# Cryptozoology: A Case Study using Molecular Markers to Identify Cryptic Species

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*Abstract:* A rancher near Hebbronville, Texas, recently discovered that an unknown large animal had attempted to gain access to a metal outbuilding on the ranch. The metal was torn and completely bitten through in several places. Because of the strength required to inflict this damage, a large animal, such as a mountain lion (*Puma concolor*) or black bear (*Ursus americanus*), were suspects. However, insufficient evidence was available to conclusively identify the culprit. We extracted DNA from hairs found at the scene and amplified a portion of the mtDNA control region. The DNA fragment was a 100% match to sequences from domestic dog (*Canis familiaris*). Thus, the mystery animal was not a rare species such as a bear or an even more exotic animal such as the mythical chupacabra, but a stray dog. Our results demonstrate that molecular techniques can serve as a useful tool for answering difficult wildlife management questions.

*Key words:* cryptozoology, molecular techniques, noninvasive sampling, rare species, species identification, wildlife genetics

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The management of wildlife that is rare, cryptic, or secretive can pose variety of challenges for biologists. In some cases, the challenge is simply to detect or verify the presence of a rare species. Other problems may require the identification of the species or individual involved in a sighting or damage event. For instance, folklore and public curiosity commonly fuels intense interest and speculation about mythical or enigmatic creatures (e.g., black panther, sasquatch, yeti, and chupacabra) in remote areas. Although these mythical animals are often disdained by mainstream science, they serve as a powerful metaphor for the difficulty in refuting or substantiating sightings involving rare or cryptic wildlife.

Historically, biologists relied on the ability to detect and identify tracks or other animal sign. Infrared cameras were an important improvement over simple detection of animal sign but are not suitable for all occasions. Recent advances in the number and types of genetic markers, as well as the automation of laboratory instrumentation, have enabled molecular techniques to be used in an ever-widening number of wildlife applications (DeYoung and Honeycutt 2005). Wildlife geneticists have played an integral role in developing and testing applications for non-invasively collected samples (Waits and Paetkau 2005). Although noninvasive sampling may be limited by the amount and quality of DNA in the sample, noninvasive methods offer a means of studying individuals or species without the need to capture or handle the individuals, a distinct advantage for rare, cryptic, or secretive species (e.g., Coltman and Davis 2006). Herein, we describe a case study in south Texas where molecular techniques were applied to a non-invasively collected sample to solve an interesting wildlife management problem.

## Methods

### Background

In summer 2007, a rancher near Hebbronville in southern Texas noticed an incident of animal damage on the property. An unknown animal had attempted to force entry into a sheet-metal outbuilding. The metal was twisted and completely bitten through in several places (Figure 1). No tracks were discernable, but a tuft

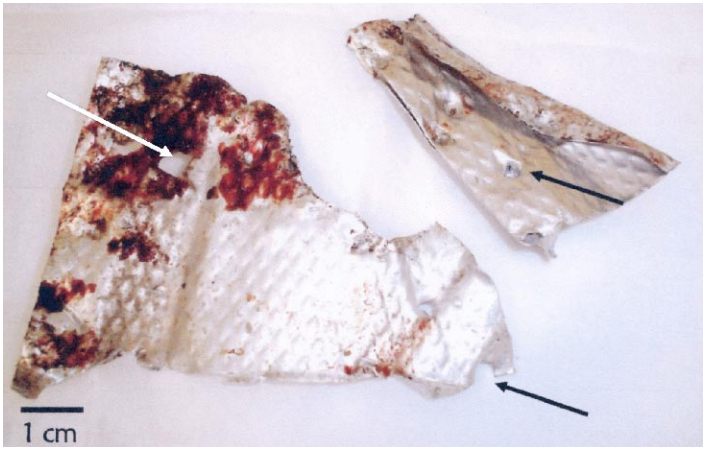


**Fig. 1.** Photo of a piece of metal siding showing tooth marks (arrows) and blood where an unknown large mammal cut its mouth on the jagged metal while attempting to access a metal outbuilding on a ranch near Hebbronville, Texas, in summer 2007.

