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Genetic Characterization of Captive Cuban Crocodiles (*Crocodylus rhombifer*) and Evidence of Hybridization With the American Crocodile (*Crocodylus acutus*)

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ABSTRACT There is a surprising lack of genetic data for the Cuban crocodile (*Crocodylus rhombifer*), especially given its status as a critically endangered species. Samples from captive individuals were used to genetically characterize this species in comparison with other New World crocodilians. Partial mitochondrial sequence data were generated from cyt-*b* (843 bp) and the $tRNA^{Pro}$- $tRNA^{Phe}$-D-loop region (442 bp). Phylogenetic analyses were performed by generating maximum parsimony, maximum likelihood, and Bayesian-based topologies. In addition, in an effort to identify species-specific alleles, ten polymorphic microsatellite loci were genotyped. Distance and model-based clustering analyses were performed on microsatellite data, in addition to a model-based assignment of hybrid types. Both mitochondrial and nuclear markers identified two distinct *C. rhombifer* genetic sub-clades (α and β); and microsatellite analyses revealed that most admixed individuals were F_2 hybrids between *C. rhombifer*-α and the American crocodile (*C. acutus*). All individuals in the *C. rhombifer*-β group were morphologically identified as *C. acutus* and formed a distinct genetic assemblage. *J. Exp. Zool. 309A:649–660, 2008.*

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Cuban crocodiles (*Crocodylus rhombifer*) are considered endangered (CITES Appendix, IUCN Red List-EN) due to their limited distribution, habitat loss, and the introduction of exotic animals into their environment (Ross, '98). The recent encroachment of humans into *C. rhombifer's* territory has limited its distributional range to about 186 square miles (300 km^2) within Cienaga de Zapata and Cienaga de Lanier in southwestern Cuba (Fig. 1). Sub-fossils of *C. rhombifer* found in Cuba are dated to the Pleistocene (Varona, '66; '84); whereas several sub-fossils found on Grand Cayman (Morgan et al., '93) and the Bahamas (Franz et al., '95) are from the Holocene, which suggests it was historically found throughout most of the Caribbean. It has been suggested that a possible contributing factor to the decline *C. rhombifer* over the past 25 years may have been the introduction of the Brown caiman (*Caiman crocodilus fuscus*) into Cienaga de Lanier (Ross, '98). Although recent reports by R. Soberón (personal communication) have indicated that this is not possible because the *C. rhombifer* population on that island was already extirpated before *C. c. fuscus* was introduced. *C. rhombifer* is also naturally sympatric with the American crocodile (*C. acutus*), but *C. acutus* has a more extensive distribution that extends from North America into South America and the Caribbean (Ramos et al., '94; Thorbjarnarson et al., 2006).

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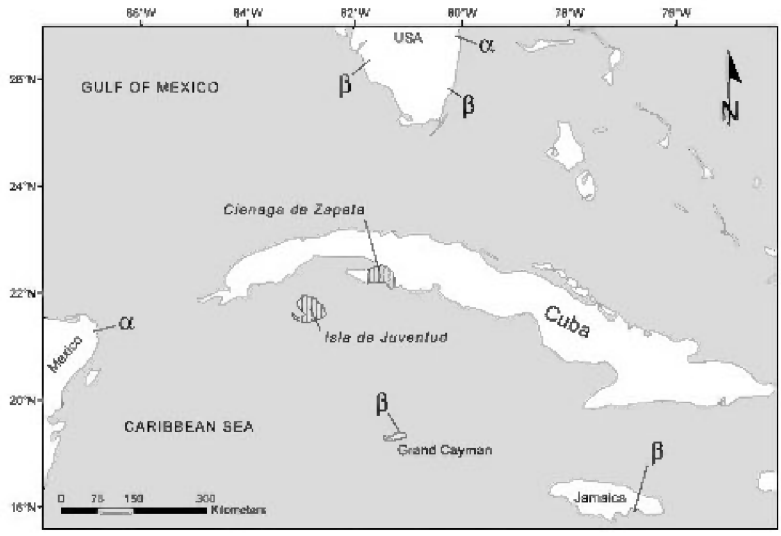

Fig. 1. Map of Cuba with localities of *C. rhombifer*-like haplotypes (α and β) found outside their present range (vertical stripes).

The Cuban crocodile's breeding season overlaps with that of *C. acutus* by a few days in the month of January (Varona, '66). The variation in length between these two animals is approximately 1.5 m with adult *C. acutus* males reaching 5 m and *C. rhombifer* reaching 3.5 m (Varona, '66); thus, making hybridization physically possible. There have been several documented cases of hybridization between crocodiles in captive populations; most pertinently between *C. acutus* and *C. rhombifer* at the Laguna del Tesoro farm in Cuba (Ross, '98), and consequently these hybrids could have been distributed to US zoological parks and private collectors.

The ability to detect hybrids is essential in identifying pure breeding populations for reintroductions into extirpated areas (Allendorf et al., 2001; FitzSimmons et al., 2002). Despite the critical status of wild *C. rhombifer* populations there has been little or no genetic data published or reported on this species; although, there is an ongoing ecological study that will include some genetic analyses (R. Ramos and O. Sanjur, personal communication). Hybrid introgression has been detected in some New World crocodilians (Hekkala, 2004; Ray et al., 2004; Rodriguez, 2007; Cedeño-Vázquez et al., 2008; Rodriguez et al., 2008), and owing to *C. rhombifer's* smaller population numbers and its frequent sympatry with *C. acutus*, the genetic integrity of this species is at risk. It has been suggested that detecting hybrids is less exhaustive when the two parental crocodiles possess different karyotypes, but detection of hybridization between individuals with similar karyotypes requires more in-depth analyses (Chavananikul et al., '94; FitzSimmons et al., 2002). Due to the chromosomal and biochemical similarity (Cohen and Gans, '70; Densmore, '83) and the relatively recent divergence (Brochu, 2000) between *C. rhombifer* and *C. acutus*, detecting hybrids based on morphological characters alone may be problematic. In this specific case, the use of molecular markers is warranted.

