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## Identification of Meat and Poultry Species in Food Products Using DNA Barcoding

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1 **Title: Identification of meat and poultry species in food products using DNA barcoding**

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25 **Abstract**

26 DNA barcoding is a promising method for the sequencing-based identification of meat  
27 and poultry species in food products. However, DNA degradation during processing may limit  
28 recovery of the full-length DNA barcode from these foods. The objective of this study was to  
29 investigate the ability of DNA barcoding to identify species in meat and poultry products and to  
30 compare the results of full-length barcoding (658 bp) and mini-barcoding (127 bp). Sixty meat  
31 and poultry products were collected for this study, including deli meats, ground meats, dried  
32 meats, and canned meats. Each sample underwent full and mini-barcoding of the cytochrome *c*  
33 oxidase subunit I (COI) gene. The resulting sequences were queried against the Barcode of Life  
34 Database (BOLD) and GenBank for species identification. Overall, full-barcoding showed a  
35 higher sequencing success rate (68.3%) as compared to mini-barcoding (38.3%). Mini-barcoding  
36 out-performed full barcoding for the identification of canned products (23.8% vs. 19.0%  
37 success), as well as for turkey and duck products; however, the primer set performed poorly  
38 when tested against chicken, beef, and bison/buffalo. Overall, full barcoding was found to be a  
39 robust method for the detection of species in meat and poultry products, with the exception of  
40 canned products. Mini-barcoding shows high potential to be used for species identification in  
41 processed products; however, an improved primer set is needed for this application.

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43 **Keywords:** Species identification, DNA sequencing, DNA barcoding, mini-barcoding, meat  
44 mislabeling, species substitution

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## 48 **1. Introduction**

49 Red meat and poultry are significant sources of protein worldwide, with over 40 billion  
50 kg produced in the United States in 2015 (USDA, 2016). Production is expected to increase in  
51 the coming years, accompanied by an increase in U.S. per capita consumption to about 100 kg by  
52 the year 2025. While meat and poultry species are generally identifiable when sold as whole  
53 cuts, processing techniques, such as grinding, smoking, curing, and/or canning, can change the  
54 appearance and sensory characteristics of the final product. The inability to visually identify  
55 species in these products, combined with variations in the retail prices for meat and poultry  
56 species, increases the potential for species substitution (Perestam, Fujisaki, Nava, & Hellberg,  
57 2017). In some instances, processing may also lead to the addition of secondary species that are  
58 not present on the label. For example, a previous study investigating mislabeling of ground meat  
59 and poultry products found undeclared species in about 20% of products sampled (Kane &  
60 Hellberg, 2016). Other studies have reported mislabeling rates of 20-70% for various meat  
61 products, including ground meat, deli meats, pet foods, and dried meats (Ayaz, Ayaz, & Erol,  
62 2006; Cawthorn, Steinman, & Hoffman, 2013; Flores-Munguia, Bermudez-Almada, & Vazquez-  
63 Moreno, 2000; Mousavi et al., 2015; Okuma & Hellberg, 2015; Ozpinar, Tezmen, Gokce, &  
64 Tekiner, 2013; Pascoal, Prado, Castro, Cepeda, & Barros-Velázquez, 2004; Quinto, Tinoco, &  
65 Hellberg, 2016).

66 There are several detrimental consequences associated with mislabeling of meat or  
67 poultry species in food products (Ali et al., 2012; Ballin, 2010). In many instances, mislabeling is  
68 a form of economic deception, such as the substitution of horsemeat for beef in the 2013  
69 European horsemeat scandal (NAO, 2013). Additionally, the presence of undeclared species in

70 food products can be harmful to consumers and pets with meat allergies and can interfere with  
71 religious practices that ban the consumption of certain animal species.

72 In order to identify the species in processed meat and poultry products, DNA or protein-  
73 based methods are often used (as reviewed in Ali et al., 2012; Ballin, 2010; M. Á. Sentandreu &  
74 Sentandreu, 2014). Commonly used methods include enzyme-linked immunosorbent assay  
75 (ELISA) (Ayaz et al., 2006; Giovannacci et al., 2004; USDA, 2005; Yun-Hwa, Woodward, &  
76 Shiow-Huey, 1995), real-time polymerase chain reaction (PCR) (Camma, Di Domenico, &  
77 Monaco, 2012; Okuma & Hellberg, 2015; Soares, Amaral, Oliveira, & Mafra, 2013; Yancy et  
78 al., 2009), PCR-restriction fragment length polymorphism (RFLP) (Doosti, Ghasemi Dehkordi,  
79 & Rahimi, 2014; Pascoal et al., 2004; Prado, Calo, Cepeda, & Barros-Velázquez, 2005), and  
80 DNA sequencing (Cawthorn et al., 2013; Kane & Hellberg, 2016; Quinto et al., 2016). ELISA  
81 and real-time PCR are rapid, targeted approaches that enable detection of species in heavily  
82 processed products, including those with species mixtures (Perestam et al., 2017). Real-time  
83 PCR is advantageous in that multiple species can be detected simultaneously and it is highly  
84 sensitive. Despite these advantages, it is limited in that a different primer set is required for each  
85 species targeted. PCR-RFLP allows for the use of universal primers and is capable of detection  
86 of species mixtures; however, it requires several post-PCR steps and it generally requires a  
87 longer DNA target as compared to real-time PCR (Ali et al., 2012). Furthermore, the analysis of  
88 PCR-RFLP results can become highly complex when multiple enzymes are used to differentiate  
89 a range of species. The application of mass spectrometry (MS) to the analysis of proteins and  
90 peptides has been proposed to overcome some of the limitations of molecular techniques (Miguel  
91 A. Sentandreu, Fraser, Halket, Patel, & Bramley, 2010; Miguel Angel Sentandreu & Sentandreu,  
92 2011; M. Á. Sentandreu & Sentandreu, 2014; von Bargen, Brockmeyer, & Humpf, 2014).

