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Characterization of Microsatellite Markers for the Siamese Crocodile and Amplification in the Closely Related Genus Crocodylus

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ABSTRACT

Twenty microsatellite markers from *Crocodylus siamensis* were characterized in 40 crocodiles: 29 *C. siamensis*, 4 *C. porosus*, 2 hybrids (*C. siamensis* + *C. porosus)* and 5 *Caiman crocodilus crocodilus*. Fourteen microsatellite markers that showed specific alleles were considered as specific markers for use in identifying the species of crocodile. These markers were: *CS*-25 for *C. siamenis; CS*-4, *CS*-26 and *CS*-30 for *C. porosus; CS*-2, *CS*-7, *CS*-10, *CS*-12, *CS*-14, *CS*-17, *CS*-22, *CS*-24 and *CS*-35 for *C. c. crocodilus;* and *CS*-20 for all species. Twelve microsatellite markers that had polymorphic alleles in the same species were considered as intra-specific markers. These markers were: *CS*-4, *CS*-5, *CS*-10, *CS*-12, *CS*-17, *CS*-18, *CS*-21, *CS*-24, *CS*-26, *CS*-30, *CS*-32 and *CS*-35. Seven microsatellite markers showed an absence of alleles in *C. c. crocodilus* and were considered as genus-specific markers. These markers were: *CS*-4, *CS*-5, *CS*-18, *CS*-21, *CS*-26, *CS*-30, and *CS*-32. The microsatellite markers mentioned above could be used for species testing and could also be evaluated to help determine parentage in situations where the maternity, paternity, or both were unknown. It was therefore recommended that more microsatellite markers be used in such situations. The use of the microsatellite markers involved in this study would broaden the scope of a breeding program, allowing progeny from adults maintained in large breeding lagoons to be tested for selection as future breeding animals.

Key words: Siamese crocodile, microsatellite markers, crocodile, crocodylus

INTRODUCTION

Microsatellites based on a variation in the number of simple sequence repeats (SSRs) have become the markers of choice for species characterization in the life sciences, including: linkage analysis (Dib *et al*., 1996), behavioral ecology (Schlötterer and Pemberton, 1998), population genetics (Goldstein and Schlötterer, 1999) and phylogeny reconstruction (Harr *et al*., 1998). Recently, many reports have demonstrated several advantages of using microsatellite markers in crocodylian systematics and population genetics. Glenn *et al*. (1998) developed nuclear microsatellite markers to address questions on the genetic population structure and the mating system

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of American alligators *(Alligator mississippiensis)* and showed that microsatellite markers could differentiate between populations of Florida and American alligators and had a higher discriminatory power of differentiation than the allozyme isozyme markers. Davis *et al*. (2001) reported a high level of genetic variation for six populations of American alligators located throughout the southeastern United States. FitzSimmons *et al*. (2001) developed 26 new microsatellite markers from the DNA of *Crocodylus acutus, C. porosus* and *Crocodylus johnstoni* to compare the population genetic structure in crocodylian families and tested loci for *Crocodylus* spp, which represented the first microsatellite loci found in *Crocodylus*.

In the IUCN Action Plan for crocodiles (Ross, 1998), mention is made of several species where information was needed on their genetic diversity and lineage. For example, population genetics surveys were needed for the critically endangered Orinoco crocodile *(C. intermedius),* to assess their level of genetic diversity to inform restocking and reintroduction programs. The Siamese Crocodile is an endangered species that formerly inhabitted the wetlands and waterways of south east Asia. Hunting and habitat loss have reduced its population in the wilds of Thailand, so that now most of the remaining Siamese crocodiles have been maintained and bred in captivity. As there has been an attempt to release pure bred Siamese crocodiles back into the wild to support conservation strategies, there was an increasing need to develop genetic markers in *Crocodylus* sp., particularly in *C. siamensis*. Although the microsatellite loci used had some overlap of allele sizes between species, it was possible to use assignment tests for differentiation. However, within this genus, most tests of cross-species PCR amplification indicated the presence of homologous microsatellite loci that were variable. Recently a study by Chaeychomsri *et al*. (2008) developed 20 new microsatellite markers for *C. siamensis* in Thailand which is the most critical species in term of the need for conservation. This paper describes the characterization of new microsatellite markers for *C. siamensis, C. porosus*, a hybrid *(C. siamensis* + *C. porosus*) and *C. c. crocodilus,* as an important tool to help establish conservation strategies and contribute to an understanding of the structure of the wild, remnant populations of this species.

