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Genetic marker for the Cuban nesting population of the hawksbill turtle, *Eretmochelys imbricata*, and its contribution to the foraging populations

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Keywords: genetic marker, natal homing, mitochondrial control region, hawksbill turtle, Cuba

Abstract: The mitochondrial control region was analysed for the population genetic structure of Cuban population of the hawksbill turtle, *Eretmochelys imbricata*. Among 30 haplotypes detected in a 480 bp fragment of the mitochondrial control region for 218 individuals in the Caribbean region, dominant haplotype in the Cuban nesting population was CB1, in company of CB2, CB3 and CB4, showing that the Cuban nesting population has an specific genetic markers. Contribution rates for the foraging samples from Doce Leguas at Zone A (south-east shelf) was 67%, indicating that the majority of hawksbill turtle migrate around a home range near the nesting site. It is interesting that the contribution rates were decreased remarkably from Zone A to Zones B at south-west shelf (43%) and D at north-east shelf (41%). The contribution rates in Zone B showed higher in Autumn (53%), than that in Spring (33%), which coincided to nesting season from September to December based on the presence of eggs in adult female individuals.

Introduction

Marine turtle is one of the well-studied animals on the molecular evolution. Avise *et al.* (1992) and Bowen *et al.* (1993) intensively studied restriction-site (RFLP) and nucleotide sequence analyses for cytochrome b region in the mitochondrial DNA (mtDNA) of the marine turtles at intraspecific and interspecific levels, and showed that cytochrome b region in the mtDNA evolution of turtles proceeds at a several-fold lower rate than the conventional vertabrate pace. Nuclear DNA analysis also showed a pattern of low nucleotide diversity (Karl *et al.*, 1992).

As loggerhead and green turtles (Witzell, 1983), adult females of the hawksbill turtle, *Eretmochelys imbricata*, migrate to a nesting sites every few years, after pelagic habitats at foraging grounds. The nesting populations are less colonial than other marine turtles, partly due to declining of population in recent decades (Carr and Meylan, 1980). Concerning to their natal homing, Broderick *et al.* (1994) first reported significant differences in mtDNA haplotype frequency between nesting areas in northeastern and northwestern Australia. Bass *et al.* (1996) documented significant mtDNA haplotype frequency shifts among seven rookeries in the Atlantic region. Espinosa *et al.* (1996) also analysed by RFLP methods based on total mtDNA and a fragment of the mtDNA control region using nesting samples from Cuba and Mexico, showing that Mexican samples contained a specific haplotype found in no other Cuban samples.

Hawksbill turtle is considered as less migratory than other marine turtles (Limpus, 1992). Tag survey in Cuba between 1989 and 1995 (Moncada,1996) showed that all the recoveries of the hawksbill tutle released in Cuban waters were within Cuban waters, while 28 recoveries of *Chelonia mydas* released in Cuban waters were consisted of 14 from within Cuban waters and 14 from other countries. Broderick *et al.* (1994) compared the mtDNA genotypic distributions observed in 4 Australian nesting populations to those from two feeding areas in northern Australia, suggesting that substantial recruitment into these foraging areas must have occurred from more distant nesting sites. Bowen *et al.* (1996) used mitochondrial control region sequence data from 7 west Atlantic nesting populations (Bass *et al.*, 1996) to estimate the contribution of regional rookeries to a foraging area at

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Mona Island in Puerto Rico, concluding that this feeding ground is not composed primarily of turtles from the adjacent nesting beach on Mona Island, but is occupied by turtles from nesting populations throughout the Caribbean region. Koike *et al.* (1997) examined a longer sequence in the control region, and were able to discern an important polymorphic sites, which allowed some haplotypes described by Bowen *et al.* (1996) and Bass *et al.* (1996) to be subdivided into new haplotypes.

In this paper we describe haplotypes for 70 individuals of the nesting populations from Doce Leguas, to set up genetic marker of the rookery of the hawksbill turtle in Cuba, with comparison to those from Perto Rico and Mexico. Subsequently contribution rate, that is, a ratio of the individuals having the Cuban haplotypes to total individuals analysed was examined using 218 individuals from 6 different foraging areas in Cuba, to estimate how nesting populations were migrating in the Caribbean sea through their life history.

Sampling of nesting and foraging populations in Cuba

For fisheries management purposes, the Cuban shelf is divided into 4 Fisheries Zones: Zone A at the south-eastern shelf, Zone B at the south-western shelf, Zone C at the north-western shelf and Zone D at the north-eastern shelf in Cuba (Fig. 1).

Doce Leguas archipelago (Fig. 2) is located at the edge of the Zone A, about 40 km south from the mainland. It is composed of tens of medium and small keys extended from east to west in around 120 km long. Sea waters temperatures in and around Doce Leguas are higher than those found elsewhere in Cuba, reaching a maximum of around 30°C in summer and drop to around 26°C in winter. Doce Leguas archipelago is fully protected and constitutes the most studied until now.

At north of their keys, interior waters are very extensive and shallow, never reach deeper than 30 meters. Coralline sand beaches are abundant in the south face of the keys, providing nesting beach to the hawksbill turtle.

On the other hand south edge shelf is narrow, protected by a vast coral reef barrier from the oceanic currents, and the depth drop sharply around 1000 meters. The coral reef barrier is abundant in sponge species, which is main diet for adult individuals of the hawksbill turtle (Anderes and Uchida, 1994).

According to the nesting surveys in Cuba (Moncada *et al.* 1997), main peaks of nesting are December for the Zone A (Doce Leguas), and September for the Zone C. About two-thirds of the nests are found at Doce Leguas, where is not caused any significant impacts from human development.

A total of 35 nesting beaches along the keys (Fig. 2) were responsible for the 65% of the Cuban total nesting effort. Nests of the hawksbill turtle happen to be in the sand beaches after the high tide zone, in beaches with low pendent dune and vegetation as their

Fig. 1. Locations of rookeries and foraging areas of the hawksbill turtle in Cuba Puerto Rico and Mexico.

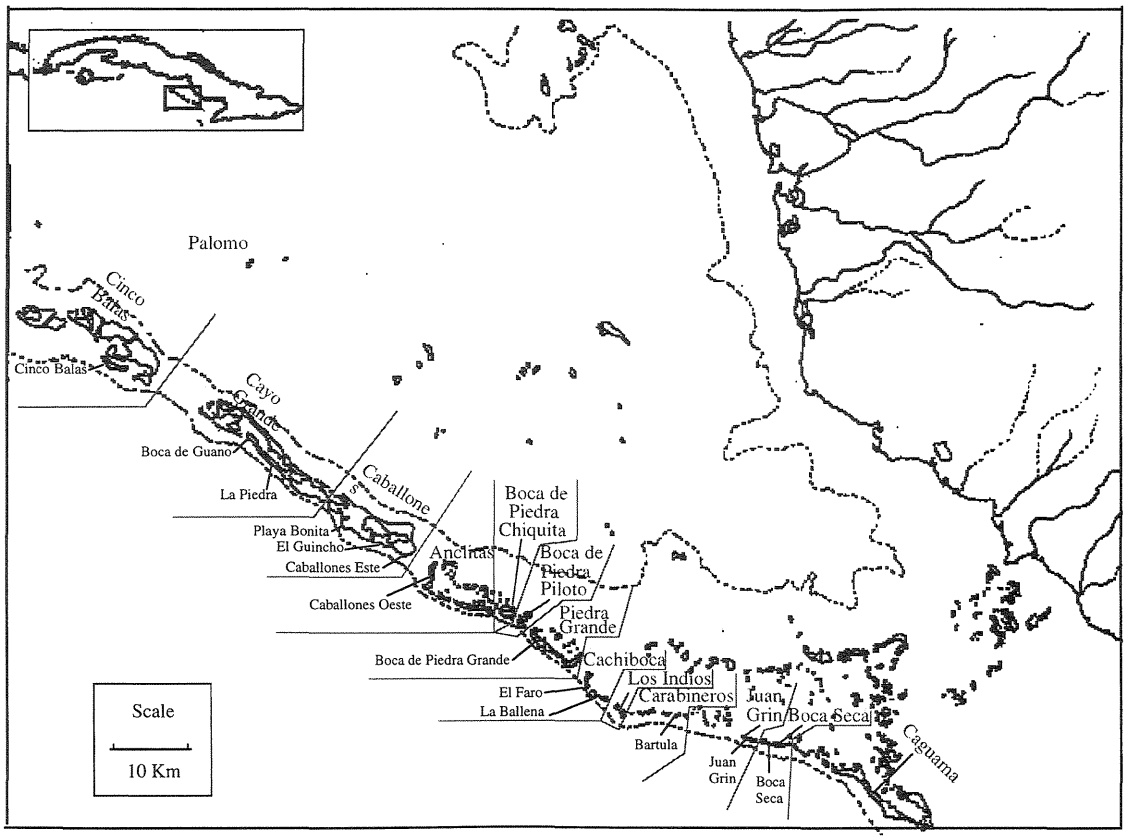

Fig. 2. Location of the nesting beaches at Doce Leguas in Cuba.

cover.

A total of 288 samples analyzed, consisting of 70 nesting samples from Doce Leguas and 218 foraging samples from 6 locations of the Cuban shelf, were offered by the Ministry of Fisheries of Cuba for the DNA project of the hawksbill turtle supported by the Japan Bekko Association.

Nesting samples were collected in the following three categories: 12 scutes samples were collected at breeding center of Isla de Pinos in 1995 (Appendix 1), which were assumed to be transported from Doce Leguas nesting site. In 1994 and 1997 the Cuban turtles research team carried out nesting surveys at Doce Leguas and collected 24 and 34 nesting samples, respectively (Appendixes 2 and 3).

In Zone A, a total of 44 foraging samples analyzed were collected by the Cuban turtle research team during tagging surveys: 23 samples from Doce Leguas in 1992 (Appendix 4), 13 samples in 1997 (Appendix 5), and 8 samples from Santa Cruz in 1993 (Appendix 6).

A total of 123 foraging samples in Zone B were collected as traditional harvest; 40 samples collected in spring of 1996 and 1997 (Appendix 7) and 75 samples collected in autumn of 1996 (Appendix 8).

In Zone D, A total of 59 foraging samples analyzed were 17 samples during tagging surveys in 1992-93 seasons from Nuevitas (Appendix 9), 15 samples also collected during tagging surveys from Las Tunas in the 1993-94 seasons (Appendix 10), 9 collected from Cayo Romano as traditional harvest (Appendix 11), and 18 samples from the Northern Sea during 1995 season (Appendix 12).

