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LETTER

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Substantial red wolf genetic ancestry persists in wild canids of southwestern Louisiana

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Tweetable abstract: Red wolf genetic ancestry in wild canids of southwestern Louisiana supports red wolf species designation and need for hybrid policy

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Abstract

Concerns over red wolf (*Canis rufus*) extinction caused by hybridization with coyotes (*C. latrans*) led to the capture and removal of remnant wild wolves from southwestern Louisiana and southeastern Texas, United States, during the 1970s. Here we show that despite decades of unmitigated hybridization, and declaration of endangered red wolves as functionally extinct in the wild, red wolf mitochondrial or nuclear DNA ancestry persists in ~55% of contemporary wild canids sampled in southwestern Louisiana. Surprisingly, one individual had 78–100% red wolf ancestry, which is within the range for 75% red wolf, red wolf backcross, or putative red wolf, depending on estimation method. Our findings bolster support for designation of red wolves as a distinct species, demonstrate a critical need for the United States Government to consider adopting an existing but unimplemented hybrid policy, and suggest that immediate reassessment of canid management and taxonomic designation in southwestern Louisiana may be warranted.

KEYWORDS

Canis rufus, Endangered Species Act, endangered, extinct, hybridization, recovery

1 | INTRODUCTION

Critically endangered red wolves (*Canis rufus*) are arguably the most imperiled wolf species in the world. The species was extirpated from the majority of its historical range and restricted to southwestern Louisiana and southeastern Texas, United States, by the 1960s as a result of persecution and habitat loss (Carley, 1975; Nowak, 2002). To thwart a presumed imminent extinction of red wolves caused by hybridization with coyotes (*C. latrans*) and small population size, the U.S. Fish and Wildlife Service (FWS) conducted intensive capture and removal efforts in the area during the 1970s, which led to the creation of a red wolf captive breeding colony (Carley, 1975; FWS, 2018c; Hinton, Chamberlain, & Rabon, 2013). Red wolves were subsequently declared functionally extinct in the wild, and a nonessential experimental population (NEP) of red wolves was established during the 1990s by releasing captive-bred wolves in northeastern North Carolina, United States. (FWS, 2018c; Stoskopf et al., 2005). Red wolf recovery and taxonomic designation have since become contentious issues, particularly in recent years (Hinton et al., 2013; Hinton, White, Rabon, & Chamberlain, 2017; Hohenlohe et al., 2017; vonHoldt et al., 2016; Waples, Kays, Fredrickson, Pacifici, & Mills, 2018).

Human-caused mortality and hybridization with coyotes have remained the primary impediments to red wolf recovery

(FWS, 2018c; Hinton et al., 2013; Hinton, White, Rabon, & Chamberlain, 2017; Stoskopf et al., 2005). Although application of an intensive placeholder strategy successfully mitigated coyote genetic introgression in the NEP to <5% (Gese & Terletzky, 2015), recent reviews questioned the long-term sustainability and necessity of this conservation action (FWS, 2018b; Wildlife Management Institute, 2014). The NEP has declined considerably in size, from 151 wolves in 2005 to 45–60 wolves in 2016 (Hinton et al., 2017), and controversial policy changes for management of the NEP were recently proposed (FWS, 2018a). The FWS was also directed by U.S. Congress to complete a reassessment of red wolf taxonomic designation by 2019 (FWS, 2018c). Furthermore, a lack of formal direction on how to treat hybrid individuals in the context of endangered species recovery has complicated red wolf conservation (vonHoldt, Brzeski, Wilcove, & Rutledge, 2018; Waples et al., 2018; Wayne & Shaffer, 2016).