MATERIALS AND METHODS

Sample collection and DNA extraction

Blood samples were collected from the anterior dorsal sinus of 40 crocodiles, namely: 29 freshwater (*C. siamensis*), 4 saltwater (*C. porosus*), 2 hybrid (*C. siamensis* + *C. porosus*) with 5 *C. c. crocodilus* as the out group. Whole blood (5 ml) was collected using a syringe rinsed with heparin. Handling and blood collection procedures were carried out in accordance with previously established ethical guidelines (Chaeychomsri *et al*., 2008).

Microsatellite analysis

Twenty microsatellite primers previously described by Chaeychomsri *et al*. (2008) were utilized for this study with primer sequences and PCR conditions shown in Table 1. All PCR reactions were carried out on an ABI 9600 thermal cycler (Perkin Elmer, Applied Biosystems Inc.). DNA samples were amplified in 25 µl reactions containing: 25 ng of DNA; 20 mM Tris-HCl (pH 8.4); 50 mM KCl; 1.5 mM $MgCl_2$; 0.2 mM of each dNTP; 0.4 mM of each primer; and 0.5 U of Taq DNA polymerase (Promega). Products were amplified under the following conditions: 94°C for 3 min; 35 cycles of 94°C for 1 min; 60-70°C depending on each primer pair (Table 1) for 1 min; followed by a final extension at 72°C for 1 min. The PCR products of each reaction mixture were separated on 6% polyacrylamide denaturing gels and bands were visualized with silver nitrate

Table 1 Microsatellite DNA primers from *C. siamensis*, 5′→ 3′ PCR priming sequences, annealing temperature (Ta), size length, number of alleles, Accession number, observed and expected heterozygosity (Ho and He).

Locus	Repeats	Primer: (5′ → 3′)	Ta (°C)	Size length (bp)	No. of allelles	Ho	He	Accession no.
CS-2	(CA_8)	F: GCAAACCTATCACCAGTTCAAGC	68	23	1	0	0	(**EF4130039**)
		R: TCTCTGTCACCTGAACCTTCCTC		23				
CS-4	(AC_{13})	F: CCATGCCCTACCACACAACTG	65	21	2	0.61	0.38	(**EF4130051**)
		R: CAAACACAAAGGCATTCAAAGATG		24				
CS-5	(AC_{16})	F: TCTCTCCTTCTTTCTGCTTGTGC	65	23	5	0.55	0.57	(**EF4130052**)
		R: GTGCAAGGTCTGTACCTGTGTGA		23				
CS-10	(GC_5AC_{17})	F: TGACAGTGGCTTTATTGAACAGG	65	23	2	0.24	0.50	(**EF4130033**)
		R: TGGACTCTCTCCTCCTGGACTTC		23				
CS-12	(AG_8)	F: GGACAGCAACAGAAAAGACAGG	60	22	2	0.21	0.12	(**EF4130034**)
		R: ATAGGAAGCGTTCGTGCTGATG		22				
CS-14	(AC_{10})	F: CCTTCATGTGGATTAGGAACAGG	60	23	1	0	0	(**EF4130035**)
		R: TAGCAGCTTGAAGTGGGTAGCAG		23				
CS-15	(TC_6)	F: TCACCTGCATATTTCTCCTTCCA	65	23	1	0	0	(**EF4130036**)
		R: GACCGGGGCTCAGTAAAACAC		21				
CS-17	(GC_5AC_{12})	F: GATCCCTCCTACACACACACG	60	21	2	0.35	0.34	(**EF4130037**)
		R: TATTAGGGCTGGACAGTCAAAGG		23				
CS-18	(A_{19})	F: GCAGAACCATAACACATGCACAG	60	23	2	0.28	0.24	(**EF4130038**)
		R: GCATTCCATAGTCCCCTCCATAG		23				
CS-20	$(C_{10}AC_5)$	F: CCTTCCGCCCAAAATAATTGC	60	21	1	0	0	(**EF4130040**)
		R: CCCTGCACTAACAAGGAGCAG		21				
CS-21	(AC_5)	F: ATTTCCACTCACAGCTTCAAACC	60	23	2	0.38	0.19	(**EF4130041**)
		R: GGATGCTTGTAGGTCTTGTTTAGC		24				
CS-22	(AC_7)	F: CTGTAGGCTGTGACAAAATCCTG	60	23	1	0	0	(**EF4130042**)
		R: CATAACCAACCAGAATGTGACTGC		24				

Table 1 (continued).

