and it contains no missing data. For future referencing and expansion of the database, contributing authors and their associated studies were cited for each sequence and the sequence metadata was also summarized in tabular format.

MATERIALS AND METHODS

Sample collection

Between 2007 and 2013, 26 blood samples

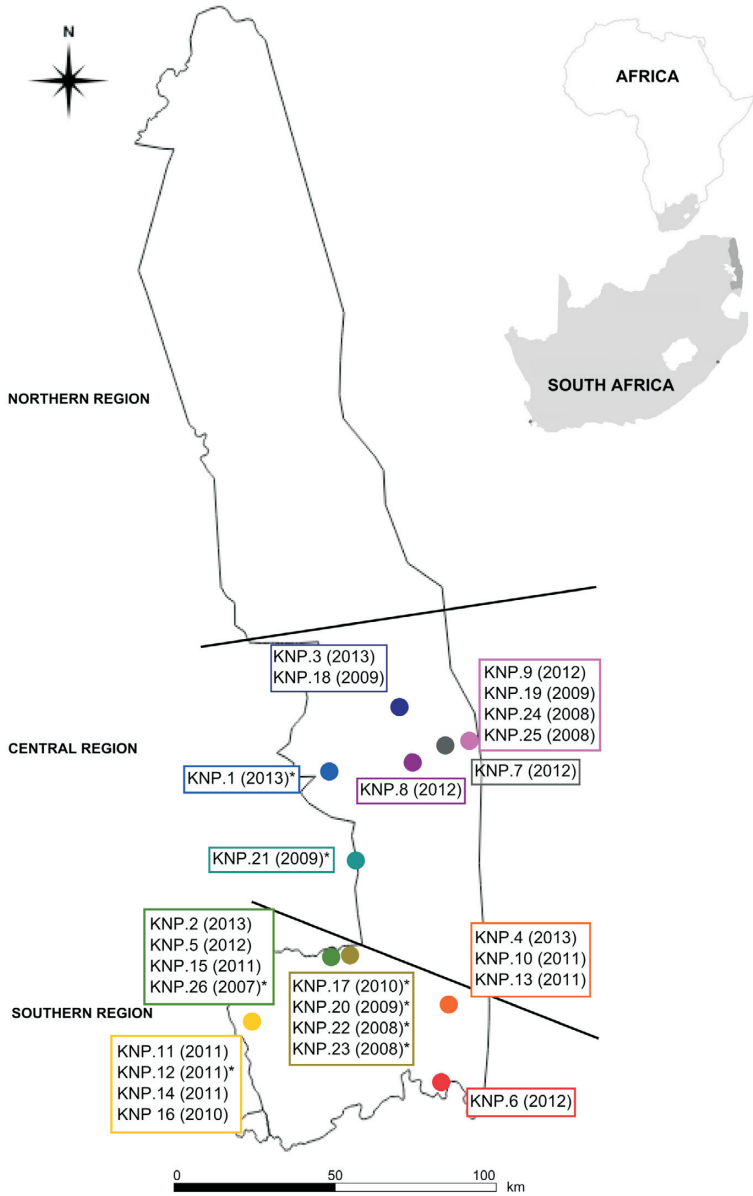


Fig. 1. Map of Kruger National Park indicating approximate sampling locations of the 26 leopards included in this study. Year of sampling is included in brackets. GPS data (exact sample location) was not available for all individuals. For these individuals sample location was inferred based on capture location (Table 1). These individuals are designated using an '*'. Insets represent the position of South Africa in Africa and the darkly shaded area within South Africa represent the scaled to size position of the Kruger National Park.

(preserved in EDTA) were opportunistically collected from free-ranging leopards that were live-trapped in the KNP, South Africa (Fig. 1; Table 1). Ethical approval for the procedure was provided by the South African National Parks (SANParks) Animal Use and Care Committee (Protocol #: 589MAPNW). All procedures including the planning and capture of leopards were undertaken by the Scientific Services and Veterinary Wildlife Services of SANParks, using methods ratified by the organization's Animal Use and Care Committee (SANParks, 2015). All blood samples were stored at –80°C in the Skukuza Biobank (SANParks, South Africa). Samples were transferred from the

Table 1. Demographic information and FIV status for the 26 free-ranging leopards sampled in Kruger National Park, South Africa.

ID #	Sex	Age	Sample year	FIV PCR	Location	Status
Ppa-KNP.1	M	Adult	2013	+	Kingfisherspruit	Wild
Ppa-KNP.2	M	Adult	2013	+	Mbabala Drainage, Skukuza	Wild
Ppa-KNP.3	F	Subadult	2013	+	Satara	Wild
Ppa-KNP.4	M	Adult	2013	–	Lower Sabie	Wild
Ppa-KNP.5	F	Adult	2012	+	Tinga, Skukuza	Wild
Ppa-KNP.6	F	Adult	2012	+	Crocodile Bridge	Wild
Ppa-KNP.7	F	Subadult	2012	–	Shishangane, Satara	Wild
Ppa-KNP.8	F	Subadult	2012	+	Satara	Wild
Ppa-KNP.9	F	Subadult	2012	–	Singita, Nwanetsi	Wild
Ppa-KNP.10	M	Adult	2011	+	Lower Sabie	Wild
Ppa-KNP.11	M	Adult	2011	+	Rest Camp, Pretoriuskop	Wild
Ppa-KNP.12	F	Adult	2011	+	Bomas, Pretoriuskop	Wild
Ppa-KNP.13	F	Adult	2011	+	Rest Camp, Lower Sabie	Wild
Ppa-KNP.14	M	Subadult	2011	+	Pretoriuskop	Wild
Ppa-KNP.15	F	Adult	2011	+	TingaNarina Lodge, Skukuza	Wild
Ppa-KNP.16	F	Adult	2010	+	Pretoriuskop	Wild
Ppa-KNP.17	M	Adult	2010	+	Skukuza	Wild
Ppa-KNP.18	M	Adult	2009	–	Satara	Wild
Ppa-KNP.19	M	Adult	2009	+	Singita, Nwanetsi	Wild
Ppa-KNP.20	M	Adult	2009	+	Skukuza	Wild
Ppa-KNP.21	M	Adult	2009	+	Mala Game Reserve	Wild
Ppa-KNP.22	M	Subadult	2008	–	State Vet Bomas, Skukuza	Wild
Ppa-KNP.23	F	Subadult	2008	–	Skukuza	Wild
Ppa-KNP.24	M	Adult	2008	–	Singita Lebombo, Nwanetsi	Wild
Ppa-KNP.25	M	Adult	2008	+	Singita, Nwanetsi	Wild
Ppa-KNP.26	M	Adult	2007	+	Sabie Sands Game Reserve	Wild

Leopard identification number (ID #); female (F); male (M); adult (>4 years); subadult (<4 years); PCR positive (+); PCR negative (–); Location (KNP section/area where leopards were sampled).

Biobank and stored at –20°C at the Division of Medical Virology, Stellenbosch University, prior to analysis.

PCR amplification of proviral DNA

Proviral DNA was automatically extracted from EDTA blood samples using a QIAGEN Blood Mini Kit and the QIACube (Qiagen, Hilden, Germany). Prior to PCR the DNA concentration (ng/µl) for each proviral DNA extraction was determined using the NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, U.S.A.). Nested PCR was performed using primers designed by Troyer *et al.* (2005) to target the conserved *pol*-RT region (577 bp) of the FIV_{Ppa} genome. All oligonucleotide primers were synthesized by Integrated DNA Technologies (IDT Inc., Coralville, Iowa, U.S.A.). First-round PCR was performed using 5 µl of extracted DNA (DNA concentration range 3.49–39.06 ng/µl) in a 50 µl reaction with a final concentration of 1x GoTaq® Flexi Buffer, 2 mM $MgCl_2$, 0.2mM concentrations of dNTPs, 0.4 µM concentration of each primer, and 2.5 units of GoTaq® DNA polymerase (Promega, Madison, WI, U.S.A.). The first round of PCR involved the pre-nested P1F and P2R primers and the cycle protocol on a GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems, Carlsbad, CA, U.S.A.) was 2 min at 94°C, 40 cycles of 20 s at 94°C, 30 s at 45°C, and 45 s at 68°C, followed by a final extension of 5 min at 68°C. The second round of PCR was performed using 1 µl of pre-nested PCR product with the nested primers P2F and P1R, and a cycle protocol of 2 min at 94°C, 30 cycles of 20 s at 94°C, 30 s at 45°C and 45 s at 68°C, followed by a final extension of 5 min at 68°C. Negative controls were run alongside all PCR reactions to ensure that reagents were not contaminated during PCR. Agarose gel electrophoresis was performed on second-round

PCR products to confirm amplification of nucleic acid fragments.

