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Identification of meat and poultry species in food products using DNA barcoding

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ABSTRACT

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

1. Introduction

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

There are several detrimental consequences associated with mislabeling of meat or poultry species in food products (Ali et al., 2012; Ballin, 2010). In many instances, mislabeling is a form of economic deception, such as the substitution of horsemeat for beef in the 2013 European horsemeat scandal (NAO, 2013). Additionally, the presence of undeclared species in food products can be harmful to consumers and pets with meat allergies and can interfere with religious practices that ban the consumption of certain animal species.

In order to identify the species in processed meat and poultry products, DNA or protein-based methods are often used (as reviewed in Ali et al., 2012; Ballin, 2010; M. Á. Sentandreu & Sentandreu, 2014). Commonly used methods include enzyme-

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linked immunosorbent assay (ELISA) (Ayaz et al., 2006; Giovannacci et al., 2004; USDA, 2005; Yun-Hwa, Woodward, & Shiow-Huey, 1995), real-time polymerase chain reaction (PCR) (Camma, Di Domenico, & Monaco, 2012; Okuma & Hellberg, 2015; Soares, Amaral, Oliveira, & Mafra, 2013; Yancy et al., 2009), PCR-restriction fragment length polymorphism (RFLP) (Doosti, Ghasemi Dehkordi, & Rahimi, 2014; Pascoal et al., 2004; Prado, Calo, Cepeda, & Barros-Velázquez, 2005), and DNA sequencing (Cawthorn et al., 2013; Kane & Hellberg, 2016; Quinto et al., 2016). ELISA and real-time PCR are rapid, targeted approaches that enable detection of species in heavily processed products, including those with species mixtures (Perestam et al., 2017). Real-time PCR is advantageous in that multiple species can be detected simultaneously and it is highly sensitive. Despite these advantages, it is limited in that a different primer set is required for each species targeted. PCR-RFLP allows for the use of universal primers and is capable of detection of species mixtures; however, it requires several post-PCR steps and it generally requires a longer DNA target as compared to real-time PCR (Ali et al., 2012). Furthermore, the analysis of PCR-RFLP results can become highly complex when multiple enzymes are used to differentiate a range of species. The application of mass spectrometry (MS) to the analysis of proteins and peptides has been proposed to overcome some of the limitations of molecular techniques (Miguel A. Sentandreu, Fraser, Halket, Patel, & Bramley, 2010; Miguel Angel Sentandreu & Sentandreu, 2011; M. Á. Sentandreu & Sentandreu, 2014; von Bargen, Brockmeyer, & Humpf, 2014). However, these methods have yet to be widely adopted, in part due to the need for costly equipment and skilled technicians (M. Á. Sentandreu & Sentandreu, 2014).

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

Conventional full-length DNA barcoding targets approximately 650 base pairs (bp) of the COI gene for species identification in well-preserved and fresh specimens (Hebert, Cywinska, et al., 2003; Hebert, Cywinska, et al., 2003). However, DNA quality can be reduced by many conditions common to food processing such as low pH, high temperatures, and high pressures (Rasmussen Hellberg & Morrissey, 2011), which makes it difficult to obtain a full-length barcode from food samples that have been heavily processed, such as canned products. Although processing of foods ultimately leads to the fragmentation of DNA, amplification of short regions of DNA may still be possible. In order to facilitate species identification in biological specimens with degraded DNA, Meusnier et al. (2008) designed a universal primer set targeting a short region of DNA within the full-length barcode. This 'mini-barcode' universal primer set was found to be capable of amplifying the target DNA fragment in 92% of species tested, including mammals, fish, birds, and insect specimens. However, the study was focused on applications in biodiversity analysis and did not specifically target species commonly used in the production of red meat or poultry. A mini-barcoding system has also been developed specifically for the identification of fish species in processed products (Shokralla, Hellberg, Handy, King, & Hajibabaei, 2015). These mini-barcodes showed a success rate of 93.2% when tested against 44 heavily processed fish products, as compared to a success rate of 20.5% with full barcoding. Although methods based on traditional DNA sequencing do not perform well with species mixtures, short genetic targets such as mini-barcodes have the potential to be combined with next-generation sequencing to allow for identification of mixed-species samples (Hellberg et al., 2016).