93 However, these methods have yet to be widely adopted, in part due to the need for costly  
94 equipment and skilled technicians (M. Á. Sentandreu & Sentandreu, 2014).

95 DNA barcoding is a sequencing-based method that has shown particular promise for the  
96 identification of animal species (Hebert, Cywinska, Ball, & DeWaard, 2003; Hebert,  
97 Ratnasingham, & deWaard, 2003). It has been adopted by the U.S. Food and Drug  
98 Administration (FDA) for use in seafood species identification (Handy et al., 2011) and has been  
99 used to successfully identify meat and poultry species in a variety of food products (Cawthorn et  
100 al., 2013; Kane & Hellberg, 2016; Quinto et al., 2016). This method relies on the use of a  
101 standardized genetic target, which for most animal species is the mitochondrial gene coding for  
102 cytochrome *c* oxidase subunit I (COI) (Hebert, Cywinska, et al., 2003; Hebert, Ratnasingham, et  
103 al., 2003). COI has been determined to be well suited for species differentiation because it  
104 exhibits a relatively low level of divergence within species and a high level of divergence  
105 between species. Furthermore, robust primer sets have been developed for the universal  
106 amplification of COI across a broad spectrum of phyla and the method is supported by a database  
107 containing DNA barcode records for close to 200,000 animal species  
108 (<http://www.boldsystems.org/>). Although DNA barcoding is more time-consuming than some of  
109 the techniques currently available, it is advantageous in that it allows for a universal approach to  
110 species identification supported by a high level of genetic information (Hellberg, Pollack, &  
111 Hanner, 2016). Furthermore, the methodology can be readily adapted for high-throughput  
112 automation.

113 Conventional full-length DNA barcoding targets approximately 650 base pairs (bp) of the  
114 COI gene for species identification in well-preserved and fresh specimens (Hebert, Cywinska, et  
115 al., 2003; Hebert, Ratnasingham, et al., 2003). However, DNA quality can be reduced by many

116 conditions common to food processing such as low pH, high temperatures, and high pressures  
117 (Rasmussen Hellberg & Morrissey, 2011), which makes it difficult to obtain a full-length  
118 barcode from food samples that have been heavily processed, such as canned products. Although  
119 processing of foods ultimately leads to the fragmentation of DNA, amplification of short regions  
120 of DNA may still be possible. In order to facilitate species identification in biological specimens  
121 with degraded DNA, Meusnier et al. (2008) designed a universal primer set targeting a short  
122 region of DNA within the full-length barcode. This ‘mini-barcode’ universal primer set was  
123 found to be capable of amplifying the target DNA fragment in 92% of species tested, including  
124 mammals, fish, birds, and insect specimens. However, the study was focused on applications in  
125 biodiversity analysis and did not specifically target species commonly used in the production of  
126 red meat or poultry. A mini-barcoding system has also been developed specifically for the  
127 identification of fish species in processed products (Shokralla, Hellberg, Handy, King, &  
128 Hajibabaei, 2015). These mini-barcodes showed a success rate of 93.2% when tested against 44  
129 heavily processed fish products, as compared to a success rate of 20.5% with full barcoding.  
130 Although methods based on traditional DNA sequencing do not perform well with species  
131 mixtures, short genetic targets such as mini-barcodes have the potential to be combined with  
132 next-generation sequencing to allow for identification of mixed-species samples (Hellberg et al.,  
133 2016).

134         Despite the potential advantages of mini-barcoding for use in the identification of meat  
135 and poultry species in heavily processed products, research into this application has not yet been  
136 carried out. Therefore, the objective of this study was to investigate the ability of DNA  
137 barcoding to identify meat and poultry species in food products and to compare the results of  
138 full-length and mini-barcoding.



139 **2. Materials and Methods**

140 *2.1 Sample collection*

141 A total of 60 different commercial products representing a variety of meat and poultry  
142 species were collected for this study. The products were purchased from online retailers and  
143 retail outlets in Orange County, CA. A variety of processed products were selected, including  
144 luncheon meats, sausages, patties, ground meats, franks, bacon, jerkies, canned meats, and pet  
145 foods. Each product was unique and products were only included in the study if they listed a  
146 single animal species on the label. Following collection, the products were labeled and  
147 catalogued, then held at their recommended storage temperatures until DNA extraction.

148 *2.2 DNA extraction*

149 DNA extraction was carried out with the DNeasy Blood and Tissue Kit (Qiagen,  
150 Valencia, CA), using modifications as described in Handy et al. (2011). Tissue samples were  
151 lysed at 56°C for 1-3 h with vortexing every ~30 min. DNA was eluted using 50 µl of pre-heated  
152 (37°C) AE buffer. The eluted DNA was stored at -20°C until PCR. A reagent blank negative  
153 control with no tissue was included in each set of DNA extractions.

154 *2.3 Polymerase chain reaction (PCR)*

155 DNA extracts from each sample underwent PCR for both full and mini-barcodes. Each  
156 reaction tube included the following components: 0.5 OmniMix Bead (Cepheid, Sunnyvale, CA),  
157 22.5 µl of molecular-grade sterile water, 0.25 µl of 10 µM forward primer or primer cocktail,  
158 0.25 µl of 10 µM reverse primer or primer cocktail, and 2 µl of template DNA. Amplification of  
159 the full barcode region was carried out using the mammalian primer cocktail described in  
160 Ivanova et al. (2012) and amplification of the mini-barcode region was carried out using the  
161 primer set described in Meusnier et al. (2008). All primers were synthesized by Integrated DNA

162 Technologies (Coralville, IA) and included M13 tails to facilitate DNA sequencing (Ivanova et  
163 al. 2012). A no template control (NTC) containing 2  $\mu$ l of sterile water was run alongside each  
164 set of reactions. PCR was carried out using a Mastercycler nexus Gradient Thermal Cycler  
165 (Eppendorf, Hamburg, Germany). Cycling conditions for full-length barcoding were followed  
166 according to Ivanova et al. (2012): 94°C for 2 min; 5 cycles of 94°C for 30 s, 50°C for 40 s, and  
167 72°C for 1 min; 35 cycles of 94°C for 30 s, 55°C for 40 s, and 72°C for 1 min; and a final  
168 extension step at 72°C for 10 min. Cycling conditions for mini-barcoding were followed  
169 according to Meusnier et al. (2008): 95°C for 2 min; 5 cycles of 95°C for 1 min, 46°C for 1 min,  
170 and 72°C for 30 s; 35 cycles of 95°C for 1 min, 53°C for 1 min, and 72°C for 30 s; and a final  
171 extension step at 72°C for 5 min. The resulting amplicons were stored at -20°C until PCR  
172 product confirmation.

