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**CASE REPORT**

**CRIMINALISTICS**

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# Identification of the Source of Ivory Idol by DNA Analysis*

**ABSTRACT:** In this study, we describe a forensic case dealing with the identification of the source of the processed ivory object by DNA analysis. Two pieces of Lord Krishna's idols from a shop were confiscated by an investigating agency of the Indian government and forwarded to us to identify the source of its origin. We succeeded in isolating DNA from both processed ivory idols by using the phenol/chloroform DNA extraction method. The extracted DNA was subjected to PCR amplification using an elephant-specific mitochondrial DNA (mtDNA) D-loop marker. DNA sequence analysis of the amplified fragment of mtDNA D-loop region confirmed that the idols were consistent with Asian elephant with 99% similarity.

**KEYWORDS:** forensic science, elephant ivory, PCR, mtDNA, DNA sequence analysis, Asian elephant

Wildlife forensic science is a rapidly growing field with the availability of successful case reports (1–3). More concern in the wildlife forensics is the nature and variety of available biological products. The extraction of DNA from the biological materials is the first important step for the entire molecular analysis. Very often, the extraction of DNA from the ancient and the processed biological samples is a challenging task. One of the research activities of our laboratory is ancient and forensic DNA study, where we frequently deal with very intricate biological materials. Very recently, two pieces of the idol of Lord Krishna (Fig. 1) were forwarded to our laboratory to find out whether these are composed of elephant ivory. A request was also made to establish whether the source of its origin was Asiatic or African elephant.

The identification of a species from the processed-animal part is very crucial in the wildlife forensic science. There are a number of molecular methods available based on mitochondrial DNA (mtDNA) markers (4–6), which have been useful to solve a number of wildlife forensic cases (1–3). The forests in the north-east and the southern parts of India are inhabited by large Asian elephant populations and therefore are prime spots for poaching of male elephant for their ivory to make ornaments and idols, which are of high trade value (7). Trading and keeping of ivory product is a wildlife offense (Wildlife Protection Act 1972); therefore, there is a grave need to confiscate and identify the objects made from ivory.

Ivory consists of dentine, deposited by calcified odontoblast cells; therefore, these cells are trapped in dentine tubules of the tusk (8), which makes ivory very tough and, hence, making isolation of DNA from these cells extremely difficult. Several studies demonstrated the successful extraction and analysis of DNA from known and confiscated ivory (9–12). In this study, we have used a conventional phenol/chloroform DNA extraction protocol (13) for extracting the DNA from the objects derived from elephant ivory. The isolated DNA is usable for polymerase chain reaction (PCR) amplification by using an elephant-specific mtDNA D-loop marker (7). The elephant-specific markers used in this study show no cross-amplification with the known DNA sample of humans, dog, deer, black buck, lion, leopard, tiger, mouse, porcupine, or dolphin (7). To confirm the wide range of specificity, *in silico* variation in


FIG. 1—*(A, B) Ivory idols of Lord Krishna (Hindu God).*

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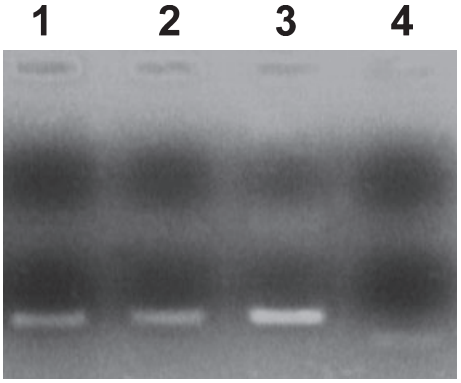


FIG. 2—*Gel image showing the PCR amplicons of: Lane 1, DNA isolated from idol A; Lane 2, DNA isolated from idol B; Lane 3, DNA of a known male Asian elephant; Lane 4, negative control.*

primer site for elephant-specific markers was also demonstrated by the inventors with vast numbers of animal species' mtDNA D-loop sequences downloaded from NCBI database (7).

## Material and Methods

### *DNA Isolation from Idols*

Small pieces (~1 cm) of ivory were broken from both the idols (Fig. 1*A*,*B*) and ground in an indigenous stainless steel grinder after vigorous washing with 2% SDS followed by rinsing in sterile double-distilled water. The ground ivory was decalcified by using a 0.5 M EDTA solution (10). An aliquot of 0.3 g of decalcified ivory powder was digested in 500 μL of lysis buffer (150 mM NaCl, 50 mM Tris, and 10 mM EDTA [pH 8.0]), 2% SDS, and 20 μL of Proteinase-K (20 mg/mL stock) and incubated at 55°C for 12–16 h with gentle rotation.

The above-digested materials were processed for DNA isolation using the phenol/chloroform method (13). The DNA solution was purified using GeneClean kit from Qiagen (Hilden, Germany).

### *PCR Amplification with Elephant-specific Primer*

The DNA samples were subjected to PCR amplification with elephant-specific primers EdlF 5′GAGGCCCTAACACAGTCAAGCAAC3′ and EdlR 5′CGTGTACGCTGGGAATTTAGGTT3′ (7). PCR amplification was carried out in a 20-μL reaction volume containing 2 μL of DNA, 100 μM each of dNTPs, 3 pM primers, 1.5 mM $MgCl_2$, 0.6 units of Taq Gold (Applied Biosystems, Foster City, CA), and 1× PCR buffer (10 mM Tris–HCl, pH 8.3, and 50 mM KCl). The PCR cycling condition used was as follows: an initial denaturation at 95°C for 10 min, followed by 40 cycles each of denaturation at 95°C for 45 sec, annealing at 58°C for 1 min, and extension at 72°C for 1.5 min. The final extension was 72°C for 10 min. The PCR products were electrophoresed on a 2% agarose gel, stained with ethidium bromide (0.5 mg/mL), and observed under UV transilluminator.

