Complex evolutionary history of felid anelloviruses

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Abstract

Anellovirus infections are highly prevalent in mammals but prior to this study only a handful of anellovirus genomes had been identified in members of the Felidae family. Here characterise anelloviruses in pumas (*Puma concolor*), bobcats (*Lynx rufus*), Canada lynx (*Lynx canadensis*), caracals (*Caracal caracal*) and domestic cats (*Felis catus*). The complete anellovirus genomes (n=220) recovered from 149 individuals were diverse. ORF1 protein sequence similarity network analyses coupled with phylogenetic analyses, revealed two distinct clusters that are populated by felid-derived anellovirus sequences, a pattern mirroring that observed for the porcine anelloviruses. Of the two-felid dominant anellovirus groups, one includes sequences from bobcats, pumas, domestic cats and an ocelot, and the other includes sequences from caracals, Canada lynx, domestic cats and pumas. Coinfections of diverse anelloviruses appear to be common among the felids. Evidence of recombination, both within and between felid-specific anellovirus groups, supports a long coevolution history between host and virus.

Introduction

Anelloviruses (also referred to as torque teno viruses) are small non-enveloped circular single-stranded negative sense DNA viruses in the *Anelloviridae* family (Biagini, 2009; Biagini et al., 2011; Lefkowitz et al., 2018). This family is currently comprised of 14 genera, all of which have constituent species that have been sampled exclusively in mammals, with the exception of gyroviruses which are associated with birds (Biagini, 2009; Biagini et al., 2011; Lefkowitz et al., 2018). Many anelloviruses have yet to be taxonomically classified. Anellovirus genomes range in size from ~2.0 to 3.9 kb and typically encode three genes referred to as ORF1, ORF2 and ORF3, the latter two of which produce several different viral proteins through alternative splicing (Kaczorowska and van der Hoek, 2020). Although anelloviruses have genomes that are among the smallest and simplest of known animal-infecting viruses, little is known about the functions of these genes. Based on the arginine-rich region found in the ORF1, which is a feature also found in the capsid proteins of distantly related ssDNA viruses in the family *Circoviridae*, it is thought this protein may be involved in replication and packaging of the viral DNA (Kaczorowska and van der Hoek, 2020).

First discovered in a human patient from Japan in 1997 (Nishizawa et al., 1997), anelloviruses have subsequently been identified in non-human primates (Catroxo et al., 2008; Hrazdilova et al., 2016; Spandole et al., 2015), pinnipeds (Crane et al., 2018; Fahsbender et al., 2017), birds (Rijsewijk et al., 2011; Sauvage et al., 2011), pigs (Aramouni et al., 2013; Bigarre et al., 2005), pandas (Zhang et al., 2017), rodents (de Souza et al., 2018; Khalifeh, 2020; Nishiyama et al., 2014), and many more hosts. Prevalence studies have revealed that anelloviruses are ubiquitous in many mammalian host populations, and present across a range of tissue types. For example, estimates of the prevalence of anellovirus infections in humans range from 5% to 90% (Kaczorowska and van der Hoek, 2020), with anelloviral DNA being detectable in blood, brain, gut tissues and faeces (Kraberger et al., 2020b; Ng et al., 2017; Pollicino et al., 2003; Tisza et al., 2020).

Although not conclusively shown to cause disease, several studies have found potential associations with; hepatitis (Al-Qahtani et al., 2016), cancer (Pan et al., 2018), a range of infections with other viruses (Biagini et al., 2003; McElvania TeKippe et al., 2012; Smits et al., 2012; Yu et al., 2020), and several other disease states. A hypothesis that is currently supported is that anelloviruses may have a presently undetermined commensal role in the biology of their hosts (Kaczorowska and van der Hoek, 2020). Given the high prevalence of anelloviruses in apparently healthy hosts and difficulties with culturing these viruses, it has remained very difficult to study interactions between these viruses and their hosts.

Despite having no known causal association with disease states, the ubiquity of infections and the small sizes of anellovirus genomes have meant that large numbers of anellovirus genomes have been characterized for several mammalian host groups. Although known to occur in felids, they have not been extensively investigated in this host group. Prior to this study only twelve anellovirus genome sequences were available in GenBank from domestic cats (*Felis catus*) in Japan (Okamoto et al., 2002), China (Zhang et al., 2016; Zhu et al., 2011), France (Biagini et al., 2007), USA (unpublished) and Czech Republic (Jarosova et al., 2015). One unpublished sequence is also available from a Brazilian Ocelot (*Leopardus pardalis*). Interestingly, these felid anellovirus sequences and that of their hosts present surprisingly incongruent phylogenies, which suggests that domestic cats, and possibly other felids too, harbour diverse anelloviruses. In order to investigate this in greater depth, we undertook a comprehensive study characterising anellovirus genomes from puma (*Puma concolor*), bobcats (*Lynx rufus*), Canada lynx (*Lynx canadensis*), caracals (*Caracal caracal*) and domestic cats. Further, we analysed these feline-derived anelloviruses to determine their diversity, recombination patterns and ancestral relationships.

Materials and Methods

Ethics statement

Mountain lion samples were obtained as part of an ongoing collaborative study with Colorado Parks and Wildlife (CPW) and provided to Colorado State University (CSU) for viral screening. Domestic cat samples were collected by collaborating shelters and sent to CSU. Blood samples from these studies have been archived and used for several studies. CSU and CPW Institutional Animal Care and Use Committees reviewed and approved this work prior to commencement (CSU IACUC protocol 05-061A). This work was performed in accordance with United States Department of Agriculture Animal Welfare Act and The Guide for the Care and Use of Laboratory Animals. CSU Public Health Assurance number is D16-00345. CSU is accredited by AAALAC International.

Bobcats from California were captured, handled, collared, and samples collected under approval of the Institutional Animal Care and Use Committee (IACUC) of the University of California, Santa Cruz (Seril1701). Scientific collecting permits were authorized by the California Department of Fish and Wildlife (Aromas, SCP-11968; Coyote Valley, SCP-13565). Further those from the Los Angeles area were approved by the University of California, Los Angeles Office of Animal Research Oversight of UCLA (Protocol ARC#2007-167-12). Scientific collecting permits were authorized by the California Department of Fish and Wildlife (SCP-9791).

Caracal handling was approved by the University of Cape Town Animal Ethics Committee (2014/V20/LS), Cape Nature (AAA007-0147-0056), and South Africa National Parks (SANParks; 2014/CRC/2014-017, 2015/CRC/2014-017, 2016/CRC/2014-017, 2017/CRC/2014-017).

Nucleic acid extraction and high-throughput sequencing

Faecal and/or blood samples were collected from domestic cats, Canada lynx, bobcats and mountain lions from North America, and blood collected from caracals from South Africa between the years of 1999-2018 (see Table 1 for details). Faecal samples were processed according to a protocol described in Steel et al. (2016). Two hundred µl of faecal sample resuspensions or blood samples were individually processed using the High Pure Viral Nucleic Acid Kit (Roche Diagnostics, USA) to extract viral DNA according to the manufacturer’s specifications. In order to target the amplification of anelloviruses, TempliPhi™ (GE Healthcare, USA) was used to preferentially amplify circular DNA through rolling-circle amplification (RCA). Circular amplified DNA was then pooled according to sample type, host and location, and used to prepare Illumina sequencing libraries with a TruSeq Nano DNA kit (Illumina, USA) and sequenced on an Illumina HiSeq 4000 at Psomagen Inc., USA. Raw reads were *de novo* assembled using metaSPAdes v3.12.0 (Bankevich et al., 2012) and contigs >1000nts analysed using BLASTx (Altschul et al., 1990) against a RefSeq viral protein database NCBI GenBank website to identify anellovirus-like contigs.

Based on the identified anellovirus-like de novo assembled contigs, back-to-back primers were designed and used with Kapa HiFi Hotstart DNA polymerase (Kapa Biosystems, USA) in a polymerase chain reaction (PCR) to recover full anellovirus genomes from individual samples. Primers used to amplify the anellovirus genomes are provided in Supplementary Data 1 and cycling conditions were applied as per manufacturer’s instructions and primer annealing temperatures. PCR products were resolved on 0.7 % agarose gels, ~2-2.7 kb amplicons were gel excised, purified, ligated into pJET 1.2 vector (Thermo Fisher Scientific, USA) and transformed into XL blue *Escherichia coli* competent cells. Recombinant plasmids with viral sequences were purified and Sanger sequenced at Macrogen Inc., Korea.

Sequence assembly, annotation and network analyses

Contigs were assembled, annotated and datasets compiled in Geneious v11.0.3 (Biomatters Ltd New Zealand). Datasets of the ORF1 protein sequences of all the anellovirus genomes recovered in this study together with those from GenBank (downloaded 1st of December 2020) were compiled and a sequence similarity network (SSN) was generated using EST-EFI (Gerlt et al., 2015) using a threshold of 75. Cytoscape (V3.8.1) (Shannon et al., 2003) was used to visualize ORF1 protein SSN. Three network clusters containing feline-derived sequences resulted from these analyses and these will hereafter be referred to as feline network cluster-1 (sequences originating from puma, bobcats and domestic cats), -2 (sequences originating from caracals, Canada lynx, puma and domestic cats) and felid rodent network cluster-1 (sequences originating from rodent species and a bobcat faecal sample).

