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### Identification of Species in Commercially Sold Game Meats using DNA Barcoding

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# Identification of Species in Commercially Sold Game Meats Using DNA Barcoding

### Abstract

Fifty-four samples of whole-cut game meats were collected from online distributors in the United States and sequenced across a 658 base-pair region of the gene coding for cytochrome c oxidase subunit I (COI). The resulting DNA sequences were identified based on top species matches in the Barcode of Life Database (BOLD) and GenBank using DNA barcoding. The results showed that 18.5% of samples were potentially mislabeled and 9.3% of samples legally contained a nearthreatened or vulnerable species and were correctly labeled. Products labeled as bison and yak were identified as domestic cattle, red deer was identified as llama and alpaca, and black bear was identified as beaver. The samples appeared to have been mislabeled due to reasons such as economic gain, cross-species breeding and product mishandling. Overall, the results of this study revealed the occurrence of game meat mislabeling in the United States and suggest the need for further evaluation of this practice.

### Introduction

### Food Fraud, A Global Concern

Food fraud, in the form of ingredient substitution and mislabeling, has been observed globally (Johnson 2014). Intentional substitution of meat species may be for economic gain or to avoid import restrictions for exotic meat. Although the reason behind substitution is important, it is equally imperative to determine if mislabeling is a factor in the continued decline of threatened or endangered species (Rasmussen and Morrissey 2009).

Game meats are animals and birds not included in the Meat and Poultry Act (FDA 2012) such as rabbit, bison and venison. Due to differences in retail prices between game meats and livestock such as beef, there is high economic motivation for species substitution to occur (ERS 2014). Although mislabeling has occurred globally in the marketplace and negatively impacted conservation efforts (D'Amato and others 2013) there is a lack of information on mislabeling of game meats in the United States.

### **DNA Barcoding**

**Buffalo identified** Unknown species DNA barcoding region A D 日 MILK

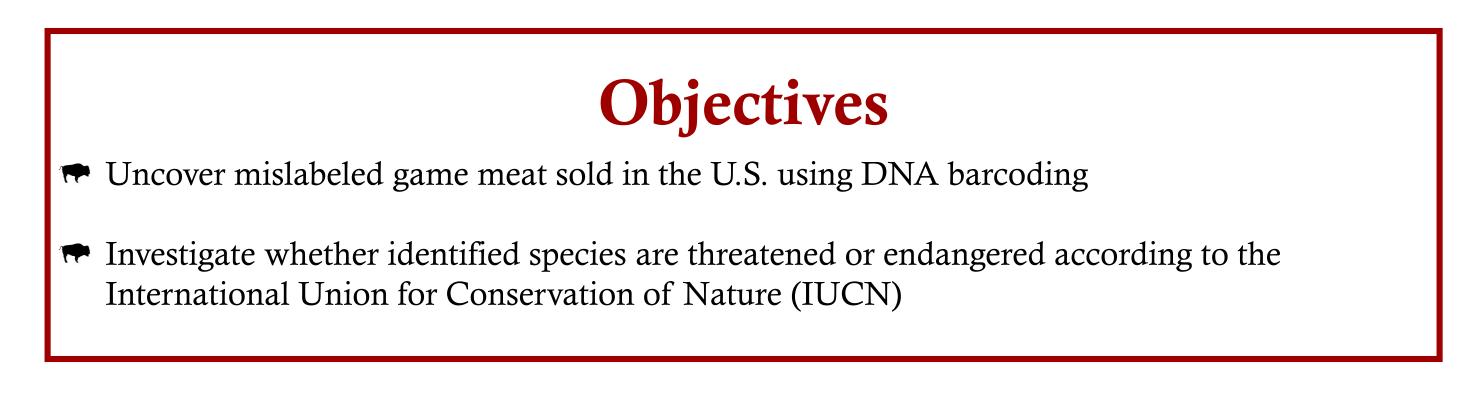
Unknown product

Universal Product Code (UPC)

Milk identified

Figure 1. DNA identifies animal species similar to grocery barcodes identifying products. Figure adapted from Google images

DNA barcoding is an organism identification system based on sequencing a universal specific genetic region (Hebert and others 2003). In animals, the gene coding for cytochrome c oxidase subunit I (COI) is used as the barcode. Similar to a Universal Product Code (UPC) present on groceries, the COI serves as a code to identify a species (Figure 1). To identify a species, the COI gene sequence is compared to a database to find the top species match.



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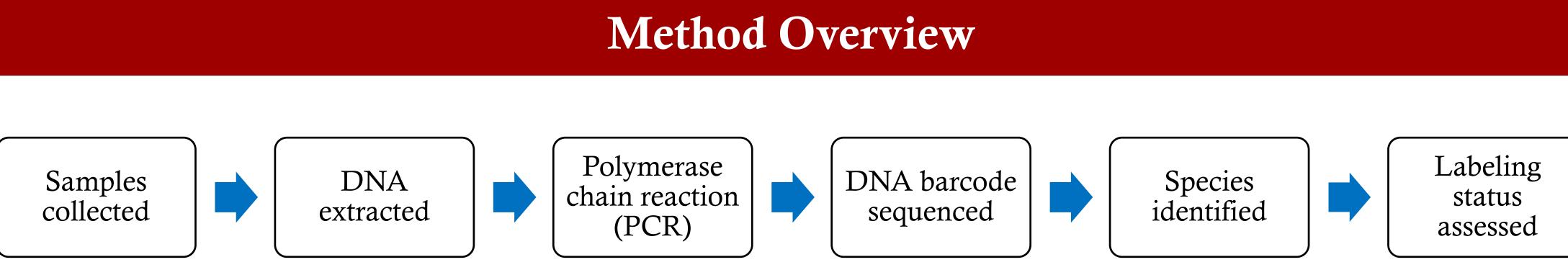


