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DNA Barcoding Reveals Mislabeling of Game Meat Species on the U.S. Commercial Market

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1 **DNA Barcoding Reveals Mislabeling of Game Meat Species on the U.S. Commercial**
2 **Market**

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16 **Abstract**

17 Game meats represent a valuable specialty market in the United States that has high
18 economic incentives associated with mislabeling. However, there is limited information
19 on this topic. The purpose of this study was to conduct a market survey of game meats sold
20 within the United States and identify instances of mislabeling using DNA barcoding.
21 Products were also examined for the presence of threatened or endangered species. Fifty-
22 four samples of whole-cut game meats were collected from online distributors in the
23 United States and sequenced across a 658 base-pair region of the cytochrome *c* oxidase
24 subunit I (COI) gene. The resulting DNA sequences were identified based on top species
25 matches in the Barcode of Life Database (BOLD) and GenBank. The results showed that
26 18.5% of samples were potentially mislabeled and 9.3% of samples legally contained a
27 near-threatened or vulnerable species and were correctly labeled. The samples appeared to
28 have been mislabeled due to reasons such as economic gain and product mishandling.
29 However, cross-species hybridization could also have contributed to the potential
30 mislabeling of bison and yak products. Although near threatened (bison) and vulnerable
31 (lion) species were identified, the products were correctly labeled and legally sold, as bison
32 populations are managed and the identified lion species is not protected by the Endangered
33 Species Act (ESA). Overall, the results of this study revealed the occurrence of game meat
34 mislabeling in the United States and suggest the need for further evaluation of this practice.

35 **Keywords:** DNA barcoding; game meat; species identification; adulteration; misbranding;
36 DNA sequencing

37

38 **1. Introduction**

39 Food fraud, in the form of ingredient substitution and mislabeling of food products,
40 has been observed globally (Cawthorn, Steinman, & Hoffman, 2013; Everstine, Spink, &
41 Kennedy, 2013). One type of food fraud is the intentional substitution of one species for
42 another, which may be carried out for reasons such as financial gain or avoidance of import
43 restrictions. Mislabeling and species substitution may also lead to illegal sales of
44 threatened (i.e., vulnerable, endangered or critically endangered) species protected by the
45 Endangered Species Act (ESA) (Fish and Wildlife Service [FWS], 2003) and disrupt
46 conservation efforts aimed at these animals (Crego-Prieto et al., 2012; International Union
47 for Conservation of Nature and Natural Resources [IUCN], 2014; Rasmussen & Morrissey,
48 2009). Game meats represent an important specialty market in the United States, with an
49 estimated value of US\$39 thousand million (National Agricultural Statistics Service
50 [NASS], 2012). According to the U.S. Food and Drug Administration (FDA), game meats
51 are defined as exotic meats, animals and birds which are not in the Meat and Poultry Act
52 (FDA, 2012). Due to differences in the retail prices between high-cost game meats and
53 lower-priced livestock, such as beef, pork and poultry, there is high economic motivation
54 for species substitution to occur (Economic Research Service [ERS], 2014). Furthermore,
55 game meats are sold as cuts or ground products, which makes it difficult to identify
56 mislabeled species based on appearance alone. There is also potential for the harvesting of
57 threatened or endangered meat species protected by the ESA that are mislabeled and sold
58 as otherwise legal game meats.

59 Game meats produced in the United States are regulated by the U.S. Department of
60 Agriculture (USDA) while game meats imported into the United States are regulated by

61 the FDA (Food Safety and Inspection Service [FSIS], 2011). While it is legal for wild
62 game to be hunted in the United States for personal consumption, the USDA requires that
63 domestically-produced game meats sold commercially are farm-raised (FDA, 2013; FSIS,
64 2011). However, unlike animals covered under the Meat and Poultry Act, mandatory
65 inspection services are not required for game meats (USDA, 2000). Imported game meat is
66 allowed provided it is an eligible product from an approved country (Animal and Plant
67 Health Inspection Service [APHIS], 2012; FSIS, 2013; USDA, 2014) and the meat does
68 not violate any U.S. regulations (FWS, 2006). Imported products must follow the Code of
69 Federal Regulations (CFR) Title 50 Part 17 which dictates that threatened and imported
70 species acquired through commerce or commercial activity are illegal and ineligible for
71 import (Endangered and Threatened Wildlife and Plants, 2014). Furthermore, according to
72 the ESA, even animals maintained in a controlled environment are prohibited for
73 commercial activity unless actions are for conservation (FWS, 2003). However, species are
74 only protected by the ESA provided that they are listed as threatened or endangered in the
75 Federal Register or included in provisions of the Convention on International Trade in
76 Endangered Species of Wild Fauna and Flora (CITES).