Recombination analyses

Sequence UoA20_55_BC (MT538139) was excluded from these analyses because it was recovered from a bobcat faecal sample and is most closely related to anelloviruses found in rodents and might have therefore been prey-animal-associated. A full genome dataset of the feline-derived anelloviruses, with the exception of UoA20_55_BC (MT538139) was aligned using MUSCLE (Edgar, 2004) and recombination analyses performed using RDP v5.5 (Martin et al., 2020). Sequences were set as circular with similar sequences auto-masked. Events were deemed as credible if they were detected by three or more of the seven recombination detection methods implemented in RDP5.5 with an associated p-value <0.05 and were supported by phylogenetic evidence.

Phylogenetic and pairwise analyses

The compiled ORF1 protein sequence dataset was aligned using MAFFT (Katoh et al., 2002) and an approximate maximum-likelihood phylogenetic tree was constructed using FastTree (Price et al., 2010) with a JTT+CAT substitution model (Jones et al., 1992; Si Quang et al., 2008), branches having less than 0.6 SH-like branch support were collapsed using TreeGraph2 v2.14 (Stover and Muller, 2010).

Full genome sequence datasets were compiled for each of the three groups of isolates identified in the network analyses shown in Figure 2. For feline network clusters 1 and 2, referred to as feline groups 1 and 2, recombination-free datasets were generated following recombination analyses. The third dataset, comprised of felid rodent network cluster-1 (a single bobcat-derived anellovirus together with rodent anellovirus sequences), referred to as rodent group 1, was aligned using MUSCLE (Edgar, 2004) but was not analysed for recombination, given how small this group is and genetically distant the members are. Maximum-likelihood phylogenies were then constructed for these three datasets. For the recombination-free sequences in the feline group 1 and 2 datasets, phylogenies were constructed using RAxML implemented in RDP5 (Martin et al., 2020) which explicitly accounts for large amounts of missing data (Stamatakis, 2014). A maximum-likelihood tree for the rodent group-1 dataset was constructed in Seaview (v4) (Gouy et al., 2010) using PhyML (Guindon et al., 2010) with the GTR+G substitution model. The phylogenetic trees were all midpoint rooted and branches with less than 0.6 bootstrap support were collapsed using TreeGraph2 v2.14 (Stover and Muller, 2010). A phylogram depicting the evolutionary history of Felidae and Viverridae was constructed with TimeTree (Hedges et al., 2015).

Pairwise identity analyses were undertaken for the ORF1 nucleotide and amino acid datasets of feline group 1, 2, and rodent group 1 with SDT v1.2 (Muhire et al., 2014).

Results and discussion

Characterisation of anelloviruses from five feline species

In this study, a total of 220 complete anellovirus genomes were determined from blood or faecal samples from five felid species. These were recovered from bobcats (n=117), Canada lynx (n=42), caracals (n=34), domestic cats (n=3), and puma (n=24). One or more anellovirus genomes were recovered and characterised from 149 individual animals: bobcats (n=78) - from Mexico (n=1) and USA (n=77); Canada lynx (n=23) - Canada (n=14), USA (n=8); caracals (n=30) - all South Africa; domestic cats (n=3) - all USA; puma (n=15) - Mexico (n=6) USA (n=9). All of these samples were collected between 1999-2018, see Table 1 for full details.

In all the anellovirus genomes the putative ORF1 and ORF2 open reading frames (ORFs) were identified and annotated. The genomes range in size from 1,829 to 2,653 nts, varying dramatically between felid species (Figure 1A). Bobcats had the smallest anellovirus genomes on average ranging from 1,829 to 2,156 nts (excluding the anellovirus which is most similar to rodent anelloviruses which is 2,352 nts, MT538139, referred to as torque teno rodfelid virus 1). The largest average genome sizes were those from caracals 2,397 – 2,586 and Canada lynx 2,429 – 2,622 nts, and the two groups with the most variable genome sizes are those from domestic cats 2,012 – 2,653 nts and pumas 1974 – 2560 nts. With the exception of one isolate previously recovered from a domestic cat in China (KX262893) (Zhang et al., 2016) which has a genome of 2,409 nts and three domestic cat isolates from the USA recovered in this study (MT538162, MT538151, MT538150) which have genomes of ~2,600 nts, all other domestic cat genomes were ~2,000 nt. Although the mountain lion isolates exhibited a broad range of sizes, only two (MT538133 and MT538082) were ~2,500 nts, while the remainder were ~2,000 nts.

Distributions of pairwise genetic distances between anelloviruses within each felid species

Anellovirus diversity within each felid species is high whether considering the ORF1 nucleotide sequence or the translated amino acid sequences (Figure 1B-F). ORF1 nucleotide pairwise identities across all the felid species were 55–100% with the distribution being slightly narrower for bobcats at 59–100%. The distribution of ORF1 amino acid pairwise distances, however, was much wider. Domestic cats and pumas harboured anelloviruses with the broadest ORF1 pairwise amino acid identity distribution, 24-99% and 21–99%, respectively. Interestingly they also make up ~8% of the felid anelloviruses recovered, the fewest anellovirus genomes recovered from all feline species. The caracal and Canada lynx-derived anellovirus translated ORF1 sequences have similar pairwise distance distributions: 35-99% and 37–99%, respectively. The translated ORF1 anellovirus sequences of bobcats, which incidentally have the most isolates recovered (~50%), share between 44–100% pairwise identity.

According to the ICTV anellovirus taxonomy proposal (Biagini et al., 2011) viruses exhibiting < 69% ORF1 pairwise nucleotide similarity can be considered distinct species. Based on this criterion, the feline anelloviruses discovered here fall into 24 tentative species groupings hereby named torque teno felid virus (TTFV) 3, 5, 7-27 (Supplementary Data 2, 3). The rodent-like anellovirus from a bobcat faecal sample (MT538139) was named torque teno rodfelid virus 1 (TTRFV-1) (Supplementary Data 4).

Anellovirus ORF1 protein phylogeny, network grouping and geographical distribution

Phylogenetic analysis of ORF1 amino acid sequences of the newly determined sequences together with all those available in GenBank (downloaded 1st December 2020) indicated that the feline sequences fall into two major phylogenetic clades that correspond with the two sequence clusters identified in the network analysis (Figure 2A). These two groups of predominantly feline sequences also contain anellovirus sequences from Japanese palm civet (*Paguma larvata*) faecal samples. Given the faecal origin of these palm civet anelloviruses and the fact that palm civets are omnivores, these could have originated in a prey animal. Interestingly, palm civets are in the Viverridae family which is in the same suborder, Feliforma*,* as the Felidae family. The most recent common ancestor of the Viverridae and Felidae likely existed between 33 and 46 MYA (Hedges et al., 2015) which could indicate that the most recent common ancestor of the civet and feline anelloviruses might have been an ancestral anellovirus that infected the common ancestor of cats and civets prior to their divergence (Figure 2A).

Feline grouping 1 is comprised of feline anellovirus sequences from bobcats and pumas from the USA and Mexico, domestic cats from Europe, Asia and the USA, and an ocelot from Brazil (Figure 2). Feline grouping 2 is comprised of feline anellovirus sequences from Canada lynx from Canada, and Alaska and Montana, USA, caracal from South Africa, domestic cats from the USA, and pumas from Mexico and the USA. Given that only two of the 24 analysed puma anellovirus sequences (MT538133 and MT538082) cluster in feline grouping 2, and that these sequences are from faecal samples, it is also possible that they are derived from felid prey animals. Bobcat and Canada lynx anelloviruses sit in two separate groupings, which is noteworthy given these two felid species are close relatives in the same genus, thought to have diverged ~3.2-5.6 MYA (Hedges et al., 2015; O'Brien and Johnson, 2007) and which presently have overlapping geographic distributions.

Lastly, an anellovirus sequence from a bobcat faecal sample groups both phylogenetically and in a network grouping with rodent-derived anellovirus sequences in rodent grouping 1. Based on this finding, we hypothesise that this sequence was derived from a rodent preyed on by the bobcat.

Domestication of cats has led to their high global prevalence; therefore, it is not surprising that the felid anelloviruses with the broadest geographic range and phylogenetic spread are those from domestic cats. Interestingly, all those identified in previous studies from the USA, Europe and Asia (except for KX262893, which sits outside both groups) (Figure 2B) fall in the same clade/network grouping, together with those from pumas and bobcats. Given the diverse nature of the domestic cat anelloviruses, their relationship with other feline anelloviruses and how underrepresented they are, more sampling is warranted to help unravel the most common ancestor. Both pumas and bobcats have overlapping geographical ranges in North America and therefore it is not unexpected that their anelloviruses fall in the same grouping. The Canada lynx anelloviruses are from samples collected in Canada and the USA, and despite dwelling in regions overlapping with puma and/or bobcats they cluster in feline grouping 2 with the caracal anelloviruses that were sampled in South Africa.

