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

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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.

42

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 μ 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 μ l of sterile water and 4 μ 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 μ l) was combined with 2 μ 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 ± 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 ± 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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- 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