77 Previous studies conducted outside the United States have uncovered instances of
78 mislabeling in processed meat and game products (Ayaz, Ayaz, & Erol, 2006; Cawthorn et
79 al., 2013; D'Amato, Alechine, Cloete, Davison, & Corach, 2013). For example, studies
80 conducted in South Africa found that 68.3-69.2% of meat products tested were mislabeled
81 (Cawthorn et al., 2013; D'Amato et al., 2013). Furthermore, a study conducted in Turkey
82 found that 22.0% of meat products tested were mislabeled, including products labeled as
83 beef identified as poultry, deer and horse (Ayaz et al., 2006). Despite the potential for

84 fraudulent mislabeling of game meats on the U.S. commercial market, there is limited
85 information on this topic, with previous studies focusing on seafood and non-game meats.
86 For example, Hsieh, Woodward and Ho (1995) tested non-game meat products sold in the
87 United States and found that 16.6% of samples were mislabeled. In a study conducted in
88 North America by Wong and Hanner (2008), 25% of the commercial seafood products
89 tested were mislabeled, including an endangered species mislabeled as a sustainable
90 counterpart. Another study reported the illegal mislabeling of shark fins belonging to the
91 white shark (*Carcharodon carcharias*), which is a protected species (Shivji, Chapman,
92 Pikitch, & Raymond, 2005). Overall, the mislabeling and exploitation of protected species
93 found in the above studies suggest the importance of investigating mislabeled game meat
94 within the United States.

95 When taxonomic features have been removed due to processing, methods based on
96 DNA or protein profiles are typically used to identify meat or seafood products at the
97 species level (Ballin, 2010; Hellberg & Morrissey, 2011). One of these methods is DNA
98 barcoding, which is based on genetic variation within a standardized genetic region. In
99 animals, this standardized region is a ~650 base-pair (bp) fragment of the gene coding for
100 cytochrome *c* oxidase subunit I (COI) (Hebert, Cywinska, Ball, & deWaard, 2003). This
101 target is a mitochondrial gene that is relatively conserved within species and exhibits
102 divergence between species, enabling samples to be identified at the species level in most
103 cases. In order to identify an unknown sample using the COI DNA barcode, the query
104 sequence is compared to a sequence database and the top species match is identified. DNA
105 barcoding has been successfully used in previous market surveys examining species

106 substitution in seafood (Smith, McVeagh, & Steinke, 2008; Wong & Hanner, 2008) and
107 meat products (D'Amato et al., 2013).

108 Given the limited information on game meat mislabeling practices in the United
109 States combined with the potential for fraud to occur, the objective of this study was to
110 perform a market survey to identify species in game meats sold within the United States
111 using DNA barcoding. Game meats were examined for instances of mislabeling as well as
112 for the presence of threatened or endangered species.

113 **2. Materials and Methods**

114 *2.1. Sample collection*

115 A total of 54 game meat products representing a variety of species were collected in
116 this study from four online retail sources in the United States (Table 1). All products were
117 received in a fresh/frozen state and upon arrival they were catalogued and stored at -80°C.
118 Prior to sampling, products were thawed overnight at 4°C. A tissue sample of ~10 mg was
119 excised with sterile scalpels and forceps and transferred to a 1.5 ml microcentrifuge tube
120 for DNA extraction.

121 *2.2. DNA extraction*

122 DNA extractions were carried out using the DNeasy Blood and Tissue Kit (Qiagen,
123 Valencia, CA), Spin-Column protocol according to modifications in Handy et al. (2011).
124 Following sample collection, the tissue samples described above were lysed with 50 µl
125 Buffer ATL and 5.56 µl Proteinase K over a period of 1-3 h at 56°C while vortexing at 30
126 min increments. Following lysis, 55.6 µl Buffer AL and 55.6 µl of 95% ethanol were
127 added to each sample tube and vortexed. The samples were transferred to columns and

128 centrifuged for 1 min at 8,000 rpm. The column membrane was washed with 140 μ l of
129 AW1 buffer and centrifuged for 1 min at 8,000 rpm followed by a second wash with 140
130 μ l of AW2 buffer and centrifugation for 3 min at 14,000 rpm. The columns were
131 transferred to a sterile 1.5 ml microcentrifuge tube prior to adding 50 μ l of AE buffer
132 preheated to 37°C. The samples were centrifuged for 1 min at 8,000 rpm to collect the
133 eluted DNA. A reagent blank with no tissue added was included alongside each set of
134 extracted samples.

