Population Genetics and Conservation of the Philippine Crocodile

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Abstract

The endemic Philippine crocodile (*Crocodylus mindorensis*) is considered to be one of the most highly threatened crocodilians in the world. Historically known to occur throughout the Philippine archipelago, wild populations are now confined to small and isolated populations on the islands of Luzon and Mindanao. Reintroduction is seen as an important element in the recovery of this species. Successful captive breeding programmes initiated in the 1980's increased the number to hundreds of captive Philippine crocodiles, many of which are candidates for reintroduction to suitable habitats. Preliminary genetic studies based on mtDNA found *Crocodylus porosus*-*C. mindorensis* hybrids in the biggest captive population which raises concerns on species integrity and suitability of the captive population for the reintroduction programme. In addition, unresolved issues on the extent of genetic differentiation among extant populations hampered recovery plans for many years.

To resolve these issues, a total of 618 wild and captive Philippine crocodiles were genotyped at 11 microsatellite loci to investigate genetic diversity and population structure. In addition, information from an existing mtDNA study was combined with the results from a Bayesian assignment test based on microsatellite loci to find evidence of hybridisation. A high degree of genetic differentiation across all populations was observed (F_{ST} = 0.29. Genetic differentiation reflected geographic structuring, with the highest F_{ST} values recorded between populations from the northern Philippines (Luzon) and southern Philippines (Mindanao). Moderate levels of genetic diversity were seen in all captive and wild populations included in the sampling, except for one captive population in Abra.

A total of 92 hybrids were identified from two captive facilities. Three of the identified hybrids in this study were part of the group released into the wild during the first reintroduction programme in 2009. These three individuals did not exhibit obvious morphological anomalies and were thought to be pure *C. mindorensis*. The results of this study have important conservation implications and will influence the management of captive and wild populations of Philippine crocodiles and the design of future reintroductions.

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I am also thankful to the following people who shared information and provided Philippine crocodile tissue samples for this study: Glenn Rebong of PWRCC; Merlijn Van Weerd of the Mabuwaya Foundation; Rainier Manalo of Conservation International; Charles Ross from Silliman University; John Aries from the University of Southern Mindanao and Sonny Dizon from Davao Crocodile Park. Thanks to Willem van de Ven, Bernard Tarun, Sammy Telan, Jesse Guerrero and other staff of the Mabuwaya Foundation who assisted in the procurement of samples from Isabela. Thank you to Chris Banks and Tom Dacey of the CSG for providing contacts and information relating to crocodile conservation and funding opportunities

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Preface

This study was part of a larger research project which examined the genetics of the Philippine crocodile (*Crocodylus mindorensis*). The project was a collaboration among the following institutions: Massey University, Omaha's Henry Doorly Zoo (HDZ), the Philippine Government's Department of Environment and Natural Resources (DENR), Palawan Wildlife Rescue and Conservation Centre (PWRCC), Mabuwaya Foundation, Silliman University and the University of Southern Mindanao (USM). The need to clarify the population genetics of the Philippine crocodile was one of the key priorities outlined in the 2005 recovery plan of the species. To address this issue, the collaboration was established to facilitate sample collection, permit processing, laboratory work, data analysis and publication of results. This study looked at the population structure and genetic variation of the Philippine crocodile as revealed by microsatellite DNA loci, while another project examined phylogeography using mitochondrial DNA markers (Tabora *et al.* 2010).

Sample Collection

Tissue and blood samples used in the study were collected or provided by the following people/institutions: Glenn Rebong (PWRCC); Rainier Manalo (Conservation International), Merlijn van Weerd, Jessie Guerrero, Bernard Tarun, Willem van de Ven (Mabuwaya Foundation); Andy Ross (Silliman University), John Tabora and Cayetano Pomares (USM); Gladys Porter Zoo; and Davao Crocodile Park. I collected 465 samples from PWRCC, which were included in the most recent CITES export permit with the help of Medel Silvosa, Glenn Rebong, Renato Cornel, Ernesto Conate, Amado Mulig, Salvador Guion, Roberto Manalang, Ferdinand Palioza, William Tabinas, Alberto Guinto and Ronnie Sumiller.

DNA Extraction and Microsatellite Genotyping

The laboratory technicians at the HDZ genetics department, John Tabora (USM) and I extracted DNA from all the samples used in this research. Microsatellite marker optimization and microsatellite genotyping were carried out by me, Shannon Engbert (HDZ) and Caroline Bailey (HDZ). The DNA extractions, polymerase chain reactions

(PCR) and microsatellite genotyping were all accomplished at the genetics laboratory at the Centre for Conservation and Research, Henry Doorly Zoo, Omaha, Nebraska, USA. Data analyses were performed at HDZ and Massey University.

Table of Contents

List of Figures

List of Tables

List of Abbreviations

AR	Allelic richness
CFI	Crocodile Farming Institute
CITES	Convention on the International Trade of Endangered Species
CSG	Crocodile Specialist Group
DAN	Nei's improved genetic distance
DENR	Department of Environment and Natural Resources
DNA	Deoxyribonucleic acid
HDZ	Henry Doorly Zoo
IAM	Infinite allele model
IUCN	International Union for the Conservation of Nature
JICA	Japan International Cooperation Agency
K	Genetic cluster
LD	linkage disequilibrium
MC	Markov chain
MNA	Mean number of alleles
mtDNA	Mitochondrial DNA
N	Number of samples
NM	Number of migrants per generation
PCR	Polymerase chain reaction
PH	Philippines
PWRCC	Palawan Wildlife Rescue and Conservation Centre
SMM	Stepwise mutation model
TPM	Two-phase mutation model
WCSP	Wildlife Conservation Society of the Philippine