Locus	Repeats	Primer: (5′ → 3′)	Ta (°C)	Size length (bp)	No. of allelles	Ho	He	Accession no.
CS-24	(AC_{26})	F: GAACCCAGGAGGCAGGAGAG	68	20	2	0.14	0.50	(**EF4130043**)
		R: CACATACACAGAACCCAGGTGTG		23				
CS-25	(AC_{9})	F: ATCCTCAGTGTCATCTGCTCACC	65	23	1	0	0	(**EF4130044**)
		R: TCTTCCCTGCTCACCTTTCTTTC		23				
CS-26	(AC_{17})	F: GCCATGTGTACTAACTGGGAAGTC	65	24	2	0.55	0.49	(**EF4130045**)
		R: GCCATTTTTAGTCAGGTTGTTGC		23				
CS-28	(AC_{15})	F: CAGTCTCCAGCACTTGGGGATAG	68	23	2	-	-	(**EF4130046**)
		R: TTGGCTACAAAGGACCAACTCAC		23				
CS-30	(AC_{15})	F: ACTCACACTACCATCTCCAGCAC	68	23	2	0.24	0.44	(**EF4130047**)
		R: TGTGTGTCATGTGCGTGAACC		21				
CS-32	(AC_{17})	F: GTACCAAGCCCCTTTAACACCTG	68	23	3	0.45	0.68	(**EF4130048**)
		R: GGGGAGAAGGAAACTAGGAGAGG		23				
CS-33	(AC_{12})	F: ATCAACTTTCAGCCTGGGATAGG	68	23	4	-	-	(**EF4130049**)
		R: ATGTCTTATTGCTTCCCTTGCAC		23				
CS-35	(AC_{17})	F: GTCTGAAAAGGGTGTTTGTG	60	20	2	0.38	0.47	(**EF4130050**)
		R: CCTAAACTGTTAGAAGCCAAG		21				

staining. The size of the microsatellite alleles was determined by comparison with molecular weight DNA/*Hinf* I Markers (Promega), with their image patterns analyzed using the Kodak 1D Digital Science V. 3.0.2: Scientific Imaging System (Eastman Kodak Company, New Haven, CT) software. Allele frequencies, the number of alleles per locus and the expected and observed heterozygocities (He and Ho) were calculated using the GENEPOP software package (Raymond and Rousset, 2001).

RESULTS

Twenty microsatellite markers were amplified by PCR to assess the level of polymorphism in *C. siamensis* (n=29), *C. porosus* (n=4), a hybrid of *C. siamensis* + *C. porosus* (n=2) and *C. c. crocodilus* (n=5) as the out group. The PCR conditions used were optimal for amplification of the microsatellite markers from *C. siamensis* and thus may not have been optimal for the other species to test all taxa. The amplifications were successful across eighteen loci (*CS*-2, *CS*-4, *CS*-5, *CS*-10, *CS*-12, *CS*-14, *CS*-15, *CS*-17, *CS*-18, *CS*-20, *CS*-21, *CS*-22, *CS*-24, *CS*-25, *CS*-26, *CS*-30, *CS*-32 and *CS*-35) for the 40 crocodiles, with two markers (*CS*-28 and *CS*-33) generating ambiguous PCR products. Polyacrylamide gel electrophoresis illustrated characteristic polymorphism in some loci; *CS*-4, *CS*-26, *CS*-25, *CS*-17, *CS*-22, *CS*-35, *CS*-20 and *CS*-5 and the results are presented in Figures 1-8.

The PCR product from locus *CS*-4 (Figure 1), *CS*-26 (Figure 2), *CS*-17 (Figure 4), *CS*-35 (Figure 6), *CS*-5 (Figure 8), *CS*-32, *CS*-10, *CS*-12, *CS*-18, *CS*-21, *CS*-24 and *CS*-30 all showed a clear polymorphic pattern in *C. siamensis*. The average observed heterozygosity (Table 1) was 0.37 (range 0.14–0.61) and the average expected heterozygosity was 0.41 (range 0.12–0.68). Furthermore, absent alleles were detected for seven loci; *CS*-4 (Figure 1), *CS*-5 (Figure 8), *CS*-18, *CS*-21, *CS*-26 (Figure 2), *CS*-30 and *CS*-32 in *C. c. crocodilus* individuals.

The PCR products from loci *CS*-4 (Figure 1), *CS*-26 (Figure 2) and *CS*-30 respectively showed a clear pattern of a single specific band (arrow) in *C. porosus*. Additionally, all products scored were similar in size to the amplicons from *C. porosus*.

The PCR product from locus *CS*-25 (Figure 3) showed a clear pattern of a single specific band (arrow) in *C. siamensis*. Additionally, all products scored were similar in size to the amplicons from *C. siamensis*.

