

# The tapa is *Sus*: DNA barcoding reveals food fraud in processed products

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## ABSTRACT

Sourcing food from wildlife is generally disallowed in Philippine law. However, there still is local demand for native bushmeat. While consumption of native species is part of indigenous cultures, some products have been commercialized and are occasionally accessible to the public. Regulation of these types of products is difficult since processing and preservation removes usable taxonomic features necessary for identification. We present a case of food adulteration in specimens labeled as “tapang baboy ramo” and “tapang usa”, assumed to have been sourced from *Sus philippensis* and *Rusa marianna*, respectively. Barcoding of the 658-bp fragment of the COI gene identifies both products as the domestic pig, *S. scrofa*. The absence of COI sequences for native pig species required the use of a 449-bp fragment of the Cytb gene. Both phylogenetic and distance-based analysis of this fragment strongly suggests that the specimens were sourced from *S. scrofa*. This is a case of adulteration of a food product and constitutes food fraud. While several agencies in the Philippines can authenticate meat products, they are limited in the species they detect. Expanding testing and databases to include native wildlife species will provide protection for wildlife and consumers alike.

*Keywords:* bushmeat, substitution, wildlife products

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## INTRODUCTION

There is a practice and demand for the consumption of bushmeat derived from Philippine wildlife (Scheffers et al. 2012). While hunting of native animals for food is generally disallowed in the Philippine Wildlife Act (2001), there are a few exceptions. Hunting is ritualistically important and a common tradition for some indigenous Filipinos (Tanalgo 2017; Villegas et al. 2021; Villegas et al. 2022). Species of wild boar, deer, bat, and monitor lizard, among others, have been traditionally consumed wherever they are found (Oliver 1995; Scheffers et al. 2012; Welton et al. 2013; Tanalgo 2017; Villegas et al. 2021; Villegas et al. 2022). There is also precedent on commercializing wildlife consumption with sustainable and legal farming, as is in the case of *Crocodylus porosus* Schneider 1801 (Mercado 2008; Manalo and Alcala 2013).

While guidelines and penalties against unregulated trade of bushmeat are in place, it still takes place in Philippine forests (Scheffers et al. 2012; Tanalgo 2017). Social media has expanded the reach of its local distributors, making the trade of these products more accessible to a wider market. A query search in Facebook Marketplace, from a user based in Quezon City, using the keywords “Baboy Ramo” (wild boar) and “Usa” (deer), returns multiple matches from sellers in Metro Manila, Central Luzon, Cagayan, and Ilocos Region. Most of the products come in the form of “tapa”, a native delicacy made by curing meat. Considering the geotag of the sellers, we presume that the products being sold and labeled “baboy ramo” are derived from *Sus philippensis* (Nehring 1886) and those labeled “usa” are *Rusa marianna* (Desmarest 1822). Both species are native to Luzon and are currently listed as vulnerable in the IUCN Red List of Threatened Species (2023). Under the DENR Administrative Order No. 2019-09, Updated National List of Threatened Philippine Fauna and their Categories, the Philippine deer is listed as endangered while the Philippine warty pig is listed as vulnerable. The exploitation of Philippine wildlife is increasingly being monitored with successful interception both online and on-ground (Canlas et al. 2017; Fischer 2021). However, it is more difficult to monitor wildlife derived products that are preserved and heavily processed since the taxonomic features of the source species can be lost (Montowska and Pospiech 2011). This presents a two-fold problem: (1) how can we determine whether the product was legally obtained and (2) how do we know if the product being sold is accurately labeled?

Food fraud is defined as false labeling for economic gain (Saadat et al. 2022). This crime is usually associated with adulteration and substitution of food items for those with inferior quality at a lower price. Techniques to detect food fraud

vary with the product in question. The identification of products that are visually homogenous and unrecognizable from their source can be challenging. However, we can resort to the application of molecular technologies. In the Philippines, DNA barcoding has been proven effective in detecting adulteration and substitution in meat and plant-derived products (Pedales et al. 2016; Sarmiento et al. 2018). While most studies involve products that are from commercially available livestock, the technology is easily translatable to applications in wildlife forensics. We present a case wherein routine DNA barcoding was used to authenticate putative “tapang usa” and “tapang baboy ramo” specimens.