Molecular markers have been used routinely to characterize threatened species and populations (Frankham et al., 2002), but genetic studies first require a point of reference to accurately assess species assignments. Mitochondrial DNA (mtDNA) is useful in constructing phylogenies and haplotype networks. However, the maternal inheritance of mtDNA limits our ability to detect hybridization to cases where there is disagreement between morphology and mtDNA assignments. Therefore, nuclear DNA (nDNA) markers must also be utilized to more accurately evaluate species designations and determine hybrid types. Developing a pure breeding stock of *C. rhombifer* will be essential in maintaining the genetic integrity of the species, which is why any potential hybridization with other species can be a problem in captive populations. The purpose of our study was to genetically characterize all available samples of captive Cuban crocodiles from US zoological institutions and to describe *C. rhombifer*-like haplotypes found in the Caribbean, Florida, and Mexico, which may present a threat to the genetic purity of other endemic crocodile species. This work will provide the foundation for future genetic

treatments of wild Cuban crocodile populations, assist in further efforts to identify hybrids outside of Cuba, and assess the utility of genetic methods in detecting inter-specific admixture within other captive populations.

MATERIALS AND METHODS

Samples

Wild *C. rhombifer* populations from Cuba could not be sampled, but zoological specimens were readily available. Whole blood or skin clips were collected from captive *C. rhombifer*, wild caught *C. acutus*, wild caught *C. moreletii* (Morelet's crocodile) and a captive *C. intermedius* (Orinoco crocodile) (see Appendix). Blood was collected via the caudal or dorsal sinus and stored in cell lysis buffer (Gorzula et al., '76; Bayliss, '87), whereas skin clips were stored in 95% ethanol. Both sets of tissue were stored at −20°C prior to DNA isolation. Total genomic DNA was extracted using the PureGene isolation kit (Gentra Systems, Minneapolis, MN), electrophoresed on a 2% agarose gel, and visualized with ethidium bromide under UV light.

Genetic markers

Mitochondrial DNA

A partial cytochrome-*b* (cyt-*b*) fragment (843 bp) was amplified from *C. rhombifer*, *C. acutus*, *C. moreletii*, and *C. intermedius* using primers crCYTBfor and crCYTBrev. Primers drL15459 (modified from Glenn et al., 2002) and CR2HA (modified from Ray and Densmore, 2002) were used to amplify the $tRNA^{Pro}$-$tRNA^{Phe}$-D-loop region (442 bp; Table 1) for only *C. rhombifer* and *C. acutus*. Polymerase chain reactions (PCR) were performed in 50 μL volumes using 0.50 μL of total genomic DNA (tDNA) (50 ng/μL), 36.25 μL of ddH_20, 10 μL of buffer (0.3 M TRIS, 0.0175 M $MgCl_2$, and 0.075 M $(NH_4)_2SO_4$), 2.0 μL of 2.5 mM dNTPs, 0.50 μL (10 mM) of forward primer, 0.50 μL (10 mM) reverse of primer and 0.25 μL (1.25 U) of Promega Taq polymerase (Promega Corp., Madison, WI). Thermocycling conditions for all primers consisted of an initial denaturation step of 2 min at 94°C, then 33 cycles of 30 sec at 94°C, 1 min at 58°C, and 45 sec at 72°C; with a final extension of 7 min at 72°C. Unincorporated dinucleotides and primers were removed from PCR products using the Qiagen PCR purification kit (Qiagen, Inc., Valencia, CA). Products were cycle sequenced using Big Dye v3.1 dye terminator (Applied Biosystems, Inc., Foster City, CA). Cycle sequence products were purified by passing through a G-50 Sephadex column (0.5 gm of Sephadex/800 μL ddH_2O), which was incubated at room temperature for 30 min and centrifuged at 3,000 rpm for 2 min to construct the column. Dried cycle sequence product was denatured in formamide and electrophoresed on an ABI 3100-*Avant* genetic analyzer (Applied Biosystems, Inc., Foster City, CA). Chromatograms were viewed and trimmed using Sequencher 4.1.4 (Gene Codes Corp. Ann Arbor, MI), and then aligned using Clustal X (Thompson et al., '97) and BioEdit 5.0.6 (Hall, '99). All newly generated sequences were accessioned into the GenBank database (EU034541-EU034627). Three sequences obtained from NCBI were used for comparison with our cyt-*b* sequence data, the Estuarine crocodile (*C. porosus*; AJ810453), the Siamese crocodile (*C. siamensis*; DQ353946) and the Nile crocodile (*C. niloticus*; AJ810452).

TABLE 1. Primer sequences used to generate mtDNA fragments

Primer	Sequence
crCYTBfor	5′ ATGACCCACCAACTACGAAAATC 3′
crCYTBrev	5′ CGAAGGGGTTTGATTAATAGGTT 3′
CrCYTBintfor[1]	5′ TAGCAACTGCCTTCATAGGCTAC 3′
drL15459	5′ AGGAAAGCGCTGGCCTTGTAA 3′
CR2HA	5′ GGGGCCACTAAAAACTGGGGGGA 3′

[1]Used only for sequencing.