Despite improved knowledge of the hybridization process, the long-term genetic consequences of red wolf-coyote hybridization generally remain poorly understood (Bohling & Waits, 2015; Hinton, Gittleman, van Manen, & Chamberlain, 2018; Wildlife Management Institute, 2014). Genetic research of canids currently inhabiting southwestern Louisiana and southeastern Texas, where the last remaining wild red wolf population resided, could provide invaluable insight into red wolf-coyote hybridization, potential outcomes of suspending the placeholder strategy in the NEP, and inform red wolf recovery actions and conservation policy (Wildlife Management Institute, 2014). Therefore, we collected contemporary genetic samples to investigate if red wolf mitochondrial (mtDNA) or nuclear (nDNA) DNA ancestry persists in canids that reside in southwestern Louisiana. We hypothesized that if red wolves and coyotes are not distinct species with behavioral or ecological isolating mechanisms, and most red wolves were removed from southwestern Louisiana during the 1970s, then limited or no red wolf ancestry would persist. Alternatively, if red wolves and coyotes are distinct species with reproductive isolating mechanisms, and multiple red wolves remained following removal efforts, then considerable levels of red wolf ancestry could persist in at least some individuals in the area.

2 | METHODS

2.1 | Sample collection

We used 54 scat and 16 hair samples that were collected noninvasively from individual canids in southwestern Louisiana during December 2015 to February 2016 via systematic scat transects and hair rub pads in a capture-recapture framework (Murphy, Augustine, Adams, Waits, & Cox, 2018b). The probability of identity for siblings ($P_{ID(sibs)}$) for nine nDNA microsatellite loci was calculated for those 70 samples by Murphy et al. (2018b) using GenAlEx v6.5 (Peakall & Smouse, 2012); five loci were required to differentiate between individuals ($P_{ID(sibs)} = 0.0082$), so samples were considered as originating from the same individual if they matched at more than five loci. A matching analysis identified 32 individuals from those 70 scat and hair samples (Murphy et al., 2018b). We augmented that data set with tissue samples collected from six dead canids (killed in vehicle collisions or by government biologists as part of coyote control efforts [Leblanc et al., 2016]) in southwestern Louisiana during the same time period. Additionally, to provide a regional comparison of genetic ancestry, we acquired tissue samples that were collected during the same period by state and federal government biologists and private landowners from 106 dead coyotes in eastern Louisiana ($n = 14$), Alabama (AL; $n = 16$), Georgia (GA; $n = 26$), Kentucky (KY; $n = 34$), Mississippi (MS; $n = 11$), and Virginia (VA; $n = 5$), United States (Figure 1). Sample collection methods conformed to jurisdictional wildlife laws and were approved by the U.S. Fish and Wildlife Service, Louisiana Department of Wildlife and Fisheries, or U.S. Department of Agriculture–Wildlife Services in accordance with standardized guidelines and policy.

2.2 | Laboratory analysis

We analyzed samples at the Laboratory for Ecological, Evolutionary and Conservation Genetics (University of Idaho, Moscow, U.S.A.), which had facilities dedicated to low quantity, low quality DNA samples. This lab housed ~1,000 reference red wolf genetic samples, including from the 14 genetic founders of the captive breeding colony who were sourced from southwestern Louisiana and southeastern Texas ~4 decades prior to our study. Additionally, the methods for genetically identifying and quantifying hybridization between red wolves and coyotes were developed at this laboratory (Adams, Kelly, & Waits, 2003a; Bohling, Adams, & Waits, 2013; Bohling & Waits, 2011; Miller, Adams, & Waits, 2003).

We extracted DNA from hair and tissue samples using a DNeasy Blood and Tissue Kit, whereas we extracted DNA from scat samples using a QIAmp Fast DNA Stool Kit (Qiagen, Inc., Hilden, Germany). We included one negative in each extraction to monitor for contamination of reagents. We attempted to generate a genotype for each sample using two multiplexes that combined for a total of 17 microsatellite loci (Bohling et al., 2013; Bohling & Waits, 2011). The first multiplex contained 0.06 μM of CXX.377, 0.07 μM of CXX.172, CXX.173 and CXX.250, 0.13 μM of CXX.109, 0.16 μM of CXX.200, 0.20 μM of AHT121, 0.60 μM of AHT103, 0.71 μM of CXX.20, 1X Qiagen Multiplex PCR Kit Master Mix, 0.5X Q solution, and 1 μL of DNA extract in a 7 μL reaction (Mellersh et al., 1997; Miller et al., 2003; Ostrander, Mapa, Yee, & Rine, 1995; Ostrander, Sprague,