Feline anellovirus phylogenetic relationships and recombination patterns

Feline grouping 1

A recombination-free phylogenetic tree of feline grouping 1 anelloviruses, including only those sequences sampled from members of the Felidae family was constructed using sequences from pumas, bobcats, domestic cats and an ocelot. These sequences fall into 12 tentative new species groupings and two that have already been established (Figure 3) (Biagini, 2009). Although anellovirus sequences from individual felid species do not form monophyletic clusters within this tree, it is nevertheless clear that anellovirus sequences sampled from particular felid species tend to cluster together. A noteworthy exception is one domestic cat sequence from the USA (JF304937), which clusters with bobcat-derived sequences in TTFV 5. Within this group it does however form a distinct lone branch and therefore it may be that as more domestic cat isolates from the USA are characterised we see the formation of a related but separate grouping.

Among the sequences from bobcats, pumas and domestic cats there were 24 recombination events detected (Figure 3, Supplementary Data 5). Out of these 24 events, 13 involved parental sequences from different felid species (i.e., inter-species recombination events) and eleven involved parents belonging to the same species (i.e. intra-species recombination events). Recombination was only detected in one of the sequences from domestic cats (EF538877, sampled in France).

Feline grouping 2

A feline grouping 2 phylogeny, with recombinant regions removed, indicated the evolutionary relationships between anellovirus sequences from caracals, lynx, bobcats, pumas and domestic cats (Figure 4). These sequences fall into 11 tentative species (Figure 4 and Supplementary Data 3). Similar to what was observed in feline group 1, the isolates cluster according to source host. A total of 17 recombination events were detected, all in the caracal and Canada lynx TTFV sequences. The Canada lynx sequences from several locations across North America (Canada, and Alaska and Montana, USA) are closely related and share evidence of common recombination events (Figure 4; Supplementary Data 3). Of the 17 recombination events, eleven occurred between anelloviruses in different species and six occurred between viruses in the same species. Eleven of the recombination events appear to have involved anelloviruses that seem to be associated with two different felid species (caracal and Canada lynx). This suggests, despite the strong associations of these viruses with the hosts from which they were isolated, the viruses infected another unsampled host or a common ancestor.

It is unusual that there are two puma-derived sequences that are part of this group, given that the other puma-derived sequences fall in feline grouping 1. These puma-associated sequences were recovered from faecal samples collected in Mexico and the state of California, USA, and therefore are either a divergent outgroup of mountain lion-infecting anelloviruses or are derived from another felid species upon which the mountain lions have preyed upon. These two puma-associated sequences are most closely related to isolates recovered from a Canada lynx (sampled in Alaska), a caracal (sampled in South Africa) and a domestic cat (sampled in the USA) (Figure 4).

Recombination hot and cold spots

The recombination events detected within the feline anellovirus genomes were not randomly distributed (Figures 3 and 4). Specifically, a statistically significant ~500nt long recombination breakpoint hotspot was evident in the non-coding region and a statistically significant cold-spot was evident throughout most of ORF1 (Figure 5). The recombination hotspot colocalizes with the GC box (Kaczorowska and van der Hoek, 2020) that is highly conserved between anelloviruses and might therefore act as a homologous region that is particularly prone to template switching during replication (Martin et al., 2011). Conversely, it is possible that the high degrees of ORF1 diversity seen even within individual TTFV species might impede homologous recombination within ORF1. Crucially, a very similar hot and cold spot pattern has been shown in anelloviruses from Weddell seals, suggesting that these patterns may be a general feature of anellovirus recombination (Fahsbender et al., 2017).

Feline rodent grouping 1

One anellovirus sequence from a bobcat faecal sample collected in Mexico was not related to the other feline derived TTFVs but instead is most closely related to anelloviruses identified from rodents (Figure 6). Phylogenetically it sits just outside a rodent clade comprised of anelloviruses from voles and mice from the UK (Nishiyama et al., 2014) and China (Du et al., 2018). This rodent-anellovirus-like sequence has an ORF1 that shares ~59-64% pairwise nucleotide identity with those from anelloviruses associated with rodents (Supplementary Data 3) and therefore we have named it torque teno rodfelid virus 1 (TTRFV-1). Given that it was obtained from a faecal sample and is most closely related to anelloviruses from rodents, it is likely that this is a virus derived from a predated rodent (Figure 6).

Co-infection dynamics

Coinfections of multiple genetically diverse anelloviruses have been reported in in humans (Okamoto et al., 1999) and also other mammals several studies (Biagini et al., 2007; Fahsbender et al., 2017; Huang et al., 2010; Kraberger et al., 2020b; Leme et al., 2013; Nishiyama et al., 2014). This was also evident for the feline TTFVs where blood samples from 17 individuals harboured between two and four distinct anellovirus species (Table 1). If viruses sampled from faecal samples are also included, an additional 21 individuals appear to harbour more than one TTFV species. Keeping in mind we cannot rule out the possibility that viruses sampled from faecal samples might have originated from prey animals. Out of the five felid species investigated, domestic cats were the only ones that did not display evidence of mixed infections involving multiple TTFV species in this study. This is however likely attributed to the low numbers of domestic cast samples analysed here (Table 1) as co-infections have been previously been shown in a domestic cat (Biagini et al., 2007).

For eight of the bobcats sampled in California we were able to obtain matching blood and faecal samples. For five of these animals, different TTFV species were detected in blood than were detected in the matched faecal samples (Table 1). There could be several possible explanations for this, including the different anellovirus species having different cell tropisms, or low viral titres in one or the other of the sample types precluding their detection in both. For three bobcats the same anellovirus species were detected in both blood and faeces suggesting that one might expect to find similar viruses in blood and faecal samples from the same animals. This expectation is reasonable given that anelloviruses are thought to be transmitted via the faecal-oral route (Kaczorowska and van der Hoek, 2020).

Concluding remarks

Anelloviruses are abundant among mammals, display high degrees of genomic diversity and appear to have complex evolutionary histories characterized by frequent recombination and potential codivergence with their host species. Specifically, anelloviruses from different groups of host species such as the primates, pinnipeds or porcine cluster together phylogenetically potentially signifying long coevolutionary histories with their host lineages (Hrazdilova et al., 2016; Spandole et al., 2015). In the case of the porcine, two distinct clusters are evident. In this study, we determine the diversity and evolutionary relationships of anelloviruses associated with members of the Felidae family by undertaking comprehensive analyses of 220 anellovirus genomes from mountain lions, bobcats, Canada lynx, caracals and domestic cats.

We determine that, as with the porcine anelloviruses, the felid anelloviruses fall into two distinct phylogenetic clades (Figure 2) If indeed the anelloviruses are codiverging with their hosts this would imply that at least two the anellovirus lineages that infected the most recent common ancestor of the felids has today yielded the feline grouping 1 and 2 lineages. Other factors most likely play a role in anellovirus evolution including the geographic distribution of the felid species (both present day and historical), and their trophic interactions. Studies involving feline foamy virus, feline immunodeficiency virus and feline leukaemia virus have indicated that predation of felids on other felids can result in cross-species virus transmissions (Chiu et al., 2019; Franklin et al., 2007; Kraberger et al., 2020a). Within both felid anellovirus groups various recombination events were detected where identified parental sequences are found infecting different felid species. While superficially this might appear to represent evidence of felid anelloviruses infecting multiple different felid species, this is not necessarily the case. Specifically, the parental sequences identified in our recombination analysis are not actual parents but rather the sequences in our sample that are most similar to the actual parents. This dynamic is best illustrated with the discovery of apparent recombinants between Canada lynx and caracal infecting anelloviruses: it is extremely implausible given the geographic separation of these species that there are any transmissions of anelloviruses between them. The actual parents of these recombinants are much more likely to be other Canada lynx or caracal infecting anelloviruses that presently remain unsampled (Figure 3, 4 and supplementary data 5).

Genome size varied greatly between anelloviruses from each felid species (Figure 1A). Although domestic cats harboured anelloviruses with a large range of genome sizes, if one disregards viruses from faecal samples that might represent prey-animal derived viruses, potentially been probable prey-animal associated viruses, anelloviruses from each group of wild felid species fell within a narrower size range. It is likely that with more sampling there may be some additional correlations between genome size and host / geographical location. With more sampling of anelloviruses in other felid species, it is likely that a clearer evolutionary picture will come to light.

The high degree of anellovirus sequence heterogeneity seen within the felids is similar to that noted for anelloviruses from primates (Kaczorowska and van der Hoek, 2020; Spandole et al., 2015), pinnipeds (Crane et al., 2018; Fahsbender et al., 2017) and swine (Blois et al., 2014; Ghosh et al., 2020; Huang et al., 2010). The feline anelloviruses fall into 24 species-level groupings (Figure 3, 4, 6; Supplementary Data 2, 3 and 4), one of which is from a bobcat faecal sample and sits within a predominantly rodent-derived anellovirus group (Figure 6).

The diversity of ORF1 nucleotide sequences found within individual felid species was >54% similarity, showing high diversity (Figure 1). This, together with fact that the recombination analysis shows that the entire ORF1 region is a recombination cold spot, is consistent with the hypothesis that there is an “arms race” between the host immune response and one or more of the proteins encoded by this ORF (such as the capsid protein): a dynamic that may have driven the diversification of ORF1 (Spandole et al., 2015).