Microsatellites

Ten polymorphic microsatellite loci (Dever and Densmore, 2001; FitzSimmons et al., 2001) were also amplified for each individual (Table 2). Primers were fluorescently labeled with WellRed dyes (Beckman Coulter, Inc., Fullerton, CA) and amplified using 12.5 μL PCR reactions, which included 0.125 μL of tDNA, 9.06 μL of ddH_2O, 2.50 μL buffer (0.3 M TRIS, 0.0175 M $MgCl_2$, and 0.075 M $(NH_4)_2SO_4$), 0.50 μL of 2.5 μM dNTPs (10 mM), 0.13 μL forward primer (10 mM), 0.13 μL reverse primer (10 mM), and 0.0625 μL (0.31 U) Promega *Taq* polymerase. PCR conditions for all primers consisted of an initial denaturation step of 2 min at 94°C, then 33 cycles of 30 sec at 94°C, 1 min at 58°C or 62°C (Table 2), and 45 sec at 72°C; with a final extension of 5 min at 72°C. Fragments were sized based on a 400 bp size

TABLE 2. Microsatellite diversity values for each genetic cluster inferred using STRUCTURE (without admixed individuals)

		C. rhombifer-I				*C. rhombifer*-II				*C. acutus*			
Locus	AT (C°)[1]	N^2	A^3	$H_O{}^4$	$H_E{}^5$	N^2	A^3	$H_O{}^4$	$H_E{}^5$	N^2	A^3	$H_O{}^4$	$H_E{}^5$
C391	58	19	1	0.00	0.00	7	5	1.00	0.78	14	5	0.57	0.68
Cj16	62	22	4	0.82	0.65	7	3	0.57	0.58	14	7	0.86	0.78
Cj18	58	21	3	0.57	0.64	7	3	0.57	0.65	14	4	0.64	0.60
Cj20	62	22	4	0.64	0.66	7	5	0.71	0.73	14	4	0.50	0.56
Cj109	62	20	3	0.80	0.63	7	4	0.29	0.50	14	6	0.50	0.82
Cj119	58	21	4	0.43	0.50	7	3	0.43	0.69	14	3	0.50	0.62
Cj131	58	21	6	0.91	0.66	7	2	0.86	0.53	14	2	0.14	0.25
Cu5-123	58	22	3	0.32	0.35	7	5	0.71	0.81	14	5	0.50	0.48
Cud68	58	18	3	0.67	0.48	7	3	0.86	0.67	14	2	0.36	0.52
Cuj131	58	20	2	0.05	0.05	7	4	0.43	0.71	14	2	0.29	0.35
mean			3.3	0.52	0.46		3.7	0.64	0.66		4.0	0.49	0.57

[1]Annealing temperature.
[2]Number of individuals sampled per locus.
[3]Number of average alleles per locus.
[4]Observed heterozygosity.
[5]Expected heterozygosity.

standard, using a CEQ8800 genetic analyzer and software (Beckman Coulter, Inc., Fullerton, CA). Using identical scoring methods, genotypes from all *C. rhombifer* samples were compared with 14 pure *C. acutus* (see Appendix) identified by Rodriguez (2007) and Rodriguez et al. (2008).

Data analysis

Sequence data

MODELTEST (Posada and Crandall, '98) was used to obtain the best-fit model of nucleotide substitution for maximum likelihood (ML), and MRMODELTEST (Nylander, 2004) was used to obtain the best-fit model of evolution for Bayesian inference (BI). Phylogenetic analyses of cyt-*b* sequence data, under the ML and maximum parsimony criteria, were performed in PAUP*4.0b10 (Swofford, 2002). A starting tree was generated by stepwise addition of taxa, with swapping performed by utilizing the tree bisection reconnection algorithm. Node support was determined by bootstrapping topologies for 1,000 replications. Additionally, a BI tree, with posterior probabilities, was constructed using MrBayes v3.1.2 (Huelsenbeck and Ronquist, 2001). Four Markov chains were implemented for 1,000,000 iterations after an initial burn-in of 100,000 iterations. To obtain finer haplotype resolution, a neighbor-joining tree was constructed in PAUP using uncorrected pairwise genetic distances based on $tRNA^{Pro}$-$tRNA^{Phe}$-D-loop sequence data. Node support was determined by bootstrapping the resulting topology for 1,000 iterations.

Microsatellite data

The program POPULATIONS v1.2.28 (Langella, '99) was employed to estimate Dc pairwise genetic distances (Cavalli-Sforza and Edwards, '67) to construct an exploratory neighbor-joining tree for all *C. rhombifer* and *C. acutus* individuals, which was visualized in TREEVIEW (Page, '96). The program STRUCTURE (Pritchard et al., 2000) was used to determine assignment probabilities to specific genetic clusters by constraining K to the number of clades suggested by the neighbor-joining topology. We assumed that purebred individuals will have high assignment probabilities (>0.97) to species genetic clusters, whereas hybrids will have intermediate assignment probabilities (<0.97). CERVUS 3.0 (Marshall et al., '98) was used to estimate measures of microsatellite diversity. If hybrids were detected, then NEWHYBRIDS (Anderson and Thompson, 2002) was used to implement a Bayesian-based algorithm, which assigns individuals into six genotypic classes. Genotype classes consist of two parental groups (*C. rhombifer* and *C. acutus*), first generation hybrids (F_1), second generation hybrids (F_2), F_1 backcrosses to *C. rhombifer* and F_2 backcrosses to *C. acutus*.

RESULTS

Sequence-based analyses

For cyt-*b* sequence data, MODELTEST and MRMODELTEST indicated the best model of nucleotide substitution was GTR+G for both ML and BI. The *C. rhombifer* species group fell within the New World crocodilian clade that included *C. niloticus* (Fig. 2). Only two haplotypes were found using cyt-*b* sequence data (α and β), which also corresponded to two haplotypes detected using $tRNA^{Pro}$-$tRNA^{Phe}$-D-loop sequences. Measures of percent uncorrected distances based on cyt-*b* sequences (Table 3), between the *C. rhombifer*-α haplotype and *C. acutus*, and between the *C. rhombifer*-β haplotype and *C. acutus* were both estimated at 5.3%. Percent divergence between the two *C. rhombifer* cyt-*b* haplotypes was 0.9%. Similarly, estimated divergences for $tRNA^{Pro}$-$tRNA^{Phe}$-D-loop sequences were 3.4% between *C. rhombifer*-α haplotypes and *C. acutus*, and 4.1% between *C. rhombifer*-β haplotypes and *C. acutus*, respectively; whereas the genetic distance between *C. rhombifer*-α and *C. rhombifer*-β haplotypes was estimated at 1.6% (data not shown).