## MATERIALS AND METHODS

### *Case History*

A faculty member of the Institute of Biological Sciences, College of Arts and Sciences, University of the Philippines Los Banos called the attention of the Biodiversity Management Bureau (BMB) noting that “tapang usa” and “tapang baboy ramo” were being sold in a local grocery store. With the assistance of technical staff of the DENR CALABARZON, one pack of each of the products was purchased and turned over to the BMB, which was eventually turned over to UPD Institute of Biology DNA Barcoding Laboratory for analysis. Samples were received on June 29, 2016 (Case Number 2017-00001). The processed meats were individually packaged in vacuum sealed plastic bags and labeled “Tapang Baboy Ramo” (wild boar tapa) and “Tapang Usa” (wild deer tapa) (Figure 1). The packages had minimal labels, no ingredient list, and only identified by the price tag and barcode. Each container had five (5) tapa slabs. Tissue samples (1x1 cm) were collected from each slab and stored in absolute ethanol for vouchering and DNA analysis.



**Figure 1. Specimens of cured meats (“Tapa”) reported to be sourced from wild boar (B) and wild deer (U). Some information on the label was blurred to conceal the origin of the product.**

## **DNA Barcoding**

DNA was extracted from tissue samples [Tapang Baboy Ramo (B1-B5) and Tapang Usa (U1-U5)] using Purelink™ Genomic DNA Mini Kit (Invitrogen™, USA) following the manufacturer's instructions. Total nucleotide concentration was measured in a Nanodrop 2000c spectrophotometer (Thermo Scientific™, USA). Nucleotide concentration ranged from 60-70 ng/μL, enough to proceed with the downstream analyses to confirm the functionality of the extracts (Piskata et al. 2017).

The barcoding region of the cytochrome oxidase I (COI) gene was amplified using the VF1/VR1 primers (Ivanova et al. 2006). We also amplified a 449-bp fragment of the cytochrome oxidase b (Cytb) to compare the specimens to sequences of native and endemic species of pigs present in the NCBI GenBank database. Amplification of the gene fragments was done in a 50 μL reaction mix consisting of: 10 μL of 5x PCR Buffer (Bioline, UK), 10 μM of forward and backward primers: VF1 (5' – TTC TCA ACC AAC CAC AAR GAY ATY GG-3')/VR1 (5' – TAG ACT TCT GGG TGG CCT AAT AAT CA – 3') for COI and Cytb F (5' – GAC ACG AAA AAT CAT CGT TG – 3')/Cytb R (5' – CCT CAG AAA GAT ATT TGT CCT CAT GG – 3') for Cytb, 5% DMSO, 2 μL of 50 mM MgCl<sub>2</sub>, 0.25 μL MyTaq Polymerase (Bioline, UK), Ultrapure Water (Invitrogen, USA) and 50 ng of DNA. The running conditions to amplify the COI fragment were as follows: (1) 94°C for 2 min, 5 cycles of 94°C for 40 s, 45°C for 40 s, and 72°C for 1 min, followed by (2) 35 cycles of 94°C for 40 s, 51°C for 40 s, and 72°C for 1 min, and a final extension at 72°C for 10 min. The Cytb fragment was amplified with the following conditions: 94°C for 8 min, 35 cycles of 92°C for 1 min, 50°C for 1 min, and 72°C for 1 min, and a final extension at 72°C for 10 min.

Amplification was confirmed in a 1% agarose gel with 1% ethidium bromide (1 mg/10 mL) and the positive reactions excised and extracted using QIAQuick Gel Extraction Kit (QIAGEN, Netherlands) following the manufacturer's protocol. The purified amplicons were sent to 1st Base Malaysia for capillary sequencing.