The representative second-round PCR products were purified using the Wizard® SV Gel and PCR Clean-Up System (Promega, Madison, WI, U.S.A.) according to the manufacturer's instructions, and subsequently sequenced using the BigDye® Terminator v3.1 Cycle Sequencing Kit and standard procedures (Applied Biosystems, Carlsbad, CA, U.S.A.). Sequencing was undertaken in both the forward and reverse direction. Sequenced products were purified using BigDye® XTerminator Purification Kit (Applied Biosystems, Carlsbad, CA, U.S.A.) and analysed on the 3130xl Genetic Analyser (Applied Biosystems, Carlsbad, CA, U.S.A.). Sequencher® Version 5.0 (Gene Codes Corporation, Ann Arbor, MI, U.S.A.) was used to edit and align the contigs. Sequences were authenticated using the National Centre for Biotechnology Information Basic Local Alignment Search Tool (BLAST) software program (Altschul, Gish, Miller, Myers & Lipman, 1990). Prior to phylogenetic analysis, multiple sequence alignment was undertaken using ClustalX Version 2.1 (Larkin *et al.*, 2007). Sequence alignment was also optimized by using Codon Alignment Version 2.1.0 (http://www.hiv.lanl.gov) and manual adjustment.

Phylogenetic analysis

To place the diversity of newly generated FIV_{Ppa} sequence data in context with the existing knowledge on FIV in all felid species, a *pol*-RT reference dataset was generated (Supporting Text; Fig. S1; Table S1 – Supplementary Material). All sequences that met the minimum requirements as outlined in the supplementary materials were phylogenetically analysed using the Maximum Likelihood method implemented in MEGA Version 6 (Tamura, Stecher, Peterson, Filipski & Kumar, 2013). To find the most suitable evolutionary model for phylogenetic inference, Model Selection (ML) within MEGA was used together with the Bayesian Information Criterion (BIC), (Nei & Kumar, 2000). Bootstrap analysis included 1000 iterations. In addition, Bayesian posterior probabilities were determined using Markov Chain Monte Carlo methods in MrBayes Version 3.2 (Huelsenbeck & Ronquist, 2001). The initial tree was random, and trees were sampled every 100 generations from a total of one million generations. Burn-in value corresponds to 25% of samples. To determine possible geographic structure among novel FIV_{Ppa} sequences, we repeated the above analyses including all 19 FIV_{Ppa} sequences obtained in the present study. Average sequence divergence and diversity values were derived from MEGA Version 6 using the optimal model of sequence evolution. Sequences were partitioned into distinct species-specific groups and then analysed to estimate the average evolutionary divergence and diversity together with the standard error for each.

RESULTS

Prevalence of FIV in leopards

Of the 26 leopard samples tested, PCR returned 19 positive samples resulting in an overall prevalence of 73%. Both male (n = 15) and female (n = 11) leopards showed an estimated prevalence of 73%. The prevalence of FIV in adult leopards (>4 years) was much higher (84%; n = 19) than the prevalence of FIV in subadult individuals (<4 years) (43%; n = 7). Reliable FIV *pol*-RT sequences of over 500 bp in length were obtained from all positive samples, and these 19 FIV_{Ppa} sequences were deposited in GenBank under accession numbers KU705335–KU705353.

Phylogenetic analysis and sequence diversity of FIV *pol*-RT sequences

For the FIV *pol*-RT reference dataset the data was trimmed at the ends to exclude all missing data. The phylogenetic analyses incorporated first a rooted approach using the FIV_{Cco} strain (Genbank accession numbers AY878196–AY878200 (Troyer *et al.*, 2005)) and since rooting in FIV is variable and thus problematic (see Carpenter *et al.* (1996); Troyer *et al.* (2005); Franklin *et al.* (2007) and Antunes *et al.* (2008)), we also employed midpoint rooting. The rooted phylogeny was based on 83 sequences comprising 320 bp in length and the final alignment of the midpoint rooted tree comprised 78 sequences of 369 bp in length. Both the rooted and unrooted trees resulted in similar overall tree topologies (see also Franklin *et al.* (2007)). To prevent duplication in presentation, we only present the analyses based on midpoint rooting. The BIC score supported the GTR+G+I as the best fit model for the midpoint rooted phylogeny. For the expanded dataset, which was also midpoint rooted (an additional 14 closely related leopard sequences were included to test for phylogeographic structure) the optimal model of evolution was T92+G+I. In both analyses all FIV_{Ppa} sequences obtained from the KNP sampling site were monophyletic with significant posterior prob-

ability (≥95%) and strong bootstrap support (ML Bootstrap = 92, Fig. 2; and ML Bootstrap = 99, Fig. 3). However, the monophyly of all FIV_{Ppa} sequences, was never retrieved in our analyses, since the monophyletic KNP clade is nested within a second clade comprising FIV from leopard, cheetah and Pallas cat (ML Bootstrap = 95; Fig. 2 and ML Bootstrap = 92; Fig. 3). In the present study, at least two distinct monophyletic lineages of FIV_{Ppa} are evident (Fig. 2; Fig. 3) albeit one of them with low bootstrap support (ML Bootstrap <70). These two lineages represent a common KNP Group (n = 20; inclusive of the previously published KNP sequence (Carpenter *et al.*, 1996)) and a group derived from leopards of unknown geographic origin (n = 4). Within the KNP group there does not appear to be any significant geographic structure related to the sampling location of the individual leopards and their associated viral sequences (Fig. 1; Fig. 3).

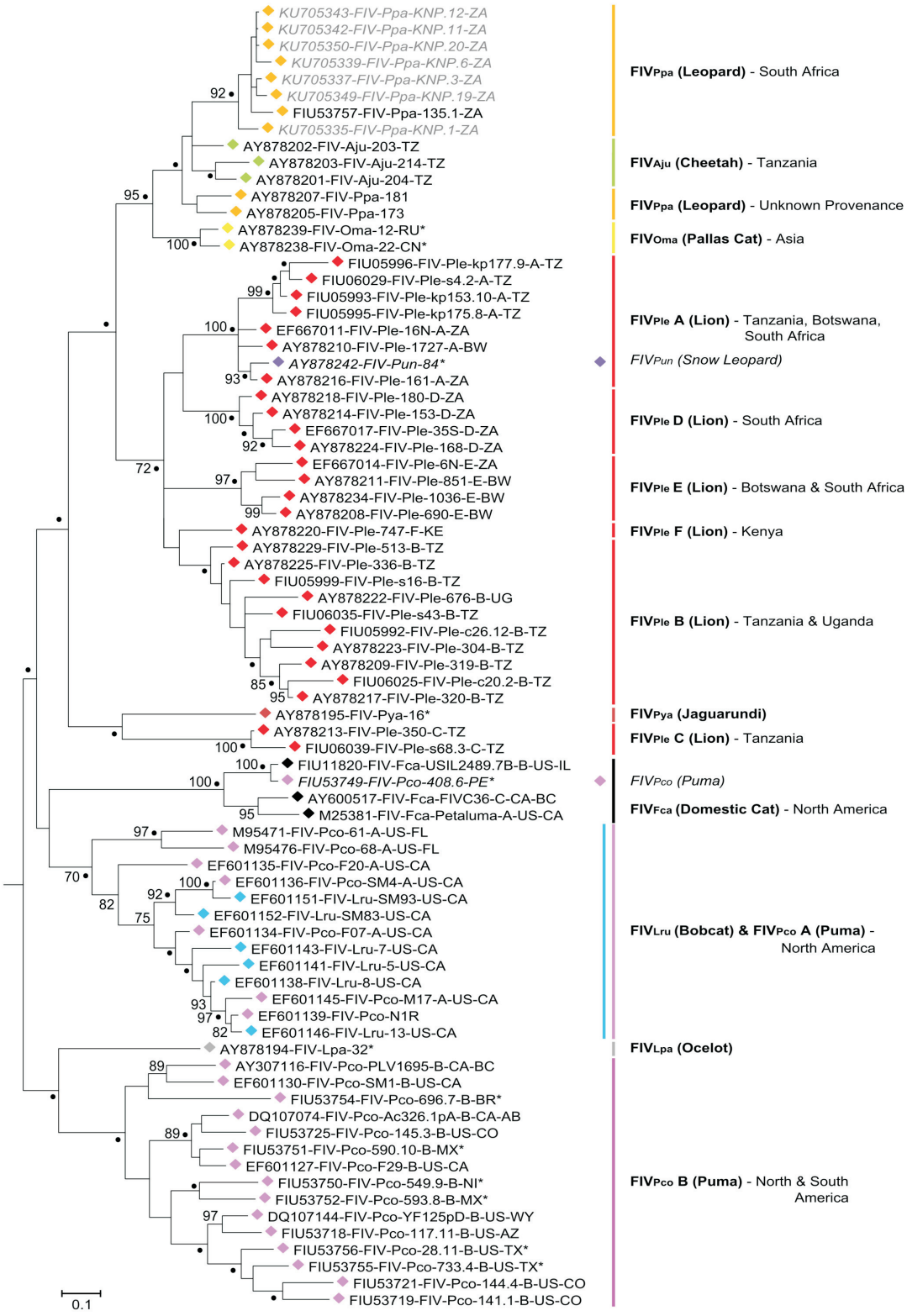
The average sequence divergence for all sequence pairs in the FIV *pol*-RT reference dataset shows a mean value of 40.7 ± 4.2% for FIV *pol*-RT. Even though the imbalance in sample sizes and limited geographic sampling can bias the comparisons between and within host species, it is interesting to note that with the data at hand the estimation of the sequence diversity based on the FIV *pol*-RT region for each species-specific group (where at least three or more sequences allowed for inclusion in the analysis) range from 7.6 ± 1.8% in FIV_{Oma} to a high of 35.4 ± 3.8% in FIV_{Pco} (Fig. 4). Analysis of divergence between each of the species-specific groups based on the FIV *pol*-RT region ranged from a low of 13.5 ± 1.9% between FIV_{Ppa} and FIV_{Aju} to a high of 68.7 ± 11.3% between FIV_{Fca} and FIV_{Pun} (Fig. 4). The genetic diversity of 7.9 ± 1.1% for the currently sampled FIV_{Ppa} is thus towards the lower end of the spectrum (genetic diversity for the KNP group is 4.1 ± 0.7% while it is 10.9 ± 2.3% for the sequences on GenBank with an unknown geographic origin). Interestingly, the genetic divergence between the two leopard clades detected in our analyses is 14.3 ± 3.8%, and is also lower than the genetic divergences between the six FIV_{Ple} subtypes which range from as low as 18.8 ± 2.7% for FIV_{Ple} subtype B and F to as high as 45.0 ± 7.0% for FIV_{Ple} subtype C and D (Fig. 4). It is also lower than the genetic divergence of 45.1 ± 5.5% for the two FIV_{Pco} subtypes (Fig. 4).