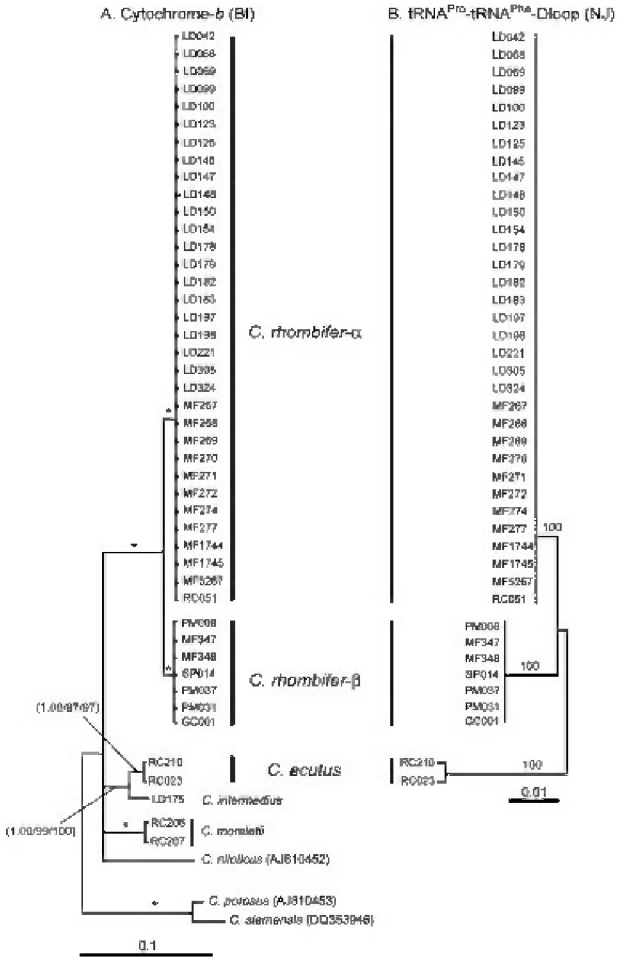

Fig. 2. (**A**) Bayesian consensus tree for cyt-*b* sequences, showing values (BI/MP/ML) where * = (1.00/100/100). (**B**) Neighbor-joining tree based on $tRNA^{Pro}$-$tRNA^{Phe}$-D-loop sequences with bootstrap support values. Two distinct subclades were inferred using both mitochondrial sequences.

Microsatellite analyses

When all the samples were pooled, a matrix of Dc distances returned three distinct clades on a neighbor-joining tree (*C. rhombifer*-I, *C. rhombifer*-II, and *C. acutus*) with several individuals clustering between clades (Fig. 3). Using the neighbor-joining tree as a guide, K was constrained to three for model-based clustering methods implemented in STRUCTURE; these genetic clusters were named *C. rhombifer*-, *C. rhombifer*-2, and *C. acutus*. Posterior assignment probabilities suggested that admixture was primarily occurring between *C. rhombifer*-I and *C. acutus* (Fig. 4), therefore, individuals with intermediate probabilities were designated as hybrids (Appendix). After the STRUCTURE results, NEWHYBRIDS was used to classify hybrid types between *C. rhombifer*-I and *C. acutus*, these individuals were mostly F_2 hybrids (Fig. 5). One individual, RC051, collected in Cancun,

TABLE 3. Uncorrected pairwise genetic distance values for cyt-b

	(1) RC051	(2) MF348	(3) RC210	(4) LD175	(5) RC206	(6) AJ810452	(7) AJ810453	(8) DQ353946
(1) *C. rhombifer*-α	–							
(2) *C. rhombifer*-β	0.009	–						
(3) *C. acutus*	0.053	0.053	–					
(4) *C. intermedius*	0.057	0.055	0.015	–				
(5) *C. moreletii*	0.052	0.052	0.045	0.046	–			
(6) *C. niloticus*	0.079	0.077	0.056	0.059	0.061	–		
(7) *C. porosus*	0.104	0.107	0.093	0.093	0.098	0.114	–	
(8) *C. siamensis*	0.121	0.121	0.107	0.107	0.110	0.123	0.021	–