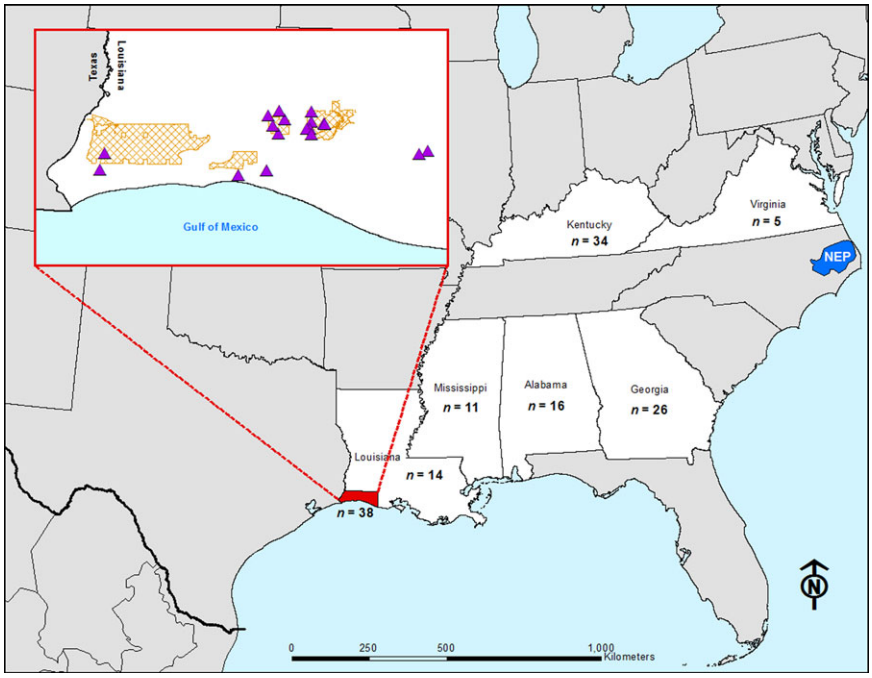

FIGURE 1 Eastern U.S.A. jurisdictions where *Canis* genetic samples (*n*) were collected during 2015–2016 (white areas). The southwestern Louisiana study area and the red wolf (*C. rufus*) nonessential experimental population (NEP) area are depicted by the red and dark blue polygons, respectively. Inset map outlined in red shows the locations (purple triangles) at which 21 individuals with red wolf mtDNA or ≥10% nDNA ancestry were sampled in southwestern Louisiana, relative to U.S. Fish and Wildlife Service National Wildlife Refuges (orange hatched polygons)

& Rine, 1993). The second multiplex contained 0.06 μM of FH2010, 0.07 μM of FH2062 and FH2054, 0.10 μM of FH2001, 0.16 μM of FH2145, 0.24 μM of FH2004, 0.36 μM of CXX.225, 0.80 μM of CXX403, 1X Qiagen Multiplex PCR Kit Master Mix, 0.5X Q solution, and 1 μL of DNA extract in a 7 μL reaction (Mellersh et al., 1997; Miller et al., 2003; Ostrander et al., 1993). We amplified tissue samples in duplicate and performed up to four and six replicate PCRs for the hair + tissue and scat samples that consistently amplified, respectively. We visualized PCR products using a 3130xl DNA Sequencer and scored allele sizes using Genemapper 3.7 (Applied Biosystems, Inc., Foster City, U.S.A.). Our assessment of sample quality and genotype screening methods followed those described by Adams and Waits (2007).

We attempted amplification of a ~320 bp fragment of the mtDNA control region for each sample using primers Thr-L and DL-H16340 (Vilà et al., 1999). Although most samples were sequenced using the Thr-L primer, 12 samples were sequenced using the H16340 primer; thus, it was necessary to trim the sequenced fragment to 294 bp for further analysis. The PCR contained 0.2 μM of each primer, 2.5 mM $MgCl_2$, 0.4 mM dNTPs, 1X Amplitaq Gold Buffer, 0.5 units of Amplitaq Gold (Applied Biosystems, Inc.), and 1 μl of DNA extract in a 15 μl reaction volume. PCR products were cleaned-up using Exo-SAPit (Affymetrix, Santa Clara, U.S.A.) according to the manufacturer's protocol. We sequenced samples in the forward direction in a quarter reaction of the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Inc). We purified sequencing products using the BigDye Xterminator Purification Kit according to the manufacturer's protocol prior to running on a 3130*xl* Genetic Analyzer (Applied Biosystems, Inc.). We analyzed and edited sequences using Sequencher 4.7 (Gene Codes Corporation, Ann Arbor, U.S.A.).