#### 173 *2.4 PCR product confirmation and DNA sequencing*

174 PCR products were confirmed using 2.0% agarose E-Gels containing ethidium bromide  
175 (Life Technologies, Carlsbad, CA). A total of 16  $\mu$ l of sterile water and 4  $\mu$ l of PCR product  
176 were loaded into each well and the gels were run for 6-8 min on an E-Gel iBase (Life  
177 Technologies). The results were captured using FOTO/Analyst Express (Fotodyne, Hartland,  
178 WI) and Transilluminator FBDLT-88 (Fisher Scientific, Waltham, MA) and visualized with  
179 FOTO/Analyst PCImage (version 5.0.0.0, Fotodyne). Next, the PCR products were purified  
180 using a 4-fold dilution of ExoSAP-IT (Affymetrix, Santa Clara, CA) in molecular-grade water.  
181 Each PCR product (5  $\mu$ l) was combined with 2  $\mu$ l of the diluted ExoSAP-IT and then placed in  
182 the thermal cycler for 15 min at 37°C followed by 15 min at 80°C. The samples were then  
183 shipped to GenScript (Piscataway, NJ) for bi-directional DNA sequencing with M13 primers.

184 Sequencing was carried out using the BigDye Terminator v3.1 Cycle Sequencing Kit (Life  
185 Technologies) and a 3730xl Genetic Analyzer (Life Technologies).

### 186 *2.5 Sequencing results and analysis*

187 All sequencing files were assembled and edited using Geneious R7 (Biomatters, Ltd.,  
188 Auckland, New Zealand) (Kearse et al. 2012). Consensus sequences were aligned using  
189 ClustalW and trimmed to the full-barcode (658 bp) or mini-barcode (127 bp) COI regions.  
190 Sequencing was only considered to be successful if the trimmed consensus sequence had < 2%  
191 ambiguities. All successful sequences were queried using the Barcode of Life Database (BOLD)  
192 Animal Identification Request Engine (<http://www.boldsystems.org/>), Public Record Barcodes.  
193 Sequences that could not be identified in BOLD were queried in GenBank using the Basic Local  
194 Alignment Search Tool (BLAST; <http://blast.ncbi.nlm.nih.gov/Blast.cgi>). The results were  
195 recorded and the common names for each species were determined using the Encyclopedia of  
196 Life (EOL) Search Engine (<http://eol.org/>).

## 197 **3. Results and Discussion**

### 198 *3.1 Full-barcoding*

199 Full-barcoding of the 60 meat and poultry products resulted in a total of 41 successful  
200 identifications (Table 1). The sequences recovered with full barcoding had an average length  
201 equal to the target barcode region of  $658 \pm 0$  bp. Full-barcode sequences also showed high  
202 quality, with an average percent high quality bases (HQ%) of  $96.4 \pm 7.0\%$  and average percent  
203 ambiguities of  $0.06 \pm 0.12\%$ . Unsuccessful samples were those that either failed to produce a  
204 DNA sequence or those that produced a poor quality or non-specific DNA sequence that did not  
205 allow for an identification to be made. Full barcoding showed strong performance for uncooked,  
206 dried (jerky), and cooked samples, with success rates of 88.9-100%. However, full barcoding did

207 not work well for canned samples, with a success rate of 19.0%. These results are in agreement  
208 with a previous study that reported a low success rate for full barcoding (20.5%) with heavily  
209 processed, shelf-stable fish products (Shokralla et al., 2015). Canning involves the use of high  
210 heat and pressure and may reduce the ability to recover a full-length barcode due to DNA  
211 fragmentation (Rasmussen Hellberg & Morrissey, 2011).

212 Full barcoding was successful in a variety of poultry products, including franks, breasts,  
213 sausage, jerky, and three canned chicken products. Among the successfully sequenced chicken  
214 products, five showed a top species match to red junglefowl (*Gallus gallus*) and the other four  
215 showed top species matches to both red junglefowl (*Gallus gallus*) and grey junglefowl (*Gallus*  
216 *sonneratii*), all with 100% genetic similarity (Table 1). Red junglefowl is considered to be the  
217 main wild ancestor of domestic chicken, with some influence from grey junglefowl (Eriksson et  
218 al., 2008; Groeneveld et al., 2010). As shown in Table 1, all nine successfully sequenced turkey  
219 products were identified as wild turkey (*Meleagris gallopavo*), with 100% genetic similarity.  
220 Sequencing was unsuccessful for a ground chicken product, two of the canned chicken products,  
221 and all four of the canned turkey products. The failure of the ground chicken product may have  
222 been due to the presence of additional, undeclared species in the product, as a sequence was  
223 assembled but it contained too many ambiguities (>2%) to pass quality control. The presence of  
224 multiple species in some ground meat products has been previously reported and may be due to  
225 cross-contamination during processing or intentional mislabeling (Hsieh, Woodward, & Ho,  
226 1995; Kane & Hellberg, 2016; Pascoal et al., 2004).

