DOI: 10.7589/JWD-D-24-00058

*Journal of Wildlife Diseases*, 61(1), 2025, pp. 262–266


# Prion Gene Sequencing in Florida Panthers (*Puma concolor coryi*) Suggests No Differential Susceptibility to Transmissible Spongiform Encephalopathy

**Elizabeth Sharkey,[1,2] David P. Onorato,[3] Melody E. Roelke-Parker,[4] Alexander Ochoa,[1,5] Melanie Culver,[6] and Robert R. Fitak[1,7]** [1]Department of Biology, Genomics & Bioinformatics Cluster, University of Central Florida, 4110 Libra Drive, Orlando, Florida 32816, USA; [2]Current address: Department of Anthropology, Washington University in St. Louis, 1 Brookings Drive, St. Louis, Missouri 63130, USA; [3]Fish and Wildlife Research Institute, Florida Fish and Wildlife Conservation Commission, 298 Sabal Palm Road, Naples, Florida 34114, USA; [4]Frederick National Laboratory for Cancer Research, Leidos Biomedical Research, Inc., 8560 Progress Drive, Fredrick, Maryland 21701, USA; [5]Current address: Department of Ecology & Evolutionary Biology, Yale University, 21 Sachem Street, New Haven, Connecticut 06520, USA; [6]US Geological Survey, Arizona Cooperative Fish and Wildlife Research Unit, and School of Natural Resources and the Environment, University of Arizona, 1064 E. Lowell Street, Tucson, Arizona 85721, USA; [7]Corresponding author (email: robert.fitak@ucf.edu)

ABSTRACT: Transmissible spongiform encephalopathy, or prion disease, poses a serious threat to wildlife; however, the susceptibility of apex predators is still being assessed. We investigated variation in the prion protein gene in Florida panthers (*Puma concolor coryi*) and found that admixture from Central American pumas probably introduced a novel, albeit benign, prion allele.

Prion diseases cause physical decline and mortality in many species, including humans, due to the accumulation of misfolded prion proteins in the central nervous system (Prusiner 1998). In North America, prion disease in free-living cervids, called chronic wasting disease (CWD), has been spreading since before the 1980s (Williams 2005). Of particular interest is whether carnivores that prey on affected ungulates are susceptible to acquiring the disease. In June 2023, the first documented case of CWD in white-tailed deer (*Odocoileus virginianus*) in Florida, US, was reported (Young 2023). It remains unclear whether CWD poses a risk to endangered Florida panthers (*Puma concolor coryi*), a subspecies of puma (*P. concolor*) and common predator of deer.

Prion disease in cats (feline spongiform encephalopathy [FSE]) has been documented in various felids (Iulini et al. 2008), including a captive puma (Willoughby et al. 1992), but there are no documented cases in free-living pumas. A study of three captive pumas found that even when fed CWD-infected prey repeatedly over many years, they did not contract prion disease (Wolfe et al. 2022). These findings suggest that certain pumas may be more susceptible to infection. It is known that some variants in the prion protein gene (*PRNP*) affect susceptibility to prion disease (Robinson et al. 2012). In carnivores, *PRNP* variants have been associated with prion disease tolerance in canids and possibly disease susceptibility in felids (Stewart et al. 2012). Although pumas examined to date had identical *PRNP* alleles (Stewart et al. 2012; Wolfe et al. 2022), all samples examined originated from Colorado, US, and may not be representative of intraspecific variability. We sequenced *PRNP* in Florida panthers and pumas from other regions of their range to determine whether variants were present that would strongly impact the structure of the prion protein involved in FSE.

We obtained *PRNP* gene sequences in silico from 21 *P. concolor* whole genomes following bioinformatic procedures described previously (Table 1; Ochoa et al. 2019; Saremi et al. 2019). Variable sites in the *PRNP* gene (accession NW_020340008.1 and region 30816895–30817651) were identified using BCFtools 1.11 (Danecek et al. 2021). Variants with quality score $\geq$20, mapping quality $\geq$30, read position and base-quality biases $>10^{-4}$, minimum allele count of 2, and not within 10 bp of an indel were retained.

