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Identification of The Seized Meat Products for Suspected Cetacea

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Abstract

For conservation purposes, unambiguous identifications of the animal species of meat products are important. All the species of Cetacea (dolphins, porpoises and whales) are listed on CITES appendices and conserved by legislation in Taiwan. The mitochondrial cytochrome b (cytb) and cytochrome c oxidase subunit I (COI) are the most commonly used loci for species identification. In this report, cytb and COI genes were used to identify the animal species of 11 seized meat products for suspected Cetacea from 7 cases. The results showed that 8 specimens from 5 cases were identified as dolphins or porpoises (conserved) and 3 specimens from 2 cases were as seals (not conserved). For each of the specimens, the results showed the same species for cytb and COI identification.

Keywords: forensic science, Cetacea, species identification, cytb, COI

Introduction

Approximate 5,000 species of animals and 28,000 species of plants are listed on the CITES (Convention on International Trade in Endangered Species of Wild Flora and Fauna) appendices, and their trade are prohibited (the species on appendix I) or under restrict control for their import/export (the species on appendices II and III). All the species of Cetacea (dolphins, porpoises and whales) are on the list of CITES appendix I or II, and also conserved by the Wildlife Conservation Act in Taiwan. For the purpose of conservation, unambiguous identifications of the meat products for suspected Cetacea are absolutely important.

DNA test of mitochondrial gene is the first choice for species identification of meat products presently. Cytochrome b (cytb) is one of the candidate genes [1-3]. Baker CS et al. used this gene to provide the genetic evidence of illegal trade in protected whales [4]. Li LH et al. analyzed the DNA sequence of 16S rRNA gene of three dolphin species (*Tursiops truncatus, Sousa chinensis* and *Steno bredanensis*) for phylogenetic study and species identification [5]. The mitochondrial control region was ever used in the identification of the species of whales, dolphins, and porpoises for DNA surveillance [6]. In this report, cytb and COI genes (cytochrome c oxidase subunit I, as the barcode for animal species identification [7]) were used to identify the species of the seized meat products for suspected Cetacea.

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Materials and methods

Case reports

Totally there were 11 specimens of seized meat products for suspected Cetacea from 7 cases collected from Mar. in 2011 to Apr. in 2013. These meat products were sold in Taiwan markets and the Conservation Police Force seized them. The specimens were provided by the Council of Agriculture (COA) for species identification.

DNA extraction and quantification

The specimen was washed with distilled water for three times. About 60 mg meat was taken from each specimen, then it was cut into small pieces for DNA extraction. DNA extraction was performed by using Tissue & Cell Genomic DNA Purification Kit (GeneMark, Taiwan).

The extracted DNA was quantified by using NanoDrop 2000 (Thermo Fisher Scientific, Delaware, USA).

PCR amplification and DNA sequencing

Two markers, cytb and COI, were amplified. PCR amplification was performed in 50 μL of reaction mixture, which contained 200 ng genomic DNA, 1X reaction buffer, 0.2 μM dNTP, 2.5 units of PCR Taq DNA polymerase (RICHBIO, Erlangen, Germany) and 0.2 μM each of primers. For cytb amplification, the primers of L14724 (4a) and H15915 (5a) were designed according to the report of Irwin DM et al. [8], and H15149 (9b) were modified from the sequences in their report. For COI amplification, LCO1490 and HCO2198 were designed according to the report of Folmer O et al [9], and the other primers (COI-F1 and COI-R1) were designed by our laboratory (Table 1). The sequences of these universal primers and amplification conditions conducted in a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Foster, CA, USA) were shown in Table 1 and Table 2. The PCR products were analyzed on a 2 % agarose gel. DNA sequencing reaction was performed by using each one of the PCR primers and the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) in the GeneAmp PCR System 9700 thermal cycler (Applied Biosystems). The AB 3730 DNA Analyzer (Applied Biosystems) was used to separate and detect the cycle sequencing products.