227 Among the three products labeled as duck, two were successfully sequenced with full-  
228 barcoding (Table 1). Both samples showed equivalent top species matches with 100% genetic  
229 similarity to two species of domesticated duck: mallard duck (*Anas platyrhyncha*) and spotbill

230 duck (*Anas poecilohyncha*). These products also had secondary matches with >98% genetic  
231 similarity to two other species of duck: Marianas mallard (*Anas superciliosa*) and American  
232 black duck (*Anas rubripes*). The multiple genetic matches are likely due to hybridization events  
233 that have occurred within the *Anas* genus (for example, see Kulikova, Zhuravlev, & McCracken,  
234 2004; Mank, Carlson, & Brittingham, 2004; Rhymer, Williams, & Braun, 1994). It is unclear as  
235 to why the third product, labeled as fresh duck wing, failed sequencing. This product resulted in  
236 a band of the expected size following gel electrophoresis, but a sequence failed to be assembled.

237 Full barcoding was successful for a variety of beef, pork, and lamb products, including  
238 ground meat, beef hot dogs, sausage, bacon, beef bologna, beef chorizo, and jerky (Table 1). On  
239 the other hand, each of the canned beef, pork, and lamb products failed sequencing. All  
240 successfully sequenced products showed a 100% genetic match to the target species, with beef  
241 products identified as cattle (*Bos taurus*), lamb products identified as domestic sheep (*Ovis*  
242 *aries*), and pork products identified as wild boar (*Sus scrofa*). Domestic pig is a subspecies of the  
243 wild boar and these two likely cannot be differentiated through DNA barcoding (Kane &  
244 Hellberg, 2016).

245 The four products with bison or buffalo on the label were successfully sequenced and  
246 identified with full barcoding. Three of the products were identified as American bison (*Bison*  
247 *bison*), with 100% genetic similarity. While American bison is the preferred common name for  
248 *B. bison*, it is also known as American buffalo (USDA, 2011). Interestingly, the fourth product  
249 was a can of dog food labeled as containing buffalo but identified through DNA barcoding as  
250 cattle (100% genetic similarity). A previous study that tested whole cuts of game meat using  
251 DNA barcoding also detected cattle in two products labeled as bison (Quinto et al., 2016). While  
252 there is an economic incentive to substitute beef for bison, these findings may have been due to

253 historical instances of interbreeding among cattle and bison (Polziehn, Strobeck, Sheraton, &  
254 Beech, 1995).

### 255 3.2 Mini-barcoding

256 Mini-barcoding resulted in successful identifications for 23 of the 60 meat and poultry  
257 products tested in this study (Table 1). Among the successfully sequenced mini-barcodes, the  
258 average length was  $125 \pm 8$  bp, which is close to the target length of 127 bp. The sequences were  
259 slightly lower quality than the full-barcode sequences, with an average HQ% of  $90.9 \pm 12.0\%$   
260 and average percent ambiguities of  $0.17 \pm 0.33\%$ . When compared on the basis of cooking  
261 methods, mini-barcoding proved to be advantageous over full barcoding for the analysis of  
262 canned products but not for uncooked, dried or cooked products. The overall success rate for  
263 mini-barcoding (38%) was much lower than that for full-length barcoding (68%). This difference  
264 appears to be due to the inability of the mini-barcode primers to bind to some of the target  
265 species, as discussed in detail later in this section.

266 Mini-barcoding outperformed full barcoding with both the turkey and duck products  
267 (Table 1). This method allowed for species identification in two of the four canned turkey  
268 products, while full barcoding was unsuccessful with all four canned products. Despite the  
269 reduced barcode coverage, mini-barcoding still allowed for identification to the species level for  
270 all successfully sequenced turkey products, with 100% genetic similarity to wild turkey (Table  
271 1). Mini-barcoding was successful with all three duck products, while full barcoding was only  
272 successful with two of the products. Similar to the results of full barcoding, the successfully  
273 sequenced samples were all identified as duck (*Anas* sp).

274 Mini-barcoding showed a slightly reduced success rate for pork samples (66.7%) as  
275 compared to full barcoding (77.8%). All samples that were successfully sequenced with mini-

276 barcoding were identified as wild boar with 100% genetic similarity, which is in agreement with  
277 the results of full barcoding. Mini-barcoding was shown to be slightly advantageous in  
278 identifying species in canned pork products, with identification in one of the two canned  
279 products that failed full-barcoding (Table 1). Mini-barcoding was unsuccessful with products  
280 labeled as pork sausage and pork chorizo, both of which were uncooked and identified through  
281 full barcoding. It is possible that these failures were due to mismatches in the mini-barcode  
282 primer binding regions, as discussed in detail below.

283         Similar to the results with pork samples, mini-barcoding showed reduced success for  
284 lamb products (25.0%) as compared to full barcoding (37.5%). Mini-barcoding was unsuccessful  
285 for all five of the canned lamb products and a jerky sample. These failures were attributed to  
286 mismatches in the mini-barcode primer-binding regions, as described below. The two uncooked  
287 lamb products were successfully sequenced with mini-barcoding. However, the reduced barcode  
288 coverage obtained with mini-barcoding had a negative effect on the ability to identify species in  
289 these products (Table 1). Both products showed a top genetic match to serow (*Capricornis* sp.)  
290 with 96% genetic similarity, whereas full barcoding showed a top match to domestic sheep for  
291 both products, with 100% similarity. Of note, these mini-barcode sequences passed quality  
292 control but had relatively low HQ% scores (64.6-80.3%) and had to be queried against GenBank  
293 because they could not be identified using BOLD. It is possible that mini-barcode sequences  
294 with better quality would provide for a stronger identification.

295         Mini-barcoding showed poor performance when tested against chicken, beef, and  
296 bison/buffalo products (Table 1). Of the 15 samples labeled as beef or bison/buffalo, only one  
297 sample (canned corned beef) was successfully sequenced and identified. This product was  
298 unsuccessful with full barcoding, but showed a top species match to cattle (96% genetic

299 similarity) with mini-barcoding. In contrast to full-barcoding, which identified chicken species in  
300 75% of the chicken products tested, mini-barcoding was unable to identify chicken in any of the  
301 products (Table 1). Interestingly, mini-barcoding did reveal the presence of sockeye salmon  
302 (*Oncorhynchus nerka*) in a canned dog food product labeled as containing only chicken (Sample  
303 10). This result was confirmed through repeat DNA extraction and sequencing. Full-barcoding of  
304 this sample indicated the presence of chicken and it is likely that the sockeye salmon was present  
305 as a secondary species. A possible explanation for the detection of salmon in the product could  
306 be contamination at the manufacturer warehouse, as this company also sells the same product in  
307 beef, duck, and salmon flavors.