### *DNA Sequencing and Sequence Analysis*

The amplicons were treated with exonuclease-1 and shrimp alkaline phosphatase (USB, Cleveland, OH) for 15 min each at 37°C and 80°C, respectively, prior to sequencing PCR. Both strands of the amplified DNA were sequenced using BigDye terminator cycle sequencing reaction kit and ABI 3730 automated DNA Analyzer

TABLE 1—*Comparison of the variable position of mtDNA D-loop sequences of idol with known sequences of Asian elephant, African elephant, Dugong (sea cow), and Great Indian rhinoceros.*

| | 06 | 07 | 08 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 21 | 22 | 23 | 24 | 26 | 27 | 29 | 32 | 33 | 37 | 52 | 53 | 54 | 55 | 56 | 57 | 65 | 67 | 68 | 69 | 70 | 71 | 72 | 73 | 74 | 75 | 76 | 77 | 78 | 79 | 80 | 81 | 82 | 84 | 85 | 89 | 91 | 94 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Idol | G | T | A | A | G | A | T | C | T | G | A | C | G | G | A | T | T | - | - | G | G | A | C | T | C | G | G | A | G | - | - | - | T | T | T | T | A | A | A | C | C | T | A | A | A | T | T | C | C | A | T | C | G |
| Asian elephant | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | - | - | . | . | . | . | . | . | . | . | . | . | - | - | - | . | . | . | . | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| African elephant | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | - | - | . | . | . | . | . | . | . | . | A | A | T | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | A |
| Sea cow | A | . | . | . | . | . | T | A | . | . | . | G | . | C | A | T | . | C | C | - | A | A | G | . | . | . | . | - | A | T | T | . | A | C | C | . | . | . | . | C | C | . | T | . | . | . | . | C | T | . |
| Indian rhinoceros | A | G | G | T | T | C | C | T | C | C | . | T | - | A | C | C | A | C | C | A | T | G | G | A | A | T | C | G | C | - | - | C | A | . | A | C | . | C | G | T | A | C | - | G | C | A | C | A | A | T | - | - |

Two-digit numbers at top indicate the position of variable nucleotide of idol, dot (.) indicates the similarity with the first (idol's) sequence, and hyphen (-) indicates the gap within the sequence.

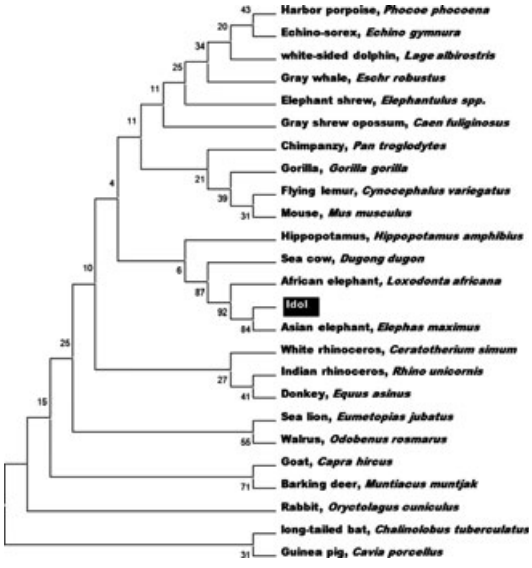


FIG. 3—*Neighbor-joining tree constructed based on aligned sequences of the partial fragment of mtDNA D-loop region of various animals, including Asian and African elephants along with ivory idols.*

(Applied Biosystems). Sequences obtained were aligned using AutoAssembler software (Applied Biosystems) to obtain the consensus sequence. The DNA sequences were aligned with mtDNA D-loop region sequences of several animal species, including African and Asian elephants using ClustalX (14). Neighbor-joining (NJ) is a bottom-up clustering method based on minimum-evolution criterion and used for the construction of phylogenetic trees (15). An NJ tree was generated for aligned sequences by bootstrapping of 1000 replication with the help of MEGA4 software (16).

## Results and Discussion

PCR amplicons of the expected size of 137 bp were obtained from DNA samples isolated from both the ivory idols (Fig. 2) using elephant-specific primer pairs confirming that these DNA samples are that of elephant species. DNA sequence variation among the various species including closely related sea cow and rhinoceros is unambiguous (Table 1) within the tested site of mtDNA D-loop region and matching 99% and 96% with Asian and African elephant, respectively. Moreover, the NJ tree constructed by using aligned sequence along with ivory idols' sequence, clustering close with the Asian elephant (Fig. 3), further confirm that the idol objects were derived from Asian elephant.

There are several studies demonstrating the isolation and amplification of DNA from the known as well as confiscated ivory (9–12). The most interesting aspect of our work is that this is the first report for the isolation and amplification of DNA from the processed ivory material. Furthermore, this report would help the identification of the source of the ivory object from the traders, in turn, which will be helpful for the conservation of declining Asian/African elephant populations. We also demonstrated that the phenol/chloroform DNA extraction method can also be an effective protocol for the analysis of DNA from ivory objects.

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