DISCUSSION

Prevalence of FIV in leopards

To date, a limited number of FIV *pol*-RT sequences have been available for leopards (n = 5). Here we provide sequence data for 19 new isolates and report a FIV_{Ppa} prevalence of 73% for the KNP leopard population. The PCR-detected prevalence is similar to the seroprevalence of 71% reported by Brown *et al.* (1993) for free-ranging leopards in KNP. These estimates are however considerably higher than the seroprevalence of 17% reported by Osofsky, Hirsch, Zuckerman & Hardy (1996) for leopards from Botswana and the seroprevalence of between 26 and 46% reported by Troyer *et al.* (2005) for leopards from across Africa. The congruence between the seroprevalence and PCR detected prevalence of FIV_{Ppa} in KNP leopards is remarkable since a direct comparison between these methods is problematic. One of the limitations of using only PCR to estimate prevalence is that individuals which have a viral load below the detectable limit of a particular PCR may be missed (Troyer *et al.*, 2004). Serological testing, on the other hand, has not been optimized for all wild felid species and the level of accuracy for this method has also been questioned (Troyer *et al.*, 2005). Making accurate prevalence predictions is thus not only reliant on large samples sizes (Biau, Kerneis & Porcher, 2008) but is dependent on sensitive and specific tests. To make a firm conclusion on the prevalence of FIV_{Ppa} in leopards, a much bigger sampling effort over a larger geographic area would be required. The latter is needed since prevalence has also been shown to vary substan-

Fig. 2. Bayesian tree of 78 FIV *pol*-RT sequences (369 bp) isolated from the 10 felid species included in the proposed FIV *pol*-RT reference dataset. Posterior probabilities ≥0.95 are indicated with '•'. ML Bootstrap values above 70% (1000 iterations) are also indicated where found. The tree is midpoint rooted. Each sequence is designated by a unique sequence name comprising of GenBank accession number, virus abbreviation, felid species from which sample was collected, individual sample ID, FIV subtype where available, and country of origin (Table S1 – Supplementary Material). Sequences isolated from captive/wild-born captive animals are designated by '*', all other sequences were isolated from wild animals. Sequences are colour coded according to the host species from which they were isolated. Novel FIV_{Ppa} sequences are indicated using grey italics font. Sequences which represent cross-species transmissions in captive settings are indicated with black italics font and shaded '♦'.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KU705342-FIV-Ppa-KNP.11-ZA
KU705350-FIV-Ppa-KNP.20-ZA
KU705339-FIV-Ppa-KNP.6-ZA
KU705337-FIV-Ppa-KNP.3-ZA
KU705349-FIV-Ppa-KNP.19-ZA
FIU53757-FIV-Ppa-135.1-ZA
KU705335-FIV-Ppa-KNP.1-ZA
AY878202-FIV-Aju-203-TZ
AY878203-FIV-Aju-214-TZ
AY878201-FIV-Aju-204-TZ
AY878207-FIV-Ppa-181
AY878205-FIV-Ppa-173
AY878239-FIV-Oma-12-RU*
AY878238-FIV-Oma-22-CN*
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FIU06029-FIV-Ple-s4.2-A-TZ
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AY878242-FIV-Pun-84*
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AY878208-FIV-Ple-690-E-BW
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AY878229-FIV-Ple-513-B-TZ
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EF601141-FIV-Lru-5-US-CA
EF601138-FIV-Lru-8-US-CA
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FIU53752-FIV-Pco-593.8-B-MX*
DQ107144-FIV-Pco-YF125pD-B-US-WY
FIU53718-FIV-Pco-117.11-B-US-AZ
FIU53756-FIV-Pco-28.11-B-US-TX*
FIU53755-FIV-Pco-733.4-B-US-TX*
FIU53721-FIV-Pco-144.4-B-US-CO
FIU53719-FIV-Pco-141.1-B-US-CO
0.1
FIVPpa (Leopard) - South Africa
FIVAju (Cheetah) - Tanzania
FIVPpa (Leopard) - Unknown Provenance
FIVOma (Pallas Cat) - Asia
FIVPle A (Lion) - Tanzania, Botswana, South Africa
FIVPun (Snow Leopard)
FIVPle D (Lion) - South Africa
FIVPle E (Lion) - Botswana & South Africa
FIVPle F (Lion) - Kenya
FIVPle B (Lion) - Tanzania & Uganda
FIVPya (Jaguarundi)
FIVPle C (Lion) - Tanzania
FIVPco (Puma)
FIVFca (Domestic Cat) - North America
FIVLru (Bobcat) & FIVPco A (Puma) - North America
FIVLpa (Ocelot)
FIVPco B (Puma) - North & South America