of hairs was snagged on the metal. The strength required to inflict this damage suggested a large animal. Large wild mammals native to southern Texas include the mountain lion (*Felis concolor*), coyote (*Canis latrans*), bobcat (*Lynx rufus*), and black bear (*Ursus americanus*; Schmidly 2004). The hairs were long and black in color, casting doubt on a native feline as a culprit because these species do not exhibit melanism (Eizirik et al. 2003), and damage of this type is uncharacteristic behavior for mountain lion or bobcat. Furthermore, bobcat and coyote seemed too small to inflict the damage, with adult coyotes ranging from 14–20 kg in weight and bobcats 5–9 kg, rarely up to 16 kg (Schmidly 2004).

Black bears are not residents of southern Texas (Schmidly 2004), but males are occasionally sighted during long-distance dispersal movements from populations near Monterrey, Mexico, <200 km to the southwest (D. G. Hewitt, Texas A&M University-Kingsville, personal communication), well within reported dispersal distances for black bears in the southwestern United States (Hellgren et al. 2005). An additional possibility would be the intentional or unintentional release of an exotic captive animal. A final possibility, though highly unlikely, would be the mythical "chupacabra" or "goatsucker," a product of local folklore, but never documented by science. Overall, a black bear seemed a plausible suspect, possessing the necessary size, strength, and pelage characteristics. Documentation of a black bear male in the area would certainly be interesting, but a female might suggest the founding of a breeding population: unverified reports of a female with cubs in the vicinity of Laredo, Texas, in past years (D. G. Hewitt, personal communication) lent additional justification for verifying the species, and possibly gender, of the individual involved in the incident. However, the lack of obvious physical evidence or additional sightings rendered the true species uncertain. We attempted a molecular analysis of the hairs left at the scene, with the goal of verifying the species of the individual involved.

## DNA Extraction and Amplification

We collected hairs snagged on the metal siding and extracted DNA from the hair follicles using a commercial kit (DNeasy tissue kit, Qiagen Genomics, Valencia, California). We amplified a portion of the mtDNA using primers developed for black bear and other carnivores (Shields and Kocher 1991) under the rationale that bears were a likely candidate, but that these molecular markers would probably amplify in most carnivores. Primers L15774 (5′-GTAAAACGACGGCCAGTACA-TGAATTGGAGGACAACCAGT-3′) and H16498 (5′-CCTGAACTAGGAACCACAT-3′) span a portion of the 3′ end of the cytochrome b gene and the 5′ end of the d-loop. We amplified mtDNA sequences using the polymerase chain reaction (PCR) in 25µl reaction volumes containing 12.5µl Amplitaq Gold PCR Master Mix (Applied Biosystems, Foster City, California), 10 pmol of each primer, and 10–50ng DNA.

Reaction conditions consisted of an initial denaturation at 94 C for 12 min followed by 32 cycles of 94 C for 50s, 61 C for 60s, 72 C for 2 min, with a final extension at 72 C for 30 min. We electrophoresed the PCR products on 1% agarose gels containing ethidium bromide and viewed under UV light to verify successful amplification. We purified products from successful reactions using an enzymatic method (ExoSAP-IT, USB Corporation, Wilmington, Maryland), then used the purified products as template for sequencing reactions using the BigDye Terminator Cycle Sequencing kit v1.1 (Applied Biosystems). Each sample was sequenced in both directions on an ABI 3130 automated DNA sequencer (Applied Biosystems).

## Data Analysis

We assembled and aligned the sequence using the computer program Sequencher 4.5 (Gene Codes, Ann Arbor, Michigan). We compared the resulting sequence against similar sequences in the GenBank sequence database repository maintained by the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) using the basic local alignment search tool (BLAST) algorithm (Altschul et al. 1990). We also constructed unrooted phylogenetic trees of similar sequences from GenBank using the computer program MEGA 4.0 (Tamura et al. 2007). We used the neighbor-joining method (Saitou and Nei 1987) based on both Kimura 2-parameter (Kimura 1980) and uncorrected p-distances, the proportion of nucleotide sites at which two sequences differ (Nei and Kumar 2000). We used 5,000 bootstrap replicates (Felsenstein 1985) to evaluate the reliability of tree branches.