135 2.3. *PCR and sequencing*

136 The mammalian primer cocktails described by Ivanova, Clare and Borisenko
137 (2012) were used to amplify a 658-bp region of the gene coding for COI. PCR was carried
138 out as described in Ivanova et al. (2012), except that OmniMix HS (Cepheid, Sunnyvale,
139 CA) lyophilized PCR reagent beads were used in place of adding individual reagents and
140 the total reaction volume was increased to 25 μ l. Each reaction included the following
141 components: 0.5 OmniMix HS PCR bead, 22.5 μ l molecular grade water, 0.25 μ l of each
142 10 μ M primer cocktail, and 2 μ l of DNA. Cycling conditions were followed according to
143 Ivanova et al. (2012): 94°C for 2 min; 5 cycles of 94°C for 30 s, 50°C for 40 s, and 72°C for
144 1 min; 35 cycles of 94°C for 30 s, 55°C for 40 s, and 72°C for 1 min; and a final extension
145 step at 72°C for 10 min. Thermocycling was carried out with a Mastercycler nexus
146 gradient thermal cycler (Eppendorf, Hamburg, Germany).

147 Confirmation of PCR was achieved as described in Hellberg, Kawalek, Van, Shen
148 and Williams-Hill (2014). PCR products (4 μ l) were loaded with dd H₂O (16 μ l) onto pre-
149 cast 2.0% E-gels (Life Technologies, Carlsbad, CA) and run for 6-10 min using an E-Gel
150 iBase (Life Technologies). Results were captured using FOTO/Analyst Express

151 (Fotodyne, Hartland, WI) and Transilluminator FBDLT-88 (Fisher Scientific, Waltham,
152 MA) and visualized with FOTO/Analyst PCImage (version 5.0.0.0, FOTODYNE).
153 Amplified products were cleaned using ExoSAP-IT (Affymetrix, Santa Clara, CA) as
154 described in Handy et al. (2011). The samples were then sent to GenScript (Piscataway,
155 NJ) for bi-directional sequencing using BigDye Terminator v3.1 Cycle Sequencing Kit
156 (Life Technologies) and an Applied Biosystems 3730xl Genetic Analyzer (Life
157 Technologies).

158 2.4. *Sequence analysis*

159 Raw sequence data was assembled and edited using Geneious R7 (Biomatters Ltd.,
160 Auckland, New Zealand). Successfully assembled consensus sequences were aligned
161 using ClustalW with the default settings in Geneious R7 and then trimmed to the COI
162 DNA barcoding region (658 bp). The length, number of ambiguities and % high quality
163 bases (HQ%) were recorded for each consensus sequence. Following quality guidelines set
164 by Handy et al. (2011), only samples with assembled bi-directional sequences that were \geq
165 500 bp with $< 2\%$ ambiguities or ≥ 500 bp single reads with $\geq 98\%$ HQ were further
166 analyzed. Nucleotide sequences meeting these requirements were searched against the
167 Species Level Barcode Records in the Barcode of Life Database (BOLD)
168 (http://www.boldsystems.org/index.php/IDS_OpenIdEngine). The top species matches
169 showing $\geq 98\%$ similarity to the query sequence were recorded. Sequences which did not
170 yield a species match in BOLD were queried in GenBank using the Basic Local Alignment
171 Search Tool (BLAST) and the top species matches were recorded. Any samples that were
172 identified as potentially mislabeled products were subjected to a second round of DNA
173 extraction and sequencing to confirm the initial result. Each identified species was queried

174 in the Encyclopedia of Life (EOL) (<http://eol.org/>) to identify the preferred common name
175 in addition to the IUCN (<http://www.iucnredlist.org/>) to determine if it was threatened or
176 endangered.

177 **3. Results and Discussion**

178 3.1. *Sequencing results*

179 Out of the 54 samples collected, a total of 22 different types of game meat were
180 represented based on the product label, with an average of 2-3 samples tested per game
181 meat type (Table 1). All 54 samples collected were successfully amplified and bi-
182 directionally sequenced to assemble a COI DNA barcode. The sequences were of high
183 quality, with an average consensus length of 657 ± 6 bp, an average ambiguity percentage
184 of $0.05 \pm 0.13\%$ and an average HQ percentage of $93.6 \pm 8.1\%$. The majority of samples
185 ($n = 51$) showed genetic matches $\geq 98\%$ to species-level entries in BOLD (Table 1). Of
186 these samples, 38 showed $\geq 98\%$ genetic similarity to sequences from just one species
187 while 13 showed this level of similarity to at least two species (discussed below). Three
188 specimens could not be identified at the species level in BOLD and were subsequently
189 searched in GenBank using BLAST. These included two products labeled as kangaroo
190 (A24 and A33) that had top species matches of 99.00% and 96.00%, respectively, to the
191 Western grey kangaroo (*Macropus fuliginosus*), and one product labeled as partridge
192 (A29) that had a top species match of 96.00% to the chukar partridge (*Alectoris chukar*).
193 Considering that divergence within species is typically $< 2\%$ for the COI DNA barcode
194 region (Hebert, Ratnasingham, & deWaard, 2003), it is possible that samples A29 and A33
195 belong to a species not yet sequenced across this genetic region. If the sequence data for a
196 particular species has not been uploaded to BOLD, the database cannot generate a species-

197 level identification (Milton, Pierossi, & Ratnasingham, 2013; Ratnasingham & Hebert,
198 2007).