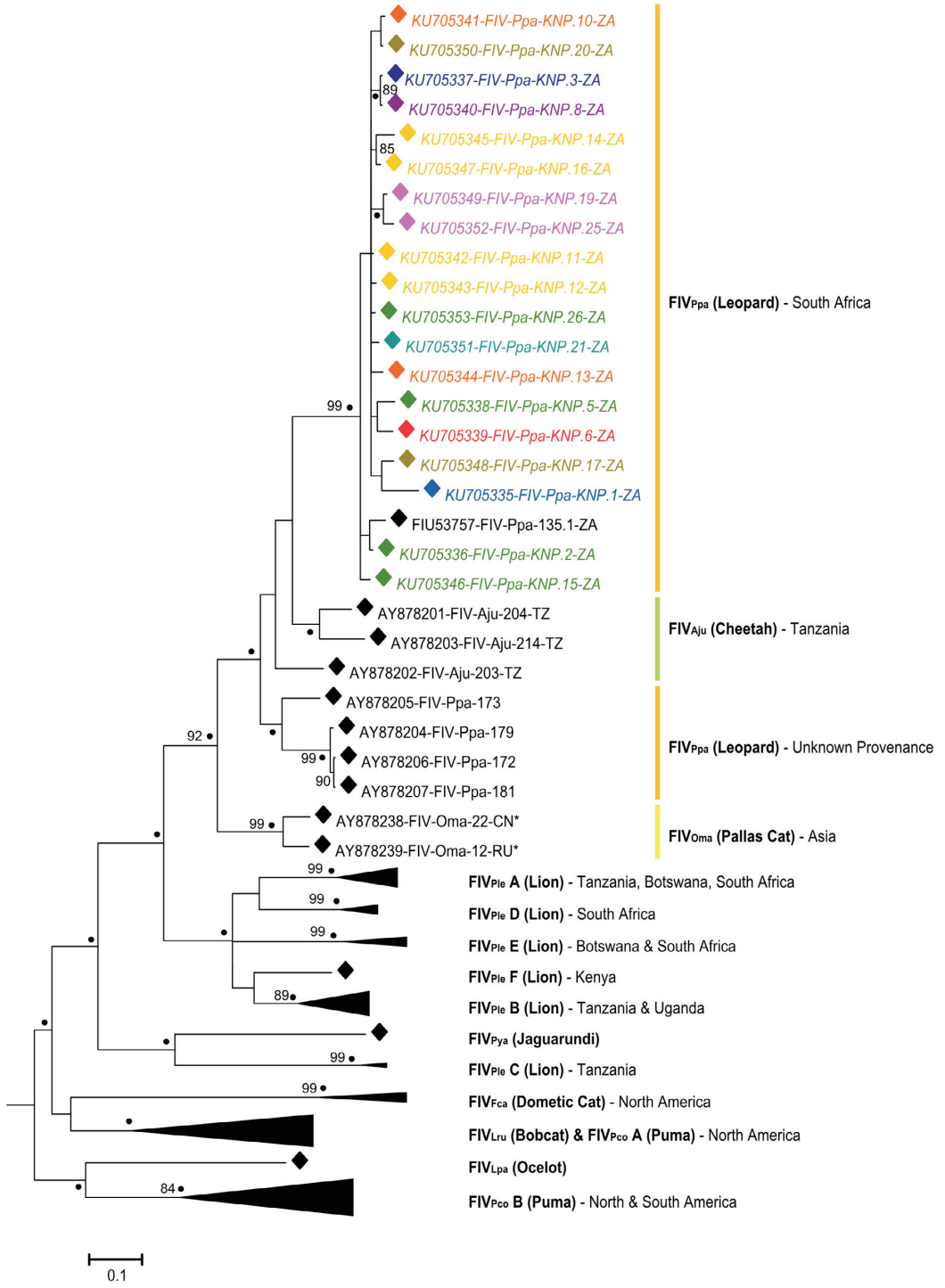

Fig. 3. Bayesian tree of 92 FIV *pol*-RT sequences (369 bp) comparing all available FIV_{Ppa} sequences ($n = 24$) to the proposed FIV *pol*-RT reference dataset. Posterior probabilities ≥0.95 are indicated with '•'. ML Bootstrap values above 70% (1000 iterations) are also indicated. The tree is midpoint rooted. Each sequence is designated as in Fig. 2. Novel FIV_{Ppa} sequences isolated from KNP leopards are colour coded to correspond with the sampling locations in Fig. 1.

Average evolutionary divergence within and between each of the 10 FIV clades										
FIV_{Lpa} *	**0 ± 0**									
FIV_{Pya} *	35.8 ± 6.2	**0 ± 0**								
FIV_{Pun} *	48.8 ± 8.4	45.3 ± 7.5	**0 ± 0**							
FIV_{Oma} *	53.4 ± 9.2	46.1 ± 7.9	36.5 ± 6.1	**7.6 ± 1.8**						
FIV_{Aju} *	41.7 ± 6.5	42.5 ± 6.8	31.2 ± 4.9	19.7 ± 3.0	**12.8 ± 2.2**					
FIV_{Ppa} *	42.6 ± 6.2	37.9 ± 6.0	32.8 ± 5.3	20.4 ± 3.1	13.5 ± 1.9	**7.9 ± 1.1**				
FIV_{Ple} *	51.0 ± 7.0	42.8 ± 6.0	26.1 ± 3.3	35.2 ± 4.4	31.9 ± 3.8	31.0 ± 3.8	**27.2 ± 2.9**			
FIV_{Pco} *	41.0 ± 5.3	43.9 ± 5.9	55.7 ± 7.4	49.0 ± 6.4	42.9 ± 5.2	41.3 ± 4.8	55.8 ± 6.6	**35.4 ± 3.8**		
FIV_{Lru} *	41.1 ± 6.4	39.4 ± 5.9	48.8 ± 7.4	38.3 ± 5.7	33.5 ± 4.5	32.6 ± 4.2	47.2 ± 5.8	37.7 ± 4.5	**16.0 ± 2.2**	
FIV_{Fca} *	51.7 ± 7.8	56.8 ± 8.7	68.7 ± 11.3	44.1 ± 6.5	44.9 ± 6.4	38.6 ± 5.2	65.9 ± 9.0	47.7 ± 5.7	44.6 ± 6.3	**20.4 ± 3.0**
FIV	**FIV_{Lpa}** *	**FIV_{Pya}** *	**FIV_{Pun}** *	**FIV_{Oma}** *	**FIV_{Aju}** *	**FIV_{Ppa}** *	**FIV_{Ple}** *	**FIV_{Pco}** *	**FIV_{Lru}** *	**FIV_{Fca}** *

FIV_{Lpa} - Ocelot - *Leopardus pardalis*	*
FIV_{Pya} - Jaguarundi - *Puma yagouaroundi*	*
FIV_{Pun} - Snow Leopard - *Panthera unica*	*
FIV_{Oma} - Pallas Cat - *Otocolobus manul*	*
FIV_{Aju} - Cheetah - *Acinonyx jubatus*	*
FIV_{Ppa} - Leopard - *Panthera pardus*	*
FIV_{Ple} - African Lion - *Panthera leo*	*
FIV_{Pco} - Puma - *Puma concolor*	*
FIV_{Lru} - Bobcat - *Lynx rufus*	*
FIV_{Fca} - Domestic Cat - *Felis catus*	*

Average evolutionary divergence within and between each of the six FIV_{Ple} subtypes						
FIV_{Ple} A	**14.4 ± 2.1**					
FIV_{Ple} B	29.7 ± 3.9	**15.4 ± 1.8**				
FIV_{Ple} C	41.8 ± 6.1	41.4 ± 5.7	**8.8 ± 2**			
FIV_{Ple} D	30.2 ± 4.3	27.4 ± 3.7	45.0 ± 7.0	**9.7 ± 1.7**		
FIV_{Ple} E	30.2 ± 4.1	27.3 ± 3.5	39.4 ± 5.6	26.2 ± 3.6	**13.8 ± 2.1**	
FIV_{Ple} F	28.2 ± 4.4	18.8 ± 2.7	34.4 ± 5.6	25.0 ± 4.0	25.9 ± 4.0	**0 ± 0**
FIV_{Ple}	**FIV_{Ple} A**	**FIV_{Ple} B**	**FIV_{Ple} C**	**FIV_{Ple} D**	**FIV_{Ple} E**	**FIV_{Ple} F**

Average evolutionary divergence within and between each of the FIV_{Pco} subtypes		
FIV_{Pco} A & FIV_{Lru}	**19.7 ± 2.3**	
FIV_{Pco} B	45.1 ± 5.5	**26.3 ± 3.0**
FIV_{Pco} & FIV_{Lru}	**FIV_{Pco} A & FIV_{Lru}**	**FIV_{Pco} B**

Fig. 4. Diversity schematic for 78 FIV *pol*-RT sequences (369 bp) used in the reference dataset showing estimates of average sequence diversity and divergence within and between the 10 FIV clades as well as the average evolutionary divergence within and between subtypes associated with FIV_{Ple}, FIV_{Pco} and FIV_{Lru}. Major FIV clades are designated in colour by an * which correspond with the colours used in Fig. 2 and Fig. 3.

tially within felid species depending on geographic area and study design (Brown *et al.*, 1993; Osofsky *et al.*, 1996; Troyer *et al.*, 2005).

The seroprevalence and PCR prevalence of above 70% detected in KNP leopards is higher than the seroprevalence of FIV detected in the seven non-domestic solitary felid species (puma, bobcat, cheetah, Pallas cat, ocelot, jaguarundi, snow leopard). Only lions which are considered to be the only truly social cats (Spencer *et al.*, 1992; Stuart & Stuart, 2007) have an overlapping or higher seroprevalence of FIV ranging from 67–100% (Olmsted *et al.*, 1992; Brown *et al.*, 1993; Troyer *et al.*, 2005; Roelke *et al.*, 2009). The relatively high prevalence of FIV in the KNP leopard population could potentially be associated with the relatively high density of leopards in KNP, which would increase the potential for intraspecific interactions and the transmission of FIV (Franklin *et al.*, 2008). Most habitat types within KNP support leopard densities between 12.7–30.3 individuals/100 km^2 (Chase Grey, Kent & Hill, 2013; Maputla, 2014) which exceeds densities in other parts of South Africa (0.6–11.11/100 km^2) (Chapman & Balme, 2010; Martins, 2011; Swanepoel, Somers & Dalerum, 2015), Botswana (6.9–7.5/100 km^2) (Steyn, 2007), Namibia (1–3.6/100 km^2) (Stein, Fuller, DeStefano & Marker, 2011), East Africa (3.8–8.5/100 km^2) (Mizutani & Jewell, 1998) and West Africa (2.7–12.1/100 km^2) (Jenny, 1996). Another possibility for the higher infection rates in KNP than elsewhere on the African continent may be related to sampling bias. In contrast to the study by Osofsky *et al.* (1996) where all leopard samples originated from legal hunting, a large percentage of the animals in the current study were animals caught in baited cage traps. It is possible that baited traps selected for animals in lower body condition (Bisi *et al.*, 2011), since these animals are most likely immune compromised and can thus benefit more through engaging in riskier behaviour in order to benefit from food incentives.