Coinfections of more than one anellovirus species add to the complexity of virus-host dynamics in the felids. When considering only virus sequences recovered from blood, 17 out of the 149 animals sampled were detectably coinfected with different anellovirus species (Table 1). Anellovirus co-infection should be considered in future studies to understand in greater depth the role these play in generating new recombinants.

As more anellovirus genomes are recovered from felids the evolutionary relationship between host and virus will be further elucidated, and this may also provide critical insight into whether these viruses are the friends or the foes of the species that they infect.

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Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Figure legends and table text

Figure 1: Pairwise distributions of anelloviruses from each species show high within host diversity. A) Plot showing genome sizes of feline derived anelloviruses recovered from the five host species (this study and previously documented) and the different sample types. **B-F)** Pairwise distribution plots of anellovirus ORF1 nucleotide and amino acid sequences for the five feline species from which anelloviruses were recovered in this and previous studies.

Figure 2: Feline anelloviruses display a complex evolutionary history. A) The approximate maximum-likelihood phylogenetic tree on the left illustrates the evolutionary relationships of ORF1 proteins from all published anellovirus genomes with those recovered from felid species in this study. Protein similarity networks are shown next to the feline anellovirus clades, with each node in the network representing the ORF1 proteins from feline and palm civet derived anelloviruses. Species of sample origin are colour coded. The phylogram on the right shows the species and genus within the Feliforma Suborder; The Felidae and Viverridae families for which anelloviruses were recovered in this study, and other previously recovered anelloviruses in these groups are

shown by a “*”. The numbers of isolates from each host is shown next to the general and Latin species names. **B)** Shows the regions from which feline and palm civet derived anelloviruses were sampled.

Figure 3: Inter- and intra-species recombination events detected in bobcat and puma anelloviruses. Recombination-free maximum-likelihood phylogeny of the sequences in feline anellovirus group 1 derived from pumas, bobcats, domestic cats and an ocelot. Anellovirus species groupings are shown in the grey bar beside the tree. Recombination events are indicated in the linearized genome schematic. Accession numbers for each sequence are coloured based on the source / host and sampling location indicated by state and/or country codes.

Figure 4: Three anelloviruses recovered from bobcat and puma faecal samples appear to be derived from prey animals. A) Recombination free maximum-likelihood phylogeny of the anelloviruses in feline group 2 sampled from caracals, Canada lynx and domestic cats. Anellovirus species groupings are displayed in the grey bar beside the tree. Detected recombination events within individual sequences are indicated in the linearized genome schematics. Accession numbers for each sequence are coloured based on the hosts from which they were sampled and locations are indicated with state and/or country codes.

Figure 5: Recombination hot- and cold-spots within anellovirus genomes from feline groupings 1 and 2. The black vertical lines above the figure indicate the positions of detected recombination breakpoints and the black line in the plot indicates breakpoint numbers falling within a 200-nucleotide sliding window. The red regions indicate the breakpoint hotspot and the blue region the cold-spot. The light and dark grey areas respectively indicate 99% the 95% confidence intervals of the expected degrees of breakpoint clustering under random recombination.

Figure 6: Maximum-likelihood phylogeny of anelloviruses in rodent grouping 1 with one bobcat-derived anellovirus from a faecal sample. Anellovirus species are shown in grey bars. Accession numbers for the sequences are coloured based on the source / host and sampling locations are indicated with state/country codes.

Table 1: Summary of sample information for all anelloviruses recovered in this study including source/host, feline demographic information, sampling location, year, type, anellovirus species grouping and accession number.

Supplementary Data 1: Details of primers used to recover anellovirus genomes
Supplementary Data 2: Pairwise analyses of the ORF1 gene for feline group 1
Supplementary Data 3: Pairwise analyses of the ORF1 gene for feline group 2
Supplementary Data 4: Pairwise analyses of the ORF1 gene for rodent feline group 1
Supplementary Data 5: Details of recombination events

Table 1: Summary of sample information for all anelloviruses recovered in this study including source/host, feline demographic information, sampling location, year, type, anellovirus species grouping and accession number.