Quality control, trimming, and assembly of sequences were performed in STADEN package (v. 2.0.0b11). COI sequences were compared to the NCBI database using the Nucleotide Basic Local Alignment Search Tool (nBLAST). This was also done with the Cytb sequences from the processed samples. Cytb sequences were available for these endemic and native pig species in the Philippines: *S. philippensis* (AY920905), *S. barbatus* (AY534297), and *S. cebifrons* (AY920906). These were retrieved from the database along with mitochondrial genome sequences of exotic pig breeds cultivated in the Philippines (Ortega et al. 2021). Alignment, distance analysis, model testing, and tree construction for the Cytb fragment were performed in MEGA-X version 10.2.6 (Kumar et al. 2018). K2P distances were summarized in a heatmap using R (R Core Team 2022).

## RESULTS AND DISCUSSION

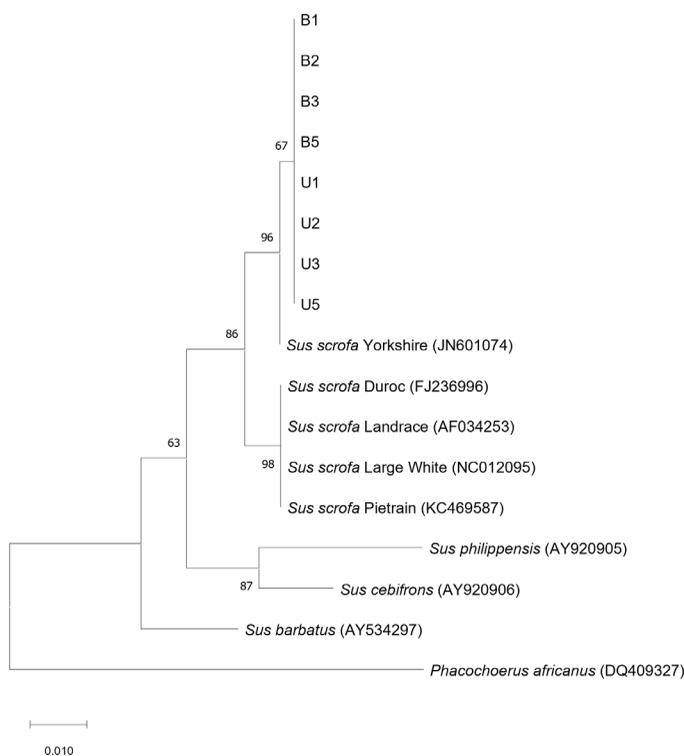
The 658-bp fragment of the COI gene was successfully amplified and sequenced for all specimens processed. The top nBLAST hits for all specimens indicate 100% identity to the domestic pig *S. scrofa* for both the Tapang Baboy Ramo (B1-B5) and Tapang Usa (U1-U5) samples (Table 1). There are six single nucleotide polymorphisms between B1-B5 and U1-U5 along the COI fragments at positions 187, 273, 286, 325, 343, and 370. This may suggest that the samples were from different maternal lineages or populations. Another nBLAST search was done for specimens U1-U5 which only includes sequences from the genus *Rusa*. The top match was for *R. unicolor* (Kerr 1792) with 79.76% identity. This confirms that the specimens labeled Tapang Usa were fraudulent and are in fact not sourced from *Rusa*.

**Table 1. Nucleotide BLAST results table comparing the COI gene fragment amplified from the Tapang Baboy Ramo (B1-B5) and Tapang Usa (U1-U5) samples (five samples each) to the global database in GenBank.**

ID	BLAST Result	%identity	Accession
B1	<i>Sus scrofa</i>	100%	MF183225
B2	<i>Sus scrofa</i>	100%	MF183225
B3	<i>Sus scrofa</i>	100%	MF183225
B4	<i>Sus scrofa</i>	100%	MF183225
B5	<i>Sus scrofa</i>	100%	MF183225
U1	<i>Sus scrofa</i>	100%	MN124249
	<i>Rusa unicolor</i>	79.76%	MF177018
U2	<i>Sus scrofa</i>	100%	MN124249
	<i>Rusa unicolor</i>	79.76%	MF177018
U3	<i>Sus scrofa</i>	100%	MN124249
	<i>Rusa unicolor</i>	79.76%	MF177018
U4	<i>Sus scrofa</i>	100%	MN124249
	<i>Rusa unicolor</i>	79.76%	MF177018
U5	<i>Sus scrofa</i>	100%	MN124249
	<i>Rusa unicolor</i>	79.76%	MF177018