199 Overall, 44 of the products were found to be correctly labeled. Inadequate data on
200 product packaging such as a missing country of origin or inconsistent naming conventions
201 of meat cuts made it difficult to uncover trends among correctly labeled products. Based
202 on the available information, game purchased whole, as a breast, or as a chuck/shoulder
203 roast was always correctly labeled. However, these observations are based on a small
204 sample size of 2-4 specimens per meat cut.

205 Thirteen of the 51 specimens identified through BOLD had multiple species
206 matches with genetic similarity $\geq 98\%$. One sample (A32) labeled as coyote was identified
207 as both coyote (*Canis latrans*) and grey wolf (*Canis lupus*) with 100.0% similarity to each
208 species. In North America, there is a continuous distribution of both coyotes and wolves,
209 allowing for interbreeding between the two species and thus preventing complete
210 distinction based on the DNA barcode (Vila et al., 1999). Four products labeled as elk
211 (A19-A21 and A50) had a 100.0% match to two species: red deer (*Cervus elaphus*) and
212 American elk (*Cervus canadensis*). Due to the lack of consensus regarding classification
213 practices and consequences of human involvement with *Cervus* species, some authors have
214 included *C. canadensis* as a subspecies of *C. elaphus* while others categorize them as two
215 separate species (Polziehn & Strobeck, 2002; Randi, Mucci, Claro-Hergueta, Bonnet, &
216 Douzery, 2001). These four products also had secondary species matches to sika deer
217 (*Cervus nippon*), sambar (*Cervus unicolor/Rusa unicolor*), and rusa (*Cervus*
218 *timorensis/Rusa timorensis*) with genetic similarities of 98.00-98.36%. These results may
219 be explained by the oftentimes inclusion of sika as a subspecies of red deer and rusa

220 descending from a similar ancestry as *R. unicolor* and *Axis porcinus* (Groves, 2006; Pitra,
221 Fickel, Meijaard, & Groves, 2004). Since the greatest genetic similarity was found with
222 red deer/American elk, the products were determined to be correctly labeled. The four
223 products labeled as elk with sole top species matches to red deer (A05, A07, A15 and A51)
224 were also determined to be correctly labeled. This was on the basis that EOL lists
225 American elk as a subspecies of *C. elaphus* and these two organisms do not show
226 sufficient divergence to be differentiated with the COI DNA barcode. In another case of
227 products matching multiple species, two specimens labeled as buffalo (A04 and A54) were
228 identified as Asian water buffalo (*Bubalus bubalis*) with 100.0% genetic similarity and as
229 water buffalo (*Bubalus arnee*) with 99.80% similarity. However, the difference in
230 nomenclature is a result of domestication and not a difference in animal species (Gentry,
231 Clutton-Brock, & Groves, 2004). Aside from hybridization and dissimilar classification
232 practices, specimens may be misidentified if the sequence data used to assign species in
233 BOLD has not been validated or is incorrect (Ratnasingham & Hebert, 2007). In addition,
234 DNA barcoding is not effective for species identification when two or more species do not
235 show sufficient genetic divergence across the selected barcode region (Ward, Costa,
236 Holmes, & Steinke, 2008).

237 The remaining samples with multiple species identified at $\geq 98\%$ genetic similarity
238 are discussed below, as none of the identified species corresponded to what was listed on
239 the label.

240 3.2. *Mislabeled products*

241 Ten of the 54 samples sequenced were determined to be potentially mislabeled
242 (Table 1). These results were confirmed by a second DNA extraction and sequencing.

243 Interestingly, six of these products were associated with economic incentives based on
244 differences in retail prices, while four products were priced lower than the list price for the
245 identified species (Table 2). In instances that lacked an economic incentive, accidental
246 mishandling by the manufacturer or the supplier may have resulted in the listed product
247 being replaced by a higher-valued species, as the substitution would have resulted in profit
248 loss.