We obtained 56 DNA or whole-blood samples originally collected by the Florida Fish and Wildlife Conservation Commission (FWC), coauthors, or collaborators. Samples included canonical Florida panthers from Southwest Florida (preintrogression panthers and their postintrogression descendants), Texas pumas (five

TABLE 1. Summary of the *PRNP* alleles recovered from puma (*Puma concolor*) in a molecular study on their susceptibility to transmissible spongiform encephalopathy. The *PRNP* sequences were obtained using either Sanger sequencing or whole-genome sequencing (WGS). The country of origin of each puma is indicated plus state or province if known. Accession numbers are for the GenBank database (https://www.ncbi.nlm.nih.gov).

| Allele | Accession | *n* (Sanger) | *n* (WGS) | Location(s) |
|---|---|---|---|---|
| PC38 | JX218980 | 45[a] | 18 | USA (California, Colorado, Texas, Florida), Mexico (Durango, Sonora) |
| PC-FL1 | OR625659 | 2[b] | 3 | USA (Florida), Brazil (Minas Gerais, São Paulo), Guatemala |
| PC-FL2 | OR860043 | 1 | 0 | USA (Florida)[c] |
| PC-MEX1 | OR860039 | 1 | 0 | Mexico (Yucatan; captive) |
| PC-ARG1 | OR860040 | 2 | 0 | Argentina, Mexico (Chiapas; captive) |
| PC-ARG2 | OR860041 | 1 | 0 | Argentina |
| PC-ARG3 | OR860042 | 1 | 0 | Argentina |
| PC-BRA1 | OR860037 | 1 | 0 | Brazil (Matto Grosso Do Sul) |
| PC-BRA2 | OR860038 | 2 | 0 | Brazil (Goiás), Venezuela |
| Total | | 56 | 21 | |

[a] One Everglades Florida panther, one canonical Florida panther, and four Texas individuals occurred in both the Sanger and WGS datasets.
[b] One Everglades Florida panther occurred in both the Sanger and WGS datasets.
[c] This sample originated from a non–Florida panther released in Florida of unconfirmed geographic origin.

females intentionally introgressed into the Florida panther population in 1995), admixed Florida panthers (resulting from genetic introgression in 1995), noncanonical Florida panthers from Everglades National Park (ENP; possible descendants of poorly documented releases of captive Central and/or South American pumas in the 1950–60s); and pumas from Central and South America (Table 1 and Fig. 1; Johnson et al. 2010). Genomic DNA was extracted using a phenol-chloroform procedure. We PCR-amplified *PRNP* with primers modified from Stewart et al. (2012): PRP-F 5′-GGTTCCAACATGAACSTAAGATG-3′ and PRP-R 5′-TAMAGGGCTGTAGGTAGACAC-3′. PCR reactions (25 µL) included 2 µL of DNA template (~1–20 ng), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.4 µM each primer, 1× GoTaq reaction buffer (Promega, Madison, Wisconsin, USA), and 1 unit of GoTaq G2 Flexi DNA polymerase (Promega). Reaction conditions were 95 C for 3 min followed by 40 cycles of 95 C for 30 s, 60 C for 30 s, 72 C for 1 min, and a final extension at 72 C for 10 min. We purified PCR products by using a DNA Clean & Concentrator kit (Zymo Research, Irvine, California, USA) and Sanger sequenced them using primers PRPseq-F 5′-CSTAAGATGCTGACGCCCTTC-3′ and PRP seq-R 5′-GACCCGTAAAAGATGAAGAAG-3′. Resulting sequences were trimmed of primers, assembled, and aligned to a known *PRNP* sequence (accession JX218980) with Geneious Prime 2022.1.1 (Biomatters Inc. 2022). To assess putative effects on the prion protein, Provean 1.1 (Choi and Chan 2015) was used to predict whether alleles were deleterious or benign, Amyco (Iglesias et al. 2019) was used to evaluate amyloid propensity, and Phyre2 (Kelley et al. 2015) was used to three-dimensionally model the translated amino acid sequences.

We identified nine *PRNP* alleles across all sequences (Table 1 and Fig. 1; Supplementary Material Table S1). Pumas from North America, including admixed Florida panthers descended from the introduced Texas, females, matched the PC38 allele (accession JX218980) previously reported in Colorado pumas (Stewart et al. 2012). A second allele, PC-FL1 (accession OR625659), was found in pumas from Brazil and Guatemala and one historical ENP Florida panther (Fig. 1A). This finding is consistent with admixture between ENP Florida panthers and Central American pumas that were released into the park (Ochoa

