

Studies on Tiger (*Panthera tigris*)
Taxonomy and Identification.

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Tyger! Tyger! burning bright

In the forest of the night

What immortal hand or eye

Could frame thy fearful symmetry?

DECLARATION

I certify that this work does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

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Thitika Kitpipit

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Thitika Kitpipit

Studies on tiger (*Panthera tigris*) taxonomy and identification.

Abstract

All subspecies of tigers (*Panthera tigris*) are listed on Appendix 1 of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), affording them the highest international legal protection. The number of tigers has declined dramatically over the last 100 years and in particular in the last two decades with the main reason for the decline being illegal poaching for body parts. Enforcement of international and national legislation requires a reliable and robust forensic test to be established. The trade in tiger body parts is primarily in the form of powders and potions preventing any morphological examination and therefore requiring a molecular approach to identify the sample in question. The five extant subspecies are classified primarily on their phenotypic appearances, although there remains debate about this number of subspecies. Based on a combination of morphological and genetic data, proposals for the number of subspecies range from two to six. This study decoded the entire mitochondrial DNA sequence two individuals of four of the five subspecies of tiger to determine tiger taxonomic classification and to develop a DNA test for the unambiguous identification to the level subspecies. The analysis included a complete mitochondrial genome characterization, nucleotide composition and pattern and codon usage; with the aim to investigate tiger inter-, intra-species variation. The comparison of DNA sequences, which included these new sequences and all reliable sequence data on GenBank, revealed very limited subspecies diversity and questions the current classification. These studies indicated the presence of only 11 tiger species-, subspecies-specific variable sites found throughout the entire mitochondrial genome. A multiplex assay was developed to analyses polymorphic bases and was able to reliably identify 15 voucher tiger samples with 100% accuracy. The sensitivity of the test was down to a level of 15,000 mitochondrial DNA copies (approximately 0.26 pg), indicating that it will work on trace amounts of tissue, bone or hair. This simple and reliable technique can be applied by forensic science laboratories with the aim of enforcing legislation protecting the trade in the last remaining tiger.

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LIST OF ABBREVIATIONS

| | |
|-------------------|----------------------------------|
| A | Adenine |
| bp | Base pair |
| cyt <i>b</i> | Cytochrome <i>b</i> |
| CO | Cytochrome Oxidase |
| C | Cytosine |
| °C | Degree Celsius |
| DNA | Deoxyribonucleic acid |
| dNTP | Deoxyribonucleotide triphosphate |
| g | Gram |
| G | Guanine |
| h | Hour |
| MgCl ₂ | Magnesium Chloride |
| T _m | Melting temperature |
| μL | Microliter |
| mg | Milligram |
| mL | Milliliter |
| mm | Millitmetre |
| min | Minute |
| mtDNA | Mitochondrial DNA |
| M | Molar |
| ND | NADH dehydrogenase |
| ng | Nanogram |
| ALT | <i>Panthera tigris altaica</i> |
| COB | <i>Panthera tigris corbetti</i> |
| SUM | <i>Panthera tigris sumatrae</i> |
| TIG | <i>Panthera tigris tigris</i> |
| % | Percentage |
| pM | Picomolar |
| pmole | Picomole |
| PCG | Protein-coding gene |
| RSCU | Relative synonymous codon usage |

LIST OF ABBREVIATIONS (Cont.)

| | |
|---------|--|
| RFLP | Restriction fragment length polymorphism |
| rpm | Revolutions per minute |
| rRNA | Ribosomal RNA |
| s | Second |
| STR | Short tandem repeat |
| SNP | Single nucleotide polymorphisms |
| CITES | The convention on international trade in endangered wild fauna and flora |
| IUCN | The international union for conservation of nature |
| TRAFFIC | The wildlife trade monitoring network |
| T | Thymine |
| tRNA | Transfer RNA |
| TBE | Tris boric acid-ethylenediaminetetra acetic acid |
| V | Voltage |
| v/v | Volume per volume |
| w/v | Weight per volume |
| WWF | World wildlife fund |