249 Five of the potentially mislabeled products (A12, A16, A17, A28 and A49) were
250 identified with 100.0% genetic similarity to a single species without secondary matches.
251 Two products labeled as bison (A12, A49) and one product labeled as yak (A28) were
252 identified as domestic cattle (*B. taurus*); a product labeled as black bear [*(Ursus*
253 *americanus*) (A16)] was identified as American beaver (*Castor canadensis*); and a product
254 labeled as pheasant [*(Phasiandae* family) (A17)] was identified as helmeted guineafowl
255 (*Numida meleagris*). The products labeled as bison, pheasant and yak each showed
256 potential for economic gain. The product labeled as pheasant and identified as guineafowl
257 had the smallest potential profit of US\$3.81/kg, while the product labeled as yak and
258 identified as domestic cattle had the largest potential profit of US\$46.59-\$57.08/kg.
259 Domestic cattle labeled as bison would have resulted in a profit of US\$8.57-\$55.43/kg
260 depending on the product type. While there is economic incentive associated with
261 mislabeling cattle as bison, these findings could be due to crossbreeding between cattle and
262 bison, which is known to occur in the wild (Polziehn, Strobeck, Sheraton, & Beech, 1995).
263 As a result of the maternal inheritance pattern of mitochondrial DNA, offspring of a male
264 bison and a female cow would have a DNA barcode matching that of cattle (Derr et al.,
265 2012; Polziehn et al., 1995). According to regulations listed in Exotic Animals and Horses,

266 9 C. F.R. § 352 (2014), products labeled as bison may refer to American bison or the
267 hybrid species cattalo. However, if cattalo is to be sold as bison, it must be a result of
268 direct breeding between American bison and cattle. Since it is unknown whether the
269 products labeled as bison, but identified as cattle, were a result of species substitution,
270 direct crossbreeding, or backcrossing, the products are considered potentially mislabeled.
271 Similarly, yak and cattle have crossbred resulting in mitochondrial cattle DNA in a species
272 resembling yak (Leslie & Schaller, 2009; Qi, Jianlin, Wang, Rege, & Hanotte, 2010).
273 However, it can be difficult to differentiate these hybridized individuals morphologically
274 and therefore mislabeling may have been unintentional, despite the potential for profit. In
275 the case of the product labeled as beaver and identified as black bear, an economic loss
276 would have resulted, indicating possible product mishandling. Interestingly, the FDA
277 previously issued a warning letter to a game meats distributor for selling misbranded black
278 bear steaks identified as brown bear (*Ursus arctos*) and misbranded black bear burgers
279 found to contain elk/red deer (*Cervus* sp.) (FDA, 2011).

280 The remaining five mislabeled samples had a genetic similarity \geq 98% to multiple
281 species, none of which corresponded to the species listed on the label. Two of the four
282 products labeled as alligator [(*Alligator* sp.) (A10 and A11)] were identified as spectacled
283 caiman (*Caiman crocodilus*) with genetic similarities of 99.54-99.69%. These products
284 also showed a secondary match to the yacare caiman (*Caiman yacare*) with genetic
285 similarity of 98.57-99.23%. According to Busack and Pandya (2001), conflicting
286 classifications have caused *C. yacare* to be considered either a separate species or a
287 subspecies of *C. crocodilus*. The distributor which sold the alligator products also sold
288 products labeled as spectacled caiman. Since replacing alligator with caiman would result

289 in profit loss (Table 2), the mislabeling may have been a result of accidental mishandling
290 or intentional substitution based on a lack of supply for the desired product.

291 Two products labeled as red deer (A31 and A41) also showed multiple species
292 matches with $\geq 98\%$ genetic similarity. One product (A31) had a primary identification of
293 llama (*Lama glama*) with genetic similarity matches of 98.62-100.0% and secondary
294 matches to guanaco (*Lama guanicoe*) and alpaca (*Lama pacos*, *Vicugna pacos*) with 98.77-
295 99.39% similarity. The second product (A41) was identified as alpaca (*L. pacos*) with
296 100.0% genetic similarity and vicuña (*Vicugna vicugna*) with 98.32% similarity.
297 Identification of multiple species for these products is a result of the domestication of
298 guanaco and vicuña to produce llama and alpaca, respectively (Barreta et al., 2013).
299 During the domestication process, hybridization occurred between llamas and alpacas
300 making it difficult to distinguish the two based on DNA barcoding. Products labeled as
301 llama and alpaca were sold by the same distributor that had the mislabeled red deer
302 products, but since llama is sold for a much higher price than alpaca, product A41 shows a
303 potential for economic gain while A31 does not. Based on the genetic similarity of llama
304 and alpaca, it is possible that both products originated from the same species or that they
305 were incorrectly identified by the supplier/distributor. Regardless of these genetic
306 similarities, both products were considered mislabeled, since none of the top species
307 matches were for red deer.