Age-related infection has previously been observed in both solitary and social felids including African lions (FIV_{Ple}) (Spencer *et al.*, 1992; Adams *et al.*, 2009), pumas (FIV_{Pco}) (Biek *et al.*, 2003) and bobcats (FIV_{Lru}) (Lee *et al.*, 2012). Based on a small samples size used in the present study, FIV prevalence in leopards in the KNP also appears to increase with age, with less than 50% of subadult leopards infected and over 80% of adult leopards infected. From our data, there also appears to be no difference in the overall prevalence of FIV between male and female leopards. The latter suggests that differences in life history between males and females (such as social contact and aggression) is not playing a large role in the transmission of FIV in leopards.

Phylogenetic analysis and sequence diversity of FIV *pol*-RT sequences

Analyses of the updated and revised FIV *pol*-RT reference dataset (Supplementary Material) includes sequences previously not included in broad scale FIV studies and thus provides the most up to date analysis of all currently published FIV strains. Troyer *et al.* (2005) previously described FIV_{Ppa} as monophyletic; we suggest here the possibility of two distinct monophyletic groups within FIV_{Ppa} and therefore the possibility of two discrete FIV_{Ppa} subtypes. By comparing the two FIV *pol*-RT lineages observed in leopards to the FIV_{Ple} subtypes observed in lions, some additional inferences can be made. Previous studies on lions suggested that subtypes diversify based on geographic location (Antunes *et al.*, 2008), such as FIV_{Ple} subtype A (southern African and East African Isolate) which forms two geographically isolated monophyletic clades (Fig. 2). Since the genetic diversity within FIV_{Ppa} is less than the divergence between previously described FIV_{Ple} subtypes it is more likely a single monophyletic FIV_{Ppa} clade as suggested by Troyer *et al.* (2005), but may be subdivided due to geographic location of the host species. Since to date only well-studied FIV strains (FIV_{Fca}, FIV_{Pco} and FIV_{Ple}) have been divided into subtypes (Fig. 2; Fig. 4), an expanded geographic sampling of leopard FIV is needed to provide more clarity.

Should the paraphyletic clustering of leopard FIV, and the low sequence divergence between FIV_{Ppa} and FIV_{Aju} hold, then it is interesting to speculate about the potential evolutionary mechanisms involved. It is well established that virus strains isolated from different species of felids often cluster according to the geographic region of host species rather than the strict phylogenetic relationships of the hosts (Franklin *et al.*, 2007; Troyer *et al.*, 2008). Our phylogenetic tree suggests that KNP leopards share a FIV strain that is closer to FIV isolated from Tanzanian cheetah than to other leopards sampled outside KNP (Fig. 3). This finding does not fully corroborate the idea that host switching among felid species is facilitated by taxa sharing overlapping geographic ranges (Franklin *et al.*, 2007; Troyer *et al.*, 2008). In the case of the KNP leopard

population, it is more parsimonious to suggest that the FIV_{Ppa} sequences isolated from leopards carry a signal of ancestral cross-species infection between leopard and cheetah since these sequences group together nested within a larger clade. However, the similar sequence diversity within FIV_{Aja} (12.8 ± 2.2%; Fig. 4) when compared to the sequence divergence between FIV_{Ppa} and FIV_{Aja} (13.5 ± 1.9%; Fig. 4), and the paraphyletic clustering of $FIV_{Ppa,}$ may also suggest that these two species-specific stains of FIV are in fact polyphyletic and not fully diverged from each other. Additional cheetah and leopard samples are clearly needed to obtain a more accurate assessment of the mechanisms involved in the evolution of leopard FIV.

At the smaller geographic scale, within the KNP sampled population, there appears to be an absence of geographic structure related to the sampling location of the individual leopards and their associated viral sequences. In five of the seven cases where FIV_{Ppa} lineages cluster together, the hosts originated from different sampling localities (Fig. 1; Fig. 3). These close evolutionary associations among the lineages is further supported by the low genetic diversity of FIV_{Ppa} within KNP (4.1 ± 0.7%).

The discovery of a high prevalence and seemingly geographically structured FIV_{Ppa} strains has some conservation relevance. It has been reported that the pathogenic effects of FIV may vary between the different strains of FIV but in some instances inferences on pathogenic potential is highly speculative (Brown *et al.*, 1994; Hofmann-Lehmann *et al.*, 1996; Pecon-Slattery *et al.*, 2008a; Adams *et al.*, 2009). As mentioned previously, there is still much speculation surrounding the pathogenic potential of FIV in wild felids. It has been suggested that wild felids may be tolerant to their species-specific FIV strains (Carpenter & O'Brien, 1995). However, more recent studies have recorded that FIV_{Ple} positive lions develop lymphocyte deficiencies, shown by a decrease in the total number of CD4+ and CD8+ T-cells and highlights the importance of monitoring the number of CD4+ cells in infected animals to examine disease progression (Bull *et al.*, 2003; Roelke *et al.*, 2006). In addition, Roelke *et al.* (2009) found that lions infected with FIV_{Ple} exhibit symptoms associated with immunodeficiency viruses (HIV, SIV and FIV_{Fca}) such as poor overall health and condition, oral lesions (gingivitis and oral papillomas), dehydration, abnormal red blood cell parameters and evidence of lymphadenopathy (enlarged lymph nodes). Considering this and the high prevalence of FIV_{Ppa} detected in leopards we could speculate that the KNP leopard population may be at risk of disease, and thus recommend long-term monitoring of the individuals identified as FIV positive in this study. This will provide new insights into disease progression in wild leopards and can aid in the conservation of this threatened large felid species.

Although our study is based on limited sampling, in a small geographic area, it highlights the need for more systematic sampling of FIV in many felid species. These data are critically needed to better understand the real distribution and prevalence of FIV, how the virus changes over time, what role cross-species infections play and most importantly whether FIV infection pose a significant conservation risk to the health of free-ranging felines.

SUPPLEMENTARY MATERIAL

Supporting text: FIV *pol*-RT Reference Dataset.

Fig. S1. Flow chart showing how the FIV *pol*-RT reference dataset was complied, indicating inclusion and exclusion criteria.

Table S1. Metadata FIV *pol*-RT Reference Dataset.

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Evaluating the diversity of the Feline Immunodeficiency Virus (FIV): a leopard perspective,

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Supporting Text – FIV *pol*-RT Reference Dataset

Materials and Methods

Developing a reference dataset of FIV pol-RT sequences for comparative analysis

All available FIV sequences isolated from non-domestic felid species were downloaded from GenBank using a search executed in Geneious Version 8 (Kearse *et al.*, 2012). The FIV sequences were then sorted by gene region, felid species from which the sequence was isolated, and author. For this study, we focused specifically on the FIV *pol*-RT gene region. To standardize the FIV *pol*-RT reference dataset, all sequences with stop codons in the first reading frame were excluded. FIV isolated from the domestic cat (FIV_{Fca}), and the novel FIV *pol*-RT sequences isolated from leopards (FIV_{Ppa}) were added. Due to the variability in sequence length, ends from the FIV *pol*-RT reference dataset were trimmed and then subjected to multiple sequence alignment (Fig. S1 – Supplementary Material) using ClustalX Version 2.1 (Larkin *et al.*, 2007). Sequence alignment was also optimized by using Codon Alignment Version 2.1.0 (http://www.hiv.lanl.gov) and manual adjustment. A 421bp fragment that included all FIV strains was generated from the alignment. Percentage identity between sequences in the database was then determined using uncorrected p-values. Sequence selection/retention was done in such a way as to maximize the variation and the geographic range of sampling. For sequences that had a percentage identity score of between 95% and 100%, only one sequence was retained, except in cases where sequences were more than 95% similar, but isolated from different felid species. To exclude all missing data, the resulting FIV *pol*-RT reference dataset was further trimmed to a final alignment length of 369bp (Fig. S1; Table S1 – Supplementary Material).

FIV sequence nomenclature

For ease of reference when interpreting phylogenetic trees, each FIV *pol*-RT sequence was designated by a unique sequence name (for example FIU53757-FIV-Ppa-135.1-ZA or FIU05996-FIV-Ple-kp177.9-A-TZ) comprising an accession number as obtained from GenBank, virus abbreviation, felid species from which sample was collected (abbreviated genus and species identifiers as a 3 letter code), individual sample ID (as designated in each individual study), FIV subtype (for felid species where FIV subtypes have previously been identified), two-letter country code as designated by the International Organisation for Standardisation (ISO 3166 Country Codes). FIV_{Ple} and FIV_{Pco} sequences that had not been assigned a subtype in their GenBank entry were identified by means of a BLAST search and assigned a subtype accordingly.