DNA extraction

In the case of scute samples, about 10 mg of powder was taken from the edge of the inner- surface of the scutes, avoiding to scrape white-wax-like residues on the surface. As soft tissues, muscle, heart and liver were taken from sacrified individuals collected from the traditional harvest sites or during research surveys. Skin samples were taken for the rear flipper of living and tagged individuals. All the soft tissues were immediatly preserved in 70% ethanol and stored at room temperature. About 1 ml of blood was taken from the cervical sinus of the living adult individuals and placed in a tube in 50 mM EDTA solution, and stored at 4°C.

About 10 mg of the scute, or about 20 mg of soft tissue in 70% ethanol, or 100 μl of blood samples was placed in 310 μl of RSB buffer, 15 μl of 10% SDS and 25 μl of 20 mg/ml Proteinase k for protein digestion, and incubated for 2 hours at 55°C on a rotator. Then nucleic acids were extracted using an IsoQuick Nucleic Acid Extraction Kit (ORCA Research Inc.; U.S.

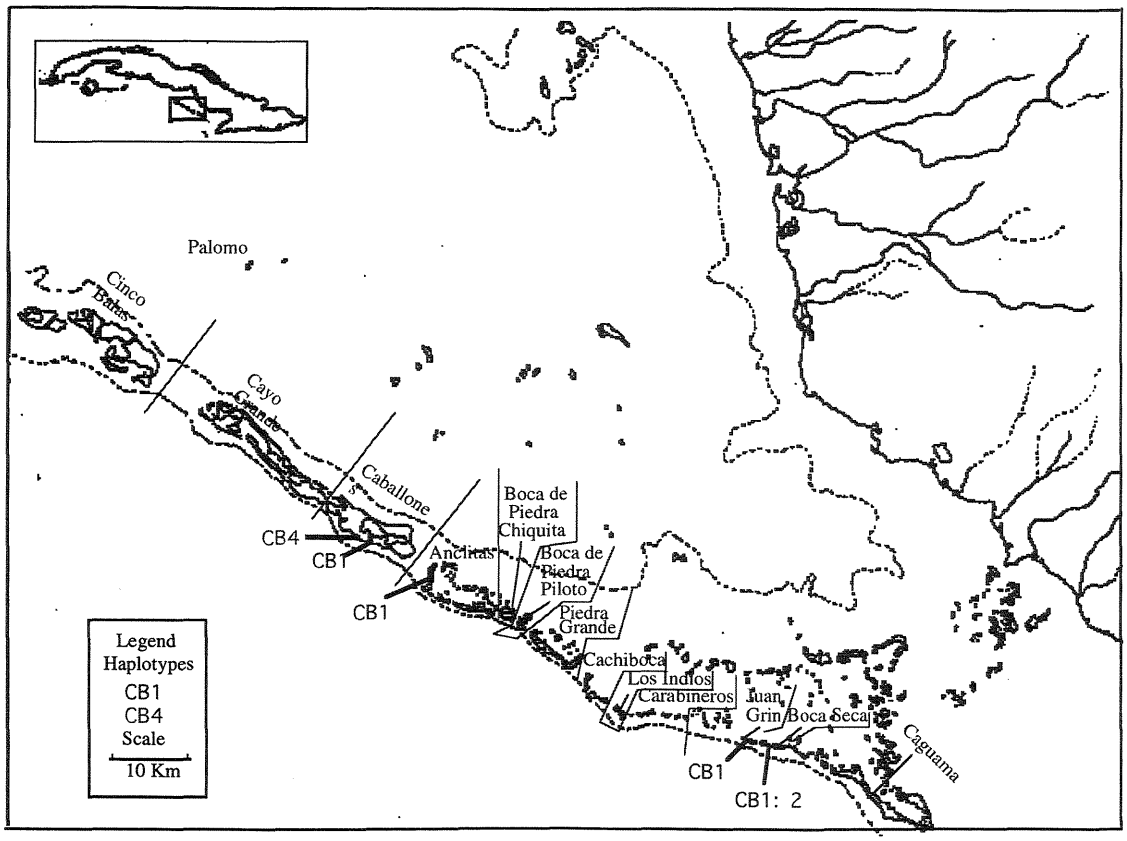

Fig. 3. Haplotypes of the nesting population in 1994 at Doce Leguas in Cuba.

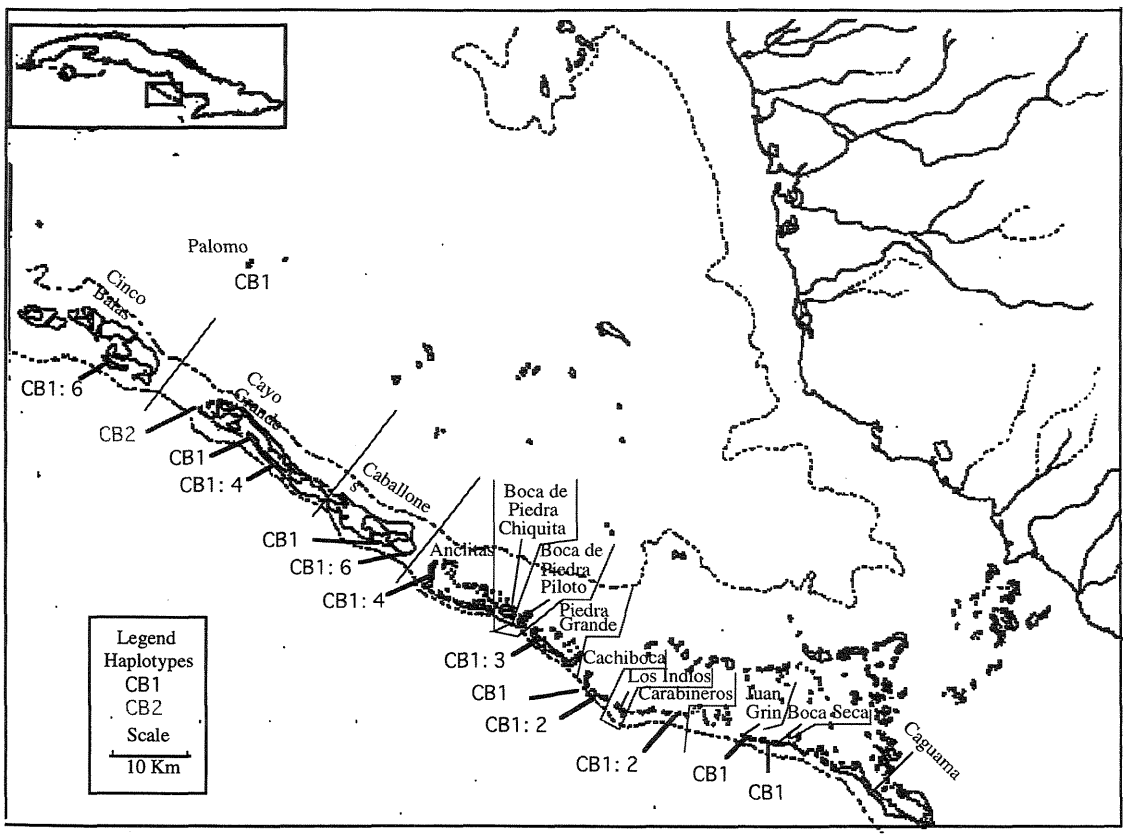

Fig. 4. Haplotypes in the nesting population in 1997 at Doce Leguas in Cuba.

Table 1. Polymorphic sites and haplotype correspondance among Koike *et al.* (1997), Bass *et al.* (1997) and Espinosa (1997)