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

2. Materials and methods

2.1. Sample collection

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

2.2. DNA extraction

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

2.3. Polymerase chain reaction (PCR)

DNA extracts from each sample underwent PCR for both full and mini-barcodes. Each reaction tube included the following components: 0.5 OmniMix Bead (Cepheid, Sunnyvale, CA), 22.5 μl of molecular-grade sterile water, 0.25 μl of 10 μM forward primer or primer cocktail, 0.25 μl of 10 μM reverse primer or primer cocktail, and 2 μl of template DNA. Amplification of the full barcode region was carried out using the mammalian primer cocktail described in Ivanova et al. (2012) and amplification of the mini-barcode region was carried out using the primer set described in Meusnier et al. (2008). All primers were synthesized by Integrated DNA Technologies (Coralville, IA) and included M13 tails to facilitate DNA sequencing (Ivanova et al., 2012). A no template control (NTC)

containing 2 μl of sterile water was run alongside each set of reactions. PCR was carried out using a Mastercycler nexus Gradient Thermal Cycler (Eppendorf, Hamburg, Germany). Cycling conditions for full-length barcoding were followed 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 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 extension step at 72 °C for 10 min. Cycling conditions for mini-barcoding were followed according to Meusnier et al. (2008): 95 °C for 2 min; 5 cycles of 95 °C for 1 min, 46 °C for 1 min, 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 extension step at 72 °C for 5 min. The resulting amplicons were stored at −20 °C until PCR product confirmation.

2.4. PCR product confirmation and DNA sequencing

PCR products were confirmed using 2.0% agarose E-Gels containing ethidium bromide (Life Technologies, Carlsbad, CA). A total of 16 μl of sterile water and 4 μl of PCR product were loaded into each well and the gels were run for 6–8 min on an E-Gel iBase (Life Technologies). The results were captured using FOTO/Analyst Express (Fotodyne, Hartland, WI) and Transilluminator FBDLT-88 (Fisher Scientific, Waltham, MA) and visualized with FOTO/Analyst PCImage (version 5.0.0.0, Fotodyne). Next, the PCR products were purified using a 4-fold dilution of ExoSAP-IT (Affymetrix, Santa Clara, CA) in molecular-grade water. Each PCR product (5 μl) was combined with 2 μl of the diluted ExoSAP-IT and then placed in the thermal cycler for 15 min at 37 °C followed by 15 min at 80 °C. The samples were then shipped to GenScript (Piscataway, NJ) for bi-directional DNA sequencing with M13 primers. Sequencing was carried out using the BigDye Terminator v3.1 Cycle Sequencing Kit (Life Technologies) and a 3730xl Genetic Analyzer (Life Technologies).

2.5. Sequencing results and analysis

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

3. Results and discussion

3.1. Full-barcoding

Full-barcoding of the 60 meat and poultry products resulted in a total of 41 successful identifications (Table 1). The sequences recovered with full barcoding had an average length equal to the target barcode region of 658 ± 0 bp. Full-barcode sequences also showed high quality, with an average percent high quality bases (HQ%) of 96.4 ± 7.0% and average percent ambiguities of 0.06 ± 0.12%. Unsuccessful samples were those that either failed to produce a DNA sequence or those that produced a poor quality or non-specific DNA sequence that did not allow for an identification to be made. Full barcoding showed strong performance for uncooked, dried (jerky), and cooked samples, with success rates of 88.9–100%. However, full barcoding did not work well for canned samples, with a success rate of 19.0%. These results are in agreement with a previous study that reported a low success rate for full barcoding (20.5%) with heavily processed, shelf-stable fish products (Shokralla et al., 2015). Canning involves the use of high heat and pressure and may reduce the ability to recover a full-length barcode due to DNA fragmentation (Rasmussen Hellberg & Morrissey, 2011).

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

Among the three products labeled as duck, two were successfully sequenced with full-barcoding (Table 1). Both samples showed equivalent top species matches with 100% genetic similarity to two species of domesticated duck: mallard duck (*Anas platyrhyncha*) and spotbill duck (*Anas poecilohyncha*). These products also had secondary matches with >98% genetic similarity to two other species of duck: Marianas mallard (*Anas superciliosa*) and American black duck (*Anas rubripes*). The multiple genetic matches are likely due to hybridization events that have occurred within the *Anas* genus (for example, see Kulikova, Zhuravlev, & McCracken, 2004; Mank, Carlson, & Brittingham, 2004; Rhymer, Williams, & Braun, 1994). It is unclear as to why the third product, labeled as fresh duck wing, failed sequencing. This product resulted in a band of the expected size following gel electrophoresis, but a sequence failed to be assembled.