2.3 | Statistical analyses

We assessed nDNA genetic differentiation by estimating pairwise F_{ST} and G''_{ST} using GenAlEx v6.5, with individuals grouped by species and sampling locale (e.g., U.S.A. state). We included the genotypes for all canids sampled in southwestern Louisiana; the coyotes sampled in Alabama, Georgia, Kentucky, eastern Louisiana, and Mississippi; 19 total red wolves, including 13 of the 14 genetic founders of the captive breeding colony, three offspring of the fourteenth genetic founder, and three additional founders who never reproduced; 38 gray wolves (*C. lupus*) from Idaho and Alaska, U.S.A.; and 38 domestic dogs (*C. lupus familiaris*; Adams, Leonard, & Waits, 2003b; Bohling et al., 2013; Bohling & Waits, 2011). We excluded the five Virginia coyotes from this analysis because of small sample size. We also conducted a principal coordinate analysis using GenAlEx 6.5 to visualize genetic distances among canid species and sampling locales.

We estimated the proportion of nDNA species ancestry (q) in sampled individuals via Bayesian assignment (Bohling et al., 2013) implemented in STRUCTURE v2.2 (Pritchard, Stephens, & Donnelly, 2000) and BAPS v5.0 (Corander, Marttinen, Sirén, & Tang, 2008). To prevent bias that can arise from including related individuals, we first used ML-Relate (Kalinowski et al., 2006) to estimate relatedness among the coyotes from Alabama, Georgia, Kentucky, eastern Louisiana, Mississippi, and Virginia, and we removed individuals with an estimated relatedness of $r \geq 0.40$; this

resulted in a final sample size of 79 coyotes. For the STRUCTURE analysis, we set the number of populations (K) *a priori* to four (i.e., red wolf, gray wolf, coyote, and domestic dog) and ran 10 replicates of the admixture model with correlated allele frequencies using a burn-in of 1×10^5 Markov chain Monte Carlo iterations followed by 4×10^5 iterations to estimate q for each individual (Bohling et al., 2013; Bohling & Waits, 2011). For the BAPS analysis, we used the same genotypes as in the STRUCTURE analysis, similarly set K *a priori* to four, used the admixture based on predefined clustering option, and ran 1×10^3 iterations to estimate q for each individual (Bohling et al., 2013). For both analyses, we used the default priors for all parameters other than K.

We considered individuals with $q \geq 0.10$ to red wolves as having red wolf ancestry (Bohling et al., 2013; Bohling & Waits, 2011; Vaha & Primmer, 2006). We analyzed all individuals that met those criteria using NewHybrids to estimate the probability of belonging to one of four hybrid categories or two parental categories (Anderson & Thompson, 2002). We specified red wolf and coyote as the two parental categories, and F1, F2, red wolf backcross, and coyote backcross as the hybrid categories. We did not include individuals with $q \geq 0.10$ to gray wolves or domestic dogs, because NewHybrids assumes that admixture is derived from only two parental groups (Anderson & Thompson, 2002; Vaha & Primmer, 2006). We assigned individuals to a particular category if the posterior probability was ≥0.50. If no categories had a posterior probability ≥0.50, then we summed the posterior probabilities among hybrid categories. If those summed hybrid posterior probabilities were ≥0.75, then we considered that individual as having an uncategorized hybrid origin.

Finally, we assessed the total number of mtDNA control region haplotypes using FaBox v1.41 (Villesen, 2007). We then compared each haplotype to the red wolf haplotype (Adams et al., 2003a). We generated a median joining network of haplotypes using Network v5 (Fluxus Technology, Ltd., Suffolk, U.K.).