Figure 2. Overview of methods utilized to identify the species of a game meat product

A total of 54 game meat products representing 22 game species were collected in this study from four online retail sources in the United States. Species identification was determined using methods shown in Figure 2. DNA extraction of  $\sim 10$  mg of tissue sample was completed with DNeasy Blood and Tissue Kit (Qiagen). The COI region of DNA was amplified with polymerase chain reaction (PCR) using a mammalian primer cocktail described by Ivanova (2012). The amplified COI was sent out for sequencing and the genetic code was queried in the Barcode of Life Database (BOLD) for species identification. Samples identified as potentially mislabeled were subjected to re-extraction and sequencing for confirmation.

## **Key Findings**

- ♥ Overall, 10 out of 54 samples (18.5%) were determined to be potentially mislabeled (Tables 1 & 2).
- ₱ 60% of potentially mislabeled products were associated with economic incentives and may have been misbranded for profit (Table 1).
- The Distributor B was most frequently associated with products potentially mislabeled for profit (Table 1).
- ➡ Samples A12 and A49 labeled as bison (Table 1) could potentially have been a result of hybridization between bison and cattle.



Figure 3. Photo of game meat purchased from an online distributor.

- ₱ 40% of potentially mislabeled products may have been a result of mishandling, as there is no economic incentive associated with mislabeling (Table 2).
- **T** Distributors associated with possible mishandling (Table 2) sold both the species listed on the product label and the identified species.
- The Distributor A was most frequently associated with products potentially mislabeled due to mishandling (Table 2).

Distributor	Sample ID	Product label	Retail price (USD)	Cut	Identified species	Retail price of identified species (USD)
В	A14	Antelope	\$41.78/kg	Center cut steak	Sika deer ( <i>Cervus nippon</i> )	\$35.16/kg
В	A12	Bison	\$19.73/kg	Stew meat	Domestic cattle ( <i>Bos taurus</i> )	\$11.16/kg
С	A49	Bison	\$70.55/kg	Rib eye steak	Domestic cattle ( <i>Bos taurus</i> )	\$15.12/kg
В	A17	Pheasant	\$13.18/kg	Leg quarters	Helmeted guineafowl ( <i>Numida meleagris</i> )	\$9.37/kg
A	A41	Red deer	\$61.73 to \$77.16/kg	Loin chop	Alpaca ( <i>Lama pacos</i> )	\$44.07/kg
D	A28	Yak	\$62.99 to \$73.48/kg	Sirloin steak	Domestic cattle ( <i>Bos taurus</i> )	\$16.40/kg

Products listed in this table indicate the retail price of the purchased product is higher than retail price of the substituted identified species resulting in potential profit gain.

Distributor	Sample ID	Product label	Retail price (USD)	Cut	Identified species	Retail price of identified species (USD)
A	A10	Alligator	\$77.14/kg	Tenderloin meat	Spectacled caiman ( <i>Caiman crocodilus</i> )	\$88.16/kg
Α	A11	Alligator	\$44.07/kg	Body and tail meat	Spectacled caiman ( <i>Caiman crocodilus</i> )	\$66.12/kg
В	A16	Black bear	\$28.55/kg	Stew meat	American beaver ( <i>Castor canadensis</i> )	\$88.16/kg
A	A31	Red deer	\$61.73 to \$77.16/kg	Loin chop	Llama ( <i>Lama glama</i> )	\$110.21/kg

Products listed in this table indicate the retail price of the purchased product is lower than the retail price of the substituted identified species resulting in potential economic loss. Since companies require profit to sustain a business, and the identified species had a lower value and were offered by the distributor, these items were suspected of being substituted due to mishandling.

### Table 2. Products potentially mislabeled due to mishandling

## Sample ID

A13, A22, A23, A52

A38

₱ 9.3% of identified species in all products were classified as near threatened or vulnerable (threatened) species according to the International Union for Conservation of Nature (IUCN) (Table 3)

← All species identified as near threatened or vulnerable (Table 3) were correctly labeled and legally sold as they are not protected by the Endangered Species Act and not listed in 50 CFR § 17 (2014)

reference Several products were found to have been substituted with a lower-valued species and were likely mislabeled for profit.

reproducts that were substituted with a higher-valued species may have been mislabeled due to inadequate traceability systems and/or mishandling by the distributor or supplier.

r Bison products identified as cattle may have been mislabeled for profit or may have been a result of hybridization.

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# Key Findings (cont'd)

### Table 3. Correctly labeled products identified as a near threatened or vulnerable species

D	Product label	Samples (n)	Top species match	Genetic similarity	Population status
2, 2	Bison	4	American bison ( <i>Bison bison</i> )	100.0%	Near threatened
	Lion	1	Lion (Panthera leo)	100.0%	Vulnerable (threatened)

## Conclusions

➡ 10 out of 54 products (18.5%) were potentially mislabeled and 5 out of 54 products (9.3%) contained a near threatened or vulnerable (threatened) species.

# Significance of Findings

The results of this study suggest that existing policies may require some amendment to identify and deter fraudulent practices, such as the implementation of mandatory inspection of game meats and verification of species labeling. Additional market research on game meat mislabeling within the United States is recommended in order to delineate trends and determine appropriate steps to improve control of this specialty food group.

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