Sequence analysis

The sequences excluding the primers were used for similarity search with those registered in GenBank through the website (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Table 1. Primers used in this study

primer name	sequence (5'→3')*
L14724 (4a)	CGAAGCTTGATATGAAAAACCATCGTTG
H15149 (9b)	TAACTGTAGCCCCTCAGAATGATATTTGTCCTCA
H15915 (5a)	AACTGCAGTCATCTCCGGTTTACAAGAC
COI-F1	RNHYTTAGTTAACAGCTAA
LCO1490	GGTCAACAAATCATAAAGATATTGG
HCO2198	TAAACTTCAGGGTGACCAAAAAATCA
COI-R1	RGGTTCRANTCCTTCCTTT

* The unusual base was coded according to the IUPAC rule. R represents the base of A or G; N of any base; H of A, C or T; and Y of C or T.

Table 2. The primer combination, condition and predicted size of the PCR amplification in this report

Locus	Primer combination	Condition		Predicted size* (bp)
cytb	L14724(4a)/ H15915(5a)	94 °C 5 min 94 °C 45 sec 50 °C 45 sec 72 °C 1 min 72 °C 7 min	35 cycles	1205
	L14724(4a)/ H15149(9b)	94 °C 5 min 94 °C 45 sec 50 °C 45 sec 72 °C 1 min 72 °C 7 min	35 cycles	464
COI	COI-F1/ COI-R1	94 °C 5 min 94 °C 45 sec 47 °C 1 min 72 °C 1 min 72 °C 7 min	35 cycles	1362
	LCO1490/ HCO2198	94 °C 5 min 94 °C 45 sec 52 °C 1 min 72 °C 1 min 72 °C 7 min	35 cycles	734

* The predicted size of the PCR product was including the primer sequences.

Results and Discussion

DNA from all the specimens was used to amplify the cytb and COI genes. Sequences of the PCR products were compared with those registered in GenBank. The most similar species and similarity were showed in Table 3. All the similarities were higher than 98 %. One specimen (Specimen no. 3) was most similar to the species of *Tursiops truncates* (Bottlenose dolphins) with the similarities of 100.0 and 99.7% for cytb and COI respectively. Three specimens (Specimen no. 1-1, 1-2 and 1-3) were most similar to the species of *Neophocaena phocaenoides* (Finless porpoise) and four specimens (Specimen no. 2, 4, 7-1 and 7-2) were similar to *Stenella attenuate* (Pantropical spotted dolphin) with high similarities respectively for both genes. These three species were all listed on the appendices of CITES and conserved by legislation in Taiwan. The other three specimens (Specimen no. 5, 6-1 and 6-2) were most similar to the species of *Phoca groenlandica* (Harp seal), which is neither the CITES species nor the conserved species by legislation in Taiwan. In this report, 5 specimens from 4 cases (Case no. 2, 3, 4 and 7) were identified as dolphins, 3 specimens from 1 case (Case no. 1) were as porpoises and 3 specimens from 2 cases (Case no. 5 and 6) were as seals. Totally, the results showed that 8 specimens from 5 cases were of conserved species and 3 specimens from 2 cases were not conserved.

Table 3. The similarity of the specimens with the most similar species in GenBank.

Case no.	Specimen no.	Scientific name (Common name)	Similarity for cytb (Accession no.)	Similarity for COI (Accession no.)
1	1-1 1-2 1-3	*Neophocaena phocaenoides* (Finless porpoise)	99.8% (HQ108406.1)	99.7% (EU496316.1)
2	2	*Stenella attenuate* (Pantropical spotted dolphin)	98.7% (EU557096.1)	98.8% (EU496336.1)
3	3	*Tursiops truncates* (Bottlenose dolphins)	100.0% (EU557093.1)	99.7% (EU557093.1)
4	4	*Stenella attenuate* (Pantropical spotted dolphin)	99.3% (EU496336.1)	99.4% (EU557096.1)
5	5	*Phoca groenlandica* (Harp seal)	99.5% (GU174609.1)	100.0% (AY377145.1)
6	6-1 6-2	*Phoca groenlandica* (Harp seal)	99.6% 99.8% (GU174609.1)	99.5% 99.5% (AM181030.1)
7	7-1 7-2	*Stenella attenuate* (Pantropical spotted dolphin)	100.0% (AF084096.1)	99.8% (EU557096.1)

The mitochondrial cytb and COI are the most commonly used loci for species identification [10]. In this report, the results showed the same species for cytb and COI identification for each of the specimens. No controversial results indicated the reliability of the established systems. The system could also be used in identification of the other commercial meat products for the purposes of conservation and investigating the frauds.

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