## Results

We obtained 495 bp of sequence from the hair sample. The BLAST search revealed that the 100 most similar sequences were canine in origin, primarily from domestic dog (Table 1). One domestic dog sequence displayed 100% sequence identity with the

**Table 1.** Summary of 100 most similar sequences generated by BLAST search on 495 bp of sequence obtained from hairs found at the scene of animal damage incident in southern Texas during summer 2007.

| Organism | *n* hits | Maximum alignment score[a] |
|---|---|---|
| *Canis familiaris* (domestic dog) | 87 | 915 |
| *Canis lupus* | 6 | 887 |
| *Canis lupus lupus* | 1 | 865 |
| *Canis lupus chanco* | 1 | 725 |
| *Canis latrans* | 5 | 704 |

a. A measure of the degree of similarity between query sequence and archived sequences. The score for a sequence alignment is calculated by summing the scores for each aligned nucleotide position and the scores for gaps (caused by mutations resulting in insertion or deletions of nucleotides) in the DNA sequence.

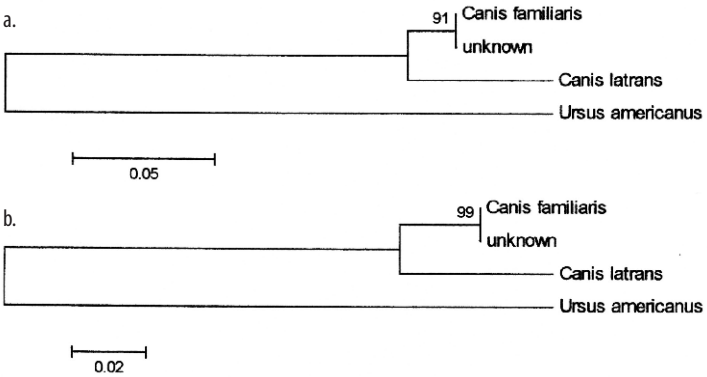


**Fig. 2.** Phylogenetic trees illustrating the position of the unknown sample from south Texas compared to *Canis familiaris*, *Canis latrans*, and *Ursus americanus*. Trees were constructed using the neighbor-joining method with 5,000 bootstrap replicates; the numerals indicate percent of bootstrap replicates that support each node. A. Tree based on the Kimura 2-parameter distance. B. Tree based on uncorrected p-distance.

unknown sample (e.g., the two sequences matched exactly). Wolf and coyote sequences displayed lower similarity scores, and no bear sequence appeared within the 100 most similar. We compared sequences from domestic dog, coyote, and black bear from the southwestern United States (GenBank accession numbers AY706503.1, DQ480511.1, and AY334363, respectively) to visualize the relationship of our unknown sample to these three species. Our unknown sample was clearly more similar to the domestic dog sequence than coyote or black bear (Figure 2).

## Discussion

Based on the DNA sequence data, we conclude that the unknown sample was not a bear or an even more exotic animal, but a domestic dog. The hair length and color did not correspond to any dogs owned by ranch personnel, so the unknown animal was probably a stray. Perhaps the most important aspect enabling the analysis was rapid detection of damage and preservation of the hair prior to DNA extraction. This resulted in amplifiable quantities of DNA that are crucial to any noninvasive study. Overall, our approach was relatively straightforward. Once the sequence data was available, we could use the large body of publicly-available data in the GenBank repository to identify the species of the animal. If indeed the animal were a bear, we would have performed an additional analysis to verify the sex of the individual.

Animals that are rare, cryptic, or secretive present a variety of difficult challenges to wildlife biologists. The recent advances in molecular techniques provide wildlife biologists and managers an additional tool for the study of these rare, cryptic, or secretive animals. The results of this study illustrate how molecular techniques can be used to provide objective answers to difficult wildlife management questions.

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