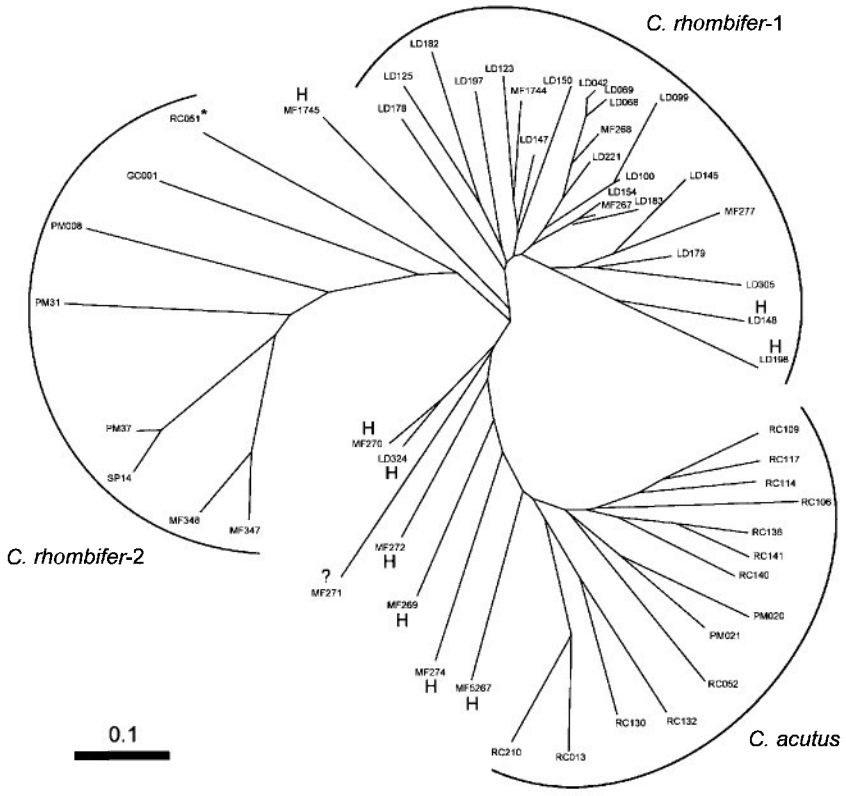

Fig. 3. Exploratory neighbor-joining tree based on Dc distances showing microsatellite distances for *C. rhombifer*, *C. acutus*, and possible *C. acutus x rhombifer* hybrids (H) constructed using POPULATIONS. A model-based analysis using STRUCTURE confirmed the same species groupings and hybrid assignments (See Fig. 4). RC051* was intermediate between both *C. rhombifer* microsatellite clusters, and MF271(?) was resolved as a possible hybrid between *C. rhombifer* and *C. palustris*.

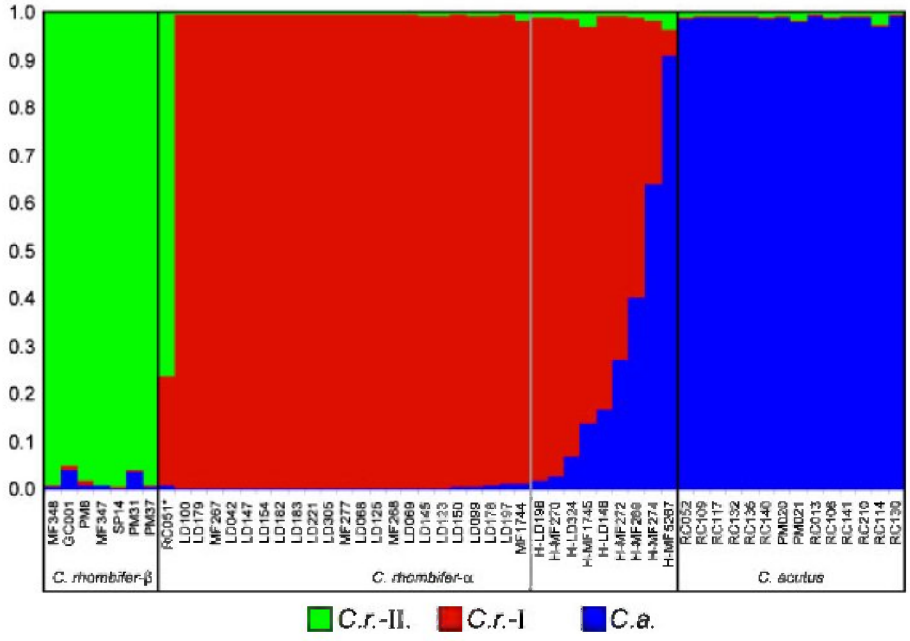

Fig. 4. A barplot of posterior probability assignments (K constrained to 3) to species groups generated in STRUCTURE based on microsatellite data and sorted by haplotype (see Fig. 2). *C.r.*-II = *C. rhombifer* genetic cluster II, *C.r.*-I = *C. rhombifer* genetic cluster I, and *C.a.* = pure *C. acutus* genetic cluster. Inferred hybrids are designated by an H.

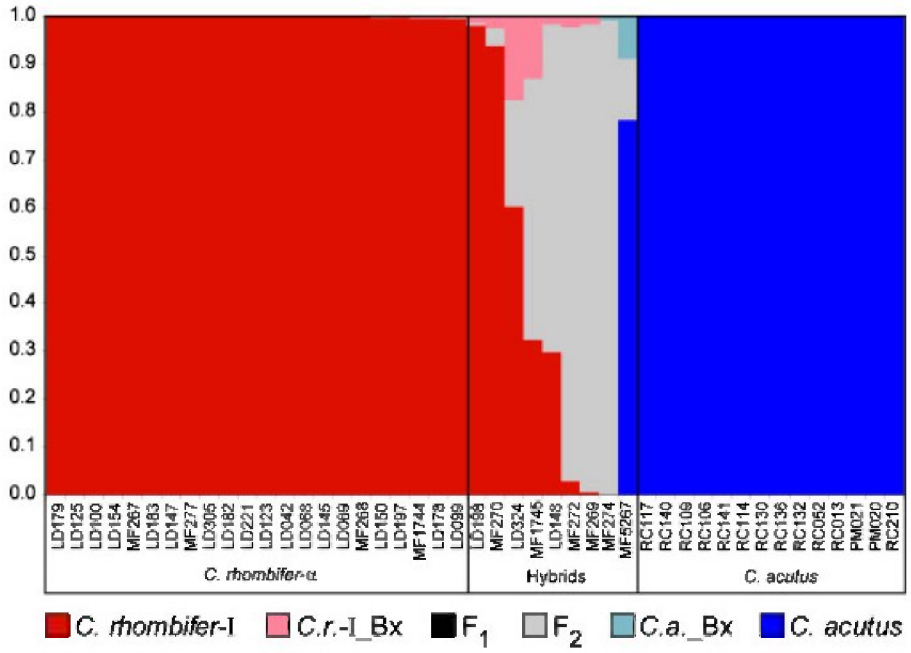

Fig. 5. A barplot of posterior probabilities for assignment to six genotype classes generated in NEWHYBRIDS (see text). The plot is partitioned into inferred pure species groups or hybrid types, F_1 = first filial generation, F_2 = second filial generation, *C.a.*_Bx = backcross to *C. acutus*, and *C.r.*-I_Bx = backcross to *C. rhombifer*-I.