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

The four products with bison or buffalo on the label were successfully sequenced and identified with full barcoding. Three of the products were identified as American bison (*Bison bison*), with 100% genetic similarity. While American bison is the preferred common name for *B. bison*, it is also known as American buffalo (USDA, 2011). Interestingly, the fourth product was a can of dog food labeled as containing buffalo but identified through DNA barcoding as cattle (100% genetic similarity). A previous study that tested whole cuts of game meat using DNA barcoding also detected

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 (*Gallus gallus*)/Grey junglefowl (*Gallus sonneratii*)	100%	Barcoding unsuccessful	N/A
02	Chicken breast, oven-roasted	Red junglefowl (*Gallus gallus*)/Grey junglefowl (*Gallus sonneratii*)	100%	Barcoding unsuccessful	N/A
03	Chicken sausage links, cooked	Red junglefowl (*Gallus gallus*)/Grey junglefowl (*Gallus sonneratii*)	100%	Barcoding unsuccessful	N/A
04	Ground chicken, uncooked	Barcoding unsuccessful	N/A	Barcoding unsuccessful	N/A
05	Chicken breast cutlets, uncooked	Red junglefowl (*Gallus gallus*)	100%	Barcoding unsuccessful	N/A
06	Chicken cat food, canned	Red junglefowl (*Gallus gallus*)	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 (*Gallus gallus*)	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 (*Gallus gallus*)/Grey junglefowl (*Gallus sonneratii*)	100%	Sockeye salmon (*Oncorhynchus nerka*)	100%
11	Chicken bologna, cooked	Red junglefowl (*Gallus gallus*)	100%	Barcoding unsuccessful	N/A
12	Chicken jerky	Red junglefowl (*Gallus gallus*)	100%	Barcoding unsuccessful	N/A
13	Turkey franks, cooked	Wild turkey (*Meleagris gallopavo*)	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 (*Meleagris gallopavo*)	100%	Wild turkey (*Meleagris gallopavo*)	100%
16	Turkey breast, oven-roasted	Wild turkey (*Meleagris gallopavo*)	100%	Wild turkey (*Meleagris gallopavo*)	100%
17	Turkey sausage patties, cooked	Wild turkey (*Meleagris gallopavo*)	100%	Wild turkey (*Meleagris gallopavo*)	100%
18	Turkey sausage, smoked	Wild turkey (*Meleagris gallopavo*)	100%	Wild turkey (*Meleagris gallopavo*)	100%
19	Turkey bacon, cooked	Wild turkey (*Meleagris gallopavo*)	100%	Wild turkey (*Meleagris gallopavo*)	100%
20	Turkey jerky	Wild turkey (*Meleagris gallopavo*)	100%	Wild turkey (*Meleagris gallopavo*)	100%
21	Turkey breast, oven-roasted	Wild turkey (*Meleagris gallopavo*)	100%	Wild turkey (*Meleagris gallopavo*)	100%
22	Ground turkey, uncooked	Wild turkey (*Meleagris gallopavo*)	100%	Wild turkey (*Meleagris gallopavo*)	100%
23	Turkey cat food, canned	Barcoding unsuccessful	N/A	Wild turkey (*Meleagris gallopavo*)	100%
24	Turkey dog food, canned	Barcoding unsuccessful	N/A	Barcoding unsuccessful	
25	Turkey cat food, canned	Barcoding unsuccessful	N/A	Wild turkey (*Meleagris gallopavo*)	100%
26	Boneless duck breast, smoked	Mallard duck (*Anas platyrhyncha*)/ Spotbill duck (*Anas poecilorhyncha*)	100%	Mallard duck (*Anas platyrhyncha*)/ Spotbill duck (*Anas poecilorhyncha*)/ Marianas Mallard (*Anas superciliosa*)	100%
27	Fresh duck wing, uncooked	Barcoding unsuccessful	N/A	Mallard duck (*Anas platyrhyncha*)/ Spotbill duck (*Anas poecilorhyncha*)/ Marianas Mallard (*Anas superciliosa*)	100%
28	Whole duck, uncooked	Mallard duck (*Anas platyrhyncha*)/ Spotbill duck (*Anas poecilorhyncha*)	100%	Mallard duck (*Anas platyrhyncha*)/ Spotbill duck (*Anas poecilorhyncha*)/ Marianas Mallard (*Anas superciliosa*)	100%
29	Thin cut beef, uncooked	Cattle (*Bos taurus*)	100%	Barcoding unsuccessful	N/A
30	Ground beef, uncooked	Cattle (*Bos taurus*)	100%	Barcoding unsuccessful	N/A
31	Roast beef, cooked	Cattle (*Bos taurus*)	100%	Barcoding unsuccessful	N/A
32	Beef hot dogs, uncured, fully cooked	Cattle (*Bos taurus*)	100%	Barcoding unsuccessful	N/A
33	Beef bologna, cooked	Cattle (*Bos taurus*)	100%	Barcoding unsuccessful	N/A
34	Beef chorizo, uncooked	Cattle (*Bos taurus*)	100%	Barcoding unsuccessful	N/A
35	Corned beef, canned	Barcoding unsuccessful	N/A	Cattle (*Bos taurus*)	96%
36	Beef jerky	Cattle (*Bos taurus*)	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 (*Sus scrofa*)	100%	Wild boar (*Sus scrofa*)	100%
41	Pork cut, uncooked	Wild boar (*Sus scrofa*)	100%	Wild boar (*Sus scrofa*)	100%
42	Pork sausage, uncooked	Wild boar (*Sus scrofa*)	100%	Barcoding unsuccessful	N/A
43	Pork bacon, smoked	Wild boar (*Sus scrofa*)	100%	Wild boar (*Sus scrofa*)	100%
44	Ham, uncured and slow-cooked	Wild boar (*Sus scrofa*)	100%	Wild boar (*Sus scrofa*)	100%
45	Pork chorizo, uncooked	Wild boar (*Sus scrofa*)	100%	Barcoding unsuccessful	N/A
46	Pork in natural juices, canned	Barcoding unsuccessful	N/A	Wild boar (*Sus scrofa*)	100%
47	All natural pork, canned	Barcoding unsuccessful	N/A	Barcoding unsuccessful	N/A
48	BBQ pork jerky	Wild boar (*Sus scrofa*)	100%	Wild boar (*Sus scrofa*)	100%
49	Lamb leg, fresh	Domestic sheep (*Ovis aries*)	100%	*Capricornis* sp.	96%
50	Ground lamb, uncooked	Domestic sheep (*Ovis aries*)	100%	*Capricornis* sp.	96%
51	Lamb pet food, canned	Barcoding unsuccessful	N/A	Barcoding unsuccessful	N/A
52	Lamb jerky	Domestic sheep (*Ovis aries*)	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 (*Bison bison*)	100%	Barcoding unsuccessful	N/A
58	Buffalo patties, uncooked	American bison (*Bison bison*)	100%	Barcoding unsuccessful	N/A
59	Buffalo jerky	American bison (*Bison bison*)	100%	Barcoding unsuccessful	N/A
60	Buffalo dog food, canned	Cattle (*Bos taurus*)	100%	Barcoding unsuccessful	N/A