sites from top of D-loop→			11	99	123	134	137	138	143	144	145	171	173	181	184	200	201	218	227	241	280	287	332	341	350	366	374	377	379	431	432	433	437	439	463
Bass's sites →			(−114)	(−25)	(−1)	10	13	14	19	20	(21)	(47)	(49)	59	62	78	79	96	105	(119)	158	(164)	210	(219)	(228)	(244)	(252)	(255)	257	309	310	(311)	315	(317)	341
Espinosa	Bass	Koike	polymorphic sites																																
1	A	CB1	C	C	A	A	A	G	A	C	T	A	A	T	T	A	C	A	G	A	C	A	T	G	A	G	A	T	A	T	G	A	G	T	G
	G	i	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	O	PR4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	G	–	–	–	–	–	–
	α	g	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	C	–	–	–	–	–	–	–
	E		**	**	**	–	–	–	G	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	H		**	**	**	–	–	–	–	–	–	–	–	–	–	–	–	–	A	–	–	–	–	–	–	–	–	–	–	–	–	T	–	–
	β		**	**	**	–	–	–	–	–	–	–	–	–	C	–	–	–	–	–	T	–	C	–	–	–	–	–	–	G	–	–	–	–	A
		a	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	G	–	–	–	–	–	–	–	–	–	–	–	–	–
		b	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	C	–	–	–	–	–	–	–	–	–	–	–	–
		f	–	–	–	–	–	–	–	–	–	G	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
		n	–	–	–	–	–	–	–	–	C	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
		ZZ	T	–	–	–	–	–	–	–	–	–	G	–	–	–	T	–	–	–	T	–	C	A	G	A	–	–	–	C	A	–	–	–	A
2	C		**	**	**	–	G	A	–	–	–	–	–	–	–	–	–	G	–	–	T	–	C	–	–	–	–	–	–	C	–	–	–	–	A
3	B	e	–	T	G	–	G	–	–	–	–	–	–	–	–	–	–	G	–	–	T	–	C	–	–	–	–	–	–	C	–	–	–	–	A
	D		**	**	**	–	G	–	–	–	–	–	–	=	–	–	–	–	–	–	T	–	–	–	–	–	–	–	–	C	–	–	–	–	A
	F	c	–	T	–	–	G	–	–	–	–	–	–	–	C	–	–	G	–	–	T	–	C	–	–	–	–	–	–	C	–	–	–	–	A
		j	–	–	G	–	G	–	–	–	–	–	–	–	C	–	–	G	–	–	T	–	C	–	–	–	–	–	–	C	–	–	–	–	A
		PR1	–	T	G	–	G	–	–	–	–	–	–	–	C	–	–	G	–	–	T	–	C	–	–	–	–	–	–	C	–	–	–	–	A
	I		**	**	**	T	G	–	–	–	–	–	–	–	C	–	–	G	–	–	T	–	C	–	–	–	–	–	–	C	–	–	–	–	A
	J		**	**	**	–	G	–	–	–	–	–	–	–	C	–	–	–	–	–	T	–	C	–	–	–	–	–	–	C	–	–	–	–	–
	K		**	**	**	–	G	–	–	–	–	–	–	–	C	–	–	–	–	–	–	–	C	–	–	–	–	–	–	C	–	–	–	–	–
	P		**	**	**	–	G	–	–	–	–	–	–	–	C	G	–	–	–	–	T	–	C	–	–	–	–	–	–	C	–	–	–	–	A
	Q	MX1	–	T	–	–	G	–	–	–	–	–	–	–	C	–	–	–	–	–	T	–	C	–	–	–	–	–	–	C	–	–	–	–	A
		MX2	–	T	G	–	G	–	–	–	–	–	–	–	C	–	–	–	–	–	T	–	C	–	–	–	–	–	–	C	–	–	–	–	A
	γ	CB2	–	T	G	–	G	–	–	–	–	–	–	–	C	–	–	–	–	–	T	–	C	–	–	–	G	–	–	C	–	–	–	–	A
		CB3	–	T	G	–	G	–	–	–	–	–	–	–	–	–	–	–	–	–	T	–	C	–	–	–	–	–	–	C	–	–	–	–	A
		CB4	–	T	G	–	G	–	–	–	T	–	–	–	C	G	–	G	–	–	T	–	C	–	–	–	–	–	–	C	–	–	–	–	A
		d	–	–	G	–	G	–	–	–	–	–	–	–	C	–	–	–	–	–	T	–	C	–	G	–	–	–	–	C	–	–	–	–	A
		h	–	T	G	–	G	–	–	–	–	–	–	–	C	–	–	G	–	–	T	–	C	–	–	–	–	–	G	C	–	–	–	–	A
		m	–	T	–	–	G	–	–	–	–	–	–	–	C	–	–	G	–	G	T	–	C	–	–	–	–	–	–	C	–	–	–	C	A
		o	–	T	G	–	G	–	–	–	–	–	–	–	C	–	–	–	–	–	T	–	C	–	–	–	–	–	G	C	–	–	–	–	A
		MX3	–	T	–	–	G	–	–	–	–	–	–	–	C	G	–	–	–	–	T	–	C	–	–	–	–	–	–	C	–	–	–	–	A
4	L	PR3	–	T	G	–	G	–	–	–	–	–	–	–	C	–	–	–	–	–	T	–	C	–	–	–	–	–	–	C	A	–	–	–	A
	M		**	**	**	–	G	–	–	–	–	–	–	–	C	–	–	–	–	–	–	–	C	–	–	–	–	–	–	–	–	–	–	–	–
	N	PR2	–	T	G	–	G	–	–	–	–	–	–	–	C	–	–	G	–	–	T	–	C	–	–	–	–	–	–	C	A	–	–	–	A
		l	–	T	–	–	G	–	–	–	–	–	–	–	C	–	–	G	–	–	T	–	C	–	–	–	–	–	–	C	–	T	–	–	A
		p	–	T	–	–	G	–	–	–	–	–	–	–	C	–	–	G	–	–	T	–	C	–	–	–	–	–	–	–	–	–	–	–	–
		q	–	T	–		G	–	–	T	–	–	–		C	–	–	–	–	–	T	–	C	–	–	–	–	–	–	C	–	–	–	–	A

MX1a, which has a 10-bp repert (GCCTCTGGTT) at 476th position, is excluded in this table

A.).

DNA amplification and sequencing

Extracted DNA were amplified by the Polymerase Chain Reaction (PCR) method. For the mitochondrial control region of the hawksbill turtle, universal primer L15926 (5'-TCAAAGCTTACACCAGTCTTGTAAACC-3') (Irwin *et al.*, 1991) and sea-turtle specific primer TCR6 (5'-GTACGTACAAGTAAAACTACCGTATGCC-3') (Norman *et al.*, 1994) were used and produced sufficient amplification of the control region. CONT1 (5'-TGTACTATTGTACATCTACTTA), CONT2 (5'-GTCACAGTAATGGGTTATTTCT) and CONT3 (5'-TTTCTCGTGATGAGCTGAAC), were designed to amplify shorter fragments for the scute samples (Koike *et al.*, in press). The PCRs were done with a Takara PCR thermal cycler MP with 30 cycles of denaturation at 94°C for 30 sec., annealing at 45°C for 45 sec., and extention at 72°C for 45 sec.

Direct sequencing was done in a Pharmacia L.K.B., R.O.B. DNA Processor (Pharmacia L.K.B. Co. Ltd.) with a Thermosequenase cycle sequencing kit (Amersham), using Cy5 fluorescence primers of the same sequences used in the PCRs. The cycle was repeated 20 times with denaturation at 94°C for 30 sec., annealing and extention at 65°C for 30 sec. Sequencing was done with an ALFred DNA Autosequencer (Pharmacia L.K.B. Co. Ltd.). Alignment of the sequence data was performed with a BioResearch/AE (Fujitsu Ltd.) based on CLUSTAL V (Higgins *et al*, 1992) and CLUSTAL W (Thompson *et al.*, 1994), with gap penalty 2. A Neighbor-Joining tree (Saitou and Nei, 1987) was constructed using BioResearch/SINCA Version 3 (Fujitsu Ltd.).

Results

Polymorphic sites and haplotypes

Twenty-eight polymorphic sites were observed in a 480 bp fragment of the mitochondrial control region for 70 nesting samples and 218 foraging samples from Cuba. The haplotype table (Table 1) defined 30 haplotypes based on 259 individuals from the Caribbean region (Koike *et al.*, 1997). These sequences were longer than those used by Bass *et al.* (1996) and, as a result, an additional 3 polymorphic sites at 11th,

Table 2. Haplotype frequencies of the foraging samples in Cuba.

Sampling Point	Samples	Haplotypes																			
	(n)	CB1	CB2	CB3	CB4	PR1	PR2	PR3	MX1	MX2	a	c	e*	g	i**	l	m	n	o	p	
Nesting samples																					
Breeding Center (95TS186-197)	12	9	2			1															
Doce Leguas (96TS101-125)	24	20	2	1	1																
Doce Leguas (96TS166-199)	34	33	1																		
TOTAL	**70**	**62**	**5**	**1**	**1**	**1**															
Foraging samples																					
Zone A (South-East)	**44**	**28**	**1**	**3**	**1**	**5**	**0**	**0**	**0**	**2**	**0**	**1**	**0**	**2**	**0**	**1**	**0**	**0**	**0**	**0**	
Doce Leguas (94TS105-127)	23	18	1	1	1	1										1					
Doce Leguas (96TS221-233)	13	5		2		1				2		1		2							
Santa Cruz (94TS154-162)	8	5				3															
Zone B (South-West)	**115**	**46**	**5**	**2**	**0**	**24**	**10**	**1**	**4**	**7**	**2**	**10**	**0**	**1**	**0**	**1**	**0**	**0**	**1**	**0**	**1*** **
Isla de Pinos: Spring (96TS234-268, 348-358)	40	9	3	1		12	3	1	4	2		4							1		
Isla de Pinos: Autumn (96TS269-347)	75	37	2	1		12	7			5	2	6		1		1					1***
Zone D (North-East)	**59**	**18**	**4**	**2**	**2**	**13**	**3**	**2**	**5**	**1**	**1**	**0**	**1**	**2**	**1**	**1**	**1**	**2**	**0**	**0**	
Nuevitas (94TS128-133, etc)	17	7			1	4		1	1		1			1			1				
Las Tunas (94134-139, 142-150)	15	4			1	4		1					1	1	1	1		1			
Cayo Romano (95TS225-234)	9	2	1			2	1		3												
Others (95TS205-224)	18	5	3	2		3	2		1	1								1			
TOTAL	**218**	**92**	**10**	**7**	**3**	**42**	**13**	**3**	**9**	**10**	**3**	**11**	**1**	**5**	**1**	**3**	**1**	**2**	**1**	**0**	**1*** **

*Haplotype e corresponds to haplotype B by Bass *et al.* (1996) from rookery in Antigua.
**Haplotype i corresponds to haplotype G by Bass *et al.* (1996) from rookery in Belize.
***Pacific haplotype

Table 3. Haplotype frequencies for three rookeries in the Caribbean sea region.

Rookery	Total	Haplotypes											
	(n)	CB1	CB2	CB3	CB4	PR1	PR2	PR3	PR4	MX1	MX1a	MX2	MX3
Doce Leguas in Cuba	70	62	5	1	1	1							
Las Coloradas in Mexico	53									48	1	3	1
Mona Island in Puerto Rico	20					12	6	1	1				

Table 4. Contribution rates of the foraging samples in Cuba.

Foraging samples	Samples	Haplotype frequencies by nesting population			
		Cuban	Puerto Rican	Mexican	Unknown
	(n)	%	%	%	%
Zone A (South-East)	**44**	**67**	**17**	**7**	**11**
Doce Leguas (94TS105-127)	23	83	4	4	9
Doce Leguas (96TS221-233)	13	54	8	15	23
Santa Cruz (94TS154-162)	8	63	38	0	0
Zone B (South-West)	**115**	**43**	**33**	**11**	**14**
Isla de Pinos: Spring (96TS234-268, 348-358)	40	33	40	15	13
Isla de Pinos: Autumn (96TS269-347)	75	53	25	7	15
Zone D (North-East)	**59**	**41**	**31**	**16**	**12**
Nuevitas (94TS128-133, etc)	17	41	29	12	18
Las Tunas (94TS134-139, 142-150)	15	33	33	7	27
Cayo Romano (95TS225-234)	9	33	33	33	0
Others (95TS205-224)	18	56	28	11	6

99th, and 123rd bp from the begging of the control region were found, indicating haplotype correspondance between our results and Bass *et al.* (1996) and Espinosa *et al.* (1996), haplotypes F and Q as reported by Bass *et al.* (1996) were subdivided into haplotypes PR1, c and j, and haplotypes MX1 and MX2, respectively.

Haplotype frequencies for nesting populations

Haplotype frequencies for nesting populations from Doce Leguas (Table 2) showed that CB1 is the dominant haplotype, making the 89% (62 of 70 individuals) of all nesting samples. Also it appears less represented haplotypes CB2, CB3 and CB4 (5, 1, and 1 individuals, respectively) from Doce Leguas.

In Table 3, haplotype frequencies for nesting populations from Cuba, Puerto Rico and Mexico (Koike *et al.* 1997) is listed, indicating that haplotype PR1 was dominant (12 of 20 individuals) in the Puerto Rican nesting population. Mexican nesting population exhibited mainly haplotype MX1 (48 of 53 individuals). This suggests that each nesting population has specific haplotypes as a genetic marker. One exception was haplotype PR1, which was the main haplotype for the Puerto Rican nesting population, but was also detected in 1 individual from the Cuban nesting population.

