

CONFIRMATION OF CUBA CROCODILE HYBRIDS USING DNA THERMAL PROFILING

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Introduction

The Cuban crocodile, *Crocodylus rhombifer*, the most endangered crocodilian of the New World, has recently been included in the Species Survival Plan of the American Association of Zoos and Aquariums (AZA). There is a need to characterize pure *C. rhombifer* individuals for captive breeding purposes and for future reintroductions into the wild. In this study, second derivative DNA thermal denaturation profiles were used to confirm *C. rhombifer* x *C. acutus* and other hybrids that were identified based on morphological characters and circumstantial evidence.

One study conducted on yeast mitochondrial DNA used first derivative DNA thermal denaturation profiles to distinguish two strains of yeast (1). We have previously used fifth derivative DNA thermal denaturation profiles to study crocodilian phylogeny. These analyses resulted in relationships congruent with those of other molecular phylogenies (2). DNA thermal profiling has recently been developed to study genetic variation within a vertebrate species (3).

The application of this technique to conservation problems is comparable to establishing a new type of DNA fingerprint for the identification and characterization of endangered individuals. DNA thermal profiling has several advantages over other molecular techniques used routinely by conservationists: a) DNA thermal profile data can be generated in less time and requires very little preparation; b) this technique circumvents the use of radioactive probes, gel electrophoresis, elaborate staining procedures, Southern blots, autoradiography, and PCR; and c) the cost of materials to run the procedure are quite small.

Methods

Total genomic DNA was extracted from 24 Cuban Crocodile individuals originating from seven zoos in

the US. DNA was melted in a Gilford Response II spectrophotometer from 60 °C to 100 °C at 0.1 °C increments. Second derivative thermal denaturation profiles were generated and the temperature corresponding to each peak was used in a parsimony analysis.

Results and Discussion

The results from parsimony analyses confirmed that five of the 24 crocodiles were Cuban Crocodile hybrids rather than pure *C. rhombifer* individuals. The individuals identified by DNA thermal profiling were as follows:

TOLEDO ZOO- Two hybrids:

Cuban Crocodile (1/2) X Nile Crocodile (1/2)

One hybrid:

Cuban Crocodile (3/4) X Nile Crocodile (1/4)

BRONX ZOO- One hybrid:

Cuban Crocodile (1/2) X Indian Mugger (1/2)

GLADYS

PORTER ZOO- One hybrid:

Cuban Crocodile (1/2) X American Crocodile (1/2)

Although no new hybrids were identified in this study using DNA thermal profile data, the fact that expected hybrids could be confirmed by the method is promising. This suggests that the method will be useful for such identifications in the future. Moreover, such profiles can serve in the development of a database that would eventually be used by zoos for captive breeding programs, as well as by law enforcement agencies to monitor the crocodilian skin trade.

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3. Akkaraju SR and Ahlquist JE. Midwest Ecology and Evolution Conference Northern Illinois University. Abstract 1994.