cattle in two products labeled as bison (Quinto et al., 2016). While there is an economic incentive to substitute beef for bison, these findings may have been due to historical instances of interbreeding among cattle and bison (Polziehn, Strobeck, Sheraton, & Beech, 1995).

3.2. Mini-barcoding

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

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

Mini-barcoding showed a slightly reduced success rate for pork samples (66.7%) as compared to full barcoding (77.8%). All samples that were successfully sequenced with mini-barcoding were identified as wild boar with 100% genetic similarity, which is in agreement with the results of full barcoding. Mini-barcoding was shown to be slightly advantageous in identifying species in canned pork products, with identification in one of the two canned products that failed full-barcoding (Table 1). Mini-barcoding was unsuccessful with products labeled as pork sausage and pork chorizo, both of which were uncooked and identified through full barcoding. It is possible that these failures were due to mismatches in the mini-barcode primer binding regions, as discussed in detail below.

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

Mini-barcoding showed poor performance when tested against chicken, beef, and bison/buffalo products (Table 1). Of the 15 samples labeled as beef or bison/buffalo, only one sample (canned corned beef) was successfully sequenced and identified. This product was unsuccessful with full barcoding, but showed a top species match to cattle (96% genetic similarity) with mini-barcoding. In contrast to full-barcoding, which identified chicken species in 75% of the chicken products tested, mini-barcoding was unable to identify chicken in any of the products (Table 1). Interestingly, mini-barcoding did reveal the presence of sockeye salmon (*Oncorhynchus nerka*) in a canned dog food product labeled as containing only chicken (Sample 10). This result was confirmed through repeat DNA extraction and sequencing. Full-barcoding of this sample indicated the presence of chicken and it is likely that the sockeye salmon was present as a secondary species. A possible explanation for the detection of salmon in the product could be contamination at the manufacturer warehouse, as this company also sells the same product in beef, duck, and salmon flavors.

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

4. Conclusions

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

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