Haplotype frequencies for foraging populations

Haplotype frequencies for the foraging population were examined to estimate the contribution rate. The contribution rate was directly calculated as a ratio of the individuals having the Cuban haplotypes to total individuals analysed, not using the maximum

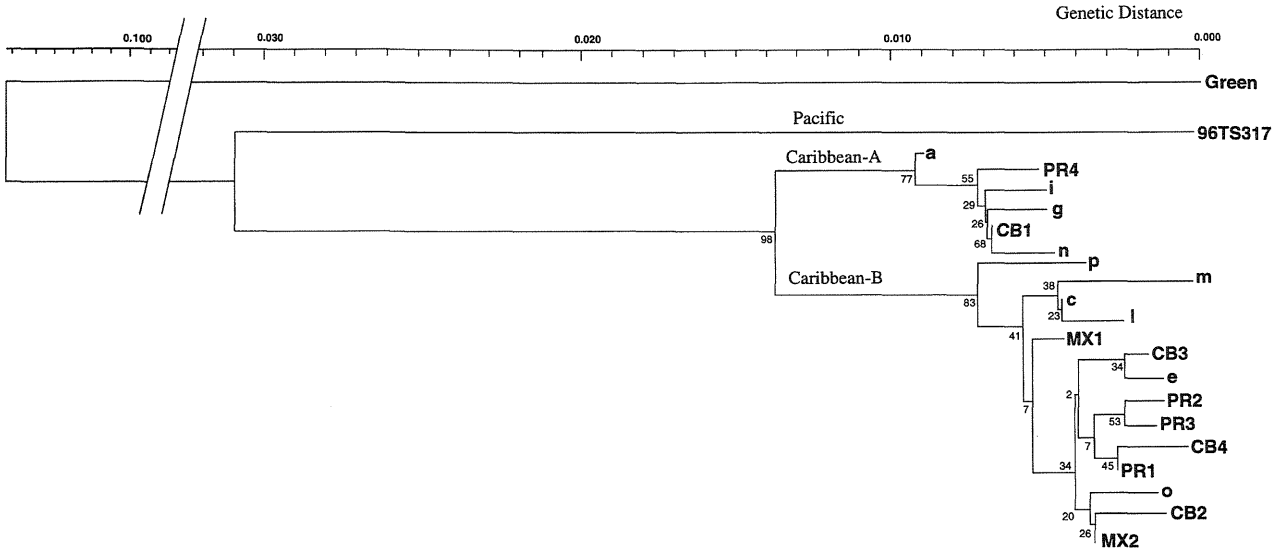

Fig. 5. Phylogenetic tree based on Neighbor-Joining method of the haplotypes found in the Cuban samples. Green turtle and one found Pacific haplotypes were used as outergroups.

likelihood method as Bowen *et al.* (1996), because haplotypes of the nesting populations we examined were not overlapped.

Foraging samples from Zone A consists of three groups: 23 and 13 samples from Doce Leguas collected in 1992 and in 1997, and 8 samples from Santa Cruz in 1993. Their contribution rates were 83%, 54% and 63%, respectively with average of 67%. Puerto Rican nesting haplotype PR1 and Mexican nesting haplotype MX2 were detected at 11% and 7%, respectively. The source-unknown haplotypes, which are not identified as the nesting haplotype examined, were c, g, o and p, reaching at 11%.

Foraging samples from Zone B were divided into samples collected in spring and those in autumn. It is noted that contribution rates were significantly different between 53% for the samples collected in autumn and 33% for those collected in spring with an average of 43%. Puerto Rican and Mexican nesting haplotypes were fully represented in this Zone with 30% and 11% respectively. Source-unknown haplotypes a, c, g, l , o were also presented in 13%. It should be noted that one indidividual was identified as an haplotype from the Pacific Region.

Foraging samples from the Zone D were those from Nuevitas, those from Las Tunas, those from Cayo Romano, and those belonging to the Northern Sea collected by the fishermen. Contribution rates were at 41%, 33%, 33% and 56%, respectively with an average of 41%. Puerto Rican and Mexican nesting haplotypes were detected at 31% and 14% of the samples. Six source-unknown haplotypes a, e, g, i, m and n were also detected at 14%.

Phylogenetic tree

A Phylogenetic tree using 19 haplotypes detected from Cuban samples was generated by the Neighbor-Joining method (Fig. 5). The green turtle (Bowen *et al.*, 1996) and pacific haplotype found in Zone B were used as an outergroup in forming the phylogenetic tree. The average genetic distances from the green turtle to the Caribbean haplotypes were 0.197.

The phylogenetic tree for the Caribbean haplotypes divided into Caribbean-A cluster consisting of haplotypes CB1, PR4 and a, b, f, g, i and n, and Caribbean-B cluster consisting of haplotype CB2 to CB3, PR1 to PR3, MX1 to MX2, v, d, e, h, j, m, o, p and zz. Bootstrap analysis indicated that the Caribbean-A cluster and Caribbean-B cluster were separated from other clusters in 77 and 83 replications, respectively, suggesting that the separations were relatively clear.

Discussion

Haplotype frequencies for nesting populations from Cuba, Puerto Rico and Mexico (Koike *et al.* 1997) indicated that each nesting population has specific haplotypes as a genetic marker. Bass *et al.* (1996) reported that 2 haplotypes (A and F) were shared among nesting areas, and all other 19 haplotypes were unique to specific colonies, identifing at least 6 female breeding stocks in the Caribbean and western Atlantic regions: Mexico, Belize, Puerto Rico, Antigua, Barbados and Brazil. Haplotype correspondance between our results and Bass *et al.* (1996) showed that haplotype F which were detected from US Virgin Island, Belize and Puerto Rico was subdivided into haplotypes PR1, c and j. Haplotypes Q detected only from Mexico were subdivided into MX1 and MX2. Haplotypes L, N and O deteced only from Puerto Rico were corresponded to haplotypes PR3, PR2 and PR4. Haplotypes B and G detected from Antigua and Belize, respectively were haplotypes e and i. Haplotypes α and γ undetected from 7 rookeries analysed were corresponded to haplotypes g and CB2, respectively. All these data suggest that each rookeries has specific haplotypes when more precise haplotypes were established by longer sequencing.

Bowen *et al.* (1996) estimated the contribution rate by the nesting population to the foraging population at Mona Island in Puerto Rico as 12.7%, by maximum likelihood analysis. The low estimate of the contribution rate reported by this authors seems mainly due to the low frequency of the main nesting haplotype; with only 1 of 15 individuals analyzed using the 1993's nesting population at Mona Island, while we detected it in 12 of 20 individuals analysed from the 1994's nesting population at Mona Island. It suggests that the female individuals of the nesting population might be not stable from year to year.

A total of 109 individuals of the Puerto Rican foraging population (Koike *et al.*, in press) included 41 individuals with the Puerto Rican nesting haplotypes PR1, PR2 and PR3 (36, 5 and 2 individuals, respectively), which represents a 41% contribution rate. The main Cuban haplotype CB1 (31 individuals), and Mexican main haplotype MX1 and MX2 (6 and 5 individuals, respectively) were also detected in the Puerto Rican foraging population. Another 10 unique haplotypes which were not identified among the nesting populations were found in the foraging population (21 individuals).

It is interesting that the contribution rates were decreased remarkably from Zone A (67%) to Zones B (43%) and D (41%). Zone A is recognized as the main nesting area of the Cuban hawksbill turtle population (Moncada *et al.*, 1997), suggesting the newly-

reproduced population from the nesting area disperse by distance. Another suitable explanation of it was offered by a tag-recovery survey (Moncada *et al.*, 1997), showing that 15 individuals marked at Doce Leguas in Zone A were all recovered at Doce Leguas with the longer term recovery (recoveries interval variating from 120 to 720 days), while 26 individuals marked at Nuevitas and Las Tunas in Zone D were all recaught elsewhere outside from there following the shore line in west-to-east direction. It seems that Doce Leguas where waters are shallow and warm has a higher degree of site fidelity.

Concerning to the seasonality, the contribution rates in Zone B were divided by season (Spring and Autumn) and showed higher contribution rate in Autumn (53%), than that in Spring (33%). Nesting season was estimated from September to December based on the presence of eggs in adult female individuals which coincided with a higher contribution rate in Autumn in Zone B.

It was found that minor Cuban nesting haplotypes CB2, 3 and 4 did not occur in cluster Caribbean-A with haplotype CB1, but in cluster Caribbean-B, indicating that a nesting population from a rookery did not always include only haplotypes from the same cluster. Considering that in the Cuban nesting population was detected 1 individual with Puerto Rican nesting haplotype PR1, it should be say that the nesting population is not completely fixed at a specific rookery, and even small, there would be a possibility of dispersal of nesting individuals from the main rookery to another rookery. Consequently, geographycal location and genetic location of the haplotype are not completely complementary, a population may exhibit several haplotypes of different genetic origins.

A number of source-unknown haplotypes were observed among the foraging populations in Cuba, indicating that further analysis on new nesting populations is needed.

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Appendix 1. Nesting samples from Doce Leguas, Zone A, collected at Breeding Center in 1995.

Lab. No.	Haplotype	Original No.	Tissue	Location	CL (cm)
95TS186	CB1	2038 (sakaiW)	Scute	Breeding Center	
95TS187	CB2	2054 (sakaiR)	Scute	Breeding Center	37.5
95TS188	PR1	2060 (sakaiR)	Scute	Breeding Center	37.6
95TS189	CB1	2061 (sakaiR)	Scute	Breeding Center	33.3
95TS190	CB2	2073 (sakaiR)	Scute	Breeding Center	36.2
95TS191	CB1	2087 (sakaiR)	Scute	Breeding Center	43.7
95TS192	CB1	2439 (sakaiR)	Scute	Breeding Center	35.6
95TS193	CB1	2441 (sakaiR)	Scute	Breeding Center	47.7
95TS194	CB1	2451 (sakaiR)	Scute	Breeding Center	32.5
95TS195	CB1	1013/5R (kobayashi)	Scute	Breeding Center	
95TS196	CB1	1013/6R (kobayashi)	Scute	Breeding Center	
95TS197	CB1	1013/7R (kobayashi)	Scute	Breeding Center	

Appendix 2. Nesting samples from Doce Leguas, Zone A, collected in 1994.