3 | RESULTS

3.1 | nDNA differentiation

We obtained consensus genotypes at 17 nDNA microsatellite loci for 38 canids sampled in southwestern Louisiana and 90 coyotes from Alabama, Georgia, Kentucky, eastern Louisiana, Mississippi, and Virginia, United States. All pairwise F_{ST} and G''_{ST} estimates were significantly different from zero ($P < 0.05$), thereby supporting restricted gene flow among species and locales (Table 1). The largest differentiation values were between red wolves and Mississippi coyotes, whereas the smallest were between gray wolves and Kentucky coyotes. For red wolf genotypes, the largest and smallest values were for Mississippi coyotes and Louisiana canids, respectively. Results from principal coordinate analysis supported F_{ST} and G''_{ST} estimates among species, with some individuals from southwestern Louisiana clustering between coyotes and red wolves (Figure 2).

3.2 | nDNA ancestry

Nineteen individuals, all from southwestern Louisiana, shared ≥10% of their nDNA ancestry with red wolves (Table 2; Figure 3). Red wolf ancestry was supported for 10 of those individuals by both estimation methods, and was supported for nine individuals by BAPS only. Red wolf ancestry values for three and 13 individuals based on STRUCTURE and BAPS analyses, respectively, were within the range of that expected for 50% red wolf or F1 hybrid (i.e., $q = 0.24$–0.43; Bohling et al., 2013; Stoskopf et al., 2005). One individual had red wolf ancestry values of 0.78 from STRUCTURE and 1.00 from BAPS, the former of which was within the range observed for 75% red wolves or red wolf backcrosses, and the latter of which was representative of a pure red wolf (Bohling et al., 2013; Stoskopf et al., 2005). We note that STRUCTURE is more likely to detect admixture and correctly assign true ancestry, whereas BAPS is less likely to misclassify pure individuals as admixed hybrids (Bohling et al., 2013).

Of the 19 individuals with at least partial red wolf nDNA ancestry, nine had ≥10% ancestry with gray wolves ($n = 7$) or domestic dogs ($n = 2$), and one other individual had >15% gray wolf and domestic dog ancestry when summed across groups (Table 2); we excluded all 10 of those individuals from NewHybrids analysis. Of the nine remaining individuals, one was uncategorized because it did not have a posterior probability ≥0.50 for any category, and the summed posterior probabilities for the hybrid categories did not reach 0.75; five other individuals were uncategorized hybrids and one individual was classified as a coyote (Table 3). However, one individual was classified as an F2 hybrid between red wolf and coyote, and the individual with 78–100% red wolf nDNA ancestry was classified as a red wolf, thereby supporting the ancestry assignment from BAPS.

3.3 | mtDNA haplotypes

We identified 16 different mtDNA sequence haplotypes in sampled individuals (Supporting Information Table S1). Ten individuals, all from southwestern Louisiana, had the red wolf haplotype (Table 2), which was not found in any individuals from eastern Louisiana or the other sampled locales. Eight of those 10 individuals also had evidence of red wolf nDNA ancestry from STRUCTURE and/or BAPS analyses. Results of a median joining network analysis indicated that four mutational steps separated the red wolf haplotype and the

TABLE 1 Estimated nDNA pairwise F_{ST} (bottom diagonal) and G''_{ST} (top diagonal) among *Canis* species and U.S.A. jurisdictions[a]

	Red Wolf	Gray Wolf	Dog	AL	GA	KY	MS	LA
Red Wolf	–	0.483	0.492	0.536	0.477	0.495	0.599	0.411
Gray Wolf	0.092	–	0.323	0.402	0.364	0.342	0.468	0.381
Dog	0.098	0.056	–	0.514	0.424	0.473	0.492	0.490
AL	0.099	0.065	0.086	–	0.076	0.121	0.146	0.085
GA	0.088	0.058	0.071	0.026	–	0.124	0.230	0.167
KY	0.085	0.050	0.072	0.027	0.025	–	0.232	0.162
MS	0.109	0.075	0.083	0.038	0.045	0.041	–	0.220
LA	0.070	0.053	0.072	0.022	0.029	0.024	0.038	–

[a]Species included were red wolf (*C. rufus*), gray wolf (*C. lupus*), domestic dog (*C. lupus familiaris*), and coyote (*C. latrans*). Coyotes are coded by the U.S.A. state where they were sampled: Alabama (AL), Georgia (GA), Kentucky (KY), Louisiana (LA), and Mississippi (MS). All values were significantly greater than zero ($P < 0.05$).

