Partial Sequence Analysis of *Cytochrome b* Gene by FINS Technique Reveals Fraud Sambar Meat in Wild Food Restaurant

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ABSTRACT

Species identification of animals in the Cervidae by sequence comparison of the Cytochrome b (Cyt b) gene using FINS method is focused in this study. The Cyt b fragments of seven species in the Cervidae, including rusa deer (Cervus timorensis), sambar deer (C. unicolor), sika deer (C. nippon), hog deer (C. porcinus), axis deer (C. axis), Eld's deer (C. eldi) and barking deer (Munticacus muntjak) were amplified by our own designed primers. The amplicons (322 bp) were sequenced and their partial sequences (263 bp) were then analyzed. Barking deer showed the highest value of genetic diversity within species (π = 0.0364; h = 0.9). The phylogenetic analysis had shown that the partial sequence of Cyt b gene can be used to classify most studied species in Cervidae accurately, except for sambar and rusa deer that cannot be differentiated. Hence, species identification of unknown meat samples was then performed by this method. Referenced Cyt b sequences of wild boar (Sus scrofa), favorite meat in wild food restaurant, and the Cyt b sequence of known sambar tissue samples were additionally compared. The genetic distances indicated that unknown meat samples were presumably wild boar. Although this method cannot differentiate sambar from rusa deer, this study

will be useful for wildlife forensic particularly when screening examination of irrelevant samples and fraud sambar meats identification are necessary.

Keywords: *Cytochrome b* gene, DNA variation, Cervidae, FINS

INTRODUCTION

Nowadays, large numbers of endangered wildlife animals listed in the CITES appendices are smuggled for illegal trades. In this case, wildlife forensic science has been used as a significant tool to prosecute the smuggler. Studies on wildlife forensic science have been reviewed (Johnson et al., 2014). Most of them have focused on species identification, for which methods based on DNA analysis are more advantageous than those of traditional morphological identification because they can be applied on different types of samples, for example, processed animal parts, derivatives within Traditional Chinese Medicine (TCMs) and objects made from animal parts (Iyengar, 2014) DNA markers used for species identification must be species-specific. These sequences can be found in both nuclear and mitochondrial DNA. However, mitochondrial DNA is more preferable because it contains high copy numbers and hardly degrades.

Many studies have shown that species-specific sequences of mitochondrial DNA, such as *ND1* (Kitpipit et al., 2012; Welton et al., 2013), *COI* (Wilson-Wilde et al., 2010), *16S rRNA* (Imaizumi et al., 2007), *Cyt b* (Jun et al., 2011), D-loop (Fumagalli et al., 2009; Gupta et al., 2011) and ITS-2 (Clarke et al., 2006) can be used to identify animal species. Molecular techniques like PCR-RFLP have been conducted to discriminate DNA polymorphisms of a target sequence for species identification, for example *Cyt b* for identification of fish species in the Cyprinidae family (Chen et al., 2011) and *16S rRNA* for sea cucumber identification (Wen et al., 2010). However, the questioned species may not be revealed when the PCR-RFLP profile of an unknown sample does not match with any of those reference species. Hence, a technique called FINS (Forensically Informative Nucleotide Sequencing) has been introduced. This technique identifies species by using phylogenetic analysis in which DNA sequence of a sample is compared with known DNA sequences in the database (Li et al., 2011)). The FINS technique has been reported as a successful method for determination of authentic seafood ingredients including, octopus (Espineira et al., 2010), jelly fish (Armani et al., 2013) and ling fish (Santaclara et al., 2014). This technique has also been applied for mislabeling investigation of pet canned food (Armani et al., 2015).