Mexico was morphologically identified as *C. acutus*, carried a *C. rhombifer*-α haplotype and exhibited evidence of admixture between *C. rhombifer*-I and *C. rhombifer*-II. Captive specimen MF271, which also carried a *C. rhombifer*-α haplotype, was resolved as a possible hybrid between *C. rhombifer* and the Mugger crocodile (*C. palustris*), and exhibited four unique alleles (Cj16, 150 bp; Cj18, 207 bp; Cj131, 226 bp; CUJ131, 193 bp) that were not found in either *C. rhombifer* group or *C. acutus*. This individual was removed from subsequent STRUCTURE and NEWHYBRIDS analyses. A similar genetic treatment of *C. palustris* will be needed to accurately determine the actual paternity of this specimen. After the removal of nine admixed individuals, RC051 and MF271, we found that 20 alleles were *C. rhombifer*-α specific, 13 alleles were *C. rhombifer*-β specific, and 17 alleles were specific for *C. acutus* (Fig. 6).

DISCUSSION

Genetic status of C. rhombifer

Biotic homogenization due to anthropogenic intervention can have serious evolutionary consequences on native species, such as changes in their global distribution (Olden et al., 2004). We have provided a genetic characterization of the Cuban crocodile using captive specimens, including some individuals with incongruent morphological and mitochondrial assignments. Phylogenetically, among New World crocodiles three separate clades were inferred from cyt-*b* sequence data (*C. rhombifer*-α and β, *C. acutus*–*C. intermedius*, and *C. moreletii*), with *C. niloticus* also grouping with the New World crocodiles (Fig. 2). Within *C. rhombifer* there were two distinct mitochondrial haplotype groups (α and β), but it is possible that greater haplotype diversity may be detected if a larger portion of the mitochondrial genome is sampled. These two haplotype groups were 0.9% divergent when comparing cyt-*b* sequences and 1.6% divergent when comparing D-loop sequences. If this pattern remains consistent, as the Caribbean is more extensively sampled for crocodiles, then *C. rhombifer*-β may represent a previously unidentified lineage. Sampling the nuclear genome (using microsatellites) we were able to detect both *C. rhombifer*-α specific alleles and *C. rhombifer*-β specific alleles, and thus two genetic clusters (*C. rhombifer*-I and *C. rhombifer*-II) were also inferred from model-based analyses of the microsatellite data. Taken together, these data may actually reflect the genetic diversity within the

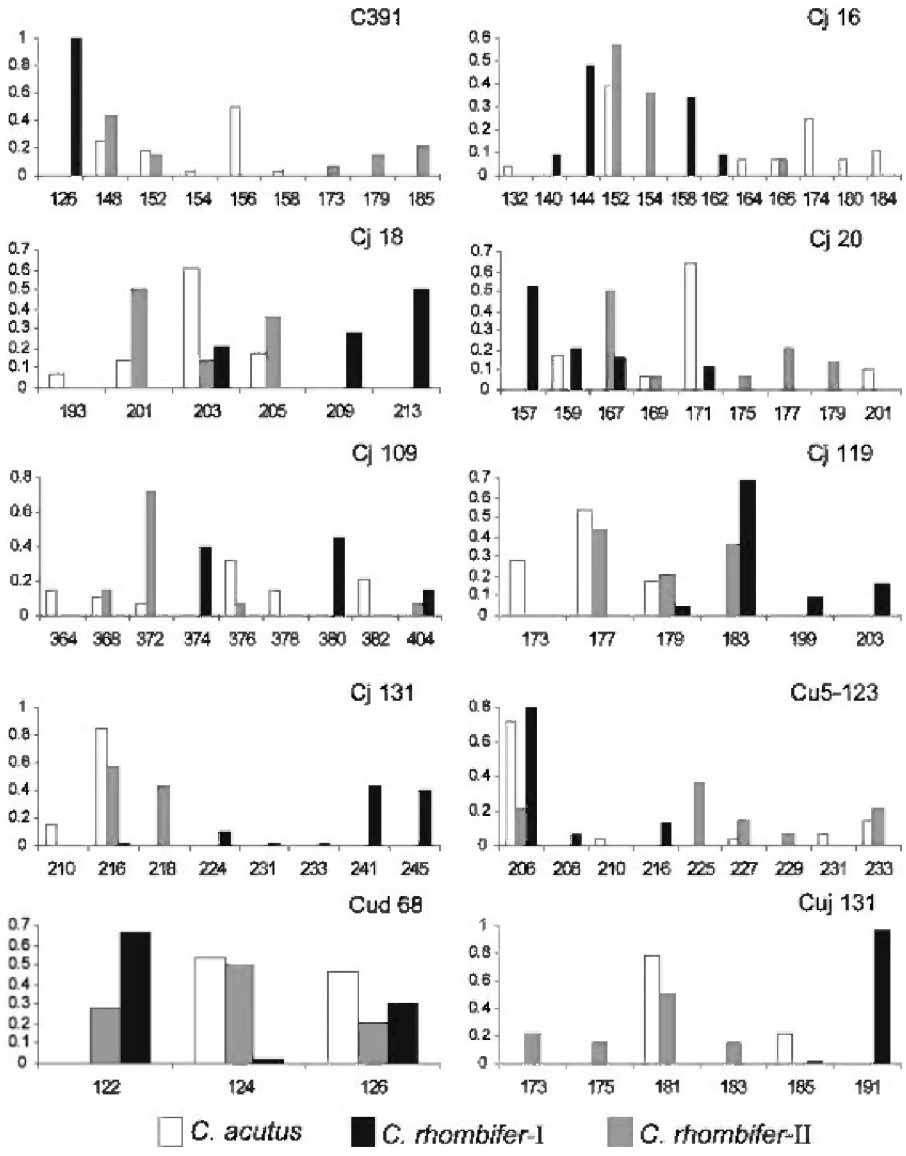

Fig. 6. Allele frequency distribution for three genetic clusters inferred by model-based clustering methods (see Fig. 3), after inferred hybrids were removed.

wild population, as the *C. rhombifer* species clade is consistently characterized as having two distinct genetic sub-groups. One of these exhibits "typical" *C. rhombifer* morphology whereas the other exhibits *C. acutus* morphology. We suspect that many crocodiles that have been "morphologically" identified as *C. acutus* in Cuba may actually belong to the *C. rhombifer*-β haplotype group. We can only speculate that β haplotypes may have been ancestrally present in Cuba, and that the current *C. rhombifer*-β group could be the result of past natural hybridization events. We cannot yet explain the evolutionary significance of *C. rhombifer*-β without reference samples from wild populations in Cuba, but action should be taken to identify these individuals and possibly remove them from captive breeding programs.