308         In order to examine mismatches in the mini-barcode primer binding regions, the full  
309 barcode sequences obtained for each species were aligned with the mini-barcode primers. While  
310 the entire reverse primer binding region could be observed, the forward mini-barcode primer  
311 overlaps with the full-barcoding forward primer and only three nucleotides could be observed  
312 from this region. Based on this comparison, the number of observable primer mismatches for a  
313 given species was found to be indirectly correlated to mini-barcoding success, as may be  
314 expected. For example, the species categories with the lowest success rates (i.e., chicken, beef,  
315 lamb, and bison/buffalo) all had between 14 and 15 mismatches in the observable mini-barcode  
316 primer binding regions. Pork, which showed a success rate of 67%, had 13 mismatches in these  
317 regions, while turkey and duck, which showed success rates of 75% and 100%, respectively,  
318 each had 12 primer mismatches. Although the mini-barcode primer set utilized in this study was  
319 originally designed to target a broad range of species, including mammals, fish, and birds  
320 (Meusnier et al., 2008), the results of this study indicate the need for an improved primer set  
321 designed specifically for amplification of meat and poultry species in commercial food products.



## 322 **Conclusions**

323 Overall, the results of this study show that full barcoding is a robust method for the  
324 identification of meat and poultry species in a variety of processed products with a single species  
325 on the label, with the exception of canned foods. Mini-barcoding out-performed full barcoding in  
326 the analysis of turkey and duck products, as well as canned products. However, the mini-barcode  
327 primers did not perform well with several of the species tested in this study, notably chicken,  
328 beef, and bison/buffalo. This result was unexpected, considering that these primers were  
329 originally designed for the universal amplification of a broad range of animal species. Therefore,  
330 future research is recommended to develop a mini-barcode primer set with greater affinity for the  
331 species used in the production of red meat and poultry. Once such a primer set is developed,  
332 additional research into the use of mini-barcoding combined with next-generation sequencing  
333 should be carried out to enable the sequencing-based identification of species mixtures in food  
334 products.

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## 340 **References**

341 Ali, M. E., Kashif, M., Uddin, K., Hashim, U., Mustafa, S., & Che Man, Y. B. (2012). Species  
342 authentication methods in foods and feeds: the present, past, and future of halal forensics.  
343 *Food Analytical Methods*, 5(5), 935-955.

- 344 Ayaz, Y., Ayaz, N. D., & Erol, I. (2006). Detection of species in meat and meat products using  
345 enzyme-linked immunosorbent assay. *Journal of Muscle Foods*, 17(2), 214-220.
- 346 Ballin, N. Z. (2010). Authentication of meat and meat products. *Meat Science*, 86, 577-587.
- 347 Camma, C., Di Domenico, M., & Monaco, F. (2012). Development and validation of fast real-  
348 time PCR assays for species identification in raw and cooked meat mixtures. *Food*  
349 *Control*(2), 400-404.
- 350 Cawthorn, D. M., Steinman, H. A., & Hoffman, L. C. (2013). A high incidence of species  
351 substitution and mislabelling detected in meat products sold in South Africa. *Food*  
352 *Control*, 32(2), 440-449.
- 353 Doosti, A., Ghasemi Dehkordi, P., & Rahimi, E. (2014). Molecular assay to fraud identification  
354 of meat products. *Journal of Food Science and Technology*, 51(1), 148-152.
- 355 Eriksson, J., Larson, G., Gunnarsson, U., Bed'hom, B., Tixier-Boichard, M., Stromstedt, L., . . .  
356 Andersson, L. (2008). Identification of the Yellow skin gene reveals a hybrid origin of  
357 the domestic chicken. *Plos Genetics*, 4(2), e1000010.
- 358 Flores-Munguia, M. E., Bermudez-Almada, M. C., & Vazquez-Moreno, L. (2000). A research  
359 note: Detection of adulteration in processed traditional meat products. *Journal of Muscle*  
360 *Foods*, 11(4), 319-325.
- 361 Giovannacci, I., Guizard, C., Carlier, M., Duval, V., Martin, J.-L., & Demeulemester, C. (2004).  
362 Species identification of meat products by ELISA. *International Journal of Food Science*  
363 *& Technology*, 39(8), 863-867.
- 364 Groeneveld, L. F., Lenstra, J. A., Eding, H., Toro, M. A., Scherf, B., Pilling, D., . . . Weigend, S.  
365 (2010). Genetic diversity in farm animals – a review. *Animal Genetics*, 41, 6-31.

366 Handy, S. M., Deeds, J. R., Ivanova, N. V., Hebert, P. D. N., Hanner, R., Ormos, A., . . . Yancy,  
367 H. F. (2011). A single-laboratory validated method for the generation of DNA barcodes  
368 for the identification of fish for regulatory compliance. *Journal of AOAC International*,  
369 94(1), 201-210.

370 Hebert, P. D. N., Cywinska, A., Ball, S. L., & DeWaard, J. R. (2003). Biological identifications  
371 through DNA barcodes. *Proceedings of the Royal Society B-Biological Sciences*,  
372 270(1512), 313-321.

373 Hebert, P. D. N., Ratnasingham, S., & deWaard, J. R. (2003). Barcoding animal life: cytochrome  
374 c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal*  
375 *Society B-Biological Sciences*, 270, S96-S99.

376 Hellberg, R. S., Pollack, S. J., & Hanner, R. H. (2016). Seafood species identification using  
377 DNA sequencing. In R. H. Hanner & A. M. Nauum (Eds.), *Seafood Authenticity and*  
378 *Traceability: A DNA-based Perspective* (pp. 113-132). San Diego, CA, USA: Academic  
379 Press/Elsevier.