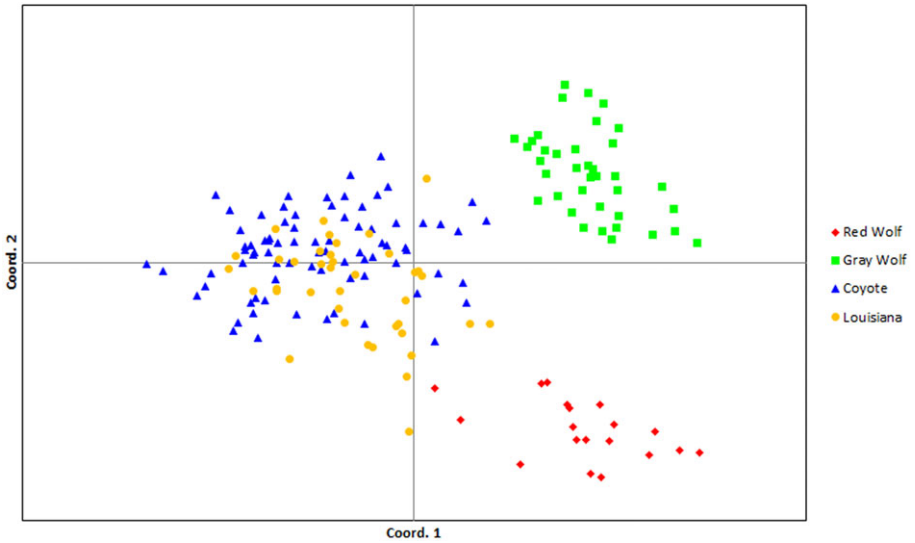

FIGURE 2 Results from principal coordinate analysis of sampled *Canis*. Individuals sampled in Louisiana, United States (yellow circles), red wolves (*C. rufus*; red diamonds), gray wolves (*C. lupus*; green squares), and coyotes (*C. latrans*; blue triangles) sampled in other southeastern U.S.A. jurisdictions are presented

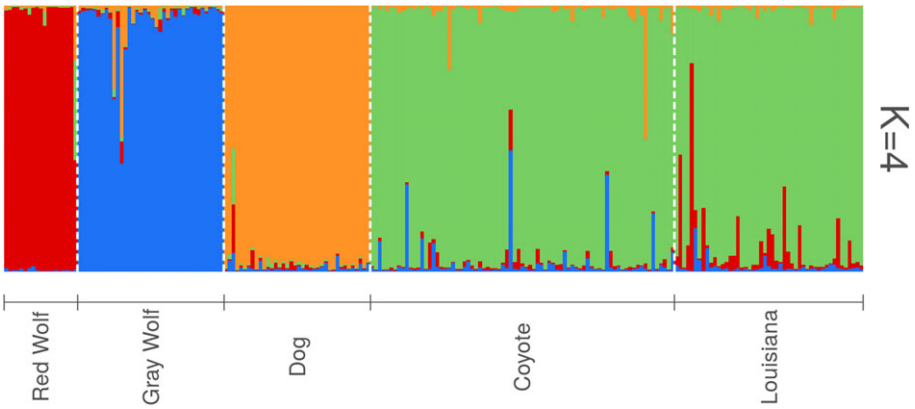

FIGURE 3 Histogram of estimated *Canis* nDNA ancestry coefficients from the STRUCTURE model of four genetic clusters ($K = 4$). Each vertical bar represents ancestry coefficients for one individual. The *K*s correspond to red wolves (*C. rufus*), gray wolves (*C. lupus*), domestic dogs (*C. lupus familiaris*), and southeastern U.S.A. coyotes (*C. latrans*)

TABLE 2 mtDNA haplotypes and estimated percent nDNA species ancestry (*q*) for all sampled *Canis* with red wolf (*C. rufus*) genetic ancestry in southwestern Louisiana, United States[a]

