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

October, 2011

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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 *b*	Cytochrome *b*
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	*Panthera tigris altaica*
COB	*Panthera tigris corbetti*
SUM	*Panthera tigris sumatrae*
TIG	*Panthera tigris tigris*
%	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