308 Finally, a product labeled as antelope [(*Bovidae* family) (A14)] showed a top
309 species match to sika deer with genetic similarities of 98.01-100.0% and secondary genetic
310 matches of 98.01-98.62% to red deer, usuri sika deer (*Cervus hortulorum*) and hokkaido
311 sika deer (*Cervus yesoensis*). The difficulty in assigning a single species may be due to

312 existing uncertainties involving deer species (Cook, Wang, & Sensabaugh, 1999; Pitra et
313 al., 2004). Early classifications relied on morphology and have been deemed inadequate.
314 The use of DNA analysis has shown molecular differences between sika, usuri sika and
315 hokkaido sika deer which are located in different geographic regions of Asia (Groves,
316 2006). Despite molecular differences between species, nomenclature assignments have not
317 been consistent and declining populations prevent complete resolution (Cook et al., 1999;
318 Groves, 2006). Furthermore, red and sika deer may share an ancestral species making it
319 difficult to discern groups. Despite these issues, none of the primary or secondary species
320 matches would have resulted in a correctly labeled antelope product. Of note, products
321 labeled as antelope and sika deer were both sold by the same distributor and the
322 replacement of antelope with sika deer would have resulted in a potential economic profit
323 of US\$6.62/kg (Table 2).

324 Each distributor was examined for the frequency of mislabeling among the
325 products sampled (Fig. 1). At least one product from each distributor was potentially
326 mislabeled, with distributors A and B having the highest numbers of potentially
327 misbranded products, at 16.0% and 57.1%, respectively. All four potentially mislabeled
328 products from distributor A were substituted with a species also offered for sale by the
329 same distributor. Since only one potentially mislabeled product from distributor A was
330 associated with economic incentives, the fraudulent products may have been due to
331 improper handling or supply issues. On the other hand, three of the four potentially
332 mislabeled products from distributor B showed profit incentives through species
333 substitution (Table 2). Overall, nine out of ten potentially mislabeled products were
334 substituted for a species the distributor also processed, with the exception being a product

335 labeled as bison (A12) sold by distributor B that was identified as domestic cattle. Though
336 not every substituted species was offered as the same cut as the expected species,
337 unintentional mix-ups may have occurred due to the inability to distinguish meats based on
338 visual inspection alone. Provided that the distributor has adequate quality systems in place,
339 incidences of mislabeling may have occurred at the supplier or farm level.

340 The overall rate of potential mislabeling found in this study (18.5%) was similar to
341 previous studies investigating non-game meat products in the United States and Turkey,
342 which reported mislabeling rates of 16.6% (Hsieh et al., 1995) and 22.0% (Ayaz et al.,
343 2006), respectively. However, these rates are much lower than those reported by studies
344 investigating meat and game products sold in South Africa [68.3-69.2%] (Cawthorn et al.,
345 2013; D'Amato et al., 2013). The higher rates of mislabeling found in the latter studies may
346 be due to differences in market regulations, inspection programs or product monitoring. In
347 comparison to seafood, the rate of mislabeling in the current study was slightly lower than
348 the rate of 25.3% reported by Wong and Hanner (2008) for North American seafood.
349 Given that seafood is one of the top food categories subject to fraud (Johnson, 2014), it
350 may be expected that seafood has a rate of mislabeling equal to or higher than game meat.

351 3.3. *Threatened and endangered species*

352 According to IUCN classifications, one sample (A38) was identified as vulnerable
353 (included in the threatened category), four samples (A13, A22, A23, A52) were identified
354 as near threatened, fifteen samples were identified as species with a population status not
355 yet assessed, and the remaining samples were identified as species with stable populations
356 (Table 1). The five products identified as a near threatened or threatened species were all
357 determined to be correctly labeled and legally sold. The four products with a status of near

358 threatened were all labeled as bison and identified as American bison (*Bison bison*) (Gates
359 & Aune, 2008). Although the bison population has increased since the steep decline in the
360 19th century, herds are currently managed under conservation programs. Despite the
361 National Agricultural Statistics Service (NASS, 2012) indicating 162,110 bison were
362 present on farms in 2012, IUCN classifications do not account for commercial herds when
363 determining if a species is threatened or endangered (Gates & Aune, 2008). The managed
364 herds allow for population control while still offering bison products for consumption. The
365 sample included in the threatened category was the product labeled and identified as lion
366 [*Panthera leo*] (A38) (Bauer, Nowell, & Packer, 2012)]. Despite being listed as a
367 threatened species by the IUCN, lion is not protected under the ESA (FWS, 2003, 2013).
368 Until this species is listed as threatened in the Federal Register, lion products sold
369 commercially do not violate conservation laws. In an attempt to conserve the lion species,
370 the FWS submitted a proposal to add *Panthera leo leo* to the list of threatened species to
371 provide protection against commercial activity (FWS, 2013). In summary, since the bison
372 population is managed and the lion is not protected by the ESA, the sale of these products
373 would not be considered illegal.