		STRUCTURE *q* (%)				BAPS *q* (%)			
Individual	**Haplotype**	**RW**	**CO**	**GW**	**DO**	**RW**	**CO**	**GW**	**DO**
LA01	RW	3.3	93.8	2.4	0.5	30	53	17	0
LA02	RW	42.8	55.3	1.2	0.7	46	47	7	0
LA04	CLA13	9.3	89.7	0.5	0.5	30	67	1	2
LA05	RW	78.0	20.6	0.4	1.0	100	0	0	0
LA06	CLA14	7.2	75.5	16.3	1.0	31	43	26	0
LA08	CLA12	23.9	75.4	0.3	0.4	32	67	0	1
LA14	RW	2.4	94.8	1.3	1.5	18	65	6	11
LA16	RW	5.3	93.5	0.5	0.7	30	66	4	0
LA17	RW	19.4	78.9	1.4	0.3	41	38	21	0
LA21	CLA10	2.2	97.2	0.4	0.2	27	65	8	0
LA25	GW/DO[b]	13.9	83.4	2.0	0.7	35	49	16	0
LA26	–	13.4	83.6	0.9	2.1	30	51	2	17
LA27	CLA10	3.2	94.9	0.9	1.0	26	57	8	9
LA29	–	31.5	67.2	0.4	0.9	48	46	3	3
LA30	–	6.6	92.0	0.9	0.5	30	55	12	3
LA31	RW	2.0	95.9	0.9	1.2	0	100	0	0
LA33	CLA10	16.3	82.3	1.1	0.3	27	62	11	0
LA34	RW	0.7	96.6	1.9	0.8	0	100	0	0
LA44	–	18.9	78.5	1.4	1.2	45	45	8	2
LA47	RW	10.7	87.8	1.1	0.4	34	58	8	0
LA49	RW	2.0	95.6	1.5	0.9	15	64	18	3

[a]Species included in analyses were coyote (*C. latrans*; CLA prefix or CO), red wolf (RW), gray wolf (*C. lupus*; GW), and domestic dog (*C. lupus familiaris*; DO).
[b]This sample was further tested with a species identification test and determined to have a gray wolf or domestic dog haplotype.

TABLE 3 Estimated posterior probabilities of parental and hybrid groups for nine individual *Canis* from southwestern Louisiana, United States, who had red wolf (*C. rufus*) genetic ancestry[a]

Individual	**RW**	**CO**	**F1**	**F2**	**RBC**	**CBC**	**Hybrid Value**	**Classification**
LA02	0.06	0.17	0.03	0.43	0.03	0.28	0.77	Hybrid
LA04	0	0.22	0.09	0.24	0.01	0.44	0.78	Hybrid
LA05	0.50	0.01	0.04	0.28	0.12	0.05	–	Red wolf
LA08	0.07	0.17	0.04	0.40	0.04	0.29	0.77	Hybrid
LA16	0	0.32	0.03	0.28	0.01	0.36	0.67	Unassigned
LA21	0	0.68	0	0.10	0	0.22	–	Coyote
LA29	0.13	0	0.08	0.55	0.18	0.06	–	F2
LA44	0.01	0.19	0.02	0.46	0.02	0.30	0.80	Hybrid
LA47	0.00	0.18	0.03	0.36	0.01	0.42	0.82	Hybrid

[a]The two parental groups were red wolf (RW) and coyote (*C. latrans*; CO), and the hybrid groups were first-generation (F1), second-generation (F2), red wolf backcross (RBC), and coyote backcross (CBC).

closest coyote haplotype in our data (Supporting Information Figure S1).

4 | DISCUSSION

Whether red wolves and coyotes hybridized where their ranges overlapped or anthropogenic habitat and landscape alterations caused the abolishment of barriers to hybridization has remained unclear (Hinton et al., 2013; Waples et a., 2018). Additionally, a lack of information regarding what may happen if the placeholder strategy is abolished and hybridization allowed to occur in the NEP was identified as a major uncertainty of the current red wolf recovery program (FWS, 2018b; Wildlife Management Institute, 2014).