380 Hsieh, Y. H. P., Woodward, B. B., & Ho, S. H. (1995). Detection of species substitution in raw  
381 and cooked meats using immunoassays. *Journal of Food Protection*, 58(5), 555-559.

382 Kane, D. E., & Hellberg, R. S. (2016). Identification of species in ground meat products sold on  
383 the U.S. commercial market using DNA-based methods. *Food Control*, 59, 158-163.

384 Kulikova, I. V., Zhuravlev, Y. N., & McCracken, K. G. (2004). Asymmetric hybridization and  
385 sex-biased gene flow between Eastern spot-billed ducks (*Anas zonorhyncha*) and  
386 mallards (*A. platyrhynchos*) in the Russian Far East. *The Auk*, 121(3), 930-949.

387 Mank, J. E., Carlson, J. E., & Brittingham, M. C. (2004). A century of hybridization: decreasing  
388 genetic distance between American black ducks and mallards. *Conservation Genetics*,  
389 5(3), 395-403.

390 Meusnier, I., Singer, G. A. C., Landry, J. F., Hickey, D. A., Hebert, P. D. N., & Hajibabaei, M.  
391 (2008). A universal DNA mini-barcode for biodiversity analysis. *BMC Genomics*, 9, 214.

392 Mousavi, S. M., Jahed Khaniki, G., Eskandari, S., Rabiei, M., Mirab Samiee, S., & Mehdizadeh,  
393 M. (2015). Applicability of species-specific polymerase chain reaction for fraud  
394 identification in raw ground meat commercially sold in Iran. *Journal of Food*  
395 *Composition and Analysis*, 40, 47-51.

396 NAO. (2013). National Audit Office. Food safety and authenticity in the processed meat supply  
397 chain. The Food Standards Agency, Department for Environment, Food & Rural Affairs,  
398 Department of Health. Report by the Comptroller and Auditor General Ordered by the  
399 House of Commons to be Printed on 9 October 2013.

400 Okuma, T., & Hellberg, R. (2015). Identification of meat species in pet foods using a real-time  
401 polymerase chain reaction (PCR) assay. *Food Control*, 50, 9-17.

402 Ozpinar, H., Tezmen, G., Gokce, I., & Tekiner, I. H. (2013). Detection of animal species in some  
403 meat and meat products by comparatively using DNA microarray and real time PCR  
404 methods. *Kafkas Universitesi Veteriner Fakultesi Dergisi*, 19(2), 245-252.

405 Pascoal, A., Prado, M., Castro, J., Cepeda, A., & Barros-Velázquez, J. (2004). Survey of  
406 authenticity of meat species in food products subjected to different technological  
407 processes, by means of PCR-RFLP analysis. *European Food Research and Technology*,  
408 218(3), 306-312.

409 Perestam, A. T., Fujisaki, K. K., Nava, O., & Hellberg, R. S. (2017). Comparison of real-time  
410 PCR and ELISA-based methods for the detection of beef and pork in processed meat  
411 products. *Food Control*, 71, 346-352.

412 Polziehn, R. O., Strobeck, C., Sheraton, J., & Beech, R. (1995). Bovine mtDNA discovered in  
413 North American bison populations. *Conservation Biology*, 9(6), 1638-1638.

414 Prado, M., Calo, P., Cepeda, A., & Barros-Velázquez, J. (2005). Genetic evidence of an Asian  
415 background in heteroplasmic Iberian cattle (*Bos taurus*): Effect on food authentication  
416 studies based on polymerase chain reaction-restriction fragment length polymorphism  
417 analysis. *Electrophoresis*, 26(15), 2918-2926.

418 Quinto, C. A., Tinoco, R., & Hellberg, R. S. (2016). DNA barcoding reveals mislabeling of game  
419 meat species on the U.S. commercial market. *Food Control*, 59, 386-392.

420 Rasmussen Hellberg, R. S., & Morrissey, M. T. (2011). Advances in DNA-based techniques for  
421 the detection of seafood species substitution on the commercial market. *Journal of*  
422 *Laboratory Automation*, 16, 308-321.

423 Rhymer, J. M., Williams, M. J., & Braun, M. J. (1994). Mitochondrial analysis of gene flow  
424 between New Zealand mallards (*Anas platyrhynchos*) and grey ducks (*A. superciliosa*).  
425 *The Auk*, 111(4), 970-978.

426 Sentandreu, M. A., Fraser, P. D., Halket, J., Patel, R., & Bramley, P. M. (2010). A proteomic-  
427 based approach for detection of chicken in meat mixes. *Journal of Proteome Research*,  
428 9(7), 3374-3383.

429 Sentandreu, M. A., & Sentandreu, E. (2011). Peptide biomarkers as a way to determine meat  
430 authenticity. *Meat Science*, 89(3), 280-285.

431 Sentandreu, M. Á., & Sentandreu, E. (2014). Authenticity of meat products: Tools against fraud.  
432 *Food Research International*, 60, 19-29.

433 Shokralla, S., Hellberg, R. S., Handy, S. M., King, I., & Hajibabaei, M. (2015). A DNA mini-  
434 barcoding system for authentication of processed fish products. *Scientific Reports*,  
435 5(Article number: 15894), 1-11.

436 Soares, S., Amaral, J. S., Oliveira, M. B. P. P., & Mafra, I. (2013). A SYBR Green real-time  
437 PCR assay to detect and quantify pork meat in processed poultry meat products. *Meat*  
438 *Science*, 94(1), 115-120.

439 USDA. United States Department of Agriculture. Identification of Animal Species in Meat and  
440 Poultry Products, MLG 17.02. Food Safety Inspection Service, Office of Public Health  
441 Science. (2005). 2. [http://www.fsis.usda.gov/wps/wcm/connect/da29aed5-acc4-4715-  
442 9b84-443f46961a05/MLg17.02.pdf?MOD=AJPERES](http://www.fsis.usda.gov/wps/wcm/connect/da29aed5-acc4-4715-9b84-443f46961a05/MLg17.02.pdf?MOD=AJPERES) Accessed 17.04.03.