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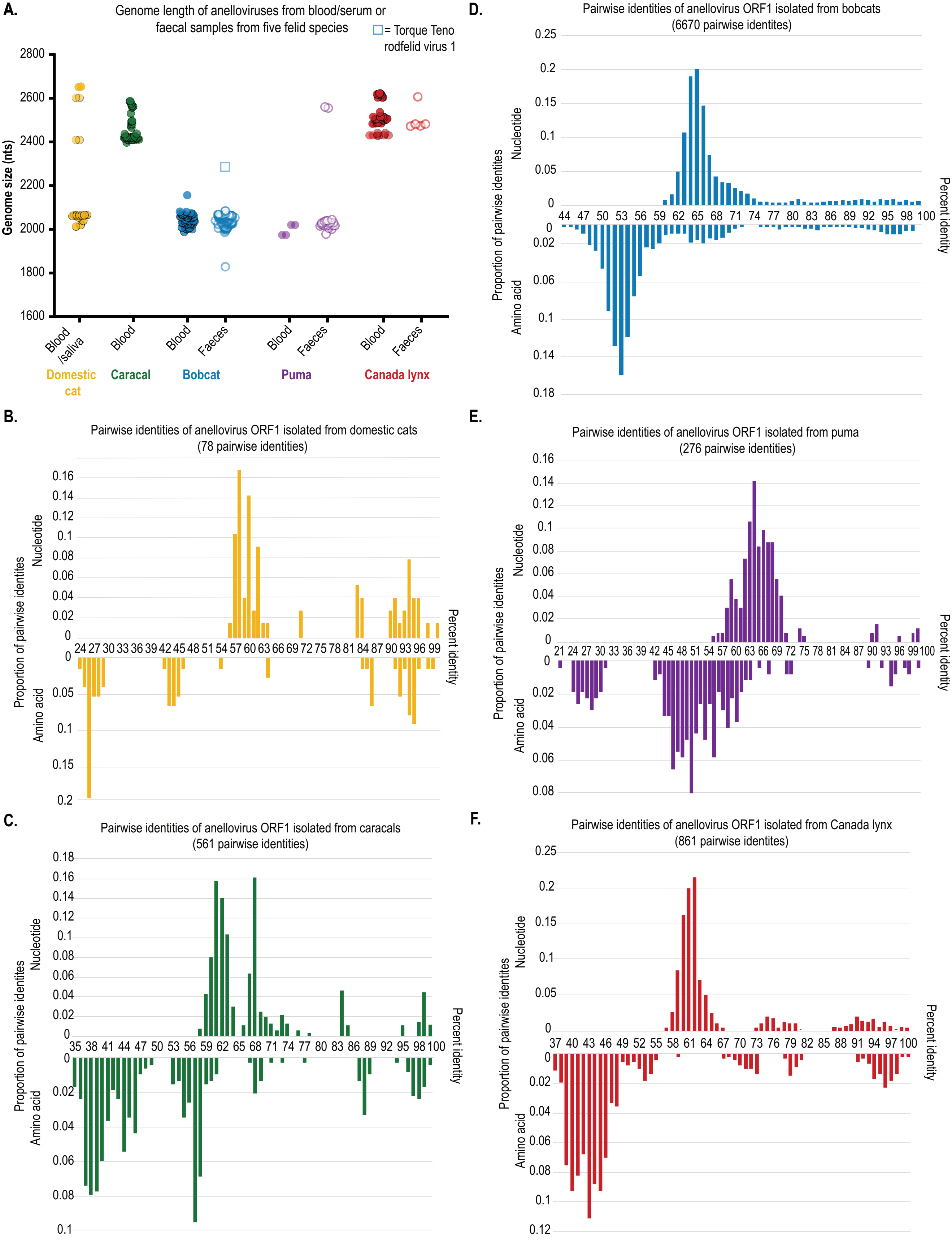
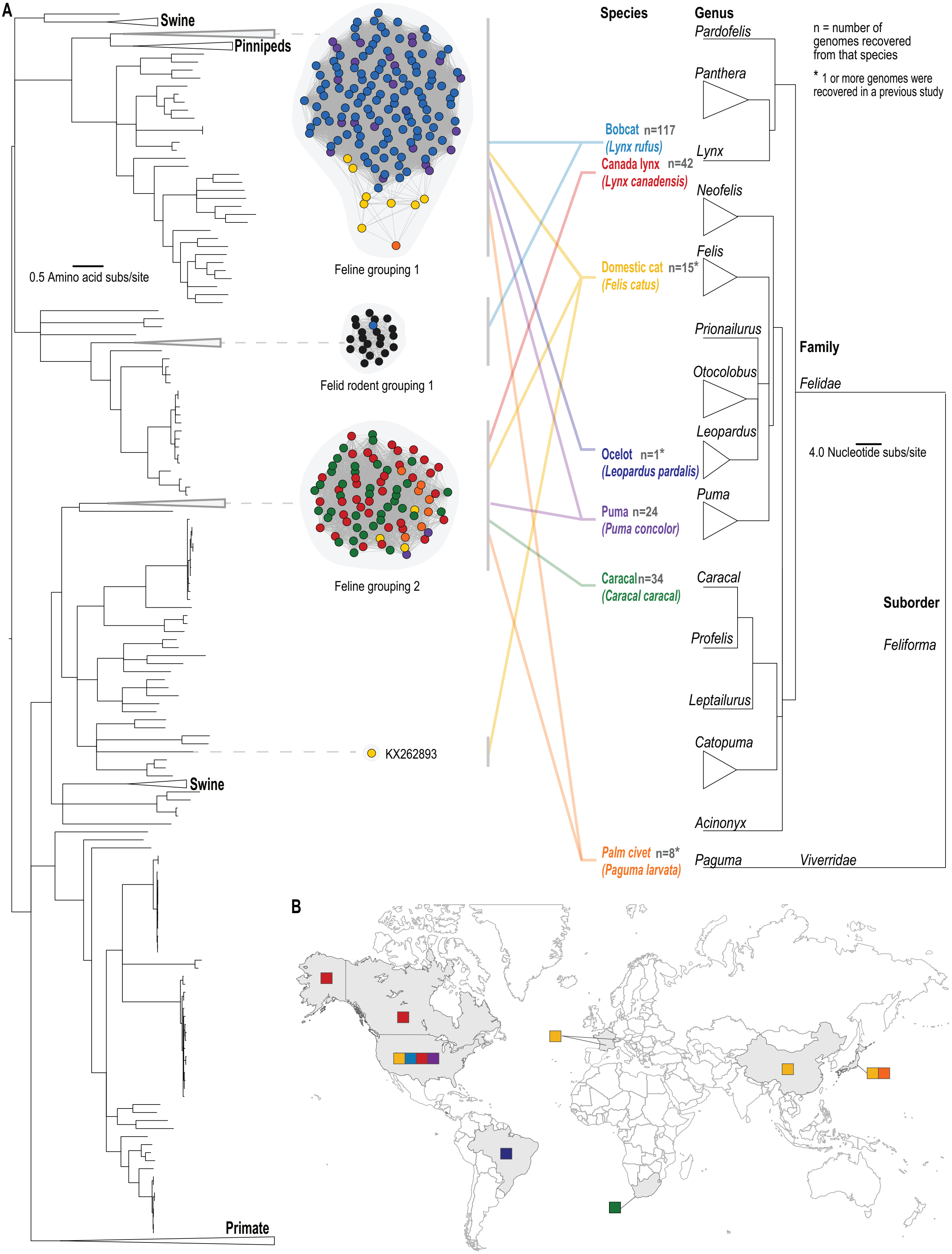
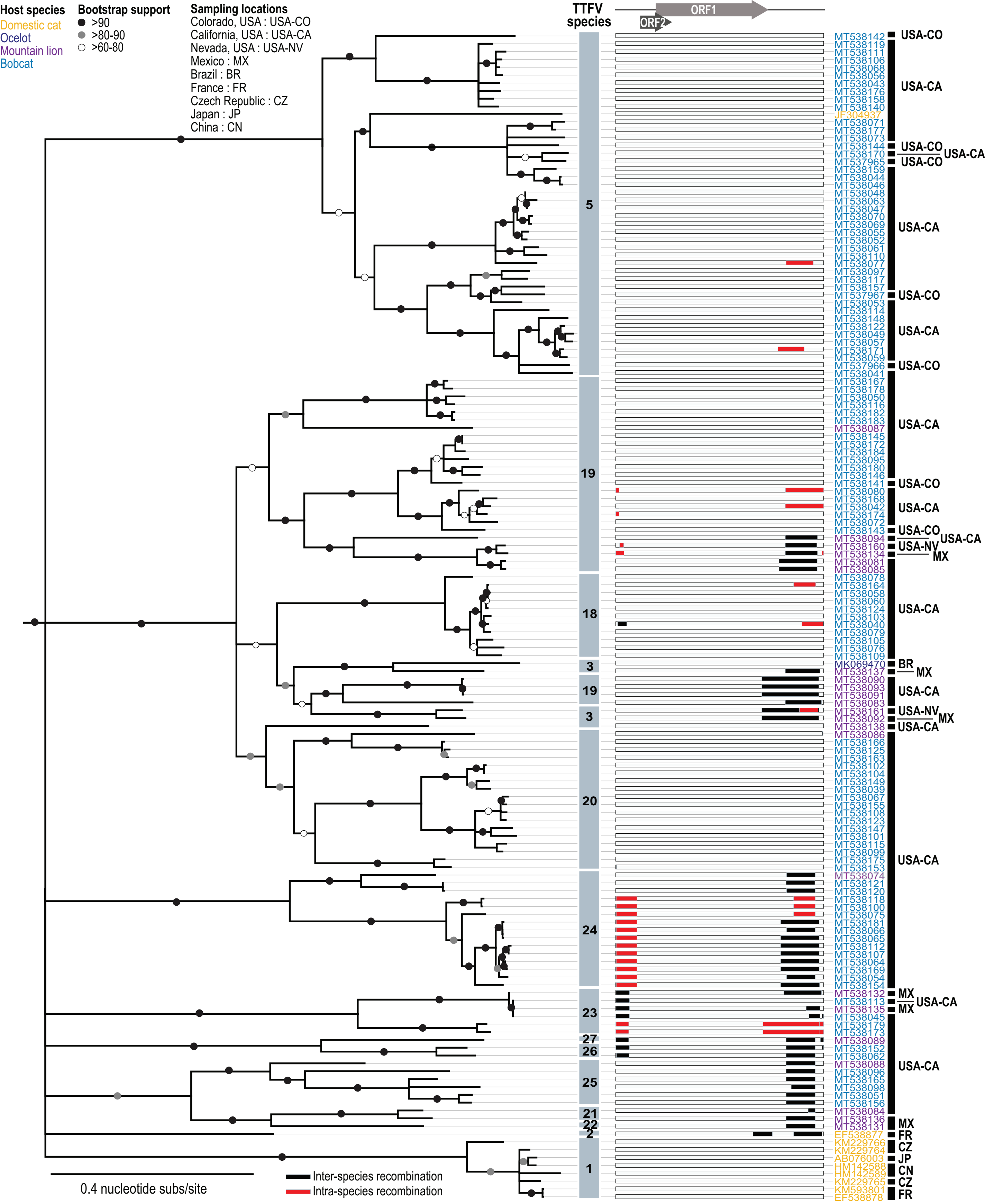

A.
Genome length of anelloviruses from blood/serum or faecal samples from five felid species
□= Torque Teno rodfelid virus 1
Genome size (nts)
2800
2600
2400
2200
2000
1800
1600
Blood /saliva
Blood
Blood
Faeces
Blood
Faeces
Blood
Faeces
Domestic cat
Caracal
Bobcat
Puma
Canada lynx
B.
Pairwise identities of anellovirus ORF1 isolated from domestic cats (78 pairwise identities)
Proportion of pairwise identites
Nucleotide
Amino acid
Percent identity
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
24 27 30 33 36 39 42 45 48 51 54 57 60 63 66 69 72 75 78 81 84 87 90 93 96 99
0
0.05
0.1
0.15
0.2
C.
Pairwise identities of anellovirus ORF1 isolated from caracals (561 pairwise identities)
Proportion of pairwise identites
Nucleotide
Amino acid
Percent identity
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
35 38 41 44 47 50 53 56 59 62 65 68 71 74 77 80 83 86 89 92 95 98 100
0
0.02
0.04
0.06
0.08
0.1
D.
Pairwise identities of anellovirus ORF1 isolated from bobcats (6670 pairwise identites)
Proportion of pairwise identites
Nucleotide
Amino acid
Percent identity
0.25
0.2
0.15
0.1
0.05
0
44 47 50 53 56 59 62 65 68 71 74 77 80 83 86 89 92 95 98 100
0
0.02
0.06
0.1
0.14
0.18
E.
Pairwise identities of anellovirus ORF1 isolated from puma (276 pairwise identities)
Proportion of pairwise identites
Nucleotide
Amino acid
Percent identity
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
21 24 27 30 33 36 39 42 45 48 51 54 57 60 63 66 69 72 75 78 81 84 87 90 93 96 99 100
0
0.02
0.04
0.06
0.08
F.
Pairwise identities of anellovirus ORF1 isolated from Canada lynx (861 pairwise identites)
Proportion of pairwise identites
Nucleotide
Amino acid
Percent identity
0.25
0.2
0.15
0.1
0.05
0
37 40 43 46 49 52 55 58 61 64 67 70 73 76 79 82 85 88 91 94 97 100
0
0.02
0.04
0.06
0.08
0.1
0.12


A
Swine
Pinnipeds
0.5 Amino acid subs/site
Feline grouping 1
Felid rodent grouping 1
Feline grouping 2
KX262893
Swine
Primate
Species
Genus
n = number of genomes recovered from that species
* 1 or more genomes were recovered in a previous study
Pardofelis
Panthera
Lynx
Neofelis
Felis
Prionailurus
Otocolobus
Leopardus
Puma
Caracal
Profelis
Leptailurus
Catopuma
Acinonyx
Paguma
Bobcat n=117
(Lynx rufus)
Canada lynx n=42
(Lynx canadensis)
Domestic cat n=15*
(Felis catus)
Ocelot n=1*
(Leopardus pardalis)
Puma n=24
(Puma concolor)
Caracal n=34
(Caracal caracal)
Palm civet n=8*
(Paguma larvata)
Family
Felidae
Viverridae
4.0 Nucleotide subs/site
Suborder
Feliforma
B