With the inclusion of the geographical locales that we sampled, contemporary canids now have been evaluated for the red wolf mtDNA haplotype in most southern U.S.A. jurisdictions within historical red wolf range (Adams et al., 2003b; Hailer & Leonard, 2008; Koblmüller et al., 2012). Southwestern Louisiana is the only area where this haplotype has been detected in wild individuals outside of the NEP in North Carolina, which supports the enduring presumption that southwestern Louisiana was part of the last remaining stronghold for red wolves (Carley, 1975; Nowak, 2002). Louisiana was among the first states in the southeastern United States that coyotes colonized (Hody & Kays, 2018), and ~12 red wolf generations have elapsed since red wolves were thought to have been removed from the wild. The proportion of red wolf genetic ancestry that persists in some contemporary wild canids in southwestern Louisiana supports hybridization with coyotes was historically mitigated by landscape, biological, or behavioral barriers, or a combination thereof. Natural mechanisms, such as size-assortative mating and aggression by larger red wolves towards coyotes (Bohling & Waits, 2015; Hinton et al., 2018), may be sufficient to preserve a portion of the red wolf gene pool despite the presence of coyotes. Implementing conservation strategies that maintain a majority of the red wolf gene pool, whether via natural or human-assisted mechanisms, likely will be important for continued red wolf recovery.

Since 1980, all large canids in southwestern Louisiana have been presumed to be coyotes, because red wolves were declared functionally extinct in the wild; however, multiple red wolves and hybrids with high red wolf ancestry clearly persisted in the area after concerted red wolf removal efforts concluded. To prevent further reductions of the remnant wild red wolf gene pool in southwestern Louisiana, we suggest that managers consider suspending coyote control efforts (e.g., Leblanc et al., 2016) until additional studies are conducted to improve our understanding of canid genetics, hybridization, and taxonomy in this area. We chose to use microsatellites because of previously established reference databases for *Canis* species, including founders of the red wolf captive breeding colony, and because microsatellites have been advantageous for studying hybridization given their high polymorphism (Bohling et al., 2013; Bohling & Waits, 2011). To provide higher resolution and more precise estimates of red wolf genetic ancestry, we suggest that an additional study of canids in southwestern Louisiana that uses thousands of single nucleotide polymorphic loci is warranted (vonHoldt et al., 2016; 2018). Additionally, because our study primarily used hair and fecal samples that were collected noninvasively in southwestern Louisiana as part of a capture-recapture study (Murphy et al., 2018b), we were limited to focusing solely on genetics. We suggest that future research should simultaneously collect both genetic and morphological data from canids in southwestern Louisiana to evaluate the relationship between genotype and phenotype.

Hybridization between imperiled and nonimperiled species is among the most challenging issues for species protection and recovery under the Endangered Species Act. The FWS developed a hybrid policy (FWS, 1996) that has been neither officially accepted nor rejected by this managing authority; consequently, no clear consensus exists on how to treat hybrids in the context of endangered species recovery (vonHoldt et al., 2018; Wayne & Shaffer, 2016). Nine to 19 individuals in southwestern Louisiana had red wolf nDNA q values greater than the minimum threshold used for classification of a red wolf-coyote hybrid in the NEP (≥12.5%; Bohling et al., 2013; Stoskopf et al., 2005). The individual canid with the highest proportion of red wolf nDNA ancestry had q values exceeding that inferred from the FWS hybrid policy for an individual that may warrant protection (i.e., ≥75%; Wayne & Shaffer, 2016), and based on BAPS and NewHybrids analyses, exceeded the threshold that has been used to classify red wolves in the NEP ($q \geq 87.5\%$; Bohling et al., 2013; Stoskopf et al., 2005). Considering the estimated canid population size for this portion of southwestern Louisiana ($N = 305$ individuals; Murphy et al., 2018b), and the percentage of sampled canids with red wolf nDNA ancestry verified by both Bayesian assignment methods (~27%), as many as ~80 individuals with red wolf genetic ancestry might currently inhabit the area. Our findings collectively support the long-standing classification of red wolves as a distinct species and highlight the importance of considering whether protection provisions afforded by the Endangered Species Act should be extended to some canids in southwestern Louisiana.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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