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Characterization of Regionally Associated Feline Immunodeficiency Virus (FIV) in Bobcats (*Lynx rufus*)

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ABSTRACT: Feline immunodeficiency virus (FIV) classically infects felid species with highly divergent species-specific FIVs. However, recent studies have detected an FIV strain infecting both bobcats (*Lynx rufus*) and pumas (*Puma concolor*) in California and Florida. To further investigate this observation, we evaluated FIV from bobcats in Florida ($n=25$) and Colorado ($n=80$) between 2008 and 2011. Partial viral sequences from five Florida bobcats cluster with previously published sequences from Florida panthers. We did not detect FIV in Colorado bobcats.

Key words: Bobcat (*Lynx rufus*), feline immunodeficiency virus, *Lentivirus*, puma (*Puma concolor*), viral evolution.

Feline immunodeficiency virus (FIV) is an enveloped retrovirus of the genus *Lentivirus* and is spread via intimate contact with an infected individual (Allison and Hoover, 2003). Each felid species harboring a FIV-like virus appears to have a species-specific strain that is highly divergent from other FIVs, demonstrating most infections occur from intraspecific transmission (VandeWoude and Apetrei, 2006). FIV sequences from pumas (*Puma concolor*) cluster into two distinct monophyletic clades, FIV-PcoA and FIV-PcoB. FIV-PcoB infects pumas across most of their range in North and South America, while FIV-PcoA was initially detected only in pumas from Florida (*Puma concolor coryii*) and southern California (Carpenter et al., 1996; Biek et al., 2003, 2006). An analysis of FIV sequences from bobcats (*Lynx rufus*) in California ($n=18$) and Florida ($n=1$) demonstrated these isolates cluster with FIV-PcoA (Franklin et al., 2007), suggesting interspecific transmission occurs between bobcats and pumas in

these regions. Franklin et al. (2007) concluded that FIV-PcoA has coevolved with bobcats and now infects pumas in these two geographic regions due to a “jump” from one host to another.

We investigated this finding by sequencing additional FIV-PcoA isolates from bobcats in Florida to determine the phylogenetic relationship of these isolates to the previously published Florida bobcat and puma isolates. Only one FIV sequence has been reported from Florida bobcats, and it was a historical sequence that had not been highly annotated (Franklin et al., 2007). We also sought to sequence FIV isolates from bobcats in Colorado, a geographic region from which no bobcat FIV sequences have been published.

Blood or tissue samples in Colorado were collected from live-captured bobcats in the Rocky Mountains west (Western Slope; $n=26$) and east (Front Range; $n=19$) of the Continental Divide between 2008 and 2011 (capture locations have been published; Bevins et al., 2012), and from Florida bobcats ($n=25$) in 2010 (Fig. 1). Additionally, mesenteric lymph node ($n=1$), spleen ($n=31$), or submandibular lymph node ($n=3$) samples were collected from 35 hunter-harvested bobcats on the Western Slope of Colorado between 2007 and 2008. Collection procedures were approved by the Colorado State University IACUC or IACUCs from collaborating institutions or agencies.

Bobcat genomic DNA was extracted from whole blood, peripheral blood mononuclear cells, or tissue samples using DNeasy Blood and Tissue Kit (Qiagen,

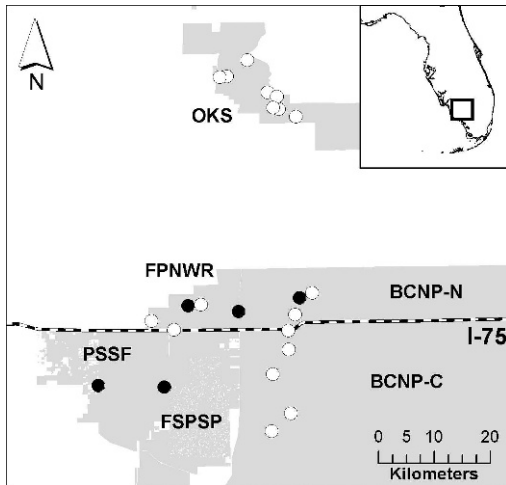


FIGURE 1. Distribution of bobcats (*Lynx rufus*) from southern Florida, sampled in 2010. Closed and open circles denote individuals with and without feline immunodeficiency virus (FIV) sequences, respectively. The dashed line indicates interstate 75 (I-75). Light gray regions indicate conservation lands. BCNP = Big Cypress National Preserve (North and Central regions); FPNWR = Florida Panther National Wildlife Refuge, FSPSP = Fakahatchee Strand Preserve State Park, OKS = Okaloacoochee Slough, PSSF = Picayune Strand State Forest.