Host species
Domestic cat
Ocelot
Mountain lion
Bobcat
Bootstrap support
>90
>80-90
>60-80
Sampling locations
Colorado, USA : USA-CO
California, USA : USA-CA
Nevada, USA : USA-NV
Mexico : MX
Brazil : BR
France : FR
Czech Republic : CZ
Japan : JP
China : CN
TTFV species
ORF1
ORF2
5
19
18
3
19
3
20
24
23
27
26
25
21
22
2
1
MT538142
MT538119
MT538111
MT538106
MT538068
MT538056
MT538043
MT538176
MT538158
MT538140
JF304937
MT538071
MT538177
MT538073
MT538144
MT538170
MT537965
MT538159
MT538044
MT538046
MT538048
MT538063
MT538047
MT538070
MT538069
MT538055
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MT538157
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MT538053
MT538114
MT538148
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MT538085
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MT538083
MT538161
MT538092
MT538138
MT538086
MT538166
MT538125
MT538163
MT538102
MT538104
MT538149
MT538039
MT538067
MT538155
MT538108
MT538123
MT538147
MT538101
MT538115
MT538099
MT538175
MT538153
MT538074
MT538121
MT538120
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MT538066
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MT538112
MT538107
MT538064
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MT538152
MT538062
MT538088
MT538096
MT538165
MT538098
MT538051
MT538156
MT538084
MT538136
MT538131
EF538877
KM229766
KM229764
AB076003
HM142588
HM142589
KM229765
KM593801
EF538878
USA-CO
USA-CA
USA-CO
USA-CA
USA-CO
USA-CA
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USA-CO
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USA-CO
USA-CA
USA-CO
USA-CA
USA-NV
MX
USA-CA
BR
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USA-CA
USA-NV
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USA-CA
USA-CA
MX
USA-CA
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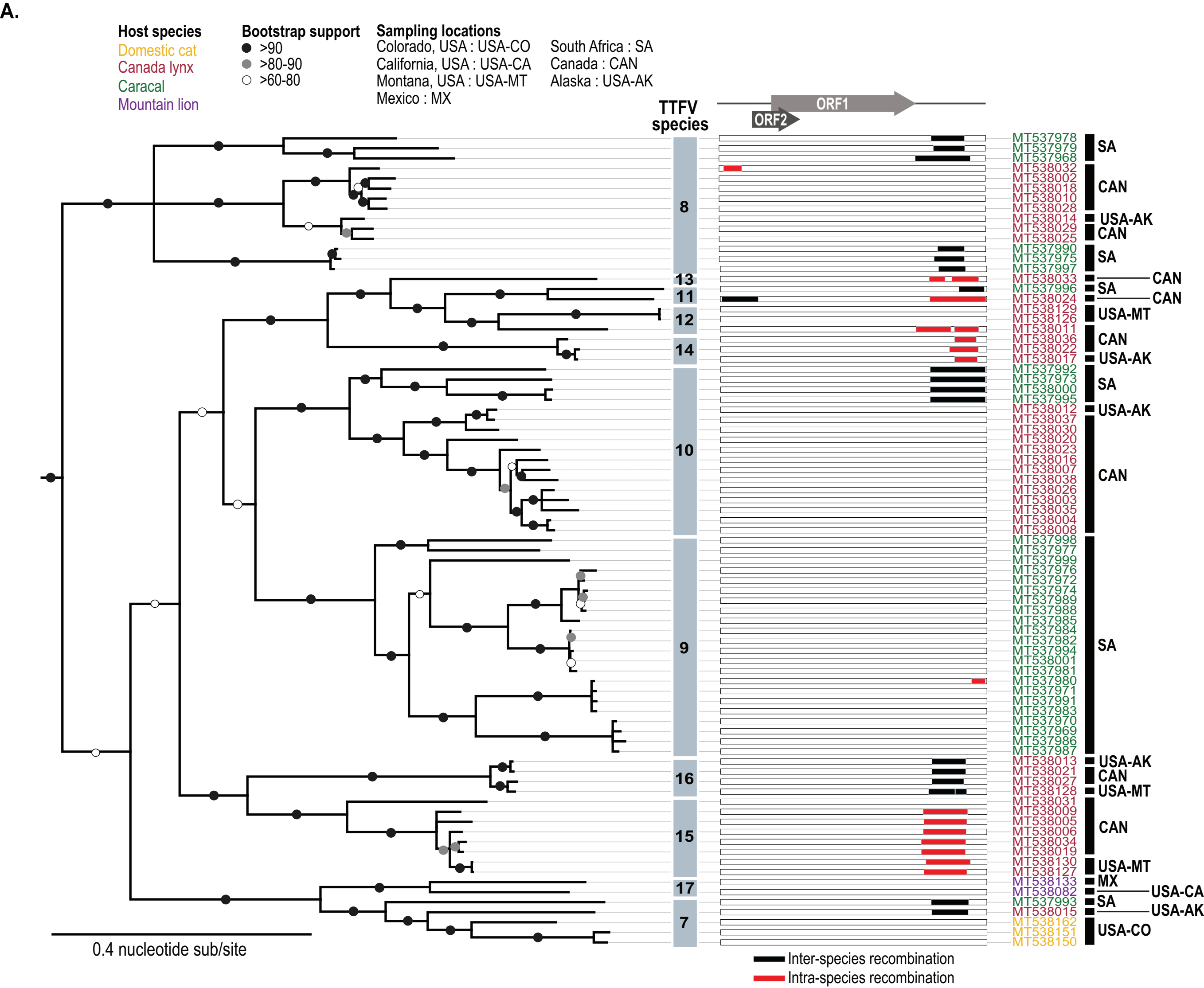
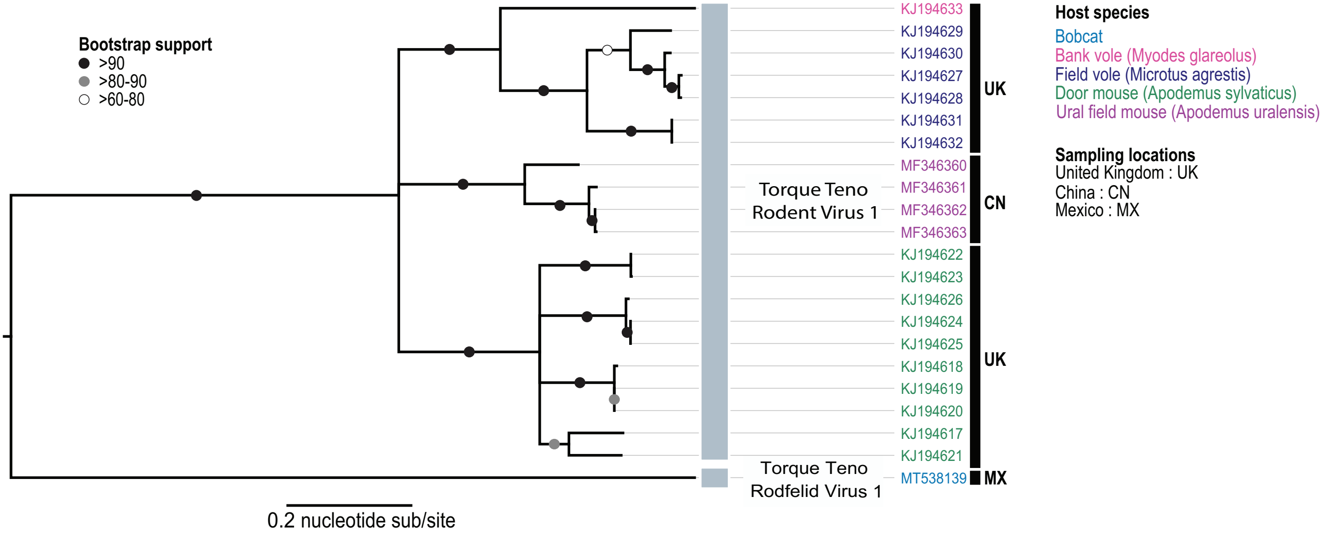
USA-CA
MX
FR
CZ
JP
CN
CZ
FR
0.4 nucleotide subs/site
Inter-species recombination
Intra-species recombination