Lab. No.	Haplotype	Original No.	Tissue	Location	Sub-zone	Date
96TS101	CB2	7	Skin	Doce Leguas		
96TS102	CB1	8	Skin	Doce Leguas		
96TS103	CB1	11	Skin	Doce Leguas		
96TS104	CB1	12	Texture	Doce Leguas		
96TS105	CB1	14	Embryo	Doce Leguas	Caballones Oeste	
96TS106	CB4	19	Texture	Doce Leguas	Playa Bonita	
96TS107	CB1	25	Skin	Doce Leguas		
96TS108	CB1	31	Texture	Doce Leguas		
96TS109	CB3	32	Texture	Doce Leguas		
96TS110	CB1	33	Texture	Doce Leguas		
96TS111	CB2	34	New born foot	Doce Leguas	Cayo Grande	1994. 12
96TS112	CB1	35	New born foot	Doce Leguas	Cayo Grande	1994. 12
96TS113	CB1	37	Embryo	Doce Leguas		
96TS114	CB1	38	New born	Doce Leguas		
96TS115	CB1	41	Embryo	Doce Leguas	Juan Grin	1994. 12
96TS116	CB1	44	Embryo	Doce Leguas	Boca Seca	1994. 12
96TS117	CB1	45	Embryo	Doce Leguas	El Gincho	1994. 12
96TS118	**	17	Embryo	Doce Leguas		
96TS119	CB1	20	Embryo	Doce Leguas		
96TS120	CB1	36	Embryo	Doce Leguas		
96TS121	CB1	39	Embryo	Doce Leguas		
96TS122	CB1	40	Embryo	Doce Leguas		
96TS123	CB1	42	Embryo	Doce Leguas		
96TS124	CB1	43	Embryo	Doce Leguas		
96TS125	CB1	44D	Embryo	Doce Leguas	Boca Seca	1994. 12

**bad-preservation

Appendix 3. Nesting samples from Doce Leguas, Zone A, collected in 1997.

Lab. No.	Haplotype	CITES No.	Tissue	Location	Sub Zone	Nest No.	Date	Notes
96TS166	CB1	3	Embryo	Doce Leguas	Cayo Palomo	P1	97. 03. 15	30 days
96TS167	CB2	4	Embryo	Doce Leguas	Pasa B. Grande	Cayo Grande	97. 03. 16	15 days
96TS168	CB1	28	Embryo	Doce Leguas	Cinco Balas	Nido-1	97. 03. 16	25-30 days
96TS169	CB1	27	Dead Newborn	Doce Leguas	Cinco Balas	Nido-2	97. 03. 16	
96TS170	CB1	30	Dead Newborn	Doce Leguas	Cinco Balas	Nido-3	97. 03. 16	
96TS171	CB1	29	Embryo	Doce Leguas	Cinco Balas	Nido-4	97. 03. 16	35-40 days
96TS172	CB1	26	Newborn	Doce Leguas	Cinco Balas	Nido-5	97. 03. 16	Born in Ship
96TS173	CB1	2	Dead Newborn	Doce Leguas	Cinco Balas	Nido-6	97. 03. 16	
96TS174	CB1	25	Dead Newborn	Doce Leguas	Boca de Guano	Nido-1	97. 03. 17	
96TS175	CB1	23	Dead Newborn	Doce Leguas	Boca de Guano	Nido-2	97. 03. 17	
96TS176	CB1	24	Embryo	Doce Leguas	Boca de Guano	Nido-4	97. 03. 17	
96TS177	CB1	22	Dead Newborn	Doce Leguas	Boca de Guano	Nido-5	97. 03. 17	
96TS178	CB1	6	Embryo	Doce Leguas	Caballones Oeste	Nido-1	97. 03. 14	
96TS179	CB1	7	Embryo	Doce Leguas	Caballones Oeste	Nido-2	97. 03. 14	
96TS180	CB1	21	Embryo	Doce Leguas	Caballones Oeste	Nido-3	97. 03. 14	
96TS181	CB1	3	Embryo	Doce Leguas	Caballones Oeste	Nido-4	97. 03. 14	
96TS182	CB1	14	Embryo	Doce Leguas	Caballones Oeste	Nido-5	97. 03. 14	
96TS183	CB1	20	Embryo	Doce Leguas	Caballones Oeste	Nido-6	97. 03. 14	
96TS184	CB1	19	Embryo	Doce Leguas	Boca de Piedra Grande	Nido-1	97. 03. 14	
96TS185	CB1	15	Embryo	Doce Leguas	Boca de Piedra Grande	Nido-3	97. 03. 14	
96TS186	CB1	9	Embryo	Doce Leguas	Boca de Piedra Grande	Nido-4	97. 03. 14	
96TS187	CB1	13	Embryo	Doce Leguas	La Caleta, La Piedra	Nido-1	97. 03. 17	
96TS188	CB1	11	Embryo	Doce Leguas	El Guincho	Nido-2	97. 03. 16	
96TS189	CB1	2	Embryo	Doce Leguas	La Bartula	Nido-1	97. 03. 12	
96TS190	CB1	1	Embryo	Doce Leguas	La Bartula	Nido-2	97. 03. 12	
96TS191	CB1	10	Embryo	Doce Leguas	La Ballena	Nido-1	97. 03. 13	
96TS192	CB1	12	Embryo	Doce Leguas	La Ballena	Nido-2	97. 03. 13	
96TS193	CB1	18	Embryo	Doce Leguas	El Faro	Nido-2	97. 03. 13	
96TS194	CB1	17	Embryo	Doce Leguas	Caballones Este	C. Este	97. 03. 15	
96TS195	CB1	8	Embryo	Doce Leguas	Caballones Este	C. Este	97. 03. 15	
96TS196	CB1	16	Embryo	Doce Leguas	Caballones Este	Nido No.	97. 03. 15	
96TS197	CB1	6	Embryo	Doce Leguas	Caballones Este	Feb-97	97. 02. 10	
96TS198	CB1	5	Embryo	Doce Leguas	Juan Grin	Nido-2	97. 03. 11	
96TS199	CB1	4	Embryo	Doce Leguas	Boca Seca	Nido-3	97. 03. 12	

Appendix 4. Foraging samples from Doce Leguas, Zone A, collected in 1992.

Lab. No.	Haplotype	Original No.	Tissue	Location	Date	Sex	Condition
94TS105	CB1	Cuba93-1-007CH	Heart, Liver	Doce Leguas	9205	unknown	70%Ethanol
94TS106	CB2	Cuba93-3-008CH	Heart, Liver	Doce Leguas	9205	unknown	70%Ethanol
94TS107	CB1	Cuba93-5-009CH	Heart, Liver	Doce Leguas	9205	unknown	70%Ethanol
94TS108	CB3	Cuba93-7-010CH	Heart, Liver	Doce Leguas	9205	unknown	70%Ethanol
94TS109	CB4	Cuba93-9-011C	Heart	Doce Leguas	9205	unknown	70%Ethanol
94TS110	CB1	Cuba93-11-031CH	Heart, Liver	Doce Leguas	9210	♂	70%Ethanol
94TS111	CB1	Cuba93-12-032CH	Heart, Liver	Doce Leguas	9210	♂	70%Ethanol
94TS112	PR1	Cuba93-13-033CH	Heart, Liver	Doce Leguas	9210	♂	70%Ethanol
94TS113	CB1	Cuba93-14-034CH	Heart, Liver	Doce Leguas	9210	♂	70%Ethanol
94TS114	CB1	Cuba93-15-035CH	Heart, Liver	Doce Leguas	9210	♂	70%Ethanol
94TS115	CB1	Cuba93-16-036CH	Heart, Liver	Doce Leguas	9210	♂	70%Ethanol
94TS116	CB1	Cuba93-17-037CH	Heart, Liver	Doce Leguas	9210	♂	70%Ethanol
94TS117	CB1	Cuba93-18-038CH	Heart, Liver	Doce Leguas	9210	♂	70%Ethanol
94TS118	CB1	Cuba93-19-039CH	Heart, Liver	Doce Leguas	9210	♂	70%Ethanol
94TS119	CB1	Cuba93-20-040CH	Heart, Liver	Doce Leguas	9210	♂	70%Ethanol
94TS120	CB1	Cuba93-21-041CH	Heart, Liver	Doce Leguas	9210	♀	70%Ethanol
94TS121	CB1	Cuba93-22-041M	Muscle	Doce Leguas	9210	♀	70%Ethanol
94TS122	CB1	Cuba93-23-042CH	Heart, Liver	Doce Leguas	9210	♀	70%Ethanol
94TS123	CB1	Cuba93-25-043CH	Heart, Liver	Doce Leguas	9210	♀	70%Ethanol
94TS124	l	Cuba93-27-044CH	Heart, Liver	Doce Leguas	9210	♂	70%Ethanol
94TS125	CB1	Cuba93-29-045CH	Heart, Liver	Doce Leguas	9210	♀	70%Ethanol
94TS126	CB1	Cuba93-31-046CH	Heart, Liver	Doce Leguas	9210	♀	70%Ethanol
94TS127	CB1	Cuba93-33-047CH	Heart, Liver	Doce Leguas	9210	♀	70%Ethanol

Appendix 5. Foraging samples from Doce Leguas, Zone A, collected in 1997.

Lab. No.	Haplotype	CITES No.	Original No.	Tissue	Location	Sub Zone	Date	SCL (cm)	SCW (cm)	Sex	Dead/ Alive	Tag
96TS221	CB1	40	H-1	Muscle	Doce Leguas	Rabihorcado	97. 03. 12	36.5	27	M	Dead	-
96TS222	MX2	36	H-2	Muscle	Doce Leguas	Rabihorcado	97. 03. 12	54	42	?	Alive	-
96TS223	CB3	37	H-4	Muscle	Doce Leguas	Rabihorcado	97. 03. 12	46.5	34	F	Dead	-
96TS224	CB1	35	H-5	Muscle	Doce Leguas	Rabihorcado	97. 03. 12	51	37.5	F	Dead	-
96TS225	CB1	42	H-1	Muscle	Doce Leguas	Rabihorcado	97. 03. 13	36	28	M	Dead	-
96TS226	c	41	H-2	Muscle	Doce Leguas	Rabihorcado	97. 03. 13	52.5	38.5	F	Dead	-
96TS227	g	39	H-1	Muscle	Doce Leguas	Palomo	97. 03. 15	45.5	34.5	M	Alive	-
96TS228	g	38	H-2	Muscle	Doce Leguas	Palomo	97. 03. 15	53	37.5	M	Dead	-
96TS229	CB1	43	H-3	Muscle	Doce Leguas	Palomo	97. 03. 15	46.5	33.5	F	Dead	-
96TS230	MX2	1	Skin	Skin	Doce Leguas	Palomo	97. 03. 15	44.5	32	?	Release	0189
96TS231	CB3	34	Skin	Skin	Doce Leguas	Palomo	97. 03. 15	58	44	?	Release	0191
96TS232	CB1	32	H-1	Skin	Doce Leguas	Boca de Guano	97. 03. 17	24	17	?	Release	2138
96TS233	PR1	33	H-2	Skin	Doce Leguas	Boca de Guano	97. 03. 17	34.5	25.5	?	Release	0190

Appendix 6. Foraging samples from Santa Cruz, Zone A, collected in 1993.