Valencia, California, USA) according to the manufacturer's instructions. Nested PCR was performed using 100–1,000 ng of genomic DNA with degenerate primers designed from a conserved section of the reverse transcriptase region of the *pol* gene as previously described (Troyer et al., 2005). While this region of the genome is relatively short, it is the most highly conserved region of the FIV genome and thus is reliably amplified by degenerate PCR primers. Other regions of the FIV genome have highly variable sequences in individual isolates. The primers we used have been shown to amplify isolates of FIV-PcoA, FIV-PcoB, and all other known FIV subtypes (Troyer et al., 2005). Twenty-three nucleotide sequences from GenBank were compiled and aligned with five new sequences we produced using the default settings in Geneious software (Drummond et al., 2011) and verified visually. Sequences were chosen to repre-

sent the geographic distribution of FIV in bobcats and pumas, and have the following accession numbers: Pco-F29 (EF601127), Pco-F15 (EF601129), Pco-F14 (EF601133), Pco-M9 (EF601132), Pco-SM4 (EF601136), Lru-1 (EF601137), Lru-12 (EF601140), Lru-5 (EF601141), Lru-7 (EF601143), Lru-SM49 (EF601153), Lru-SM117 (EF601154), Lru-SM59 (EF601155) (Franklin et al., 2007); Pco-61-3 (U53753), Pco-117 (U53718), Pco-333 (U53742), Pco-28 (U53756), Pco-141 (U53719), Pco-145 (U53725) (Carpenter et al., 1996); and Pco-182 (M95470), Pco-68 (M95476), Pco-75 (M95478) (Olmstead et al., 1992). Domestic cat FIV sequences FIV-Fca (U11820) (Sodora et al., 1995) and FIV-petaluma (M25381) were also included. Sequences were trimmed at 5' and 3' ends resulting in a sequences data set with fragments of identical length (319 bases). A phylogenetic tree was constructed with MEGA5 using the maximum likelihood method (Tamura et al., 2011). The initial tree for the heuristic search was obtained using the BIONJ method with MCL distance matrix. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories [+G, parameter=0.936]). The rate variation model allowed for some sites to be evolutionarily invariable (44%).

None of the 80 bobcat samples from Colorado were positive for FIV by PCR, which is in contrast to 10–20% documented antibody-prevalence (Bevins et al., 2012). Five of 25 bobcats from Florida were FIV-positive by PCR (20% prevalence). Prevalence of FIV, detected by PCR, in Florida bobcats is similar to previous reports from Ventura County (Franklin et al., 2007). All positive Florida bobcats were adult males captured around highway I-75 (Fig. 1). No Florida bobcats in the most northern region sampled were PCR-positive for FIV (OKS; $n=8$; Fig. 1).

Sequence analysis demonstrated that FIV isolates amplified from Florida bobcats are FIV-PcoA and cluster with the previously published Florida bobcat and

