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USE OF DNA BARCODING TO CONTROL THE ILLEGAL WILDLIFE TRADE: A CITES CASE REPORT FROM PAKISTAN

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ABSTRACT

Illegal wildlife trade is a great threat to the conservation efforts made worldwide to save wildlife species and their parts. Use of molecular methods, including DNA barcoding, is gaining acceptance to detect cross-border movement of endangered species. Here we report the utility of DNA barcoding in the detection of smuggling of an endangered turtle species from Pakistan. The consignment labeled as "fish meat" was intercepted at a Pakistani port and was tested for its source using DNA Barcoding with fish-specific primers. Sequences from the samples from this consignment matched (99%) with those from *Lissemys punctata* (Indian flap-shelled turtle), a species listed by the Convention on International Trade in Endangered Species (CITES). This report highlights the problem of smuggling protected species under false pretenses and the importance of DNA barcoding in stopping such illegal trade.

Keywords: Molecular taxonomy, freshwater turtle, *Lissemys punctata*, CITES

INTRODUCTION

Since the notification of empowering Sindh Wildlife Department to protect the turtles and tortoise of the order Chelonia has been issued, many consignments of turtles have been confiscated. Recently, over 200 black pond turtles, destined for the Bangkok black market, were confiscated at the Karachi port. Lately, the smuggling of turtles of the same species was foiled at the Chinese border, which were then repatriated to Pakistan and released in their native habitat. Now the smugglers have devised new means of carrying out their illicit practice, as instead of live turtles they smuggle the turtle parts by labeling them as fish meat, a legal trade item. In March 2015, a consignment of turtles including shells, bones, skulls, and dried meat, under the label "fish meat," was intercepted at Karachi port. The shipment weighing about 1900 kg, roughly comprised of 4000 turtles, was worth approximately sixty million USD. The species in question are turtles native to the Indus River and listed in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (Appendix II). The poaching, catching, trapping, netting, and using their parts, whole or derivatives, trading, transport, and export is strictly prohibited as per the Sindh Wildlife Protection Ordinance, 1972, as well as the Pakistan Trade Control of Wild Fauna and Flora Act, 2012. The consignment was claimed to comprise of fish meat, but the shape, color, and co-items (shells, bones, skulls) made the consignment suspicious. However, a firm identification of the contents was difficult. Thus, DNA Barcoding was employed to identify the species-source of the contents as has been practiced for the identification of many sea

food products (Wong et al., 2008; Li et al., 2011; Pappalardo et al., 2015; de Brito et al., 2015; Leal et al., 2015). This report presents DNA barcoding as a widely applicable, rapid, cost effective, and authentic test to cope with illegal wildlife trade.

METHODOLOGY

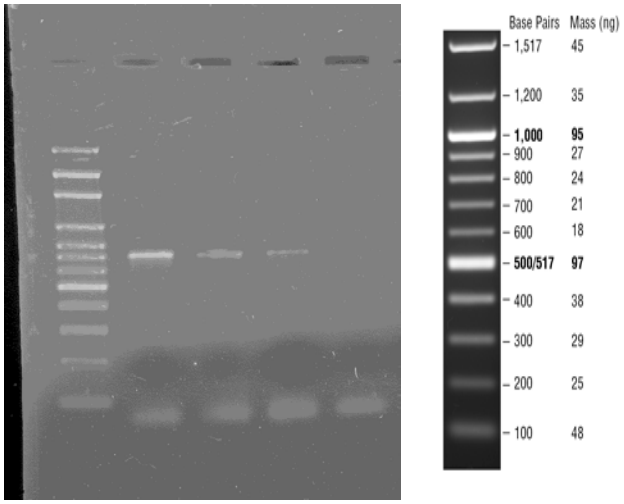
Genomic DNA was extracted from the meat tissues following published protocols (Anonymous, 2005). The DNA barcode region (approximately 700 bp) of the COI gene was amplified by using universal primers, FishF1 (5'-TCAACCAACCACAAAGACATTGGCAC-3') and FishR1 (5'-TAGACTTCTGGGTGGCCAAAGAATCA-3') (Ward et al., 2005). The 25 µL PCR reaction comprised of 2.5µL of 10X PCR buffer, 3µL of 25mM $MgCl_2$, 0.2mM each dNTP, 1.25µL of 10µM each primer, 2.5 U of Taq polymerase, 100ng of DNA template, and PCR water. Amplification was performed following PCR cycling protocol (pre-denaturation at 95°C for 5 minutes, 35 cycles of 95 °C for 1 min, 55°C for 1 min, and 72°C for 2min, post extension at 72°C for 5min) and the PCR products were examined on 1.5% agarose gel. The amplicons were sequenced by the Sangers Method (Sanger *et al*., 1977). The obtained sequences (Chromatograms: Supplementary Material 1, Sequence FASTA Format: Supplementary Material 2), were aligned using NCBI's BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

RESULTS AND DISCUSSION

The ~700 bp barcode amplicons (Fig. 1) sequenced from the unidentified tissue samples collected from the smuggled consignment, showed 99% similarity with those from *Lissemys punctata* (Accession No. KF894768.1, JN794087.1, JN416995.1, HQ329775.1). This indicates that samples under study belonged to the Indian flap-shelled turtle, *L. punctata*. Based on the content information included with the consignment, we used fish-specific primers but the PCR product turned out to be from a turtle. It was not a surprise, as these primers target a broader taxonomic range of fish (Ward, 2009; Ning et al., 2015; Chandra et al., 2015) as well as turtles (Reid et al., 2011). DNA Barcoding is gaining wide acceptance not just because of its validity to identify animal species based on sequence matches (Hebert et al. 2003), but also due to the convenience of amplification with the same set of primers for a broader taxon range. The freshwater turtle *L. punctata*, commonly known as the Indian flap-shelled turtle, is native to South Asia (Bangladesh, India, Myanmar, Nepal, Pakistan and Sri Lanka) and the barcode sequences further confirmed its identity and presence in Pakistan. The species is on CITES Appendix II, which includes species for which trade must be controlled in order to avoid utilization incompatible with their survival.

In conclusion, as the illegal wildlife trade in the developing and under-developed countries is on the rise, a major reason of failure to curb the transportation of protected species is non-availability of scientific tools for the correct species identification. This creates a loophole for the corrupt individuals in the relevant law enforcing agencies to pass the illegal consignments and makes the smuggling of protected species, labeled as non-protected species, difficult to control. The availability of advanced scientific tools, like DNA barcoding, can prove best 'cop' to overcome these difficulties because of its quick and valid outcomes.

L------1------2------3------4



(a) **(b)**

Figure 1: Amplified DNA of approximately 700bp resolved on 1.5% agarose gel: (a): Lane L, 100bp DNA ladder, Lane 1-3, 700 bp COI amplicon, Lane C, no template control. (b): 100 bp DNA Ladder index.

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