374 The observed incidence of threatened species identified in products analyzed in this
375 study (1.85%) is similar to previous market studies examining mislabeled threatened fish
376 and seafood species. Wong and Hanner (2008) found that 1.10% of the 91 North American
377 seafood products analyzed in their study were from a threatened species. Another market
378 survey reported that 0.58% of 1,215 seafood items collected in the United States were
379 endangered or critically endangered (Warner, Timme, Lowell, & Hirshfield, 2013). On the
380 other hand, one study focused on just one product type found that 18.25% out of 400 whale

381 meat products analyzed from Japan and Korea were from protected stocks (Dalebout,
382 Lento, Cipriano, Funahashi, & Baker, 2002).

383 **4. Conclusion**

384 Overall, the results of this study demonstrate the occurrence of mislabeling of
385 commercially-sold game meat products in the United States, with 18.5% of the game meats
386 analyzed determined to be potentially mislabeled. Although one of the products tested
387 contained a species from a threatened population, the product was correctly labeled and
388 legally sold. An examination of the potentially misbranded products suggested the
389 possibility of intentional mislabeling for economic gain for over half of the misbranded
390 products. The other products appear to have been misbranded for other reasons, such as
391 inadequate traceability systems and/or mishandling by the distributor or supplier. It is also
392 possible that products may appear to be mislabeled due to cross-species hybridization or
393 inconsistencies with classification. The results of this study suggest that existing policies
394 may require some amendment to identify and deter such practices, such as the
395 implementation of mandatory inspection of game meats and verification of species
396 labeling. Additional market research on game meat mislabeling within the United States is
397 recommended in order to delineate trends and determine appropriate steps to improve
398 control of this specialty food group.

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445 [idx?SID=b88acb3201f2467a1e26dfd31094629c&node=pt50.2.17&rqn=div5](http://www.ecfr.gov/cgi-bin/text-idx?SID=b88acb3201f2467a1e26dfd31094629c&node=pt50.2.17&rqn=div5)

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453 [5#se9.2.352_11](http://www.ecfr.gov/cgi-bin/text-idx?SID=b5ac9450b7c79a8d6a011f22af451a40&mc=true&node=pt9.2.352&rgn=div5#se9.2.352_11)

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558 **Figure Captions**

559 **Figure 1.** Summary of products by distributor showing the percentage of correctly labeled
560 and mislabeled game meat products tested

561 **Table 1.** Summary of samples collected and analyzed in this study. Top species matches and % genetic similarity were determined using the
 562 Barcode of Life Database (BOLD), unless otherwise noted

Sample ID	Product label	Samples (n)	Top species match	Genetic similarity	Population status ^a
A27, A44	Alligator	2	American alligator (<i>Alligator mississippiensis</i>)	100.0%	Stable
A10	Alligator	1	Spectacled caiman (<i>Caiman crocodilus</i>) ^b	99.54%	Stable
A11	Alligator	1	Spectacled caiman (<i>Caiman crocodilus</i>) ^b	99.69%	Stable
A03	Alpaca	1	Alpaca (<i>Lama pacos</i>)	99.54%	Not assessed
A30	Antelope	1	Nilgai antelope (<i>Boselaphus tragocamelus</i>)	99.69%	Stable
A14	Antelope	1	Sika deer (<i>Cervus nippon</i>) ^b	100.0%	Stable
A02	Beaver	1	American beaver (<i>Castor canadensis</i>)	99.80%	Stable
A13, A22, A23, A52	Bison	4	American bison (<i>Bison bison</i>)	100.0%	Near threatened
A12, A49	Bison	2	Domestic cattle (<i>Bos taurus</i>) ^b	100.0%	Not assessed
A16	Black bear	1	American beaver (<i>Castor canadensis</i>) ^b	100.0%	Stable
A06	Bobcat	1	Bobcat (<i>Lynx rufus</i>)	100.0%	Stable
A04, A54	Buffalo	2	Asian water buffalo (<i>Bubalus bubalis</i>)	100.0%	Not assessed
A01, A08, A09	Camel	3	Dromedary camel (<i>Camelus dromedarius</i>)	100.0%	Not assessed
A32	Coyote	1	Coyote (<i>Canis latrans</i>); Grey wolf (<i>Canis lupus</i>) ^c	100.0%	Stable