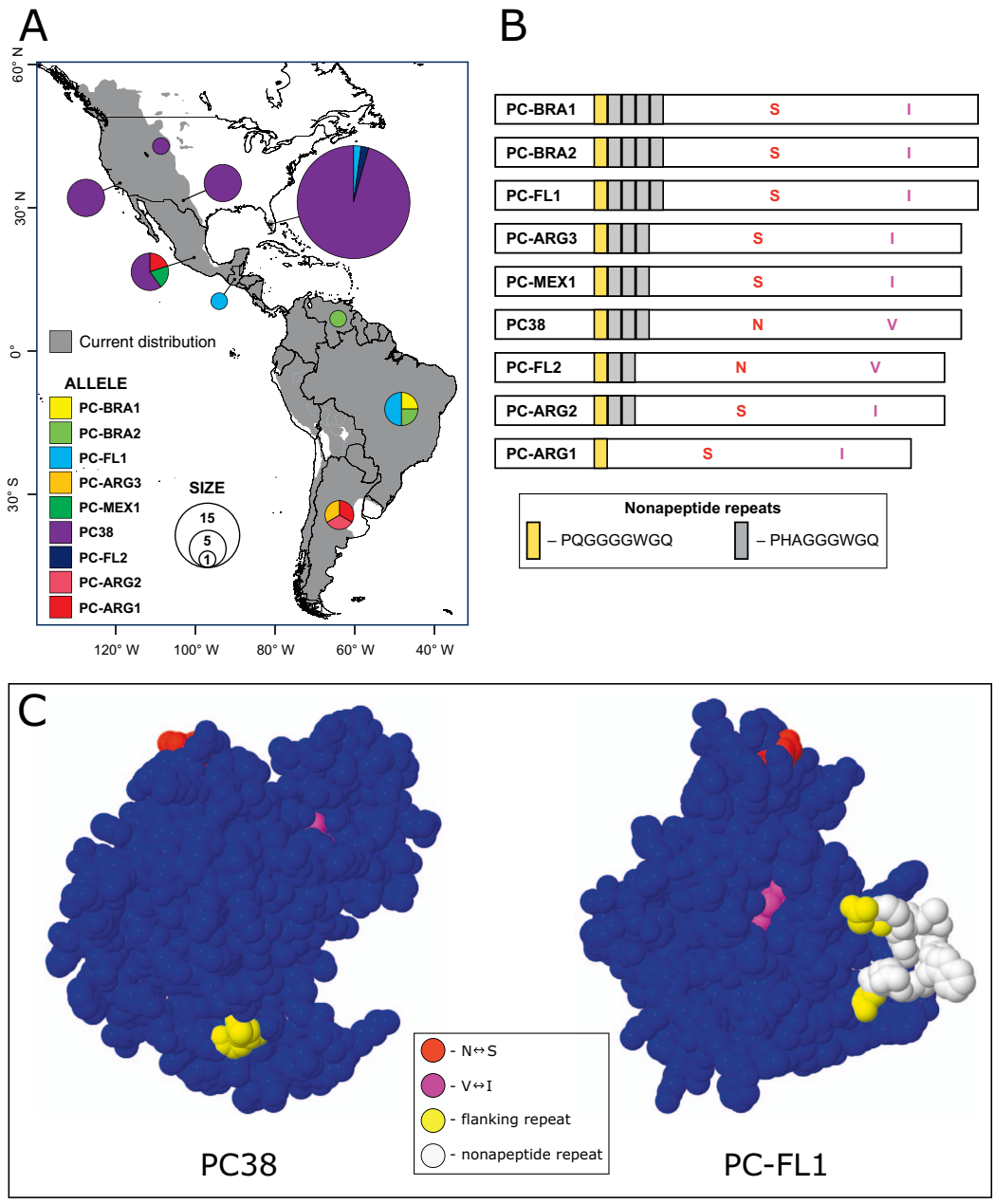


FIGURE 1. Summary of the nine *PRNP* alleles recovered from puma (*Puma concolor*) in a molecular study on their susceptibility to transmissible spongiform encephalopathy. (A) Map of the geographic distribution of *PRNP* alleles (pie charts) and *P. concolor* distribution. (B) Diagram of the translated amino acid sequences for each *PRNP* allele. The length of each amino sequence is drawn to scale (longest is 260 amino acids), and both the nonapeptide repeat structure and nonsynonymous polymorphisms (using amino acid abbreviations) are shown. (C) Predicted prion protein structure for two alleles found in Florida panthers (*P. c. coryi*; PC38 and PC-FL1) that differ at nonsynonymous sites. The variable amino acids are color coded according to the legend provided (using amino acid abbreviations).

et al. 2019). Allele PC-FL2 closely matched PC38, but lacked one nonapeptide repeat.

The nine alleles differed at eight positions: five synonymous sites, two nonsynonymous sites, and a nonapeptide repeat polymorphism (Fig. 1B). The single-nucleotide and nonapeptide polymorphisms were confirmed by both Sanger and whole-genome sequencing in six individuals (Table 1). Whole-genome sequencing could not accurately genotype the nonapeptide polymorphism in the historical ENP individual, which is expected considering the reference allele is PC38 and genotyping large indels is problematic with short reads. In Florida panthers, the nonsynonymous polymorphisms between PC38 or PC-FL2 and PC-FL1 were predicted to be of benign or neutral effect (PROVEAN scores −2.29 and −0.11). Allele PC-FL1 had a higher Amyco score (0.39) than PC38 (0.36), indicating a slightly higher propensity to form amyloid plaques, but still not aggregation prone. Allele PC-FL1 also had no noticeable impact on protein conformation (Fig. 1C). The nonapeptide repeat [9QG–$(9HA)_n$] varied between zero and four copies of the $(9HA)_n$ nonapeptide and is consistent with polymorphisms observed in six other felid genera (Stewart et al. 2012). It is currently unclear in felids whether nonapeptide repeat counts influence the propensity for and/or timing of onset of FSE symptoms.