Lab. No.	Haplotype	Original No.	Tissue	Location	Date	Sex	Condition
94TS154	PR1	Cuba93-79-092?	?	Santa Cruz	9311	J	70%Ethanol
94TS155	CB1	Cuba93-81-093?	?	Santa Cruz	9311	♂	70%Ethanol
94TS156	CB1	Cuba93-84-094?	?	Santa Cruz	9311	♀	70%Ethanol
94TS157	PR1	Cuba93-86-095B	Blood	Santa Cruz	9311	J	Blood
94TS158	missing	Cuba93-87-096B	Blood	Santa Cruz	9311	J	Blood
94TS159	CB1	Cuba93-88-097B	?	Santa Cruz	9311	♀	Blood
94TS160	PR1	Cuba93-89-098?	?	Santa Cruz	9311	♀	70%Ethanol
94TS161	CB1	Cuba93-91-099?	?	Santa Cruz	9311	♂	70%Ethanol
94TS162	CB1	Cuba93-93-100B	Blood	Santa Cruz	9311	unknown	Blood

Appendix 7. Foraging samples from Isla de Pinos, Zone B, collected in the Spring of 1996.

Lab. No.	Haplo type	CITES No.	Original No.	Tissue	Location	Sub Zone	Date	SCL (cm)	SCW (cm)	Wt (Kg)	Sex	Dead/ Alive	Tag	Eggs	Folic	Weigth(g)				
																Shell	Marg	Hoof	Plast	Total
96TS234	PR1	25	IP 4	Scraped Shell	I. Pinos	-	1996. 3. 16	66	51	44	F	Dead	No	-	-	1025	200	50	200	1475
96TS235	MX1	26	IP 5	Scraped Shell	I. Pinos	-	1996. 3. 16	72	52	38	F	Dead	No	-	-	1100	160	50	150	1460
96TS236	MX1	27	IP 6	Scraped Shell	I. Pinos	-	1996. 3. 23	63.4	44	28	F	Dead	No	-	-	300	100	50	100	550
96TS237	-	28	IP 7	Scraped Shell	I. Pinos	-	1996. 3. 23	86	60	84	F	Dead	No	-	-	1300	272	100	300	1972
96TS238	CB1	29	IP 8	Scraped Shell	I. Pinos	-	1996. 3. 23	73	49	42	M	Dead	No	-	-	1000	175	50	150	1375
96TS239	PR1	30	IP 9	Scraped Shell	I. Pinos	-	1996. 3. 23	81	57	60	M	Dead	No	-	-	1150	250	100	225	1725
96TS240	PR2	31	IP 10	Scraped Shell	I. Pinos	-	1996. 3. 30	49.2	35.3	12	M	Dead	No	-	-	400	125	50	140	715
96TS241	PR1	32	IP 11	Scraped Shell	I. Pinos	b	1996. 4. 3	81	57	56	F	Dead	No	-	-	900	200	72	50	1222
96TS242	PR3	33	IP 12	Scraped Shell	I. Pinos	a	1996. 4. 10	86	61	82	F	Dead	No	-	Y	1600	300	112	350	2362
96TS243	CB2	34	IP 13	Scraped Shell	I. Pinos	b	1996. 4. 10	68	50	40	M	Dead	No	-	-	1300	200	50	250	1800
96TS244	PR1	35	IP 14	Scraped Shell	I. Pinos	b	1996. 4. 10	70	50	40	F	Dead	No	-	-	950	200	38	100	1288
96TS245	PR1	36	IP 15	Scraped Shell	I. Pinos	b	1996. 4. 10	74	55	54	F	Dead	No	-	-	1050	200	50	150	1450
96TS246	CB1	37	IP 16	Scraped Shell	I. Pinos	b	1996. 4. 11	70	50.5	40	F	Dead	No	-	-	700	150	50	80	980
96TS247	c	38	IP 17	Scraped Shell	I. Pinos	a	1996. 4. 12	59	44	26	F	Dead	No	-	-	612	150	50	150	962
96TS248	MX2	39	IP 18	Scraped Shell	I. Pinos	a	1996. 4. 12	78	60	82	F	Dead	No	-	Y	1900	400	150	272	2722
96TS249	CB1	40	IP 19	Scraped Shell	I. Pinos	b	1996. 4. 13	76	55	57	F	Dead	No	-	Y	1350	300	100	250	2000
96TS250	CB1	41	IP 20	Scraped Shell	I. Pinos	a	1996. 4. 13	69	49	38	F	Dead	No	-	-	950	150	50	150	1300
96TS251	PR1	42	IP 21	Scraped Shell	I. Pinos	a	1996. 4. 15	77	56	40	M	Dead	No	-	-	750	100	75	75	1000
96TS252	CB2	43	IP 22	Scraped Shell	I. Pinos	b	1996. 4. 15	84	66	73	F	Dead	No	-	26 mm	1250	200	150	200	1800
96TS253	CB3	44	IP 23	Scraped Shell	I. Pinos	a	1996. 4. 20	87.2	62.3	80	F	Dead	No	-	Y	1250	250	100	300	1900
96TS254	MX2	45	IP 24	Scraped Shell	I. Pinos	a	1996. 4. 22	75.6	54.3	59	F	Dead	No	-	-	1850	350	100	250	2550
96TS255	MX1	46	IP 25	Scraped Shell	I. Pinos	a	1996. 4. 23	68	50	34	F	Dead	No	-	Y	650	175	50	150	1025
96TS256	-	47	IP 26	Scraped Shell	I. Pinos	a	1996. 4. 24	77	51.6	58	F	Dead	No	-	Y	1050	200	75	200	1525
96TS257	c	48	IP 27	Scraped Shell	I. Pinos	a	1996. 4. 24	70.5	50	42	F	Dead	No	-	-	1050	175	50	150	1425
96TS258	CB1	49	IP 28	Scraped Shell	I. Pinos	b	1996. 4. 25	73	49.8	40	F	Dead	No	-	-	1150	200	50	250	1650
96TS259	PR1	50	IP 29	Scraped Shell	I. Pinos	a	1996. 4. 26	43.7	30.6	9.5	F	Dead	No	-	-	200	45	15	45	305
96TS260	PR2	51	IP 30	Scraped Shell	I. Pinos	a	1996. 4. 27	78	56	56	M	Dead	No	-	-	930	160	65	110	1265
96TS261	CB2	52	IP 31	Scraped Shell	I. Pinos	a	1996. 4. 29	65.5	48.2	34	M	Dead	No	-	-	630	120	50	140	940
96TS262	PR1	53	IP 32	Scraped Shell	I. Pinos	b	1996. 4. 29	72.5	45.6	40	F	Dead	No	-	-	590	105	40	90	825
96TS263	PR1	54	IP 33	Scraped Shell	I. Pinos	b	1996. 5. 2	69.8	51.6	42	F	Dead	No	-	-	805	180	60	190	1235
96TS264	PR1	55	IP 34	Scraped Shell	I. Pinos	b	1996. 5	64.5	47	30	F	Dead	No	-	-	1090	140	60	180	1470
96TS265	PR2	56	IP 35	Scraped Shell	I. Pinos	b	1996. 5	78	55.5	80	F	Dead	No	-	Y	1290	260	100	210	1860
96TS266	-	57	IP 36	Scraped Shell	I. Pinos	b	1996. 5	80.5	57	55	F	Dead	No	-	-	1960	355	95	235	2645
96TS267	CB1	58	IP 37	Scraped Shell	I. Pinos	b	1996. 5	63.5	47	28	M	Dead	No	-	-	950	145	55	160	1310
96TS268	-	59	IP 38	Scraped Shell	I. Pinos	b	1996. 5	74	52.5	54	F	Dead	No	-	Y	1195	225	70	210	1700
96TS348	c	142	1151	Skin	I. Pinos	b	1997. 3						1151	-	-					
96TS349	c	143	287	Skin	I. Pinos	b	1997. 4. 18	66	48	32	F		No	-	-					
96TS350	CB1	144	277	Skin	I. Pinos	b	1997. 4. 14	77	57	62	F		No	-	20 mm					
96TS351	CB1	145	284	Skin	I. Pinos	b	1997. 4. 16	71.6	53	46	F		No	-	-					
96TS352	CB1	146	4	Skin	I. Pinos	b	1997. 4						No	-	-					
96TS353	MX1	147	281	Skin	I. Pinos	b	1997. 4. 14	85	61	73	F		No	-	25 mm					
96TS354	o	148	286	Skin	I. Pinos	b	1997. 4. 18	73	53	46	F		No	-	-					
96TS355	PR1	149	275	Skin	I. Pinos	b	1997. 4. 10	82	57	63	F		No	-	Y					
96TS356	PR1	150	274	Skin	I. Pinos	b	1997. 4. 10	72	51	42	F		No	-	-					

Appendix 8. Foraging samples from Isla de Pinos, Zone B, collected in the Autumn of 1996.