Results

FIV pol-RT reference dataset

The overall number of FIV sequences included in the proposed updated FIV *pol*-RT reference dataset comprised 78 sequences (Fig. S1; Table S1 – Supplementary Material). Noticeable differences between the updated FIV *pol*-RT reference dataset and the one used by Troyer *et al.* (2005) is the inclusion of FIV_{Ple} subtype E sequences isolated by Adams, van Vuuren, Bosman, Kania, and Kennedy (2011), six FIV_{Lru} sequences isolated from bobcats (Franklin *et al.*, 2007), one previously published leopard (FIV_{Ppa}) sequence (Carpenter *et al.*, 1996) and seven novel FIV_{Ppa} sequences isolated from South African leopards from the KNP as found herein. In the present dataset, it was decided to exclude the FIV_{Ccr} sequences isolated from spotted hyena to limit missing data (the FIV_{Ccr} sequences in GenBank are <350bp).

Discussion

FIV pol-RT reference dataset

Reviewing all previously published FIV *pol*-RT sequences isolated from wild felids, and the development of an updated FIV *pol*-RT reference dataset was prompted by the absence of an updated predefined reference dataset against which to compare novel FIV sequences of leopard. The variability in length of the FIV *pol*-RT sequences and the resulting alignment was the limiting factor in the development of the proposed FIV *pol*-RT reference dataset (Fig. 2; Fig. S1– Supplementary Material). For well-studied species such as the domestic cat, African lion, bobcat and puma there is an abundance of FIV *pol*-RT sequences of variable lengths (Olmsted *et al.*, 1989a; Olmsted, Hirsch, Purcell, & Johnson, 1989b; Talbott *et al.*, 1989; Phillips *et al.*, 1990; Olmsted *et al.*, 1992; Brown, Yuhki, Packer, & O'Brien, 1994; Langley *et al.*, 1994; Sodora *et al.*, 1995; Carpenter *et al.*, 1996; Biek *et al.*, 2003; de Rozieres *et al.*, 2004; Troyer *et al.*, 2005; Poss *et al.*, 2006; Franklin *et al.*, 2007; Antunes *et al.*, 2008; Adams *et al.*, 2011; Lee *et al.*, 2012), while for the less well-studied species such as leopard, cheetah, Pallas cat, ocelot, jaguarundi, snow leopard and spotted hyena there are few FIV *pol*-RT sequences and the fragment length is generally short (Carpenter *et al.*, 1996; Troyer *et al.*, 2005; Brown *et al.*, 2010). Final sequence selection was therefore determined by the shortest sequences and the final alignment was less than 400bp. In addition, we chose to exclude FIV *pol*-RT sequences isolated from spotted hyena, firstly as they are not Felidae (Johnson *et al.*, 2006; Stuart & Stuart, 2007), and secondly their inclusion would further reduce the size/length of the final alignment. As both additional sequences and longer sequences become available for all felid species, the proposed FIV *pol*-RT reference dataset will need to be updated. Sequences isolated from jaguarundi, in particular, should be entered into GenBank using the revised species name *Puma*

yagouaroundi formerly *Herpailurus yagouaroundi* and the updated virus abbreviation FIV_{Pya}. For this study, the single jaguarundi sequence is designated using FIV_{Pya} as per the revised species name.

The FIV *pol*-RT reference dataset advances our current knowledge on FIV given that phylogenetic analysis of all previously published FIV *pol*-RT sequences revealed cases where identical sequences isolated from the same individual felid occur multiple times in GenBank under different accession numbers (Table S1 – Supplementary Material). Unlike HIV, previous studies have not made use of a standardised naming system for all FIV sequences. We would like to propose the inclusion of essential information when constructing phylogenetic trees that would aid in the subsequent interpretation of phylogenetic trees. We suggest as outlined in the Materials and Methods, that each FIV *pol*-RT sequence is designated by a unique sequence name for inclusion in the phylogenetic analysis.

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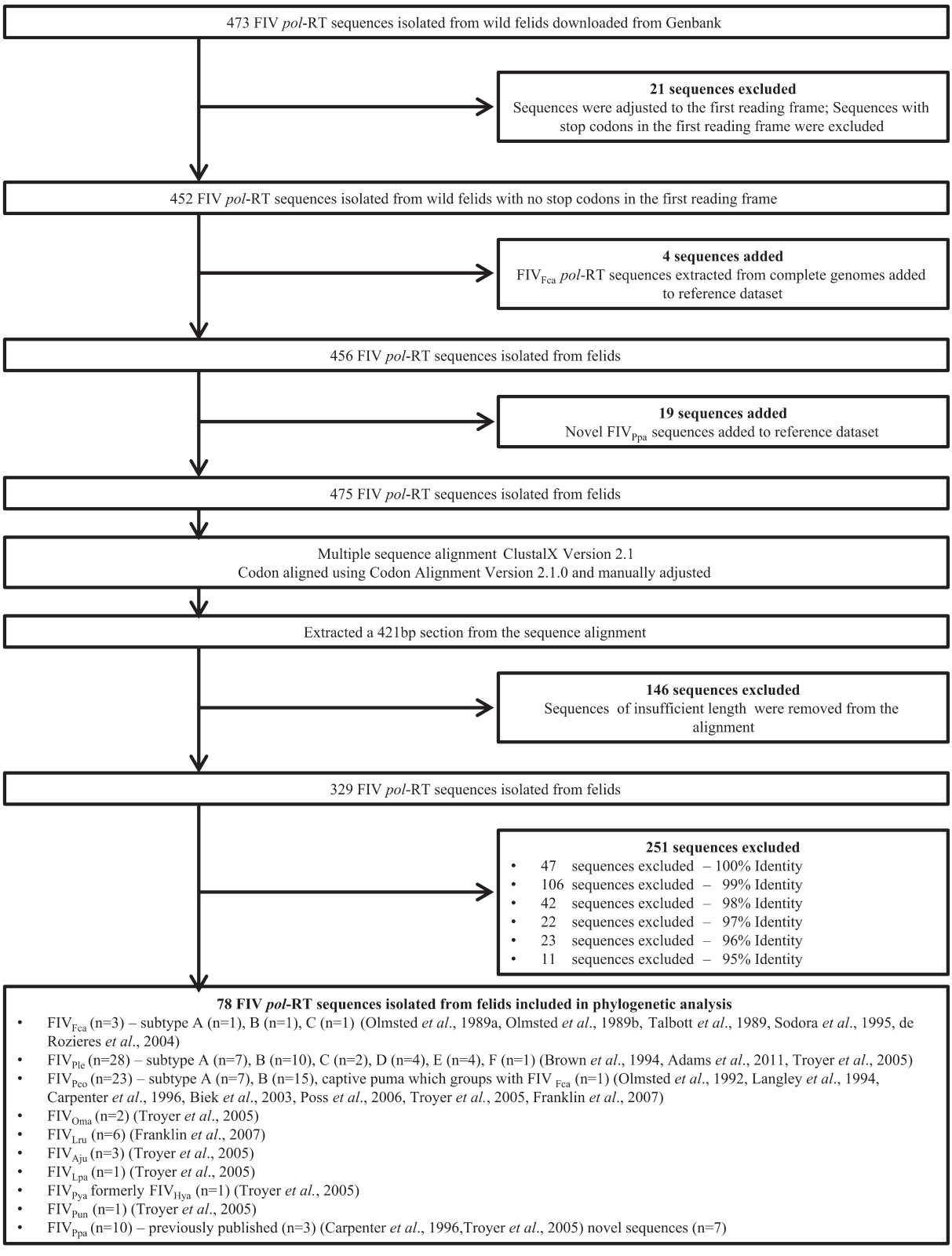

Fig. S1. Flow chart showing how the FIV *pol*-RT reference dataset was complied, indicating inclusion and exclusion criteria.

