Ascertaining Species Of Origin From A Confiscated Meat Using Dna Forensics

Abstract

Poaching of wildlife for local consumption and commercial purposes is a major threat to the loss of world's biodiversity which has even resulted to the extirpation of the numerous species at local or regional scale. Law enforcement agencies have been working in the implementation of Wildlife Protection Act to combat illegal trade of wildlife for parts and products all across the world. Nevertheless, several times confiscated materials have been altered and fabricated so much that their morphological identification often got difficult. In last few decades, DNA technology has revolutionized species identification in forensic investigations and has shown its power in firm identification of those fabricated materials. We received a confiscated material, appeared to be chopped raw meat with no intact morphological identity. The accused was caught by the officials of the State Forest Department after he put pictures on social media depicting his involvement in cooking and eating of animal meat, suspected to be of wildlife origin. On homology search, we found a 99% identity of the confiscated material with Asian Palm Civet (Paradoxurus hermaphroditus) and neighbour joining trees based on genetic distances clustered the query sequence with the Asian Palm Civet with 100% bootstrap support. The present study exhibits hope for ascertaining species identification from the processed meat in reliable assessment for wildlife forensics. This case study extends, the role of the authenticated references as available on public database, highlighting the application of DNA forensics in identifying species even from the phenotypically altered materials.

Keywords: Wildlife forensics; DNA barcoding; Asian Palm Civet; 12S rRNA; CytB gene.

Introduction

Poaching of wild animals for meat, skin, bone and other body parts is one of the most ancient cultures on the way of human civilization and evolution. This regular practices with the inclusion of commercial greed of mankind has caused the loss of biodiversity and brought several species extinct like Javan Rhino, Western Black Rhino, Passenger pigeon or near extinct like Pink Headed Duck, Hangul. Although, all wild animals in India are protected by Wildlife (Protection) Act, 1972. However, illegal poaching and trade for wildlife parts and products are still silently on and several animals are being poached every year.

Examination in forensics based on morphological and immunological clues has limited power in

ascertaining species and individual identity using conventional methods. In wildlife forensics, species identification from parts and products which often got altered and processed several folds, become challenging using conventional methods even for a trained and skilled biologist. Often, specimens received for identification are either too old and or degraded or altered into pieces that ascertaining species identity become very difficult. Therefore, DNA-based identifications become useful tool for investigation of confiscated wildlife samples with altered morphological identity. Mitochondrial genes like CoI (Hebert et al. 2003; Khedkar et al. 2016) and CytB, 16S rRNA and 12S rRNA have been used for catering the need of species identification from the confiscated materials and have been proven reliable for species identification from variety of samples with compromised morphological identity. The present study was aimed to identify the species of origin from an altered confiscated material in order to submit a technical advisory to prosecute legal trials in the court of law and for the implementation of the Wildlife (Protection) Act, 1972 of India.

Case presentation

A person from Palta (North 24 Parganas District), of West Bengal state, India was suspected to poach an animal of wild origin as he was caught by the forest department after he uploaded few photos of himself in the act of killing and preparing to cook a wild animal on social media. The State Forest Department arrested him just after immediate hype on social media under the violation of Wildlife (Protection) Act, 1972. Divisional Forest Officer of North 24 Parganas, West Bengal, confiscated the meat, presumed of wildlife origin and sent to Zoological Survey of India, Kolkata for species identification so that legal proceeding could be initiated. Materials and methods: We took a sub sample of meat, chopped into small pieces, coded as “ZSI/FI/2017/11” and washed overnight with 1X sterile Phosphate Buffer Saline (PBS). Isolation of genomic DNA was carried out through Phenol-Chloroform-Isoamyl alcohol (PCI) method. Universal primers of Cytochrome B and 12S rRNA genes, were used to amplify these genes for species level identification. PCR products were cleaned up using Exo-SAP treatment and Cycle sequencing was carried out independently for forward and reverse primer using Big Dye terminator cycle sequencing kit v3.1 (Applied Biosystems, USA). Sequencing was performed on Genetic analyzer 3730. Sequence quality was checked manually nucleotide by nucleotide and validated using Sequencher v4.7 (www.genecode.com). Resulted sequences were submitted to NCBI/BOLD database. Homologous sequences were downloaded from NCBI/BLAST search and multiple sequence alignment for all the downloaded sequences was carried out with MUSCLE alignment tool in MEGA6 (Tamura et al., 2013). For phylogenetic construction, we identified the best fit model based on the BIC value for all the downloaded and generated sequences and neighbour joining (NJ) trees were constructed in MEGA6 using Tamura-Nei model with gamma distribution.

Results

NA from the confiscated meat sample successfully isolated using PCI method and visualized on 1% agarose ge. PCR amplified fragment of both CytB and 12S rRNA genes visualised (on 2% agarose gel) as separate single DNA bands between 250bp and 500bp band of the 1kb ladder. On BLAST search for both the genes, the query sequences showed to have 99 percent identity with the sequences of Asian Pam Civet (Paradoxurus hermaphroditus). The genetic distance matrix for both the genes revealed that the suspected confiscated material have closest genetic similarity (Nei's DA 0.011 for CytB gene) and (Nei's DA 0.012 for 12S rRNA) with the known sequences of Asian Palm Civet, Paradoxurus hermaphroditus and NJ trees demonstrated that query sequences clustered tightly with the known sequences of Asian Pam Civet (Paradoxurus hermaphroditus) with 100% bootstrap support. Hence, this got confirmed by the collective evidences like homology search on NCBI/Genbank database, genetic distances and phylogentic tree analysis that the confiscated material is of Asian Palm Civet Origin Paradoxurus hermaphroditus which is scheduled species (Schedule II) and protected under Wildlife (Protection) Act, 1972 and widely distributed in the State of West Bengal. The case report on species identification was submitted to the authority of the Forest Department of West Bengal.

Discussion

Although, several earlier studies have shown the applicability of barcoding genes for rapid and reliable forensic identification, the present study has shown a significant role in identifying species from raw meat which did not have any morphological identity (such as hair, nail or skull) left. Hence, the current study generated new hope by showing that documenting species identification is possible even for raw meat for reliable assessment of wildlife forensic investigations. Declaration of interest: Authors declare that they have no conflict of interest.