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Genetic Diversity of the Hawksbill Turtle in the Indo-Pacific and Caribbean Regions

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ABSTRACT. – **Genetic diversity of the hawksbill turtle (*Eretmochelys imbricata*) in the Indo-Pacific and Caribbean regions was examined using 15 nesting samples from Okinawa and Ishigaki Island, Japan, and Seribu Islands, Indonesia, and 106 foraging samples from Okinawa Prefecture, Japan, Philippines, Solomon Islands, Fiji, Maldives, and Seychelles. A phylogenetic tree using the 24 haplotypes detected in the Indo-Pacific samples and 29 haplotypes from Caribbean samples was divided into Indo-Pacific and Caribbean haplotypes. Some similar characteristics were recognized in the genetic structure between the Indo-Pacific and Caribbean hawksbills. Due to the low number of nesting samples in Okinawa, contribution rates could not be calculated, and so haplotype diversity (h) for 104 individuals from the Yaeyama Archipelago (0.776) were compared with those of 115 individuals from Isla de Pinos, Cuba (0.777) and 106 individuals from Mona Island, Puerto Rico (0.791), suggesting that these three foraging populations exhibit comparable high haplotype diversities, consisting of 40–50% of the most common haplotype, 15–30% of the second and third most common haplotypes, and accompanied by several less common haplotypes. Network analysis indicated that in the Indo-Pacific A cluster there were 12 haplotypes with 1-substitution differences radiating from haplotype 1, and in the Caribbean A cluster, 7 haplotypes radiated from CU1 with 1-substitution differences. The Caribbean B cluster had a complicated network consisting of multiple 1-substitution differences. These 1-substitution differences suggest a recent common ancestor for each cluster.**

KEY WORDS. – **Reptilia; Testudines; Cheloniidae; *Eretmochelys imbricata*; sea turtle; genetics; genetic diversity; mitochondrial control region; network analysis; Caribbean Sea; Indian Ocean; Pacific Ocean; Japan; Indonesia**

Mitochondrial DNA (mtDNA) has been widely used for molecular phylogenetic studies. Because mtDNA is maternally inherited and does not recombine, mtDNA sequence analysis can provide precise estimates of divergence within a gene phylogeny. Furthermore, mtDNA has a high mutation rate, about 10 times that of nuclear DNA, and nucleotide substitutions therefore accumulate quickly even between species that diverged quite recently. In particular, sequences within the mtDNA control region evolve rapidly, and have proven useful for resolution of population structure (Avise, 1994).

The mitochondrial control region has proven very useful for identifying the nesting origin of migratory animals (Bowen and Avise, 1996). Hawksbill turtles are thought to migrate from foraging grounds to nesting rookeries every two to three years (Miller, 1997). Nesting populations of this species are less colonial than other marine turtles, and it is often difficult to survey them. Examination of mtDNA haplotype frequencies between nesting areas in northeastern and northwestern Australia (Broderick et al., 1994) indicated significant differences. Bowen et al. (1996) used mtDNA control region sequence data from seven western Atlantic nesting populations (Bass et al., 1996) to estimate the contribution of regional rookeries to a foraging area at Mona Island in Puerto Rico. Koike et al. (1998) and Díaz-Fernández et al. (1999) also analyzed mtDNA haplotype frequencies using nesting samples from Cuba, Mexico, and Puerto Rico, which indicated that each nesting population had specific haplotypes which may be useful as genetic markers.

In this paper, we describe genetic diversity for some populations of the hawksbill turtle, *Eretmochelys imbricata*, in the Indo-Pacific and Caribbean regions, based on sequence data of the left domain in the mitochondrial control region.

MATERIALS AND METHODS

A total of 136 samples were analyzed from the Indo-Pacific region, of which 15 were nesting samples. The latter consisted of: one hatchling from Okinawa (1996); 5 samples from Ishigaki Island (two hatchlings from Osaki beach, 1997, which were assumed to come from different clutches, and three breeding individuals originally from a single nest at Arasaki beach); and 9 hatchlings from different clutches at Segama Island (Seribu Islands, Indonesia) collected in 1996 by the Department of Forestry.

The 106 foraging samples from Okinawa Prefecture consisted of: 100 samples from turtles taken by traditional

harvest around the Yaeyama Archipelago; 4 samples from dead individuals at Ishigaki Island; and 2 samples from turtles found dead on Okinawa. Another 15 foraging samples of scutes imported before Japan lifted its CITES reservation on *E. imbricata* were offered by the Japan Bekko Association: 2 from the Philippines, 4 from the Solomon Islands, 1 from Fiji, 1 from the Maldives, and 7 from the Seychelles.

Haplotype data for phylogenetic tree and network analysis and frequency for the foraging populations from Isla de Pinos in Cuba and Mona Island in Puerto Rico were described in Díaz-Fernández et al. (1999).

About 10 mg of scute or about 20 mg of soft tissue (in 70% ethanol) was placed in 310 µl of RSB buffer, 15 µl of 10% SDS, and 25 µl of 20 mg/ml Proteinase K, and incubated for 2 hrs at 55°C on a rotator for protein digestion. Nucleic acids were then extracted using an Iso Quick Nucleic Acid Extraction Kit (ORCA Research Inc., USA).