Overall, it appears that 1) managed genetic restoration has maintained the disease-resistant PC38 allele in Florida panthers; and 2) introgression of Central American pumas into ENP introduced a novel, probably benign, *PRNP* allele that is either nonexistent or rare in extant Florida panthers. Insofar that other North American pumas are resistant to FSE (Wolfe et al. 2022), our results suggest that Florida panthers are not at any elevated risk of acquiring FSE, but ongoing monitoring of CWD in Florida and elsewhere, considering the recent documentation of CWD in Florida white-tailed deer, may assist with long-term conservation.

We thank the FWC and National Park Service for samples and Alex Erwin for reviewing this manuscript. This study was supported by the NSF Research Experiences for Undergraduates project 2149866. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the US Government.

## SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at http://dx.doi.org/10.7589/JWD-D-24-00058.

## LITERATURE CITED

Biomatters Inc. 2022. Geneious Prime v2022.1.1. https://www.geneious.com/download/previous-versions/. Accessed November 2024.

Choi Y, Chan AP. 2015. PROVEAN web server: A tool to predict the functional effect of amino acid substitutions and indels. *Bioinformatics* 31: 2745–2747.

Danecek P, Bonfield JK, Liddle J, Marshall J, Ohan V, Pollard MO, Whitwham A, Keane T, McCarthy SA, et al. 2021. Twelve years of SAMtools and BCFtools. *Gigascience* 10:giab008.

Iglesias V, Conchillo-Sole O, Batlle C, Ventura S. 2019. AMYCO: Evaluation of mutational impact on prion-like proteins aggregation propensity. *BMC Bioinformatics* 20:24.

Iulini B, Cantile C, Mandara MT, Maurella C, Loria GR, Castagnaro M, Salvadori C, Porcario C, Corona C, et al. 2008. Neuropathology of Italian cats in feline spongiform encephalopathy surveillance. *Vet Pathol* 45:626–633.

Johnson WE, Onorato DP, Roelke ME, Land ED, Cunningham M, Belden RC, McBride R, Jansen D, Lotz M, et al. 2010. Genetic restoration of the Florida panther. *Science* 329:1641–1645.

Kelley LA, Mezulis S, Yates CM, Wass MN, Sternberg MJE. 2015. The Phyre2 web portal for protein modeling, prediction and analysis. *Nat Protoc* 10:845–858.

Ochoa A, Onorato DP, Fitak RR, Roelke-Parker ME, Culver M. 2019. *De novo* assembly and annotation from parental and $F_1$ puma genomes of the Florida panther genetic restoration program. *G3 (Bethesda)* 9:3531–3536.

Prusiner SB. 1998. Prions. *Proc Natl Acad Sci USA* 95: 13363–13383.

Robinson SJ, Samuel MD, O'Rourke KI, Johnson CJ. 2012. The role of genetics in chronic wasting disease of North American cervids. *Prion* 6:153–162.

Saremi NF, Supple MA, Byrne A, Cahill JA, Coutinho LL, Dalén L, Figueiró HV, Johnson WE, Milne HJ, et al. 2019. Puma genomes from North and South America provide insights into the genomic consequences of inbreeding. *Nat Commun* 10:4769.

Stewart P, Campbell L, Skogtvedt S, Griffin KA, Arnemo JM, Tryland M, Girling S, Miller MW, Tranulis MA, et al. 2012. Genetic predictions of prion disease susceptibility in carnivore species based on variability of the prion gene coding region. *PLoS One* 7:e50623.

Williams ES. 2005. Chronic wasting disease. *Vet Pathol* 42:530–549.

Willoughby K, Kelly DF, Lyon DG, Wells GA. 1992. Spongiform encephalopathy in a captive puma (*Felis concolor*). *Vet Rec* 131:431–434.

Wolfe LL, Fox KA, Griffin KA, Miller MW. 2022. Mountain lions (*Puma concolor*) resist long-term dietary exposure to chronic wasting disease. *J Wildl Dis* 58:40–49.

Young RA. 2023. *Establishment of a chronic wasting disease (CWD) management zone and special regulations for deer. Executive Order Number EO 23-19*. Florida Fish and Wildlife Conservation Commission. https://myfwc.com/media/31851/eo-23-19.pdf. Accessed July 2023.

*Submitted for publication 2 April 2024.*
*Accepted 19 August 2024.*