Hybridization in crocodiles

In Vietnam, captive *C. rhombifer* and *C. siamensis* have been deliberately hybridized (Thang, '94). Hybridization was also reported to have occurred between captive *C. rhombifer* and

C. acutus in breeding pens of the Laguna del Tesoro farm in Cuba (Ross, '98), as well as in the wild (Ramos et al., '94). Varona ('66) suggested that in Cuba at least some admixture was taking place between *C. acutus* and *C. rhombifer*, because several specimens exhibited morphological characters typical of both species, but clearly outside the normal range of variation found in *C. acutus*. However, among our samples we found that only MF271 and MF5267 exhibited anomalous morphology and that all F_2 hybrids appeared to exhibit *C. rhombifer* morphology. We found that hybridization events involving captive *C. rhombifer* were invariably between the *C. rhombifer*-α group and *C. acutus*. All hybrid individuals had *C. rhombifer*-like mtDNA, which suggests that in captivity hybridization is typically between a female *C. rhombifer* and a male *C. acutus*. These results are consistent and congruent with those of FitzSimmons et al. (2002).

Anthropogenic perturbation and natural migration events may pose a potential threat to the genetic integrity of *C. acutus* populations in the Caribbean, Florida, and Mesoamerica. Further, given the large proportion of admixture between *C. acutus* and *C. moreletii* (Cedeño-Vázquez et al., 2008; Rodriguez et al., 2008), populations of true crocodiles in Mexico may be threatened by an additional source of hybrid introgression from *C. rhombifer*. For example, RC051, which was found in waterways near Cancun, Mexico, carried a *C. rhombifer*-α haplotype and was morphologically identified as *C. acutus*; however, it was ultimately designated as an admixture between *C. rhombifer*-I and *C. rhombifer*-II by the nuclear data.

Conservation and management implications

Hybridization ultimately presents a management problem for New World crocodilians and complicates the identification of species based on morphology alone. A genetic evaluation (using both mtDNA and nDNA) in conjunction with a morphometric characterization can provide a more accurate view of an individual's ancestry than either method alone. As an example, a crocodile marked for reintroduction into Cat Tien National Park (Vietnam) was genetically identified as a *C. rhombifer* × *siamensis* hybrid and was subsequently prevented from being released into the wild population (FitzSimmons et al., 2002). In order for reintroductions of native species to be successful, only purebred individuals should be released back into their native habitats (Allendorf et al., 2001). Our work has provided an initial genetic assessment of the critically endangered Cuban crocodile. We hope these data can be used to identify pure individuals for breeding stock, which should be considered if repopulation of extirpated areas (e.g. Cienaga de Lanier) is to take place.

An evaluation of current Cuban crocodile stocks is warranted to ensure a purebred captive breeding line, especially considering that out of seven US captive stocks surveyed five had some level of genetic admixture. Identification of hybrids using morphology may be problematic given that only two individuals studied were morphologically anomalous. Additionally, thorough genetic assessments of wild Cuban and American crocodile populations in the Caribbean are needed to provide a better genetic reference for assignment tests, to quantify the amount of potential genetic admixture between genetically differentiated groups, and to help clarify the evolutionary implications of the *C. rhombifer*-β sub-clade. The increase in ease and the concomitant decrease in cost of generating and analyzing molecular genetic data with statistical model-based analyses can be practical and informative for both management and conservation efforts. These methods are especially important for the conservation of endangered fauna with limited distributions, such as the Cuban crocodile.

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APPENDIX

Samples used in this study, source of samples and group assignments for each data set.

