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HOG-NOSED SKUNKS (*CONEPATUS LEUCONOTUS*) ALONG THE GULF OF MEXICO: POPULATION STATUS AND GENETIC DIVERSITY

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Abstract—We examined 12 hog-nosed skunks (*Conepatus leuconotus*) from southern Texas and central Tamaulipas, Mexico, during 2004–2009. We amplified the entire mitochondrial-DNA control region (D-loop) from three individuals. Results indicated that these sequences were from distinct maternal lineages with 15 variable sites. However, when reduced and compared to previously published sequences, we observed a low overall pairwise difference of 0.1% ($SE = 0.1\%$). Our results suggest that hog-nosed skunks are persisting along the Gulf Coast region of southern Texas and northern Mexico.

Resumen—Examinamos 12 zorrillos de lomo blanco (*Conepatus leuconotus*) del sur de Texas y de la región central de Tamaulipas, México, durante 2004–2009. Amplificamos en su totalidad la región control de ADN mitocondrial (D-loop) de tres individuos. Los resultados indicaron que estas secuencias fueron de linajes maternos distintos con 15 sitios variables. Sin embargo, cuando se redujeron y se compararon con las secuencias previamente publicadas, se observó una diferencia general baja de 0.1% ($EE = 0.1\%$). Nuestros resultados sugieren que el zorrillo de lomo blanco persiste en la región costera del golfo de México del sur de Texas y del norte de México.

Hog-nosed skunks (*Conepatus leuconotus*) are adapted for digging and rooting (Patton, 1974; Davis and Schmidly, 1994), and they have black pelage with one white dorsal stripe extending from forehead to tail (Dragoo, 2009; Dragoo and Sheffield, 2009). The historical distribution of hog-nosed skunks encompassed much of the southwestern United States and Mexico; however, current status of populations largely is unknown (Dragoo et al., 2003, 2004; Dragoo, 2009; Dragoo and Sheffield, 2009). Numerous reports suggested that populations, particularly along the Gulf Coast of Texas, have experienced severe declines associated with modifications of habitat (Schmidly, 1983; Dragoo et al., 2003; Dragoo, 2009), use of pesticides (Dragoo et al., 2003; Dragoo and Sheffield, 2009), and competition by wild boars (*Sus scrofa*; Dragoo et al., 2003). The lack of records of hog-nosed skunks from southern Texas has promoted speculation that populations have been extirpated (Dragoo et al., 2003).

Dragoo et al. (2003) evaluated the taxonomy of *Conepatus* using morphometric and genetic characteristics; their study supported one species of *Conepatus* with three potential subspecies. However, only one partially sequenced individual from the Gulf Coast region was included, a museum specimen collected in 1947 from Brooks County, Texas. Our objectives were to conduct roadside surveys to document occurrence of hog-nosed skunks in the Gulf Coast region, and to explore genetic relationships among specimens encountered.

During February 2005–November 2007, we searched for road-killed hog-nosed skunks in southern Texas. We conducted surveys on Texas Highway 281 in Jim Wells, Brooks, and Hidalgo counties. This area has level-to-rolling topography, open prairies, and numerous species of shrubs and cacti. Soils are clays to sandy loams with many profiles representing differences in drainage and moisture-holding capacities (Correll and Johnston, 1979). For each road-killed hog-nosed skunk, we took photographs, recorded latitude and longitude, and sampled tissues when feasible. We identified eight road-killed hog-nosed skunks (Table 1) and collected tissues from three of them. In addition, we live-trapped one, camera-trapped two, and identified one road-killed hog-nosed skunk in central Tamaulipas, Mexico, during 2004–2009 (Table 1). We took photographs, recorded latitude and longitude, and collected a tissue sample from the live-trapped hog-nosed skunk in Mexico.

We isolated mitochondrial DNA from three of four tissues collected (Table 1) using a commercial kit (DNeasy, Qiagen Genomics, Valencia, California). We used the polymerase chain reaction (PCR) to amplify the entire mitochondrial-DNA control region (D-loop) fol-

TABLE 1—Hog-nosed skunks (*Conepatus leuconotus*) observed in southern Texas and Tamaulipas, Mexico, with method of detection, latitude and longitude, and date of observation.