A.
Host species
Domestic cat
Canada lynx
Caracal
Mountain lion
Bootstrap support
● >90
● >80-90
○ >60-80
Sampling locations
Colorado, USA : USA-CO
California, USA : USA-CA
Montana, USA : USA-MT
Mexico : MX
South Africa : SA
Canada : CAN
Alaska : USA-AK
TTFV species
ORF1
ORF2
8
13
11
12
14
10
9
16
15
17
7
MT537978
MT537979
MT537968
MT538032
MT538002
MT538018
MT538010
MT538028
MT538014
MT538029
MT538025
MT537990
MT537975
MT537997
MT538033
MT537996
MT538024
MT538129
MT538126
MT538011
MT538036
MT538022
MT538017
MT537992
MT537973
MT538000
MT537995
MT538012
MT538037
MT538030
MT538020
MT538023
MT538016
MT538007
MT538038
MT538026
MT538003
MT538035
MT538004
MT538008
MT537998
MT537977
MT537999
MT537976
MT537972
MT537974
MT537989
MT537988
MT537985
MT537984
MT537982
MT537994
MT538001
MT537981
MT537980
MT537971
MT537991
MT537983
MT537970
MT537969
MT537986
MT537987
MT538013
MT538021
MT538027
MT538128
MT538031
MT538009
MT538005
MT538006
MT538034
MT538019
MT538130
MT538127
MT538133
MT538082
MT537993
MT538015
MT538162
MT538151
MT538150
SA
CAN
USA-AK
CAN
SA
CAN
SA
CAN
USA-MT
CAN
USA-AK
SA
USA-AK
CAN
SA
USA-AK
CAN
USA-MT
CAN
USA-MT
MX
USA-CA
SA
USA-AK
USA-CO
0.4 nucleotide sub/site
Inter-species recombination
Intra-species recombination


Bootstrap support
● >90
● >80-90
○ >60-80
KJ194633
KJ194629
KJ194630
KJ194627
KJ194628
KJ194631
KJ194632
MF346360
MF346361
MF346362
MF346363
KJ194622
KJ194623
KJ194626
KJ194624
KJ194625
KJ194618
KJ194619
KJ194620
KJ194617
KJ194621
MT538139
Torque Teno Rodent Virus 1
Torque Teno Rodfelid Virus 1
UK
CN
UK
MX
Host species
Bobcat
Bank vole (Myodes glareolus)
Field vole (Microtus agrestis)
Door mouse (Apodemus sylvaticus)
Ural field mouse (Apodemus uralensis)
Sampling locations
United Kingdom : UK
China : CN
Mexico : MX
0.2 nucleotide sub/site

Sample ID	Host	Sex	Sampling location	Sample date	Sample type	Virus	Accession #
55	Bobcat	unknown	Colorado, USA	unknown	Blood	TTFV5	MT537965
85	Bobcat	unknown	Colorado, USA	unknown	Blood	TTFV5	MT537966
105	Bobcat	unknown	Colorado, USA	unknown	Blood	TTFV5	MT537967
LSF3	Bobcat	F	California, USA	2003	Faeces	TTFV20	MT538039
						TTFV18	MT538040
						TTFV5	MT538041
LSF4	Bobcat	M	California, USA	2004	Faeces	TTFV19	MT538042
						TTFV5	MT538043
						TTFV5	MT538044
						TTFV23	MT538045
						TTFV5	MT538046
LSF9	Bobcat	unknown	California, USA	unknown	Faeces	TTFV5	MT538049
						TTFV19	MT538050
LSF12	Bobcat	M	California, USA	2008	Faeces	TTFV25	MT538051
						TTFV5	MT538052
						TTFV5	MT538053
LSF14	Bobcat	M	California, USA	2008	Faeces	TTFV24	MT538054
						TTFV5	MT538055
LSF19	Bobcat	M	California, USA	2010	Faeces	TTFV5	MT538056
LSF30	Bobcat	M	California, USA	2010	Faeces	TTFV18	MT538060
						TTFV5	MT538061
						TTFV26	MT538062
LSF32	Bobcat	F	California, USA	2010	Faeces	TTFV24	MT538064
LSF33	Bobcat	F	California, USA	2010	Faeces	TTFV24	MT538065
						TTFV24	MT538066
LSF44	Bobcat	M	California, USA	2011	Faeces	TTFV5	MT538073
LSF48	Bobcat	F	California, USA	2014	Faeces	TTFV24	MT538074
LSF55	Bobcat	unknown	California, USA	2014	Faeces	TTFV24	MT538075
LSF56	Bobcat	unknown	California, USA	2014	Faeces	TTFV18	MT538076
LSF58	Bobcat	F	California, USA	2011	Faeces	TTFV5	MT538077
LSF59	Bobcat	M	California, USA	2011	Faeces	TTFV18	MT538078
LSF61	Bobcat	M	California, USA	2011	Faeces	TTFV18	MT538079
LSF62	Bobcat	M	California, USA	2011	Faeces	TTFV19	MT538080
LSF123	Puma	M	California, USA	2008	Faeces	TTFV19	MT538081
LSF125	Puma	M	California, USA	2010	Faeces	TTFV19	MT538083
						TTFV21	MT538084
LSF126	Puma	F	California, USA	2015	Faeces	TTFV19	MT538085
LSF128	Puma	unknown	California, USA	2014	Faeces	TTFV20	MT538086
						TTFV19	MT538087
						TTFV25	MT538088
LSF129	Puma	unknown	California, USA	2011	Faeces	TTFV27	MT538089
						TTFV19	MT538090
						TTFV19	MT538091
LSF132	Puma	unknown	California, USA	unknown	Faeces	TTFV3	MT538092
						TTFV19	MT538093
LSF134	Puma	unknown	California, USA	2012	Faeces	TTFV19	MT538094
LSF140	Bobcat	unknown	California, USA	2012	Faeces	TTFV19	MT538095
LSF141	Bobcat	M	California, USA	2017	Faeces	TTFV25	MT538096
						TTFV5	MT538097
LSF147	Bobcat	M	California, USA	2017	Faeces	TTFV25	MT538098
						TTFV20	MT538099
LSF48	Bobcat	F	California, USA	2014	Faeces	TTFV24	MT538100
LSF68	Bobcat	M	California, USA	2012	Faeces	TTFV20	MT538101
LSF69	Bobcat	unknown	California, USA	unknown	Faeces	TTFV20	MT538102
LSF70	Bobcat	unknown	California, USA	2012	Faeces	TTFV18	MT538103
LSF72	Bobcat	unknown	California, USA	2012	Faeces	TTFV20	MT538104
LSF73	Bobcat	unknown	California, USA	2012	Faeces	TTFV18	MT538105
LSF77	Bobcat	unknown	California, USA	2010	Faeces	TTFV5	MT538106
LSF89	Bobcat	unknown	California, USA	unknown	Faeces	TTFV24	MT538107
						TTFV20	MT538108
LSF91	Bobcat	unknown	California, USA	2009	Faeces	TTFV18	MT538109
LSF101	Bobcat	unknown	California, USA	2010	Faeces	TTFV5	MT538110
LSF107	Bobcat	unknown	California, USA	2010	Faeces	TTFV5	MT538111
LSF112	Bobcat	unknown	California, USA	2010	Faeces	TTFV24	MT538112
LSF117	Bobcat	unknown	California, USA	2011	Faeces	TTFV23	MT538113
LSF140	Bobcat	unknown	California, USA	2012	Faeces	TTFV5	MT538114
LSF145	Bobcat	M	California, USA	2017	Faeces	TTFV20	MT538115
LSF150	Bobcat	F	California, USA	2017	Faeces	TTFV19	MT538116
LSF153	Bobcat	F	California, USA	unknown	Faeces	TTFV5	MT538117
LSF154	Bobcat	M	California, USA	2017	Faeces	TTFV24	MT538118
LSF156	Bobcat	M	California, USA	2009	Faeces	TTFV5	MT538119
LSF165	Bobcat	M	California, USA	2011	Faeces	TTFV5	MT538122
LSF170	Bobcat	M	California, USA	2010	Faeces	TTFV18	MT538124
LSF176	Bobcat	unknown	California, USA	2011	Faeces	TTFV20	MT538125