Sample #	Morphology	Source	MtDNA[1]	MtDNA[2]	Msat[3]	Msat[4]
LD042	*C. rhombifer*	St. Augustine Zoo	α	α	*C.r.*-1	*C.r.*-I
LD068	*C. rhombifer*	St. Augustine Zoo	α	α	*C.r.*-1	*C.r.*-I
LD069	*C. rhombifer*	St. Augustine Zoo	α	α	*C.r.*-1	*C.r.*-I
LD099	*C. rhombifer*	St. Augustine Zoo	α	α	*C.r.*-1	*C.r.*-I
LD100	*C. rhombifer*	St. Augustine Zoo	α	α	*C.r.*-1	*C.r.*-I
LD123	*C. rhombifer*	Gladys Porter Zoo	α	α	*C.r.*-1	*C.r.*-I
LD125	*C. rhombifer*	Private property	α	α	*C.r.*-1	*C.r.*-I
LD145	*C. rhombifer*	Private property	α	α	*C.r.*-1	*C.r.*-I
LD147	*C. rhombifer*	Private property	α	α	*C.r.*-1	*C.r.*-I
LD148	*C. rhombifer*	St. Augustine Zoo	α	α	*C.r.*-1	*Hyb*
LD150	*C. rhombifer*	Jumbo Lair	α	α	*C.r.*-1	*C.r.*-I
LD154	*C. rhombifer*	Jumbo Lair	α	α	*C.r.*-1	*C.r.*-I
LD178	*C. rhombifer*	St. Augustine Zoo	α	α	*C.r.*-1	*C.r.*-I
LD179	*C. rhombifer*	St. Augustine Zoo	α	α	*C.r.*-1	*C.r.*-I
LD182	*C. rhombifer*	Toledo Zoo	α	α	*C.r.*-1	*C.r.*-I
LD183	*C. rhombifer*	Toledo Zoo	α	α	*C.r.*-1	*C.r.*-I
LD197	*C. rhombifer*	Bronx Zoo	α	α	*C.r.*-1	*C.r.*-I
LD198	*C. rhombifer*	Bronx Zoo	α	α	*C.r.*-1	*Hyb*
LD221	*C. rhombifer*	Bronx Zoo	α	α	*C.r.*-1	*C.r.*-I
LD305	*C. rhombifer*	Private property	α	α	*C.r.*-1	*C.r.*-I
LD324	*C. rhombifer*	Bronx Zoo	α	α	*Hyb*	*Hyb*
MF267	*C. rhombifer*	St. Augustine Zoo	α	α	*C.r.*-1	*C.r.*-I
MF268	*C. rhombifer*	St. Augustine Zoo	α	α	*C.r.*-1	*C.r.*-I
MF269	*C. rhombifer*	Bronx Zoo	α	α	*Hyb*	*Hyb*
MF270	*C. rhombifer*	Bronx Zoo	α	α	*Hyb*	*Hyb*
MF271	*Anomalous*	Bronx Zoo	α	α	*Hyb*	–
MF272	*C. rhombifer*	Toledo Zoo	α	α	*Hyb*	*Hyb*
MF274	*C. rhombifer*	Toledo Zoo	α	α	*Hyb*	*Hyb*
MF277	*C. rhombifer*	Busch Gardens	α	α	*C.r.*-1	*C.r.*-I
MF1744	*C. rhombifer*	Gladys Porter Zoo	α	α	*C.r.*-1	*C.r.*-I
MF1745	*C. rhombifer*	Gladys Porter Zoo	α	α	*Hyb*	*Hyb*
MF5267*	*Anomalous*	Jupiter, FL	α	α	*Hyb*	*Hyb*
RC051*	*C. acutus*	Cancun, Mexico	α	α	*C.r.*-2	*C.r.*-II
GC001*	*C. acutus*	Grand Cayman Island	β	β	*C.r.*-2	*C.r.*-II
MF347*	*C. acutus*	Jamaica	β	β	*C.r.*-2	*C.r.*-II
MF348*	*C. acutus*	Jamaica	β	β	*C.r.*-2	*C.r.*-II
PM008	*C. acutus*	Busch Gardens	β	β	*C.r.*-2	*C.r.*-II
PM031*	*C. acutus*	Imperial River, FL	β	β	*C.r.*-2	*C.r.*-II
PM037*	*C. acutus*	Snapper Creek Canal, FL	β	β	*C.r.*-2	*C.r.*-II
SP014	*C. acutus*	Private property	β	β	*C.r.*-2	*C.r.*-II
PM020*	*C. acutus*	North Key Largo, FL	–	*C. acutus*[5]	*C.a.*	*C.a.*
PM021*	*C. acutus*	North Key Largo, FL	–	*C. acutus*[5]	*C.a.*	*C.a.*
RC013*	*C. acutus*	Yucatan, Mexico	–	*C. acutus*[6]	*C.a.*	*C.a.*
RC052*	*C. acutus*	Quintana Roo, Mexico	–	*C. acutus*[6]	*C.a.*	*C.a.*
RC106*	*C. acutus*	Quintana Roo, Mexico	–	*C. acutus*[6]	*C.a.*	*C.a.*
RC109*	*C. acutus*	Quintana Roo, Mexico	–	*C. acutus*[6]	*C.a.*	*C.a.*
RC114*	*C. acutus*	Quintana Roo, Mexico	–	*C. acutus*[6]	*C.a.*	*C.a.*
RC117*	*C. acutus*	Quintana Roo, Mexico	–	*C. acutus*[6]	*C.a.*	*C.a.*
RC130*	*C. acutus*	Quintana Roo, Mexico	–	*C. acutus*[6]	*C.a.*	*C.a.*
RC132*	*C. acutus*	Quintana Roo, Mexico	–	*C. acutus*[6]	*C.a.*	*C.a.*
RC136*	*C. acutus*	Quintana Roo, Mexico	–	*C. acutus*[6]	*C.a.*	*C.a.*
RC140*	*C. acutus*	Quintana Roo, Mexico	–	*C. acutus*[6]	*C.a.*	*C.a.*
RC141*	*C. acutus*	Quintana Roo, Mexico	–	*C. acutus*[6]	*C.a.*	*C.a.*
RC210*	*C. acutus*	Yucatan, Mexico	*C. acutus*	*C. acutus*[6]	*C.a.*	*C.a.*
RC023*	*C. acutus*	Quintana Roo, Mexico	*C. acutus*	*C. acutus*[6]	–	–
LD175	*C. intermedius*	Private property	*C. intermedius*	–	–	–

Sample #	Morphology	Source	MtDNA[1]	MtDNA[2]	Msat[3]	Msat[4]
RC206*	*C. moreletii*	Quintana Roo, Mexico	*C. moreletii*	*C. moreletii*	–	–
RC207*	*C. moreletii*	Yucatan, Mexico	*C. moreletii*	*C. moreletii*	–	–

*Wild caught individuals.
[1]Based cyt-*b* sequence data (see Fig. 2A).
[2]Based on $tRNA^{Pro}$- $tRNA^{Phe}$-Dloop sequence data (see Fig. 2B).
[3]Distance-based analysis of microsatellite data (see Fig. 3).
[4]Model-based analysis of microsatellite data (see Fig. 4).
[5]Rodriguez (2007).
[6]Cedeño-Vázquez et al. (2008).

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