Skunk	State	County	Detection	Latitude and longitude	Date
1	Texas	Hidalgo	Road-kill	26.7074°N, 98.1108°W	2 September 2005
2	Texas	Brooks	Road-kill	26.8460°N, 98.1272°W	2 June 2005
3	Texas	Brooks	Road-kill	26.9182°N, 98.1372°W	1 July 2005
4	Texas	Brooks	Road-kill	26.9339°N, 98.1387°W	2 February 2005
5[a]	Texas	Brooks	Road-kill	27.0651°N, 98.1429°W	2 October 2005
6[b]	Texas	Brooks	Road-kill	27.1544°N, 98.1508°W	7 July 2005
7	Texas	Brooks	Road-kill	26.9643°N, 98.1365°W	14 January 2006
8	Texas	Brooks	Road-kill	26.9441°N, 98.1379°W	9 November 2007
9[c]	Tamaulipas	—	Live-trap	23.4379°N, 97.9712°W	12 November 2004
10	Tamaulipas	—	Road-kill	23.5531°N, 97.9719°W	11 February 2009
11	Tamaulipas	—	Camera-trap	23.9994°N, 98.5435°W	4 March 2009
12	Tamaulipas	—	Camera-trap	23.9994°N, 98.5537°W	8 October 2009

[a] HNS-02, GenBank accession JF262199.
[b] HNS-01, GenBank accession JF262198.
[c] HNS-MEX, GenBank accession JF262200.

lowing Dragoo et al. (1993, 2003) using primers L16272 (5′-TACACTGGTCTTGTAAACC-3′) and H1008 (5′-AAGGCTAGGACCAAACCT-3′). We amplified D-loop sequences in 25 μL reaction-volumes containing 12.5 μL Amplitaq Gold PCR Master Mix (Applied Biosystems, Foster City, California), 10 pmol of each primer, and 10–50 ng of template DNA. Reactions consisted of initial denaturation at 94°C for 12 min followed by 32 cycles of 94°C for 50 s, 61°C for 60 s, 72°C for 2 min, and a final extension at 72°C for 30 min. We electrophoresed PCR products on 1% agarose gels containing ethidium bromide and viewed them under ultraviolet light to verify successful amplification. We used an enzymatic method (ExoSAP-IT; USB Corporation, Wilmington, Maryland) to purify PCR products from successful reactions, which we included as template for sequencing reactions using the BigDye Terminator Cycle Sequencing kit version 1.1 (Applied Biosystems, Foster City, California). We sequenced each sample in both directions on an ABI 3130xl automated DNA sequencer (Applied Biosystems, Foster City, California) and deposited sequences into GenBank (accessions JF262198- JF262200).

We obtained 1,162 base-pairs of sequence spanning most of the D-loop region for each sample with 1,142 base-pairs available for analysis. We used ClustalX version 2 (Larkin et al., 2007) to align sequences and DnaSP version 5 (Librado and Rozas, 2009) to estimate D-loop sequence and nucleotide polymorphism, i.e., number of unique sequences and number of variable sites among sequences, respectively. We used MEGA version 4 (Tamura et al., 2007) to evaluate pairwise proportional differences among sequences (i.e., divergence of sequences). We also reduced sequences to 335 base-pairs and created a neighbor-joining tree using p-distance to visualize relationships among our sequences to the three geographically closest sequences (i.e., Kimble and Brooks counties, Texas, and Tamaulipas, Mexico) from Dragoo et al. (2003).

Our three complete sequences resolved into distinct haplotypes with 15 variable sites and a mean divergence of 0.9% ($SE = 0.2\%$). We then included two geographically close and complete sequences (e.g., Kimble County and Tamaulipas, Mexico) from Dragoo et al. (2003). Combined, the five sequences represented distinct haplotypes with 26 polymorphic sites and a mean divergence of sequences of 1.2% ($SE = 0.2\%$).

Our three reduced sequences resolved into two distinct haplotypes, exhibiting one variable site and a mean divergence of 0.2% ($SE = 0.2\%$). Using p-distance and 1,000 bootstraps, our sequences grouped closely with the sequences from Kimble County, Tamaulipas, and Brooks County reported by Dragoo et al. (2003). Collectively, the six reduced sequences exhibited one variable site and a mean divergence of 0.1% ($SE = 0.1\%$).

Our research confirms persistence of populations of hog-nosed skunks in the Gulf Coast region of southern Texas and central Tamaulipas. Our complete sequences represented three differentiated maternal lineages along the Gulf Coast. However, when reduced and compared to previously published lineages in Dragoo et al. (2003), only one of our haplotypes was distinct (HNS-02). Our additional data from the Gulf Coast region supported the taxonomic conclusions of Dragoo et al. (2003). Populations of hog-nosed skunks in southern Texas and northeastern Mexico might be more stable than previously suggested (e.g., Dragoo et al., 2003; Dragoo and Sheffield, 2009). Alternatively, populations may be re-expanding into formerly occupied areas. Additional studies of reproduction, survival, habitat, and genetics of hog-nosed skunks along the Gulf of Mexico are needed.

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