UoA1	Puma	unknown	Sonora, Mexico	2013	Faeces	TTFV22	MT538131
UoA2	Puma	unknown	Sonora, Mexico	2014	Faeces	TTFV23	MT538132
UoA3	Puma	unknown	Sonora, Mexico	2014	Faeces	TTFV19	MT538134
UoA4	Puma	unknown	Sonora, Mexico	2014	Faeces	TTFV23	MT538135
						TTFV21	MT538136
UoA9	Puma	unknown	Sonora, Mexico	2014	Faeces	TTFV3	MT538137
						TTFV3	MT538138
x183	Bobcat	M	California, USA	2000	Blood	TTFV5	MT538140
x262	Bobcat	M	Colorado, USA	2008	Blood	TTFV19	MT538141
x269	Bobcat	M	Colorado, USA	2008	Blood	TTFV5	MT538142
x271	Bobcat	M	Colorado, USA	2008	Blood	TTFV19	MT538143
x272	Bobcat	M	Colorado, USA	2008	Blood	TTFV5	MT538144
x906	Bobcat	F	California, USA	2007	Blood	TTFV19	MT538145
						TTFV19	MT538146
						TTFV20	MT538147
					Faeces	TTFV5	MT538047
						TTFV5	MT538048
x913	Bobcat	M	California, USA	2007	Blood	TTFV5	MT538148
x1172	Bobcat	F	California, USA	2010	Blood	TTFV20	MT538149
x1294	Bobcat	M	California, USA	2009	Blood	TTFV26	MT538152
X1296	Bobcat	F	California, USA	2009	Blood	TTFV20	MT538153
					Faeces	TTFV24	MT538120
						TTFV24	MT538121
x1299	Bobcat	M	California, USA	2010	Blood	TTFV24	MT538154
						TTFV20	MT538155
x1301	Bobcat	M	California, USA	2010	Blood	TTFV25	MT538156
X1303	Bobcat	F	California, USA	2010	Blood	TTFV5	MT538157
X1307	Bobcat	M	California, USA	2010	Blood	TTFV5	MT538158
						TTFV5	MT538159
					Faeces	TTFV5	MT538057
X1350	Puma	F	Nevada, USA	2010	Blood	TTFV19	MT538160
						TTFV3	MT538161
x1508	Bobcat	F	California, USA	2011	Blood	TTFV20	MT538163
x1509	Bobcat	F	California, USA	2010	Blood	TTFV18	MT538164
					Faeces	TTFV18	MT538058
						TTFV5	MT538059
x1511	Bobcat	M	California, USA	2010	Blood	TTFV25	MT538165
						TTFV20	MT538166
x1513	Bobcat	F	California, USA	2010	Blood	TTFV19	MT538167
					Faeces	TTFV20	MT538123
x1514	Bobcat	M	California, USA	2010	Blood	TTFV19	MT538168
	Bobcat		California, USA		Faeces	TTFV5	MT538063
x1516	Bobcat	F	California, USA	2010	Blood	TTFV24	MT538169
						TTFV5	MT538170
x1520	Bobcat	M	California, USA	2010	Blood	TTFV5	MT538171
x1522	Bobcat	F	California, USA	2010	Blood	TTFV19	MT538172
						TTFV23	MT538173
x1523	Bobcat	M	California, USA	2010	Blood	TTFV19	MT538174
						TTFV20	MT538175
x1525	Bobcat	F	California, USA	2010	Blood	TTFV5	MT538176
x1529	Bobcat	M	California, USA	2010	Blood	TTFV5	MT538177
					Faeces	TTFV20	MT538067
						TTFV5	MT538068
x1532	Bobcat	F	California, USA	2010	Blood	TTFV19	MT538178
					Faeces	TTFV5	MT538069
						TTFV5	MT538070
						TTFV5	MT538071
x1535	Bobcat	M	California, USA	2011	Blood	TTFV23	MT538179
					Faeces	TTFV19	MT538072
x1542	Bobcat	F	California, USA	2011	Blood	TTFV19	MT538180
x1543	Bobcat	M	California, USA	2011	Blood	TTFV24	MT538181
x1574	Bobcat		California, USA	2009	Blood	TTFV19	MT538182
						TTFV19	MT538183
x1576	Bobcat	M	California, USA	2011	Blood	TTFV19	MT538184
CCB9	Caracal	M	Western Cape, South Africa	2014	Blood	TTFV8	MT537968
CCB10	Caracal	M	Western Cape, South Africa	2016	Blood	TTFV9	MT537969
CCB16	Caracal	F	Western Cape, South Africa	2016	Blood	TTFV9	MT537970
CCB21	Caracal	M	Western Cape, South Africa	2017	Blood	TTFV9	MT537971
CCB22	Caracal	F	Western Cape, South Africa	unknown	Blood	TTFV9	MT537972
CCB23	Caracal	F	Western Cape, South Africa	2015	Blood	TTFV10	MT537973
						TTFV9	MT537974
CCB25	Caracal	M	Western Cape, South Africa	2015	Blood	TTFV8	MT537975
CCB27	Caracal	M	Western Cape, South Africa	2015	Blood	TTFV9	MT537976
CCB28	Caracal	M	Western Cape, South Africa	2015	Blood	TTFV9	MT537977
CCB29	Caracal	M	Western Cape, South Africa	2015	Blood	TTFV8	MT537978

CCB31	Caracal	F	Western Cape, South Africa	2015	Blood	TTFV8	MT537979
CCB36	Caracal	M	Western Cape, South Africa	2015	Blood	TTFV9	MT537980
CCB37	Caracal	F	Western Cape, South Africa	2015	Blood	TTFV9	MT537981
CCB38	Caracal	M	Western Cape, South Africa	2015	Blood	TTFV9	MT537982
CCB39	Caracal	M	Western Cape, South Africa	2016	Blood	TTFV9	MT537983
CCB40	Caracal	M	Western Cape, South Africa	2015	Blood	TTFV9	MT537984
CCB41	Caracal	M	Western Cape, South Africa	2015	Blood	TTFV9	MT537985
CCB42	Caracal	F	Western Cape, South Africa	2016	Blood	TTFV9	MT537986
CCB43	Caracal	M	Western Cape, South Africa	2016	Blood	TTFV9	MT537987
CCB44	Caracal	M	Western Cape, South Africa	2016	Blood	TTFV9	MT537988
CCB46	Caracal	F	Western Cape, South Africa	2016	Blood	TTFV9	MT537989
CCB48	Caracal	F	Western Cape, South Africa	2016	Blood	TTFV8	MT537990
CCB49	Caracal	F	Western Cape, South Africa	2016	Blood	TTFV9	MT537991
CCB52	Caracal	M	Western Cape, South Africa	2016	Blood	TTFV10	MT537992
						TTFV7	MT537993
CCB55	Caracal	F	Western Cape, South Africa	2016	Blood	TTFV9	MT537994
CCB56	Caracal	M	Western Cape, South Africa	2016	Blood	TTFV10	MT537995
				2016	Blood	TTFV11	MT537996
CCB59	Caracal	F	Western Cape, South Africa	2018	Blood	TTFV8	MT537997
CCB60	Caracal	M	Western Cape, South Africa	2017	Blood	TTFV9	MT537998
				2017	Blood	TTFV9	MT537999
CCB62	Caracal	M	Western Cape, South Africa	2018	Blood	TTFV10	MT538000
CCB63	Caracal		Western Cape, South Africa	2018	Blood	TTFV9	MT538001
CLB2	Canada lynx	F	Quebec, Canada	2003	Blood	TTFV8	MT538002
						TTFV10	MT538038
CLB3	Canada lynx	F	Quebec, Canada	2004	Blood	TTFV10	MT538003
CLB4	Canada lynx	F	Quebec, Canada	2004	Blood	TTFV10	MT538004
						TTFV15	MT538005
CLB5	Canada lynx	F	Quebec, Canada	2004	Blood	TTFV15	MT538006
CLB7	Canada lynx	F	Quebec, Canada	2002	Blood	TTFV10	MT538007
CLB8	Canada lynx	F	Quebec, Canada	2002	Blood	TTFV10	MT538008
CLB9	Canada lynx	F	Colorado, USA	2011	Blood	TTFV15	MT538009
CLB10	Canada lynx	F	Quebec, Canada	2002	Blood	TTFV8	MT538010
						TTFV12	MT538011
CLB11	Canada lynx	M	Alaska, USA	1999	Blood	TTFV10	MT538012
						TTFV16	MT538013
						TTFV8	MT538014
						TTFV7	MT538015
CLB12	Canada lynx	F	Colorado, USA	2011	Blood	TTFV10	MT538016
CLB13	Canada lynx	M	Alaska, USA	1999	Blood	TTFV14	MT538017
CLB14	Canada lynx	M	Yukon, Canada	1999	Blood	TTFV8	MT538018
						TTFV15	MT538019
CLB16	Canada lynx	F	Colorado, USA	2006	Blood	TTFV10	MT538020
						TTFV16	MT538021
						TTFV14	MT538022
CLB17	Canada lynx	F	British Columbia, Canada	2005	Blood	TTFV10	MT538023
						TTFV11	MT538024
						TTFV8	MT538025
CLB18	Canada lynx	M	British Columbia, Canada	2005	Blood	TTFV10	MT538026
						TTFV16	MT538027
CLB20	Canada lynx	M	British Columbia, Canada	2005	Blood	TTFV8	MT538028
						TTFV8	MT538029
CLB21	Canada lynx	M	Yukon, Canada	2006	Blood	TTFV10	MT538030
						TTFV15	MT538031
						TTFV8	MT538032
						TTFV13	MT538033
						TTFV15	MT538034
CLB22	Canada lynx	M	Yukon, Canada	2006	Blood	TTFV10	MT538035
						TTFV14	MT538036
CLB24	Canada lynx	M	Yukon, Canada	2010	Blood	TTFV10	MT538037
LSF125	Puma	M	California, USA	2010	Faeces	TTFV17	MT538082
MAF4	Canada lynx	M	Montana, USA	2018	Faeces	TTFV12	MT538126
MAF5	Canada lynx	M	Montana, USA	2018	Faeces	TTFV15	MT538127
MAF11	Canada lynx	F	Montana, USA	2018	Faeces	TTFV16	MT538128
MAF12	Canada lynx	M	Montana, USA	2018	Faeces	TTFV12	MT538129
						TTFV15	MT538130
UoA2	Puma	unknown	Sonora, Mexico	2014	Faeces	TTFV17	MT538133
X1259	Domestic Cat	M	Colorado, USA	2010	Blood	TTFV7	MT538150
X1498	Domestic Cat	M	Colorado, USA	2011	Blood	TTFV7	MT538162
X1272	Domestic Cat	F	Colorado, USA	2010	Blood	TTFV7	MT538151
UoA20	Bobcat	unknown	Sonora, Mexico	2014	Faeces	TTRodFV 1	MT538139