The PCR products from loci *CS*-2, *CS*-7, *CS*-10, *CS*-12, *CS*-14, *CS*-17 (Figure 4), *CS*-22 (Figure 5), *CS*-24 and *CS*-35 (Figure 6) showed a clear pattern of a single specific band (arrow) in *C. c. crocodilus*. Additionally, all products scored were similar in size to the amplicons from *C. c. crocodilus*.

The PCR product from locus *CS*-20 (Figure 7) showed a clear pattern of a single specific band (arrow) in *C. c. crocodilus* (arrow A), in *C. porosus* (arrow B) and in *C. siamensis* (arrow C). Additionally, all products scored were similar in size to the amplicons for each species.

The results showed that Ho was very low in *C. siamensis* (0.14-0.62) and revealed that most of the *C. siamensis* individuals sampled were inbred from the one population, or had been purebred in the crocodile farm. The PCR product size of the loci *CS*-4, *CS*-5, *CS*-10, *CS*-20, *CS*-25, *CS*-26, *CS*-30, *CS*-32 and *CS*-33 could be used to identify *C. siamensis* and *C. porosus* and the hybrid of *C. siamensis* and *C. porosus*. These results showed a difference in allele size in *C. siamensis*, *C. porosus* and *C. c. crocodilus* and some loci showed an absence of alleles in *C. c. crocodilus* (Figures 1, 2 and 8).

DISCUSSION

Even with these preliminary results, the

Figure 1 A sample of allelic polymorphism observed at locus *CS*-4. From left to right: lanes 1–6, 9-26, 29 and 33-36 *C. siamensis*, lanes 27-28 hybrid (*C. siamensis* + *C. porosus)*, lanes 7-8 and 37-38 *C. porosus*, lanes 30-32 and 39-40 *C. c. crocodilus* and control = wild *C. siamensis*.

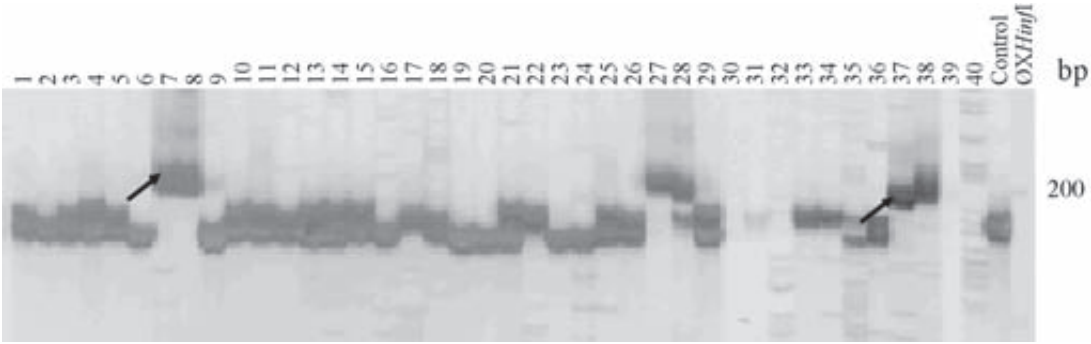

Figure 2 A sample of allelic polymorphism observed at locus *CS*-26. From left to right: lanes 1–6, 9-26, 29 *C. siamensis* and 33-36 *C. siamensis*, lanes 27-28 hybrid (*C. siamensis* + *C. porosus)*, lanes 7-8 and 37-38 *C. porosus*, lanes 30-32 and 39-40 *C. c. crocodilus* and control = wild *C. siamensis*.

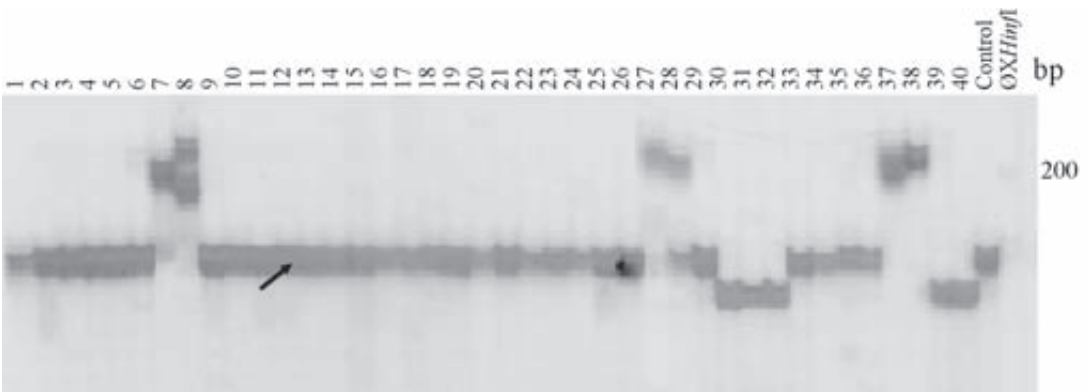

Figure 3 A sample of allelic polymorphism observed at locus *CS*-25. From left to right: lanes 1-6, 9-26, 29 *C.* and 33-36 *C. siamensis*, lanes 27-28 hybrid (*C. siamensis* + *C. porosus)*), lanes 7-8 and 37-38 *C. porosus*, lanes 30-32 and 39-40 *C. c. crocodilus* and control = wild *C. siamensis*.