443 USDA. United States Department of Agriculture, Food Safety Inspection Service. Bison from  
444 Farm to Table. (2011). [http://www.fsis.usda.gov/wps/wcm/connect/d996bade-c1a4-  
445 490d-bed7-eddedf277403/Bison\\_from\\_Farm\\_to\\_Table.pdf?MOD=AJPERES](http://www.fsis.usda.gov/wps/wcm/connect/d996bade-c1a4-490d-bed7-eddedf277403/Bison_from_Farm_to_Table.pdf?MOD=AJPERES) Accessed  
446 16.11.18.

447 USDA. USDA Agricultural Projections to 2025. Office of the Chief Economist, World  
448 Agricultural Outlook Board, U.S. Department of Agriculture. Prepared by the  
449 Interagency Agricultural Projections Committee. Long-term Projections Report OCE-  
450 2016-1, 99 pp. (2016). <https://www.usda.gov/oce/commodity/projections/> Accessed  
451 16.11.21.

452 von Bargaen, C., Brockmeyer, J., & Humpf, H.-U. (2014). Meat authentication: a new HPLC–  
453 MS/MS based method for the fast and sensitive detection of horse and pork in highly  
454 processed food. *Journal of agricultural and food chemistry*, 62(39), 9428-9435.

455 Yancy, H. F., Washington, J. D., Callahan, L., Mason, J. A., Deaver, C. M., Farrell, D. E., . . .  
456 Myers, M. J. (2009). Development, evaluation, and peer verification of a rapid real-time  
457 PCR method for the detection of animal material. *Journal of Food Protection*, 72(11),  
458 2368-2374.

459 Yun-Hwa, P. H., Woodward, B. B., & Shiow-Huey, H. (1995). Detection of species substitution  
460 in raw and cooked meats using immunoassays. *Journal of Food Protection*, 58(5), 555-  
461 559.

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- Full DNA barcoding identified meat or poultry species in 68% of products tested
- Full DNA barcoding could detect all seven types of meat and poultry species tested
- DNA mini-barcoding identified meat or poultry species in 38% of products tested
- Mini-barcoding was advantageous for detection of some species in canned products
- Mini-barcoding performed poorly with chicken, beef, and buffalo/bison products



**Table 1.** Detailed results for all commercial meat and poultry products (n = 60) tested in this study with full and mini-barcoding. Each product was unique and only listed a single animal species on the label.

Sample ID	Product Description	Full Barcode Results		Mini Barcode Results	
		Top Species Match	Genetic Similarity	Top Species Match	Genetic Similarity
01	Chicken franks, cooked	Red junglefowl ( <i>Gallus gallus</i> )/Grey junglefowl ( <i>Gallus sonneratii</i> )	100 %	Barcoding unsuccessful	N/A
02	Chicken breast, oven-roasted	Red junglefowl ( <i>Gallus gallus</i> )/Grey junglefowl ( <i>Gallus sonneratii</i> )	100 %	Barcoding unsuccessful	N/A
03	Chicken sausage links, cooked	Red junglefowl ( <i>Gallus gallus</i> )/Grey junglefowl ( <i>Gallus sonneratii</i> )	100 %	Barcoding unsuccessful	N/A
04	Ground chicken, uncooked	Barcoding unsuccessful	N/A	Barcoding unsuccessful	N/A
05	Chicken breast cutlets, uncooked	Red junglefowl ( <i>Gallus gallus</i> )	100 %	Barcoding unsuccessful	N/A
06	Chicken cat food, canned	Red junglefowl ( <i>Gallus gallus</i> )	100 %	Barcoding unsuccessful	N/A
07	Chicken dog food, canned	Barcoding unsuccessful	N/A	Barcoding unsuccessful	N/A
08	Chicken Vienna sausage, canned	Red junglefowl ( <i>Gallus gallus</i> )	100 %	Barcoding unsuccessful	N/A
09	White chicken chunks in water, canned	Barcoding unsuccessful	N/A	Barcoding unsuccessful	N/A
10	Chicken chunks for dogs, canned	Red junglefowl ( <i>Gallus gallus</i> )/Grey junglefowl ( <i>Gallus sonneratii</i> )	100 %	Sockeye salmon ( <i>Oncorhynchus nerka</i> )	100 %
11	Chicken bologna, cooked	Red junglefowl ( <i>Gallus gallus</i> )	100 %	Barcoding unsuccessful	N/A
12	Chicken jerky	Red junglefowl ( <i>Gallus gallus</i> )	100 %	Barcoding unsuccessful	N/A
13	Turkey franks, cooked	Wild turkey ( <i>Meleagris gallopavo</i> )	100 %	Barcoding unsuccessful	N/A
14	Oven-roasted turkey, canned	Barcoding unsuccessful	N/A	Barcoding unsuccessful	N/A