Table S1: Metadata FIV *pol*-RT Reference Dataset

Sequence Name	Accession Number	ID / Isolate	Virus	Subtype	Species Name	Common Name	Country	Reserve/Area	Wild / Captive	Reference
M25381-FIV-Fca-Petaluma-A-US-CA	M25381 - CG	Petaluma	FIV_{Fca}	A	*Felis catus*	Domestic Cat	USA	Petaluma, California		Olmsted *et al*, 1989a, 1989b; Talbott *et al*, 1989
FIU11819-FIV-Fca-USIL2489.7B-B-US-IL	FIU11820 - CG	USIL2489_7B	FIV_{Fca}	B	*Felis catus*	Domestic Cat	USA	Chicago, Illinois		Sodora *et al*, 1995
AY600516-FIV-Fca-FIVC36-C-CA-BC	AY600517 - CG	FIV-C36	FIV_{Fca}	C	*Felis catus*	Domestic Cat	Canada	British Colombia		de Rozieres *et al*, 2004
AY878242-FIV-Pun-84*	AY878242	Pun 84	FIV_{Pun}		*Panthera uncia*	Snow Leopard			Wild-born captive	Troyer *et al*, 2005; Personal Communication
AY878195-FIV-Pya-16*	AY878195	Hya 16	FIV_{Pya} formerly FIV_{Hya}		*Puma yagouaroundi* formerly *Herpailurus yagouaroundi*	Jaguarundi			Wild-born captive	Troyer *et al*, 2005; Personal Communication
AY878194-FIV-Lpa-32*	AY878194	Lpa 32	FIV_{Lpa}		*Leopardus pardalis*	Ocelot			Wild-born captive	Troyer *et al*, 2005; Personal Communication
AY878238-FIV-Oma-22-CN*	AY878238	Oma 22	FIV_{Oma}		*Otocolobus manul*	Pallas Cat	China		Captive-born	Troyer *et al*, 2005
AY878239-FIV-Oma-12-RU*	AY878239	Oma 12	FIV_{Oma}		*Otocolobus manul*	Pallas Cat	Russia		Wild-born captive	Troyer *et al*, 2005
FIU53757-FIV-Ppa-135.1-ZA	FIU53757	Ppa 135-1	FIV_{Ppa}		*Panthera pardus*	Leopard	South Africa	Kruger NP	Wild	Carpenter *et al*, 1996
AY878205-FIV-Ppa-173	AY878205	Ppa 173	FIV_{Ppa}		*Panthera pardus*	Leopard			Wild	Troyer *et al*, 2005
AY878207-FIV-Ppa-181	AY878207	Ppa 181	FIV_{Ppa}		*Panthera pardus*	Leopard			Wild	Troyer *et al*, 2005
KU705335-FIV-Ppa-KNP.1-ZA	KU705335	Ppa-KNP.1	FIV_{Ppa}		*Panthera pardus*	Leopard	South Africa	Kruger NP	Wild	Novel Sequence
KU705337-FIV-Ppa-KNP.3-ZA	KU705337	Ppa-KNP.3	FIV_{Ppa}		*Panthera pardus*	Leopard	South Africa	Kruger NP	Wild	Novel Sequence
KU705339-FIV-Ppa-KNP.6-ZA	KU705339	Ppa-KNP.6	FIV_{Ppa}		*Panthera pardus*	Leopard	South Africa	Kruger NP	Wild	Novel Sequence
KU705342-FIV-Ppa-KNP.11-ZA	KU705342	Ppa-KNP.11	FIV_{Ppa}		*Panthera pardus*	Leopard	South Africa	Kruger NP	Wild	Novel Sequence
KU705343-FIV-Ppa-KNP.12-ZA	KU705343	Ppa-KNP.12	FIV_{Ppa}		*Panthera pardus*	Leopard	South Africa	Kruger NP	Wild	Novel Sequence
KU705349-FIV-Ppa-KNP.19-ZA	KU705349	Ppa-KNP.19	FIV_{Ppa}		*Panthera pardus*	Leopard	South Africa	Kruger NP	Wild	Novel Sequence
KU705350-FIV-Ppa-KNP.20-ZA	KU705350	Ppa-KNP.20	FIV_{Ppa}		*Panthera pardus*	Leopard	South Africa	Kruger NP	Wild	Novel Sequence

Sequence Name	Accession Number	ID / Isolate	Virus	Subtype	Species Name	Common Name	Country	Reserve/Area	Wild / Captive	Reference
AY878201-FIV-Aju-204-TZ	AY878201	Aju 204	FIV_{Aju}		*Acinonyx jubatus*	Cheetah	Tanzania	Serengeti NP	Wild	Troyer *et al*,2005
AY878202-FIV-Aju-203-TZ	AY878202	Aju 203	FIV_{Aju}		*Acinonyx jubatus*	Cheetah	Tanzania	Serengeti NP	Wild	Troyer *et al*, 2005
AY878203-FIV-Aju-214-TZ	AY878203	Aju 214	FIV_{Aju}		*Acinonyx jubatus*	Cheetah	Tanzania	Serengeti NP	Wild	Troyer *et al*,2005
FIU05993-FIV-Ple-kp153.10-A-ZA	FIU05993	llvkp153-10	LLV	A	*Panthera leo*	Lion	South Africa	Kruger NP	Wild	Brown *et al*,1994
FIU05995-FIV-Ple-kp175.8-A-ZA	FIU05995	llvkp175-8	LLV	A	*Panthera leo*	Lion	South Africa	Kruger NP	Wild	Brown *et al*, 1994
FIU05996-FIV-Ple-kp177.9-A-ZA	FIU05996	llvkp177-9	LLV	A	*Panthera leo*	Lion	South Africa	Kruger NP	Wild	Brown *et al*, 1994
FIU06029-FIV-Ple-s4.2-A-TZ	FIU06029	llvs4-2	LLV	A	*Panthera leo*	Lion	Tanzania	Serengeti NP	Wild	Brown *et al*,1994
AY878210-FIV-Ple-1727-A-BW	AY878210	Ple 1727	FIV_{Ple}	A	*Panthera leo*	Lion	Botswana		Wild	Troyer *et al*,2005
AY878216-FIV-Ple-161-A-ZA	AY878216	Ple 161	FIV_{Ple}	A	*Panthera leo*	Lion	South Africa		Wild	Troyer *et al*,2005
EF667011-FIV-Ple-16N-A-ZA	EF667011	FIVple 16N	FIV_{Ple}	A	*Panthera leo*	Lion	South Africa	Kruger NP	Wild	Adams *et al*,2011
FIU05999-FIV-Ple-lm16-B-TZ	FIU05999	llvlm16	LLV	B	*Panthera leo*	Lion	Tanzania	Lake Manyara	Wild	Brown *et al*,1994
FIU05992-FIV-Ple-c26.12-B-TZ	FIU05992	llvc26-12	LLV	B	*Panthera leo*	Lion	Tanzania	Ngorogongoro Crater	Wild	Brown *et al*,1994
FIU06025-FIV-Ple-c20.2-B-TZ	FIU06025	llvc20-2	LLV	B	*Panthera leo*	Lion	Tanzania	Ngorogongoro Crater	Wild	Brown *et al*,1994
FIU06035-FIV-Ple-s43-B-TZ	FIU06035	llvs43	LLV	B	*Panthera leo*	Lion	Tanzania	Serengeti NP	Wild	Brown *et al*,1994
AY878209-FIV-Ple-319-B-TZ	AY878209	Ple 319	FIV_{Ple}	B	*Panthera leo*	Lion	Tanzania		Wild	Troyer *et al*, 2005
AY878217-FIV-Ple-320-B-TZ	AY878217	Ple 320	FIV_{Ple}	B	*Panthera leo*	Lion	Tanzania		Wild	Troyer *et al*, 2005
AY878222-FIV-Ple-676-B-UG	AY878222	Ple 676	FIV_{Ple}	B	*Panthera leo*	Lion	Uganda		Wild	Troyer *et al*,2005
AY878223-FIV-Ple-304-B-TZ	AY878223	Ple 304	FIV_{Ple}	B	*Panthera leo*	Lion	Tanzania		Wild	Troyer *et al*,2005
AY878225-FIV-Ple-336-B-TZ	AY878225	Ple 336	FIV_{Ple}	B	*Panthera leo*	Lion	Tanzania		Wild	Troyer *et al*, 2005
AY878229-FIV-Ple-513-B-TZ	AY878229 AY549258	Ple 513	FIV_{Ple}	B	*Panthera leo*	Lion	Tanzania		Wild	Troyer *et al*, 2004; Troyer *et al*, 2005