initial application of these primers appeared promising. These data from populations of *C. siamensis, C. porosus* and *C. c. crocodilus* indicated that several loci would be quite useful in studies to identify the species of crocodile. In a captive population of *C. siamensis,* it appeared that there would be enough genetic variation to determine paternal exclusion in clutches. It is hoped that these primers will enable the application of genetic investigations in other studies of the order Crocodylia. As in other species, these genetic markers would offer the most powerful insight when they were combined with available demographic or behavioral data. Additionally, a combined approach using both mtDNA and microsatellite markers was considered to be the most effective means of elucidating questions on gene flow and population history. It is hoped that the availability of these microsatellite markers would allow comparative studies of mating systems and population structure among crocodilians.

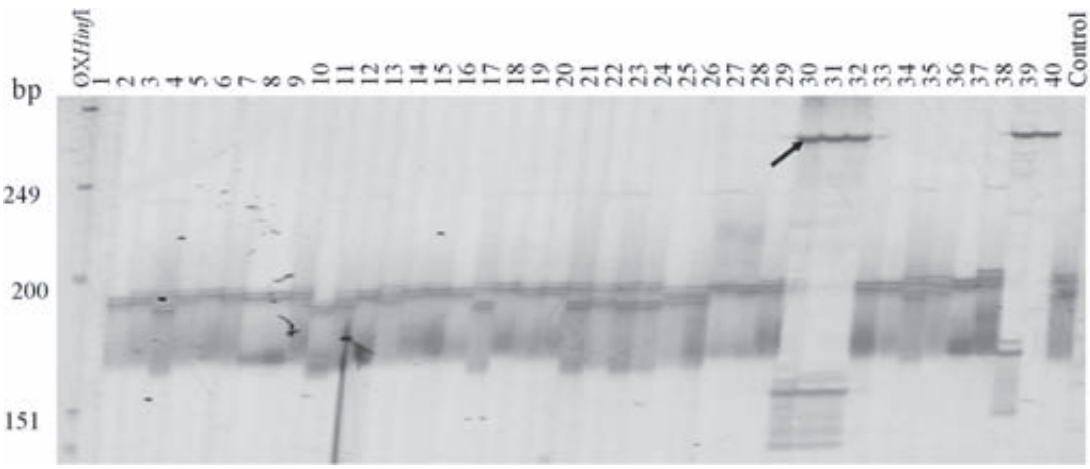

Figure 4 A sample of allelic polymorphism observed at locus *CS*-26. From left to right: lanes 1–6, 9-26, 29 and 33-36 *C. siamensis*, lanes 27-28 hybrid (*C. siamensis* + *C. porosus)*, lanes 7-8 and 37-38 *C. porosus*, lanes 30-32 and 39-40 *C. c. crocodilus* and control = wild *C. siamensis*.

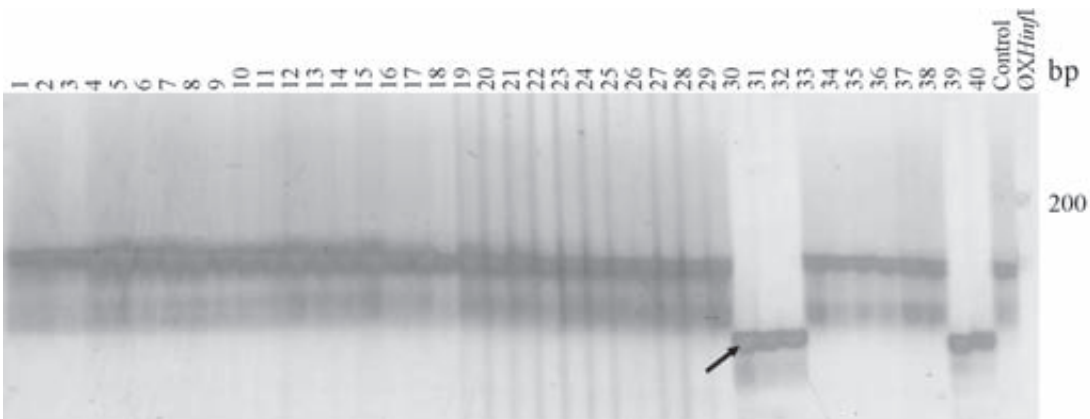

Figure 5 A sample of allelic polymorphism observed at locus *CS*-22. From left to right: lanes 1–6, 9-26, 29 *C.* and 33-36 *C. siamensis*, lanes 27-28 hybrid (*C. siamensis* + *C. porosus)*, lanes 7-8 and 37-38 *C. porosus*, lanes 30-32 and 39-40 *C. c. crocodilus* and control = wild *C. siamensis*.