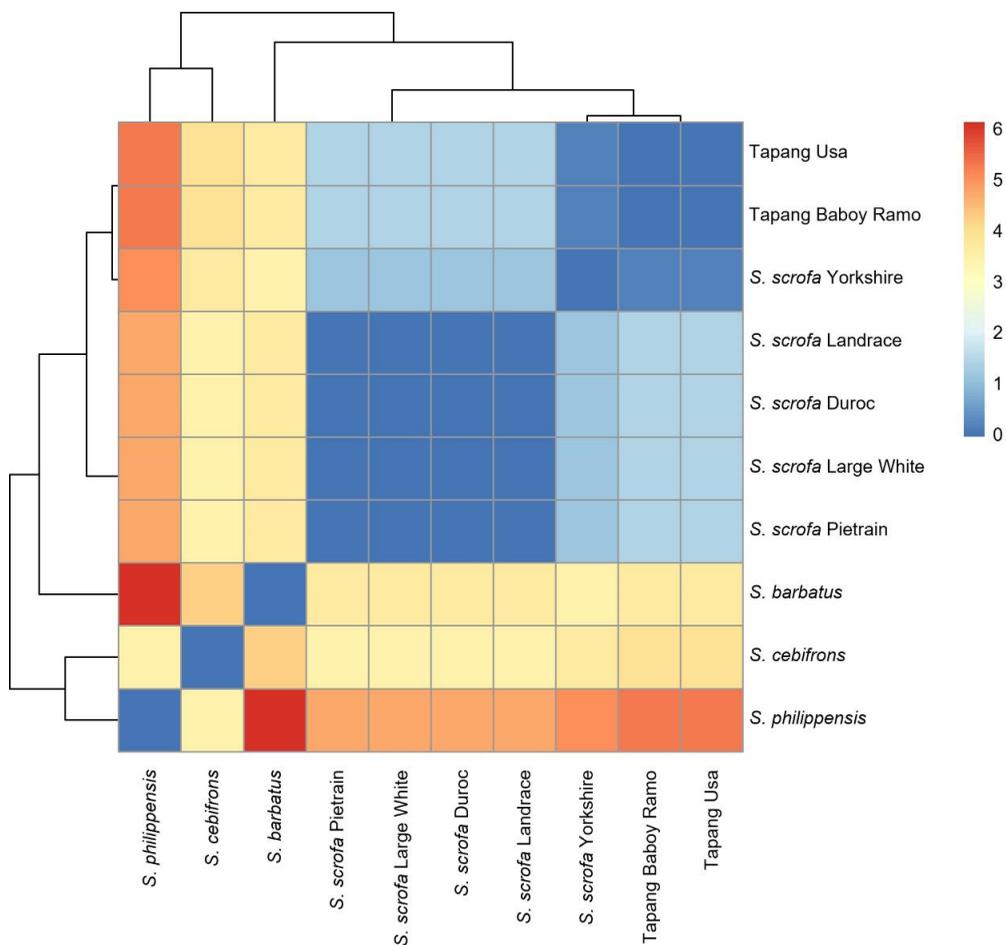
There was still the question of whether these samples were from commercially available breeds of *S. domestica* or were native species of pigs. While the fragment of the COI gene is the standard for DNA barcoding in animals (Hebert et al. 2003; Ahmed et al. 2022), mitochondrial genomes and COI barcodes for Philippine species of pigs are lacking in GenBank and therefore, we cannot compare the

sequenced samples to reference. The *Cytb* gene is a good alternative as it exhibits low intraspecific variation and has been argued to be more suitable for species identification in mammals (Teletchea et al. 2008; Tobe et al. 2009; Tobe et al. 2011). Additionally, *Cytb* sequences for *S. philippensis*, *S. cebifrons*, and *S. barbatus* are available in the database and for this reason, we sequenced a 449-bp fragment of the gene. Only two samples (B4 and U4) failed to amplify but analyses for the other specimens continued. All sequenced samples have identical *Cytb* sequences. The closest nucleotide BLAST hit for the fragment is *S. scrofa* breed Yorkshire (99.78% identity). We then compared the samples to published *Cytb* sequences of native species along with five breeds of the domestic pig (Ortega et al. 2021). The constructed gene tree clusters all sequenced samples with *S. scrofa* (Figure 2). The support for the clustering of the samples with *Sus scrofa* is relatively high (86%) but the analysis shows poor support (63%) for the separation of the endemic *S. philippensis* and *S. cebifrons* cluster with the domestic pigs.