Lab. No.	Haplo type	CITES No.	Original No.	Tissue	Location	Sub Zone	Date	SCL (cm)	SCW (cm)	Wt (Kg)	Sex	Dead/ Alive	Tag	Eggs	Folic	Weigth(g)				
																Shell	Marg	Hoof	Plast	Total
96TS269	c	60	IP 39	Scraped Shell	I. Pinos	b	1996. 8. 19	68.5	51.5	35	F	Dead	No	-	-	1050	250	50	225	1575
96TS270	CB1	61	IP 40	Scraped Shell	I. Pinos	b	1996. 8. 19	67	47.5	34	F	Dead	No	-	-	925	175	45	225	1370
96TS271	CB1	62	IP 42	Scraped Shell	I. Pinos	b	1996. 8. 28	41	32	6	?	Dead	No	-	-	200	75	15	50	340
96TS272	CB1	63	IP 43	Scraped Shell	I. Pinos	b	1996. 8. 29	73	51.5	38	M	Dead	No	-	-	1050	110	50	75	1285
96TS273	PR1	64	IP 44	Scraped Shell	I. Pinos	b	1996. 8. 29	73.3	53.6	42	F	Dead	No	-	-	925	75	40	100	1140
96TS274	CB1	65	IP 45	Scraped Shell	I. Pinos	b	1996. 8. 31	65	53	34	?	Dead	No	-	-	1100	125	75	225	1525
96TS275	PR1	66	IP 46	Scraped Shell	I. Pinos	b	1996. 8. 31	67	54	36	F	Dead	No	-	-	1000	200	50	200	1450
96TS276	CB1	67	IP 47	Scraped Shell	I. Pinos	b	1996. 9. 3	77	50.5	38	F	Dead	No	-	-	1300	150	60	125	1635
96TS277	-	68	IP 48	Scraped Shell	I. Pinos	b	1996. 9. 3	66	46.7	28	F	Dead	No	-	-	850	125	40	110	1125
96TS278	CB1	69	IP 49	Scraped Shell	I. Pinos	b	1996. 9. 4	60.7	45.3	22	F	Dead	No	-	-	600	140	25	125	890
96TS279	CB1	70	IP 50	Scraped Shell	I. Pinos	b	1996. 9. 5	58.2	43.3	22	F	Dead	No	-	-	625	125	25	150	925
96TS280	CB1	71	IP 51	Scraped Shell	I. Pinos	b	1996. 9. 5	73.8	55	46	F	Dead	No	-	-	1360	200	50	150	1760
96TS281	CB2	72	IP 52	Scraped Shell	I. Pinos	b	1996. 9. 5	85.9	62	58	F	Alive	No	Y	-	1525	375	125	225	2250
96TS282	CB1	73	IP 53	Scraped Shell	I. Pinos	b	1996. 9. 6	65.5	48.3	34	F	Dead	No	-	-	825	100	40	150	1115
96TS283	g	74	IP 54	Scraped Shell	I. Pinos	b	1996. 9. 8	77.3	57.8	48	F	Dead	No	-	-	1975	300	100	300	2675
96TS284	PR1	75	IP 55	Scraped Shell	I. Pinos	b	1996. 9. 8	78.8	59.9	54	F	Dead	No	-	-	800	100	50	75	1025
96TS285	CB1	76	IP 56	Scraped Shell	I. Pinos	b	1996. 9. 3	71.5	55	36	F	Dead	No	-	-	1300	200	50	175	1725
96TS286	PR1	77	IP 57	Scraped Shell	I. Pinos	b	1996. 9. 3	76.7	60.7	50	F	Dead	No	-	-	700	100	25	100	925
96TS287	CB1	78	IP 58	Scraped Shell	I. Pinos	a	1996. 9. 9	73.3	52	37	F	Dead	No	-	-	925	150	100	100	1275
96TS288	CB1	79	IP 60	Scraped Shell	I. Pinos	b	1996. 9. 10	53	39	14	F	Dead	No	-	-	450	50	10	50	560
96TS289	CB1	80	IP 61	Scraped Shell	I. Pinos	b	1996. 9. 10	61.5	46.5	24	F	Dead	No	-	-	560	75	25		660
96TS290	CB1	81	IP 62	Scraped Shell	I. Pinos	b	1996. 9. 10	58	44	20	F	Dead	No	-	-	550	125	50	125	850
96TS291	CB1	82	IP 63	Scraped Shell	I. Pinos	b	1996. 9. 10	74	54	40	F	Dead	No	-	-	1050	200	50	150	1450
96TS292	CB1	83	IP 64	Scraped Shell	I. Pinos	b	1996. 9. 10	72.3	53	38	F	Dead	No	-	-	825	125	75	125	1150
96TS293	MX2	84	IP 65	Scraped Shell	I. Pinos	a	1996. 9. 11	69	51	40	F	Dead	No	-	-	1175	125	50	150	1500
96TS294	PR1	85	IP 66	Scraped Shell	I. Pinos	b	1996. 9. 11	80	62	57	F	Dead	No	Y	-	1475	125	100	200	1900
96TS295	CB1	86	IP 67	Scraped Shell	I. Pinos	b	1996. 9. 11	69	48.2	33	F	Dead	No	-	-	1075	125	50	150	1400
96TS296	a	87	IP 70	Scraped Shell	I. Pinos	a	1996. 9. 13	69.8	49	28	F	Dead	No	-	-	700	150	50	150	1050
96TS297	a	88	IP 71	Scraped Shell	I. Pinos	b	1996. 9. 13	76.3	57.3	47	F	Dead	No	-	-	1500	250		275	2025
96TS298	CB1	89	IP 74	Scraped Shell	I. Pinos	a	1996. 9. 15	69.8	51.8	37	F	Dead	No	-	-	1100	200	25	225	1550
96TS299	CB1	90	IP 78	Scraped Shell	I. Pinos	b	1996. 9. 15	83	60.3	64	F	Dead	No	-	Y	800	125	125	175	1225
96TS300	PR1	91	IP 79	Scraped Shell	I. Pinos	b	1996. 9. 15	81.2	54.7	53	F	Dead	No	-	Y	2825	150		125	3100
96TS301	CB1	92	IP 85	Scraped Shell	I. Pinos	a	1996. 9. 18	74.5	57.3	48	F	Dead	No	-	-	1400	250	100	275	2025
96TS302	CB1	93	IP 87	Scraped Shell	I. Pinos	b	1996. 9. 19	77	57	50	M	Dead	No	-	-	1100	200	100	100	1500
96TS303	CB1	94	IP 89	Scraped Shell	I. Pinos	a	1996. 9. 20	74	55.5	40	F	Dead	No	-	-	1050	175		150	1375
96TS304	CB1	95	IP 113	Scraped Shell	I. Pinos	a	1996. 9. 28	70.1	54.6	42	F	Dead	No	-	-	925	175	50	175	1325
96TS305	CB1	96	IP 120	Scraped Shell	I. Pinos	a	1996. 9. 30	74.7	53.9	43	M	Dead	No	-	-	1300	150	50	125	1625
96TS306	c	97	IP 127	Scraped Shell	I. Pinos	a	1996. 10. 2	70	54	38	F	Dead	No	-	-	900	200	50	250	1400
96TS307	c	98	IP 128	Scraped Shell	I. Pinos	a	1996. 10. 4	71.5	52.2	38	M	Dead	No	-	-	750	150	50	150	1100
96TS308	PR2	99	IP 130	Scraped Shell	I. Pinos	a	1996. 10. 4	74.8	55.5	46	F	Dead	No	-	-	1250	175	75	150	1650
96TS309	CB1	100	IP 135	Scraped Shell	I. Pinos	b	1996. 10. 4	71.3	52	38	M	Dead	No	-	-	875	200	75	175	1325
96TS310	PR1	101	IP 137	Scraped Shell	I. Pinos	a	1996. 10. 5	70	49.8	34	F	Alive	No	-	-	1200	150	50	200	1600
96TS311	MX2	102	IP 138	Scraped Shell	I. Pinos	b	1996. 10. 9	73	53	44	F	Dead	No	-	-	900	200	75	250	1425
96TS312	c	103	IP 139	Scraped Shell	I. Pinos	b	1996. 10. 9	69	50	37	F	Dead	No	-	-	1250	250		200	1700
96TS313	CB1	104	IP 141	Scraped Shell	I. Pinos	a	1996. 10. 9	71	55	41	F	Dead	No	-	-	950	200	50	200	1400
96TS314	CB1	105	IP 142	Scraped Shell	I. Pinos	a	1996. 10. 9	73	56	52	F	Dead	No	-	-	1550	300	75	350	2275
96TS315	PR2	106	IP 143	Scraped Shell	I. Pinos	a	1996. 10. 10	66.2	49.5	30	M	Dead	No	-	-	950	175	50	150	1325
96TS316	CB1	107	IP 144	Scraped Shell	I. Pinos	a	1996. 10. 10	59.3	44.6	24	F	Dead	No	-	-	550	100	25	150	825
96TS317	Pacific	108	IP 145	Scraped Shell	I. Pinos	a	1996. 10. 10	63.7	47.2	29	F	Dead	No	-	-	750	190	50	175	1165
96TS318	CB1	109	IP 146	Scraped Shell	I. Pinos	a	1996. 10. 10	68.8	48.8	30	F	Dead	No	-	-	800	150	50	200	1200
96TS319	l	110	IP 148	Scraped Shell	I. Pinos	b	1996. 10. 11	84	61	70	F	Dead	No	-	-					0
96TS320	CB1	111	IP 149	Scraped Shell	I. Pinos	b	1996. 10. 12	60	43	20	F	Dead	No	-	-	600	100	25	75	800
96TS321	MX2	112	IP 150	Scraped Shell	I. Pinos	b	1996. 10. 12	80	58	56	F	Dead	No	-	-	700	125	100	200	1125
96TS322	c	113	IP 151	Scraped Shell	I. Pinos	a	1996. 10. 14	77	55	53	F	Dead	No	-	-	1025	200	75	175	1475
96TS323	PR2	114	IP 152	Scraped Shell	I. Pinos	a	1996. 10. 14	65	47	27	F	Dead	No	-	-	850	150	50	225	1275
96TS324	PR2	115	IP 153	Scraped Shell	I. Pinos	a	1996. 10. 14	42	29	23	F	Dead	No	-	-	985	75	25	200	1285
96TS325	CB2	116	IP 156	Scraped Shell	I. Pinos	b	1996. 10. 15	77	57	48	F	Dead	No	-	-	1000	175		125	1300
96TS326	MX2	117	IP 159	Scraped Shell	I. Pinos	a	1996. 10. 21	80	58	50	F	Dead	No	-	-	2950	300	100	200	3550
96TS327	MX2	118	IP 160	Scraped Shell	I. Pinos	b	1996. 10. 21	55	41	19	F	Dead	No	-	-	450	75	25	50	600
96TS328	CB1	119	IP 161	Scraped Shell	I. Pinos	a	1996. 10. 22	65	49	22	M	Dead	No	-	-	725	150	50	150	1075
96TS329	PR2	120	IP 162	Scraped Shell	I. Pinos	a	1996. 10. 22	78	56	44	F	Dead	No	-	-	1200	225	75	300	1800
96TS330	CB3	121	IP 163	Scraped Shell	I. Pinos	a	1996. 10. 24	75.5	52.5	43	F	Dead	No	-	-	1250	250	75	250	1825
96TS331	PR1	122	IP 164	Scraped Shell	I. Pinos	a	1996. 10. 24	57.5	40.6	22	M	Dead	No	-	-	600	75	25		700
96TS332	CB1	123	IP 165	Scraped Shell	I. Pinos	a	1996. 10. 24	76.8	55.4	49	F	Dead	No	-	-	1150	200	75	200	1625
96TS333	PR1	124	IP 166	Scraped Shell	I. Pinos	a	1996. 10. 24	68	48	30	F	Dead	No							
96TS334	PR2	125	IP 167	Scraped Shell	I. Pinos	a	1996. 10. 24	77.4	55	54	F	Dead	No	-	-	1550	200	75	175	2000
96TS335	CB1	126	IP 168	Scraped Shell	I. Pinos	b	1996. 10. 24	57	44	20	F	Dead	No	-	-	600	125	25	100	850
96TS336	PR2	127	IP 169	Scraped Shell	I. Pinos	b	1996. 10. 25	74.3	52.5	43	F	Dead	No	-	-	1175	250	50	250	1725
96TS337	-	128	IP 170	Scraped Shell	I. Pinos	a	1996. 10. 28	78	58	59	F	Dead	No	-	-	1550	300	100	250	2200
96TS338	CB1	129	IP 171	Scraped Shell	I. Pinos	a	1996. 10. 28	70	60	54	F	Dead	No	-	-	900	200	100	150	1350
96TS339	c	130	IP 172	Scraped Shell	I. Pinos	a	1996. 10. 28	69	51	42	F	Dead	No	-	-	800	200	50	150	1200
96TS340	PR1	131	IP 173	Scraped Shell	I. Pinos	a	1996. 10. 28	69	51	44	F	Dead	No	-	-	850	100	25	200	1175
96TS341	CB1	132	IP 174	Scraped Shell	I. Pinos	b	1996. 10. 28	69	51	37	F	Dead	No	-	-	1050	100	50	150	1350
96TS342	-	133	IP 175	Scraped Shell	I. Pinos	b	1996. 10. 28	71.5	50	39	F	Dead	No	-	-	900	125	50	100	1175
96TS343	-	134	IP 176	Scraped Shell	I. Pinos	b	1996. 10. 28	66	48.5	33	M	Dead	No	-	-	800	100	50	150	1100
96TS344	PR1	135	IP 177	Scraped Shell	I. Pinos	b	1996. 10. 28	75	56	47	F	Dead	No	-	-	1700	350	75	150	2275
96TS345	PR1	136	IP 178	Scraped Shell	I. Pinos	b	1996. 10. 28	78	53.5	55	F	Dead	No	-	-	1400	250	75	250	1975
96TS346	CB1	137	IP 179	Scraped Shell	I. Pinos	b	1996. 10. 28	73	54	40	M	Dead	No	-	-	1025	225	75	150	1475
96TS347	CB1	138	IP 187	Scraped Shell	I. Pinos	b	1996. 10. 31	76	57	46	F	Dead	No	-	-	1100	125	50	125	1400