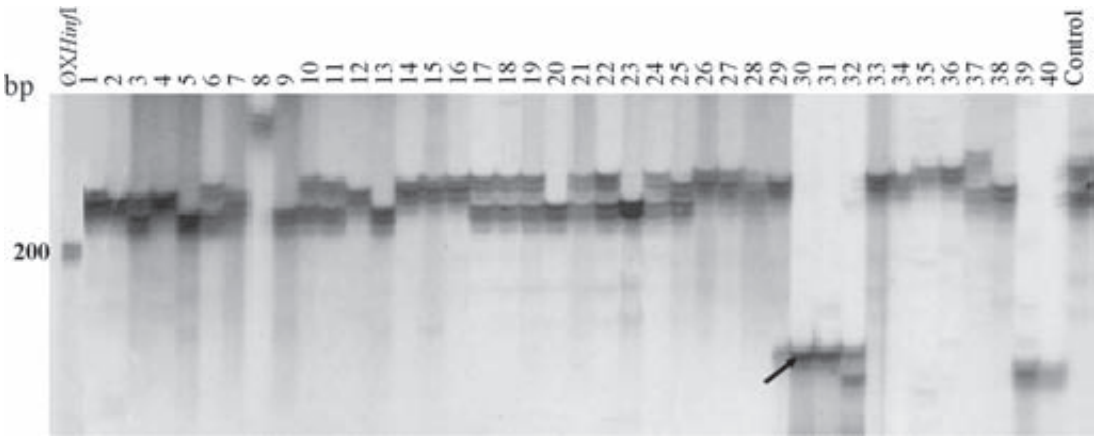

Figure 6 A sample of allelic polymorphism observed at locus *CS*-35. From left to right: lanes 1–6, 9-26, 29 and 33-36 *C. siamensis*, lanes 27-28 hybrid (*C. siamensis* + *C. porosus)*, lanes 7-8 and 37-38 *C. porosus*, lanes 30-32 and 39-40 *C. c. crocodilus* and control = wild *C. siamensis*.

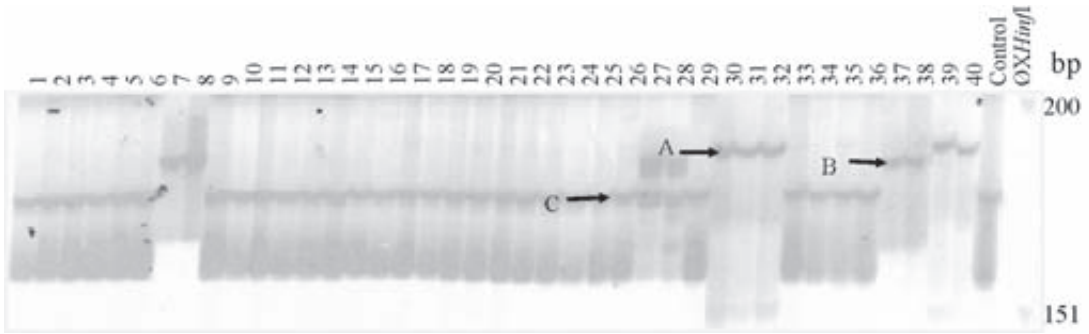

Figure 7 A sample of allelic polymorphism observed at locus *CS*-20. From left to right: lanes 1–6, 9-26 and 33-36 *C. siamensis*, lanes 27-28 hybrid (*C. siamensis* + *C. porosus)*, lanes 7-8 and 37-38 *C. porosus*, lanes 30-32 and 39-40 *C. c. crocodilus* and control = wild *C. siamensis*.