**Figure 2. Maximum-likelihood tree (HKY+G nucleotide substitution model) for the 413-bp alignment of the cytochrome b (*Cytb*) gene. The common warthog *Phacochoerus africanus* (Gmelin 1788) was used as the outgroup taxon. Scale bar = 1 nucleotide substitution/100 nucleotides.**

Since the tapa specimens were recovered in Luzon, it is likely that the products might have been sourced from *S. philippensis*, a species native to the region (Oliver 1995). Variation greater than 3.0% for a different fragment of the Cytb region was able to unambiguously identify species of *Sus* (Gupta et al. 2013). K2P distances within the samples and domestic pig Cytb sequences ranged from 0-1.47% (Figure 3). Tapa samples from *S. philippensis* have a distance of 5.34%, well within the range of interspecific variation of 3.49-6.18% among the sequences. Both phylogeny and distance-based analyses strongly suggest that the tapa specimens were indeed sourced from domestic pigs.



**Figure 3. Heatmap and dendrogram of K2P distances (in percentage) of the tapa samples compared to native *Sus* species and breeds of domestic pigs. Legend = %distance.**

We have determined that the products did not come from either wild *S. philippensis* or *R. marianna*. This is a case of adulteration as defined in Section 4.3.h of the Meat Inspection Act (2004). However, the possibility that the products were from a *S. philippensis* and *S. scrofa* hybrid cannot be entirely dismissed due to the unreliability of the mitochondrial DNA to detect hybridization in pigs (Banayo et al. 2023). While it is unlikely that the tapas were directly sourced from native wildlife, this is still a confirmed case of food fraud. Several regulatory bodies in the Philippines are mandated to monitor incidences and implement charges for these activities through the Food Safety Act (2013) and in this case, the Meat Inspection Act of the Philippines (2004). The National Meat Inspection Service does provide molecular biology-based identification to detect adulteration in food. Their detection services, however, exclude native wildlife and are limited to the DNA of livestock as well as “hot meat” sourced from rats, dogs, and cats (Fortajada et al. 2021).

DNA barcoding is one of the most widely used techniques in meat authentication in the world (Ballin 2010; Saadat et al. 2022). Both *S. philippensis* and *R. marianna* are poorly represented in the GenBank database. COI barcodes are absent for both species while Cytb sequences of native pig species are limited. Expanding the breadth of the database to include multiple markers will allow us to monitor cases of food fraud accurately for these types of specialty meats. More studies are needed to build such a reference library and to determine the population genetics of wild, endemic species of the Philippines. While there are more advanced techniques that are available for the detection of animal DNA in processed foods (Ha et al. 2017; Batule et al. 2020), the data generated can only be made useful if reference sequences are available.

The increasing reach and commercialization of wildlife derived products require further monitoring. The techniques to identify these cases are readily available and we encourage partnerships between enforcement agencies and molecular laboratories in the Philippines to stop illegal bushmeat trade and ensure food safety in the country.

## **CONCLUSION**

Using routine DNA barcoding, we were able to detect food fraud in processed meat specimens. The success of extraction and amplification of both COI and Cytb markers were not as drastically affected by the preservation medium of the tapa specimens, with the Cytb fragment only failing to amplify for 2 of the specimens. Phylogenetic and distance-based analyses confirmed that the specimens labeled “tapang usa” and “tapang baboy ramo” were from *Sus scrofa* and did not come from wild-caught

native species. This case of dishonesty in labeling is classified as adulteration of the food product. The technology for meat authentication is available. Specialized cases involving the authentication of wildlife derived bushmeat requires the expansion of the current barcode database of native species to ensure proper identification.

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