15	Turkey breakfast sausage links, uncooked	Wild turkey ( <i>Meleagris gallopavo</i> )	100 %	Wild turkey ( <i>Meleagris gallopavo</i> )	100 %
16	Turkey breast, oven-roasted	Wild turkey ( <i>Meleagris gallopavo</i> )	100 %	Wild turkey ( <i>Meleagris gallopavo</i> )	100 %
17	Turkey sausage patties, cooked	Wild turkey ( <i>Meleagris gallopavo</i> )	100 %	Wild turkey ( <i>Meleagris gallopavo</i> )	100 %
18	Turkey sausage, smoked	Wild turkey ( <i>Meleagris gallopavo</i> )	100 %	Wild turkey ( <i>Meleagris gallopavo</i> )	100 %
19	Turkey bacon, cooked	Wild turkey ( <i>Meleagris gallopavo</i> )	100 %	Wild turkey ( <i>Meleagris gallopavo</i> )	100 %
20	Turkey jerky	Wild turkey ( <i>Meleagris gallopavo</i> )	100 %	Wild turkey ( <i>Meleagris gallopavo</i> )	100 %
21	Turkey breast, oven-roasted	Wild turkey ( <i>Meleagris gallopavo</i> )	100 %	Wild turkey ( <i>Meleagris gallopavo</i> )	100 %
22	Ground turkey, uncooked	Wild turkey ( <i>Meleagris gallopavo</i> )	100%	Wild turkey ( <i>Meleagris gallopavo</i> )	100 %
23	Turkey cat food, canned	Barcoding unsuccessful	N/A	Wild turkey ( <i>Meleagris gallopavo</i> )	100 %
24	Turkey dog food, canned	Barcoding unsuccessful	N/A	Barcoding unsuccessful	
25	Turkey cat food, canned	Barcoding unsuccessful	N/A	Wild turkey ( <i>Meleagris gallopavo</i> )	100 %
26	Boneless duck breast, smoked	Mallard duck ( <i>Anas platyrhyncha</i> )/ Spotbill duck ( <i>Anas poecilorhyncha</i> )	100%	Mallard duck ( <i>Anas platyrhyncha</i> )/ Spotbill duck ( <i>Anas poecilorhyncha</i> )/Marianas Mallard ( <i>Anas superciliosa</i> )	100 %
27	Fresh duck wing, uncooked	Barcoding unsuccessful	N/A	Mallard duck ( <i>Anas platyrhyncha</i> )/ Spotbill duck ( <i>Anas poecilorhyncha</i> )/Marianas Mallard ( <i>Anas superciliosa</i> )	100 %
28	Whole duck, uncooked	Mallard duck ( <i>Anas platyrhyncha</i> )/ Spotbill duck ( <i>Anas poecilorhyncha</i> )	100%	Mallard duck ( <i>Anas platyrhyncha</i> )/ Spotbill duck ( <i>Anas poecilorhyncha</i> )/Marianas Mallard ( <i>Anas superciliosa</i> )	100 %
29	Thin cut beef, uncooked	Cattle ( <i>Bos taurus</i> )	100%	Barcoding unsuccessful	N/A
30	Ground beef, uncooked	Cattle ( <i>Bos taurus</i> )	100%	Barcoding unsuccessful	N/A
31	Roast beef, cooked	Cattle ( <i>Bos taurus</i> )	100%	Barcoding unsuccessful	N/A
32	Beef hot dogs, uncured, fully cooked	Cattle ( <i>Bos taurus</i> )	100%	Barcoding unsuccessful	N/A
33	Beef bologna, cooked	Cattle ( <i>Bos taurus</i> )	100%	Barcoding unsuccessful	N/A
34	Beef chorizo, uncooked	Cattle ( <i>Bos taurus</i> )	100%	Barcoding unsuccessful	N/A

35	Corned beef, canned	Barcoding unsuccessful	N/A	Cattle ( <i>Bos taurus</i> )	96%
36	Beef jerky	Cattle ( <i>Bos taurus</i> )	100%	Barcoding unsuccessful	N/A
37	Beef pet food, canned	Barcoding unsuccessful	N/A	Barcoding unsuccessful	N/A
38	Beef pet food, canned	Barcoding unsuccessful	N/A	Barcoding unsuccessful	N/A
39	Beef pet food, canned	Barcoding unsuccessful	N/A	Barcoding unsuccessful	N/A
40	Ground pork, uncooked	Wild boar ( <i>Sus scrofa</i> )	100%	Wild boar ( <i>Sus scrofa</i> )	100%
41	Pork cut, uncooked	Wild boar ( <i>Sus scrofa</i> )	100%	Wild boar ( <i>Sus scrofa</i> )	100%
42	Pork sausage, uncooked	Wild boar ( <i>Sus scrofa</i> )	100%	Barcoding unsuccessful	N/A
43	Pork bacon, smoked	Wild boar ( <i>Sus scrofa</i> )	100%	Wild boar ( <i>Sus scrofa</i> )	100%
44	Ham, uncured and slow-cooked	Wild boar ( <i>Sus scrofa</i> )	100%	Wild boar ( <i>Sus scrofa</i> )	100%
45	Pork chorizo, uncooked	Wild boar ( <i>Sus scrofa</i> )	100%	Barcoding unsuccessful	N/A
46	Pork in natural juices, canned	Barcoding unsuccessful	N/A	Wild boar ( <i>Sus scrofa</i> )	100%
47	All natural pork, canned	Barcoding unsuccessful	N/A	Barcoding unsuccessful	N/A
48	BBQ pork jerky	Wild boar ( <i>Sus scrofa</i> )	100%	Wild boar ( <i>Sus scrofa</i> )	100%
49	Lamb leg, fresh	Domestic sheep ( <i>Ovis aries</i> )	100%	<i>Capricornis</i> sp.	96%
50	Ground lamb, uncooked	Domestic sheep ( <i>Ovis aries</i> )	100%	<i>Capricornis</i> sp.	96%
51	Lamb pet food, canned	Barcoding unsuccessful	N/A	Barcoding unsuccessful	N/A
52	Lamb jerky	Domestic sheep ( <i>Ovis aries</i> )	100%	Barcoding unsuccessful	N/A
53	Lamb pet food, canned	Barcoding unsuccessful	N/A	Barcoding unsuccessful	N/A
54	Lamb pet food, canned	Barcoding unsuccessful	N/A	Barcoding unsuccessful	N/A
55	Lamb pet food, canned	Barcoding unsuccessful	N/A	Barcoding unsuccessful	N/A
56	Lamb pet food, canned	Barcoding unsuccessful	N/A	Barcoding unsuccessful	N/A
57	Ground bison, uncooked	American bison ( <i>Bison bison</i> )	100%	Barcoding unsuccessful	N/A
58	Buffalo patties, uncooked	American bison ( <i>Bison bison</i> )	100%	Barcoding unsuccessful	N/A
59	Buffalo jerky	American bison ( <i>Bison bison</i> )	100%	Barcoding unsuccessful	N/A
60	Buffalo dog food, canned	Cattle ( <i>Bos taurus</i> )	100%	Barcoding unsuccessful	N/A