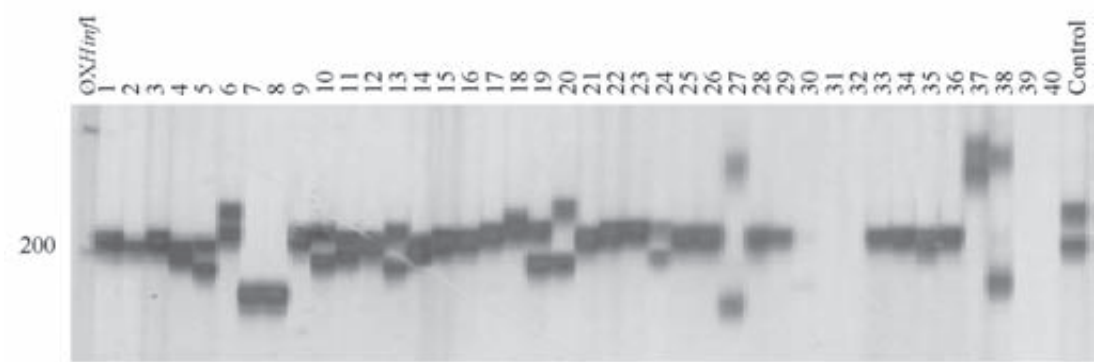

Figure 8 A sample of allelic polymorphism observed at locus *CS*-5. From left to right: lanes 1–6, 9-26, 29 *C. siamensis* and 33-36 *C. siamensis*, lanes 27-28 hybrid (*C. siamensis* + *C. porosus)*, lanes 7-8 and 37-38 *C. porosus*, lanes 30-32 and 39-40 *C. c. crocodilus* and control = wild *C. siamensis*.

The observed heterozygosity (Ho) was very low in *C. siamensis* ranging from 0.14-0.61. This indicated that most of *C. siamensis* have been inbred from the one population, or had been purebred in the crocodile farm. Thus, selection for individuals with higher heterozygosity as is typically recommended (Moritz, 1999) would be possible for planned reintroduction into a National Park. This strategy theoretically, would provide a greater range of options on which selection could be based, if indeed variation at the dinucleotide microsatellite loci provided a surrogate measure of variation in coding regions of the genomic DNA. Whether or not this assumption were met, selecting individuals with a higher heterozygosity (if all other concerns were equal) would remain a risk minimization strategy.

Within crocodile farms, intentional hybridization has been used to produce better-quality skins, or faster-growing offspring, but the inclusive fitness was unknown. Knowing the species status of individuals within these mixed populations would allow for such comparisons, if breeders could be paired in breeding pens. For the reintroduction effort, it would be important to confirm that the captive population consisted mostly of purebred C. *siamensis,* with some hybrid F1 and F2 individuals rather than an undetected admixture (Allendorf *et al*., 2001). In contrast, a captive population in Thailand may have had greater admixture between *C. siamensis* and *C. porosus,* as it included F2 hybrids and hybrid back crosses to both parental species (Chavananikul *et al*., 1994).

These results have been useful in identifying captive hybrids of the Siamese crocodile, *C. siamensis*. Using microsatellites to determine species status has followed on from previous genetic work that used karyotyping to determine hybrid *C. siamensis* x *C. porosus* in crocodile farms of Thailand (Chavananikul *et al*., 1994). These two species differ in chromosome number and have substantially different karyotypes, and so purebreds, F1 hybrids and F2 hybrids could readily be distinguished. However, *C. siamensis* and *C. porosus* each have a different arrangement of chromosome types and a more detailed analysis would be required to recognize hybrids.

Of equal concern in any reintroduction and augmentation program would be the possibility of breaking up locally-adapted gene complexes through outbreeding among different source populations (Storfer, 1999). If this were true for *C. siamensis*, then it would remain an inherent problem for this and other crocodilian reintroductions, because crocodile farms typically have individuals originating from widely-scattered populations. Records may not have been kept, individuals might not have been marked for identification and breeders would be typically put into large communal pens. One could argue that to avoid the problem of outbreeding depression, only the most productive lineages should be selected for reintroduction (if indeed this information is known). However, this would run the risk of selecting individuals who were best suited to captive rather than wild situations. The extreme contrast between outbreeding depression versus hybrid vigour is something that undoubtedly occurs in making comparisons.

CONCLUSION

This study evaluated 20 microsatellite markers from the Siamese crocodile (*C. siamensis*) and their transferablility to other Crocodylus species. Eighteen microsatellite-designed primers provided reliable amplifications. These microsatellite markers were evaluated in a captive breeding crocodile farm for species testing.

Fourteen microsatellite markers (77% of the total microsatellite loci) showed specific alleles that were considered as specific markers for the identification of the species of crocodile. There was one marker (*CS*-25) for *C. siamenis*, three

markers (*CS*-4, *CS*-26 and *CS*-30) for *C. porosus*, nine markers (*CS*-2, *CS*-7, *CS*-10, *CS*-12, *CS*-14, *CS*-17, *CS*-22, *CS*-24 and *CS*-35) for *C. c. crocodilus* and one marker (*CS*-20) that could be used to identify all species.