Sequence Name	Accession Number	ID / Isolate	Virus	Subtype	Species Name	Common Name	Country	Reserve/Area	Wild / Captive	Reference
FIU06039-FIV-Ple-s68.3-C-TZ	FIU06039	llvs68-3	LLV	C	*Panthera leo*	Lion	Tanzania	Serengeti NP	Wild	Brown *et al*, 1994
AY878213-FIV-Ple-350-C-TZ	AY878213 AY549238	Ple 350	FIV_{Ple}	C	*Panthera leo*	Lion	Tanzania		Wild	Troyer *et al*, 2004; Troyer *et al*, 2005
AY878214-FIV-Ple-153-D-ZA	AY878214	Ple 153	FIV_{Ple}	D	*Panthera leo*	Lion	South Africa		Wild	Troyer *et al*, 2005
AY878218-FIV-Ple-180-D-ZA	AY878218	Ple 180	FIV_{Ple}	D	*Panthera leo*	Lion	South Africa		Wild	Troyer *et al*, 2005
AY878224-FIV-Ple-168-D-ZA	AY878224	Ple 168	FIV_{Ple}	D	*Panthera leo*	Lion	South Africa		Wild	Troyer *et al*, 2005
EF667017-FIV-Ple-35S-D-ZA	EF667017	FIVple 35S	FIV_{Ple}	D	*Panthera leo*	Lion	South Africa	Kruger NP	Wild	Adams *et al*, 2011
AY878208-FIV-Ple-690-E-BW	AY878208	Ple 690	FIV_{Ple}	E	*Panthera leo*	Lion	Botswana		Wild	Troyer *et al*, 2005
AY878211-FIV-Ple-851-E-BW	AY878211	Ple 851	FIV_{Ple}	E	*Panthera leo*	Lion	Botswana		Wild	Troyer *et al*, 2005
AY878234-FIV-Ple-1036-E-BW	AY878234	Ple 1036	FIV_{Ple}	E	*Panthera leo*	Lion	Botswana		Wild	Troyer *et al*, 2005
EF667014-FIV-Ple-6N-E-ZA	EF667014	FIVple 6N	FIV_{Ple}	E	*Panthera leo*	Lion	South Africa	Kruger NP	Wild	Adams *et al*, 2011
AY878220-FIV-Ple-747-F-KE	AY878220	Ple 747	FIV_{Ple}	F	*Panthera leo*	Lion	Kenya		Wild	Troyer *et al*, 2005
M95471-FIV-Pco-61-A-US-FL	M95471 FIU53753 AY878237 (PLU03982 - CG)	PLV-14 / Pco 61	PLV	FIV_{Pco} A /FIV_{Lru}	*Puma concolor*	Puma	USA	Everglades NP, Florida	Wild	Olmsted *et al*, 1992; Carpenter *et al*, 1996; Troyer *et al*, 2005; Langley *et al*, 1994
M95476-FIV-Pco-68-A-US-FL	M95476	PLV-18 / Pco 68	PLV	FIV_{Pco} A /FIV_{Lru}	*Puma concolor*	Puma	USA	Big Cypress Swamp, Florida	Wild	Olmsted *et al*, 1992; Franklin *et al*, 2007
EF601134-FIV-Pco-F07-A-US-CA	EF601134	Pco-F07	FIV_{Pco}	FIV_{Pco} A /FIV_{Lru}	*Puma oncolor*	Puma	USA	California (SDRC)	Wild	Franklin *et al*, 2007
EF601135-FIV-Pco-F20-A-US-CA	EF601135	Pco-F20	FIV_{Pco}	FIV_{Pco} A /FIV_{Lru}	*Puma concolor*	Puma	USA	California (SDRC)	Wild	Franklin *et al*, 2007
EF601136-FIV-Pco-SM4-A-US-CA	EF601136	Pco-SM4	FIV_{Pco}	FIV_{Pco} A /FIV_{Lru}	*Puma concolor*	Puma	USA	California (OC)	Wild	Franklin *et al*, 2007
EF601139-FIV-Pco-N1R	EF601139	Pco-N1R	FIV_{Pco}	FIV_{Pco} A /FIV_{Lru}	*Puma concolor*	Puma	USA		Wild	Franklin *et al*, 2007
EF601145-FIV-Pco-M17-A-US-CA	EF601145	Pco-M17	FIV_{Pco}	FIV_{Pco} A /FIV_{Lru}	*Puma concolor*	Puma	USA	California (SDRC)	Wild	Franklin *et al*, 2007

Sequence Name	Accession Number	ID / Isolate	Virus	Subtype	Species Name	Common Name	Country	Reserve/Area	Wild/ Captive	Reference
FIU53718-FIV-Pco-117.11-B-US-AZ/NV	FIU53718	FIV Pco 117-11	FIV_{Pco}	B	*Puma concolor*	Puma	USA	Arizona/Nevada	Wild	Carpenter *et al*, 1996
FIU53719-FIV-Pco-141.1-B-US-CO	FIU53719	FIV Pco 141-1	FIV_{Pco}	B	*Puma concolor*	Puma	USA	Colorado	Wild	Carpenter *et al*, 1996
FIU53721-FIV-Pco-144.4-B-US-CO	FIU53721	FIV Pco 144-4	FIV_{Pco}	B	*Puma concolor*	Puma	USA	Colorado	Wild	Carpenter *et al*, 1996
FIU53725-FIV-Pco-145.3-B-US-CO	FIU53725	FIVPco 145-3	FIV_{Pco}	B	*Puma concolor*	Puma	USA	Colorado	Wild	Carpenter *et al*, 1996
FIU53749-FIV-Pco-408.6-PE*	FIU53749	FIV Pco 408-6	FIV_{Pco}	Fca	*Puma concolor*	Puma	Peru	Lima Peru Zoo	Captive	Carpenter *et al*, 1996
FIU53750-FIV-Pco-549.9-B-NI*	FIU53750	FIV Pco 549-9	FIV_{Pco}	B	*Puma concolor*	Puma	Nicaragua	Juigalpa Zoo	Captive	Carpenter *et al*, 1996
FIU53751-FIV-Pco-590.10-B-MX*	FIU53751	FIV Pco 590-10	FIV_{Pco}	B	*Puma concolor*	Puma	Mexico	Sonoran Ecological Center	Captive	Carpenter *et al*, 1996
FIU53752-FIV-Pco-593.8-B-MX*	FIU53752	FIV Pco 593-8	FIV_{Pco}	B	*Puma concolor*	Puma	Mexico	Parque Zoologico Centenario	Captive	Carpenter *et al*, 1996
FIU53754-FIV-Pco-696.7-B-BR*	FIU53754 AY878236	FIV Pco 696-7	FIV_{Pco}	B	*Puma concolor*	Puma	Brazil	Goiania Zoo	Captive	Carpenter *et al*, 1996; Troyer *et al*, 2005
FIU53755-FIV-Pco-733.4-B-US-TX*	FIU53755	FIV Pco 733-4	FIV_{Pco}	B	*Puma concolor*	Puma	USA	Houston Zoo, Texas	Captive	Carpenter *et al*, 1996
FIU53756-FIV-Pco-28.11-B-US-TX*	FIU53756	FIV Pco 28-11	FIV_{Pco}	B	*Puma concolor*	Puma	USA	San Antonio ZoologicalGardens and Auarium, Texas	Captive	Carpenter *et al*, 1996
AY307116-FIV-Pco-1695-B-CA-BC	AY307116 (DQ192583 - CG)	PLV1695	PLV	B	*Puma concolor*	Puma	Canada	Vancouver Island	Wild	Poss *et al*, 2003; Poss *et al*, 2006
DQ107074-FIV-Pco-Ac326.1pA-B-CA-AB	DQ107074	Ac326.1pA	FIV_{Pco}	B	*Puma concolor*	Puma	Canada	Alberta	Wild	Biek *et al*, 2006
DQ107144-FIV-Pco-YF125pD-B-US-WY/MT	DQ107144	YF125pD	FIV_{Pco}	B	*Puma concolor*	Puma	USA	Yellowstone NP, Wyoming/Montana	Wild	Biek *et al*, 2006
EF601127-FIV-Pco-F29-B-US-CA	EF601127	Pco-F29	FIV_{Pco}	B	*Puma concolor*	Puma	USA	California (SDRC)	Wild	Franklin *et al*, 2007
EF601130-FIV-Pco-SM1-B-US-CA	EF601130	Pco-SM1	FIV_{Pco}	B	*Puma concolor*	Puma	USA	California (OC)	Wild	Franklin *et al*, 2007
EF601138-FIV-Lru-8-US-CA	EF601138	Lru 8	FIV_{Lru}	FIV_{Pco} A /FIV_{Lru}	*Lynx rufus*	Bobcat	USA	California (OC)	Wild	Franklin *et al*, 2007
EF601141-FIV-Lru-5-US-CA	EF601141	Lru 5	FIV_{Lru}	FIV_{Pco} A /FIV_{Lru}	*Lynx rufus*	Bobcat	USA	California (OC)	Wild	Franklin *et al*, 2007
EF601143-FIV-Lru-7-US-CA	EF601143	Lru 7	FIV_{Lru}	FIV_{Pco} A /FIV_{Lru}	*Lynx rufus*	Bobcat	USA	California (OC)	Wild	Franklin *et al*, 2007
EF601146-FIV-Lru-13-US-CA	EF601146	Lru 13	FIV_{Lru}	FIV_{Pco} A /FIV_{Lru}	*Lynx rufus*	Bobcat	USA	California (OC)	Wild	Franklin *et al*, 2007
EF601151-FIV-Lru-SM93-US-CA	EF601151	Lru SM93	FIV_{Lru}	FIV_{Pco} A /FIV_{Lru}	*Lynx rufus*	Bobcat	USA	California (OC)	Wild	Franklin *et al*, 2007
EF601152-FIV-Lru-SM83-US-CA	EF601152	Lru SM83	FIV_{Lru}	FIV_{Pco} A /FIV_{Lru}	*Lynx rufus*	Bobcat	USA	California (OC)	Wild	Franklin *et al*, 2007