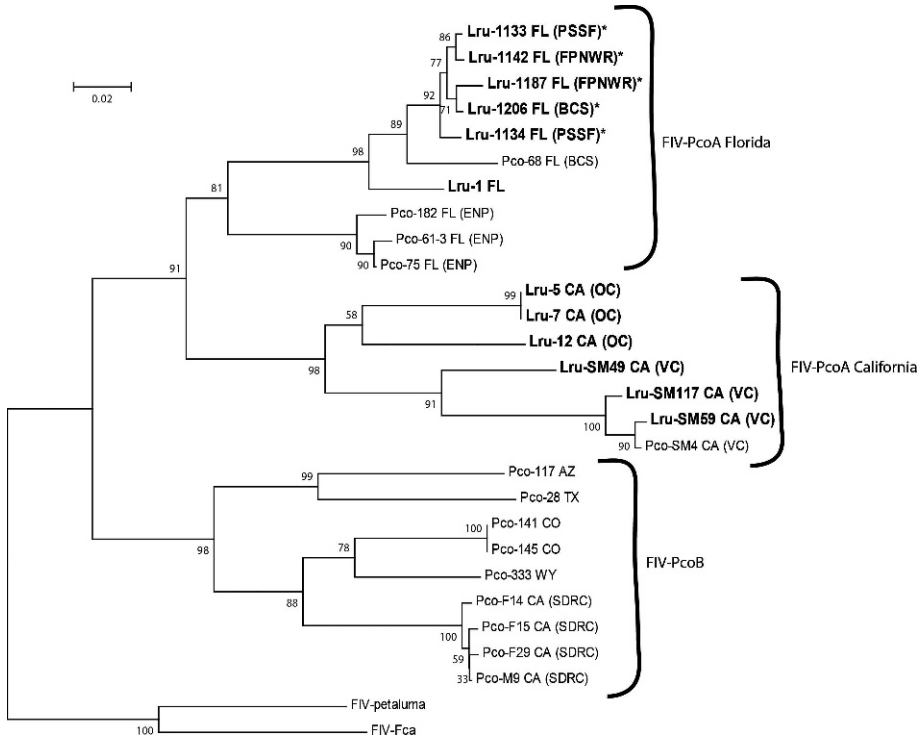


FIGURE 2. Maximum likelihood tree, using the general time reversible model, demonstrates the relationship between previously and newly characterized sequences of the conserved region of the *pol* gene of feline immunodeficiency virus from pumas (*Puma concolor*) and bobcats (*Lynx rufus*). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Sequences in bold type are bobcats; sequences in regular type are pumas. Asterisk indicates newly characterized sequence. For California: OC = Orange County; SDRC = San Diego and Riverside counties; VC = Ventura County. For Florida: BCS = Big Cypress Swamp; ENP = Everglades National Park; FPNWR = Florida Panther National Wildlife Refuge; PSSF = Picayune Strand State Forest.

panther isolates (Olmstead et al., 1992; Carpenter et al., 1996; Fig. 2). Furthermore, Florida FIV sequences are similar to others in the same geographic location, regardless of the species from which they were isolated. Two well-supported clusters (Florida and California) appear within the FIV-PcoA clade (Fig. 2). Within these clusters, FIV isolates group at an even finer spatial scale. Florida isolates from more northern regions (PSSF, FPNWR, BCNP; Fig. 1) are more similar to isolates from Everglades National Park (Fig. 2). Similarly, California isolates group into distinct Ventura County and Orange County clusters (Franklin et al., 2007). A recent analysis of southern California bobcat FIVs failed

to reveal FIV monophylogeny on either side of a major freeway, even though host genetics were divided on this basis (Lee et al., 2012). FIV isolates infecting domestic cats represents a monophyletic clade that is highly divergent from isolates infecting pumas and bobcats, reiterating previous findings (Troyer et al., 2005; VandeWoude and Apetrei, 2006; Fig. 2). These studies may collectively inform the scale at which phylogeography of FIVs occur.

FIV-PcoA has not been found in pumas or bobcats outside of Florida or California, and FIV of any clade has yet to be sequenced from bobcats outside of these two regions (Franklin et al., 2007). There are three possible explanations for the lack of FIV-positive samples from Colorado: 1)

FIV proviral load in these animals is too low to detect by PCR, 2) the bobcats are not infected with FIV, or 3) the primers used in the PCR failed to amplify a highly divergent, yet unidentified, FIV subtype. The latter explanation is unlikely because the primers used in this study have detected diverse FIVs in many felid species from around the world (Troyer et al., 2005). Given our ability to consistently amplify FIV isolates from bobcats and pumas elsewhere in the US, we would likely have detected the virus if Colorado bobcats were infected with FIV-PcoA or FIV-PcoB, even at a low proviral load. The fact that we were unable to identify FIV in 80 bobcat samples from Colorado suggests that bobcats in this region do not carry the virus, and that pumas in this region also do not carry this strain because they are infected only after contact with FIV-positive bobcats. The reasons for such a significant geographic disparity are unknown and warrant further study, particularly given serologic evidence of exposure (Bevins et al., 2012).

Florida and California have experienced rapid human population growth and widespread urban development, resulting in fragmenting suitable habitat and potentially increasing contact rates and competition for resources. These changes may consequently increase disease transmission within and between species (Harris and Atkins, 1991; Hoctor et al., 2000; Franklin et al., 2007). Habitat modification is a leading determinant of cross-species transmission of pathogens (Deem et al., 2001; Patz et al., 2004; Aguirre and Tabor, 2008). Encounters between bobcats and pumas would most likely be aggressive and asymmetric, providing an opportunity for transmission of FIV from bobcats to pumas (Currier, 1983; Knick, 1990; Koehler and Hornocker, 1991). Increased interaction leading to cross-species transmission of FIV from bobcats to pumas in Florida and California, coupled with relatively high FIV prevalence in bobcats in these areas, is a

possible explanation for the occurrence of FIV-PcoA in pumas in these regions. The reasons that FIV infection of bobcats could not be detected in Colorado are unclear and require additional study.

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