Twelve microsatellite markers (66% of the total microsatellite loci) showed polymorphic alleles in the same species that were considered as intra-specific markers. They were: *CS*-4, *CS*-5, *CS*-10, *CS*-12, *CS*-17, *CS*-18, *CS*-21, *CS*-24, *CS*-26, *CS*-30, *CS*-32 and *CS*-35. These markers might be useful in paternity testing and hybridization breeding.

Seven microsatellite markers (38% of the total microsatellite loci) showed as an absent allele in *C. c. crocodilus* and were considered as genus-specific markers. These markers: *CS*-4 (Figure 1), *CS*-5, *CS*-18, *CS*-21, *CS*-26, *CS*-30 and *CS*-32 might be useful in family testing.

It was therefore recommended that more microsatellite markers be used for species identification and family testing. The use of these microsatellite markers would broaden the scope of a breeding program, allowing progeny from adults maintained in large breeding lagoons to be tested for selection as future breeding animals.

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LITERATURECITED

Allendorf, F. W., R. F. Leary, P. Spruell and J. K. Wenburg. 2001. The problems with hybrids: setting conservation guidelines. **Trends. Ecol. Evol.** 16: 613-622.

Chaeychomsri, W., P. Tabthipwon, N. Noparatnaraporn and V. Siripholvat . 2008. Development of Microsatellite Markers for Siamese Crocodile (*Crocodylus siamensis*). **Kasetsart J. (Nat. Sci.)** 42(2): 256-262.

Chavananikul, V., S. Wattanodorn and P. Youngprapakorn. 1994. Karyotypes of 5 species of crocodiles kept in Samutprakan crocodile farm and zoo, pp. 58-62. *In* **Proceeding of the 12th Working Meeting Crocodile Specialist Group**, 2-6 May, 1994. Pattaya.

Davis, L. M., T. C. Glenn, R. M. Elsey, H. C. Dessauer and R. H. Sawyer. 2001. Multiple paternity and mating patterns in the American alligator, Alligator mississippiensis. **Mol. Ecol.** 10: 1011-1024.

Dib, C., S. Faure, C. Fizames, D. Samson, N. Drouot, A. Vignal, P. Millasseau, S. Marc, J. Kazan , E. Seboun, M. Lathrop, G. Gyapay, J. Morissette and J. Weissenbach. 1996. A comprehensive genetic map of the human genome based on 5,264 microsatellites. **Nature** 380: 152-154.

FitzSimons, N. N., S. Tanksley, M. R. Forstner, E. E. Louis, R. Daglish, J. Gratten and S. Davis. 2001. Microsatellite markers for Crocodylus: new genetic tools for population genetics, mating system studies and forensics, pp. 51-57 *In* G. Grigg, F. Seebacher and C.E. Franklin, (eds.). **Crocodilian Biology and Evolution.** Surey Beatty and Sons, Chipping Norton, Australia.

Glenn, T. C., H. C. Dessauer and M. Braun. 1998. Characterization of microsatellite DNA loci in American Alligators. **Copeia** 1998: 591-602.

Goldstein, D. B. and C. Schlötterer. 1999. **Microsatellites: Evolution and Applications**. Oxford University Press, Oxford.

Harr, B., S. Weiss, J. R. David, G. Brem and C. Schl÷tterer. 1998. A microsatellite-based multilocus phylogeny of the *Drosophila melanogaster* species complex. **Curr. Biol**. 8: 1183–1186.

Moritz, C. 1999. Conservation units and translocations: strategies for conserving evolutionary processes. **Heriditas** 13: 217-228.

Raymond, M. and F. Rousset. 2001. **Genepop on the Web** [Online]. Available at http://wbiomed.curtin.edu.au/genepop (cited 7 May 2001; modified 2 Feb. 2004; verified 19 Feb. 2004). Laboratiore de Genetique et Environment, Montpellier, France.

Ross, J. P. 1998. Crocodiles, **Status Survey and Conservation Action Plan** (online). 2nd edition IUCN/SSC Crocodile Specialist Group. IUCN, Gland, Switzerland. 167 pp. Available: http://www.flmnh.ufl.edu/natsci/herpetology/act-plan/plan1998a.htm

Schlötterer, C. and J. Pemberton. 1998. The use of microsatellites for genetic analysis of natural populations-a critical review, pp. 71–86 *In* R. Desalle and B. Schierwater, (eds.). **Molecular approaches to ecology and evolution.** Birkhäuser , Basel, Switzerland.

Storfer, A. 1999. Gene flow and local adaptation in a sunfish-salamander system. **Behavioral Ecology and Sociobiology** 46: 273-279.