A15, A05	Elk	2	Red deer (<i>Cervus elaphus</i>)	100.0%	Stable
A07	Elk	1	Red deer (<i>Cervus elaphus</i>)	99.85%	Stable
A51	Elk	1	Red deer (<i>Cervus elaphus</i>)	99.69%	Stable
A19, A20, A21, A50	Elk	4	Red deer (<i>Cervus elaphus</i>); American elk (<i>Cervus canadensis</i>) ^c	100.0%	Stable; Not assessed
A34	Emu	1	Emu (<i>Dromaius novaehollandiae</i>)	100.0%	Stable
A24	Kangaroo	1	Western grey kangaroo (<i>Macropus fuliginosus</i>) ^d	99.00%	Stable
A33	Kangaroo	1	Western grey kangaroo (<i>Macropus fuliginosus</i>) ^d	96.00%	Stable
A38	Lion	1	Lion (<i>Panthera leo</i>)	100.0%	Vulnerable (threatened)
A43	Muskrat	1	Common muskrat (<i>Ondatra zibethicus</i>)	99.85%	Stable
A29	Partridge	1	Chukar partridge (<i>Alectoris chukar</i>) ^d	96.00%	Stable
A42	Partridge	1	Chukar partridge (<i>Alectoris chukar</i>)	99.54%	Stable
A40	Partridge	1	Red-legged partridge (<i>Alectoris rufa</i>)	99.84%	Stable
A26, A36, A48	Pheasant	3	Ring-necked pheasant (<i>Phasianus colchicus</i>)	100.0%	Stable
A17	Pheasant	1	Helmeted guineafowl (<i>Numida meleagris</i>) ^b	100.0%	Stable
A35	Raccoon	1	Northern raccoon (<i>Procyon lotor</i>)	99.69%	Stable
A31	Red deer	1	Llama (<i>Lama glama</i>) ^b	100.0%	Not assessed
A41	Red deer	1	Alpaca (<i>Lama pacos</i>) ^b	100.0%	Not assessed
A39, A45	Turtle	2	Common snapping turtle (<i>Chelydra serpentina</i>)	100.0%	Stable

A18, A25, A46	Wild boar	3	Wild boar (<i>Sus scrofa</i>)	100.0%	Stable
A47	Wild boar	1	Wild boar (<i>Sus scrofa</i>)	99.84%	Stable
A53, A55	Yak	2	Yak (<i>Bos grunniens</i>)	100.0%	Not assessed
A28	Yak	1	Domestic cattle (<i>Bos taurus</i>) ^b	100.0%	Not assessed

563 ^aAccording to the International Union for Conservation of Nature (IUCN)

564 ^bPotentially mislabeled

565 ^cSequences for a single sample matched multiple species with 100.0% similarity

566 ^dNo sequence matches were available in BOLD for this sample. The top species match from GenBank is reported

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Table 2. Retail price comparisons of potentially mislabeled game meats. Retail prices were obtained from online distributors unless otherwise noted

Distributor	Sample ID	Product label	Retail price (USD)	Cut	Identified species	Retail price of identified species (USD)	Notes
A	A10	Alligator	\$77.14/kg	Tenderloin meat	Spectacled caiman (<i>Caiman crocodilus</i>)	\$88.16/kg	N/A
A	A11	Alligator	\$44.07/kg	Body and tail meat	Spectacled caiman (<i>Caiman crocodilus</i>)	\$66.12/kg	Price of identified species is for "Caiman Meat." Body and tail meat was not offered.
B	A14	Antelope	\$41.78/kg	Center cut steak	Sika deer (<i>Cervus nippon</i>)	\$35.16/kg ^a	N/A
B	A12	Bison	\$19.73/kg	Stew meat	Domestic cattle (<i>Bos taurus</i>)	\$11.16/kg ^{a,b}	Price of identified species is for boneless beef stew.
C	A49	Bison	\$70.55/kg	Rib eye steak	Domestic cattle (<i>Bos taurus</i>)	\$15.12/kg ^{a,b}	Price of identified species is for uncooked beef steaks.
B	A16	Black bear	\$28.55/kg	Stew meat	American beaver (<i>Castor canadensis</i>)	\$88.16/kg	Beaver as stew meat was not an available cut by this distributor. Price is for stew meat from another online distributor
B	A17	Pheasant	\$13.18/kg	Leg quarters	Helmeted guineafowl (<i>Numida meleagris</i>)	\$9.37/kg ^a	N/A
A	A31	Red deer	\$61.73 to \$77.16/kg	Loin chop	Llama (<i>Lama glama</i>)	\$110.21/kg	Prices of identified species are for llama strip loin steak.

							No loin chop cut offered.
A	A41	Red deer	\$61.73 to \$77.16/kg	Loin chop	Alpaca (<i>Lama pacos</i>)	\$44.07/kg ^a	Prices of identified species are for alpaca strip loin steak. No loin chop cut offered.
D	A28	Yak	\$62.99 to \$73.48/kg	Sirloin steak	Domestic cattle (<i>Bos taurus</i>)	\$16.40/kg ^{a,b}	Price of identified species is for choice sirloin steak.

582 ^aProduct has an economic incentive to be sold mislabeled

583 ^bBased on the average retail price listed by the USDA Economic Research Service for January 2014 - August 2014 (ERS, 2014)