Appendix 9. Foraging samples from Nuevitas, Zone D, collected in 1992-93.

Lab. No.	Haplotype	Original No.	Tissue	Location	Date	Sex	Condition
94TS128	a	Cuba93-35-059H	Heart	Nuevitas	9210	♂	70%Ethanol
94TS129	PR1	Cuba93-37-060M	Muscle	Nuevitas	9210	♂	70%Ethanol
94TS130	CB4	Cuba93-38-062C	Heart	Nuevitas	9210	♂	70%Ethanol
94TS131	g	Cuba93-40-063H	Liver	Nuevitas	9210	unknown	70%Ethanol
94TS132	PR3	Cuba93-41-064C	Heart	Nuevitas	9210	unknown	70%Ethanol
94TS133	CB1	Cuba93-42-065C	Heart	Nuevitas	9210	unknown	70%Ethanol
94TS140	m	Cuba93-55-076CH	Heart, Liver	Nuevitas	9311	♀	70%Ethanol
94TS141	CB1	Cuba93-57-077CH	Heart, Liver	Nuevitas	9311	♀	70%Ethanol
94TS151	CB1	Cuba93-75-089CH	Heart, Liver	Nuevitas	9311	unknown	70%Ethanol
94TS152	PR1	Cuba93-77-090CH	Heart, Liver	Nuevitas	9311	unknown	70%Ethanol
94TS153	CB1	Cuba93-78-091M	Muscle	Nuevitas	9311	unknown	70%Ethanol
94TS163	PR1	Cuba93-94-101B	Blood	Nuevitas	9311	♀	Blood
94TS164	PR1	Cuba93-96-102B	Blood	Nuevitas	9311	♀	Blood
94TS165	MX1	Cuba93-98-104CH	Heart, Liver	Nuevitas	9311	♀	70%Ethanol
94TS166	CB1	Cuba93-99-105CH	Heart, Liver	Nuevitas	9311	♀	70%Ethanol
94TS167	CB1	Cuba93-100-106CH	Heart, Liver	Nuevitas	9311	unknown	70%Ethanol
94TS168	CB1	Cuba93-101-107CH	Heart, Liver	Nuevitas	9311	♂	70%Ethanol

Appendix 10. Foraging samples from Las Tunas, Zone D, collected in 1993-94.

Lab. No.	Haplotype	Original No.	Tissue	Location	Date	Sex	Condition
94TS134	PR3	Cuba93-44-070CH	Heart, Liver	Las Tunas	9311	♂	70%Ethanol
94TS135	e	Cuba93-46-071CH	Heart, Liver	Las Tunas	9311	♀	70%Ethanol
94TS136	CB1	Cuba93-48-072CH	Heart, Liver	Las Tunas	9311	♀	70%Ethanol
94TS137	PR1	Cuba93-50-073CH	Heart, Liver	Las Tunas	9311	♂	70%Ethanol
94TS138	PR1	Cuba93-52-074CH	Heart, Liver	Las Tunas	9311	♀	70%Ethanol
94TS139	i	Cuba93-53-075CH	Heart, Liver	Las Tunas	9311	♀	70%Ethanol
94TS142	CB1	Cuba93-60-078H	Heart	Las Tunas	9311	♀	70%Ethanol
94TS143	PR1	Cuba93-61-079CH	Heart, Liver	Las Tunas	9311	♀	70%Ethanol
94TS144	g	Cuba93-63-080CH	Heart, Liver	Las Tunas	9312	♀	70%Ethanol
94TS145	l	Cuba93-65-081CH	Heart, Liver	Las Tunas	9312	♀	70%Ethanol
94TS146	CB4	Cuba93-66-082CH	Heart, Liver	Las Tunas	9312	♀	70%Ethanol
94TS147	CB1	Cuba93-68-083CH	Heart, Liver	Las Tunas	9401	♀	70%Ethanol
94TS148	PR1	Cuba93-70-084CH	Heart, Liver	Las Tunas	9312	♀	70%Ethanol
94TS149	CB1	Cuba93-72-085CH	Heart, Liver	Las Tunas	9401	♀	70%Ethanol
94TS150	n	Cuba93-74-086M	Muscle	Las Tunas	9312	♀	70%Ethanol

Appendix 11. Foraging samples from Cayo Romano, Zone D, collected by Fishermen in 1995.

Lab. No.	Haplotype	Original No.	Tissue	Location	Comments
95TS225	CB2	951025/1 (sakai)	Hoof	Cayo Romano	Collected by fishermen
95TS226	-	951025/2 (sakai)	Hoof	Cayo Romano	Collected by fishermen
95TS227	CB1	951025/3 (sakai)	Hoof	Cayo Romano	Collected by fishermen
95TS228	MX1	951025/4 (sakai)	Hoof	Cayo Romano	Collected by fishermen
95TS229	PR1	951025/5 (sakai)	Hoof	Cayo Romano	Collected by fishermen
95TS230	MX1	951025/6 (sakai)	Hoof	Cayo Romano	Collected by fishermen
95TS231	PR1	951025/7 (sakai)	Hoof	Cayo Romano	Collected by fishermen
95TS232	CB1	951025/8 (sakai)	Hoof	Cayo Romano	Collected by fishermen
95TS233	MX1	951025/9 (sakai)	Hoof	Cayo Romano	Collected by fishermen
95TS234	PR2	951025/10 (sakai)	Hoof	Cayo Romano	Collected by fishermen

Appendix 12. Foraging samples from Zone D, collected by Fishermen in 1995.

Lab. No.	Haplotype	Original No.	Tissue	Location	Coments
95TS205	PR1	951024A/1 (sakai)	Hoof	Northern Sea	Collected by fishermen
95TS206	-	951024A/2 (sakai)	Hoof	Northern Sea	Collected by fishermen
95TS207	CB3	951024A/3 (sakai)	Hoof	Northern Sea	Collected by fishermen
95TS208	MX2	951024A/4 (sakai)	Hoof	Northern Sea	Collected by fishermen
95TS209	CB1	951024A/5 (sakai)	Hoof	Northern Sea	Collected by fishermen
95TS210	CB1	951024A/6 (sakai)	Hoof	Northern Sea	Collected by fishermen
95TS211	PR1	951024A/7 (sakai)	Hoof	Northern Sea	Collected by fishermen
95TS212	CB2	951024A/8 (sakai)	Hoof	Northern Sea	Collected by fishermen
95TS213	CB2	951024A/9 (sakai)	Hoof	Northern Sea	Collected by fishermen
95TS214	n	951024A/10 (sakai)	Hoof	Northern Sea	Collected by fishermen
95TS215	CB3	951024B/1 (sakai)	Hoof	Northern Sea	Collected by fishermen
95TS216	MX1	951024B/2 (sakai)	Hoof	Northern Sea	Collected by fishermen
95TS217	PR2	951024B/3 (sakai)	Hoof	Northern Sea	Collected by fishermen
95TS218	PR1	951024B/4 (sakai)	Hoof	Northern Sea	Collected by fishermen
95TS219	CB2	951024B/5 (sakai)	Hoof	Northern Sea	Collected by fishermen
95TS220	PR2	951024B/6 (sakai)	Hoof	Northern Sea	Collected by fishermen
95TS221	CB1	951024B/7 (sakai)	Hoof	Northern Sea	Collected by fishermen
95TS222	CB1	951024B/8 (sakai)	Hoof	Northern Sea	Collected by fishermen
95TS223	CB1	951024B/9 (sakai)	Hoof	Northern Sea	Collected by fishermen
95TS224	-	951024B/10 (sakai)	Hoof	Northern Sea	Collected by fishermen