Extracted DNA was amplified by the Polymerase Chain Reaction (PCR) method. Universal primer L15926 (5-TCAAAGCTTACACCAGTCTTGTAAACC-3) (Kocher et al., 1989) and sea-turtle specific primer TCR6 (5-GTACGTACAAGTAAAACTACCGTATGCC-3) (Norman et al., 1994) were used and produced sufficient amplification of the mitochondrial control region of the hawksbill turtle. The PCR was done with an ASTEC PC-800/Thermolyne Amplytron II with 30 cycles of denaturation at 94°C for 30 sec, annealing at 45°C for 45 sec, and extension at 72°C for 45 sec.

Direct sequencing was carried out in a R.O.B. DNA Processor (Pharmacia L.K.B. Co. Ltd.) with a Thermo-sequenase cycle sequencing kit (Amersham), using Cy5 fluorescent labeled primers with the same sequences used in the PCR. The cycle was repeated 20 times with denaturation at 94°C for 30 sec, annealing and extension at 65°C for 30 sec. Sequencing was done with an ALFred DNA Auto-sequencer (Pharmacia L.K.B. Co. Ltd.).

Alignment of the sequence data was performed with a BioResearch/AE program (Fujitsu Ltd.) based on CLUSTAL V (Higgins et al., 1992) and CLUSTAL W (Thompson et al., 1994). A Neighbor-joining tree (Saitou and Nei, 1987) and bootstrap value (Saitou and Imanishi, 1989) was constructed using BioResearch/SINCA program version 3 (Fujitsu Ltd.). Network analysis was undertaken with Split Tree 2.4 (Huson, 1997). Haplotype diversity (h) was calculated using the formula: $h = 2n(1-\sum x_i^2)/(2n-1)$ (Nei and Tajima, 1981).

RESULTS

From the 136 Indo-Pacific samples, 40 substitution sites defining 24 haplotypes were observed in 481 bp fragments (Table 1). Haplotype Pac was previously identified in one individual of the foraging sample from Isla de Pinos, Cuba (Díaz-Fernández, 1999), as a haplotype having a

Table 1. Haplotype table for the 24 haplotypes detected in hawksbill turtles in the Indo-Pacific region. * Haplotype Pac was found only in the sample from the Caribbean region (Díaz-Fernández et al., 1999). Italic letters indicate transversions; = indicates indels. Haplotypes are divided into three clusters as indicated by the phylogenetic tree (Fig. 1).

	Polymorphic sites																																								
								1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	3	3	3	3	3	3	4	4	4
	2	2	5	6	6	7	8	0	1	2	3	3	4	4	4	6	7	8	9	9	0	0	3	3	3	4	5	5	6	6	8	9	1	3	6	7	7	8	3	3	4
Haplotypes	0	4	8	8	9	9	6	0	5	4	6	8	5	6	8	0	4	5	4	8	0	5	0	4	8	2	1	8	8	9	5	3	0	9	7	5	8	0	2	9	0
1	A	=	A	G	A	G	T	T	T	G	C	A	C	T	C	A	A	T	T	T	A	A	A	A	G	A	C	C	T	A	A	A	A	T	G	A	T	A	C	G	T
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*A*	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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3	G	T	-	A	*C*	*T*	C	C	-	A	-	-	T	C	T	-	-	-	C	C	-	*C*	-	-	A	G	-	T	C	-	-	G	-	-	-	-	-	G	T	-	-
7	G	T	-	A	*C*	*T*	C	C	-	A	-	-	T	C	T	-	-	-	C	C	-	*C*	-	-	A	G	-	T	C	G	-	-	-	-	-	-	-	G	T	-	-
8	G	T	-	A	*C*	*T*	C	C	-	A	-	-	T	C	T	-	G	-	C	C	-	*C*	-	-	A	G	-	T	C	-	-	G	-	-	-	-	-	G	T	-	-
15	G	T	-	A	*C*	*T*	C	-	-	A	-	-	T	C	T	-	-	-	C	C	-	*C*	-	-	A	G	-	T	C	-	-	G	-	-	-	-	-	G	T	-	-
17	G	T	-	A	*C*	*T*	C	C	-	A	-	-	T	C	-	-	-	-	C	C	-	*C*	-	-	A	G	-	T	C	-	-	-	-	-	-	-	-	G	T	-	-
23	G	T	-	-	*C*	*T*	C	-	-	A	-	G	T	C	-	G	-	-	-	C	-	*C*	-	-	-	G	-	T	C	-	-	-	G	C	A	G	-	-	T	*T*	-
24	G	T	-	-	*C*	*T*	C	-	-	A	-	G	T	C	-	-	-	-	-	C	-	*C*	-	-	-	G	-	T	C	-	-	-	G	C	A	G	-	-	T	*T*	-

Table 2. Haplotype distribution among the nesting and foraging samples of hawksbill turtles from the Indo-Pacific region.

	total	Haplotypes																							
Localities	*n*	1	2	4	5	6	9	10	11	12	13	14	16	18	19	20	21	22	3	7	8	15	17	23	24
Nesting samples																									
Okinawa, Japan	1																		1						
Ishigaki Island, Japan	5						5																		
Seribu Islands, Indonesia	9											9													
Foraging samples																									
Okinawa, Japan	2																		2						
Yaeyama Islands, Japan	104	46	2	1	3	4	7	3	1	2	1	4	1	3	1	1	1	1	14	4	1	2	1		
Philippines	2	2																							
Solomon Islands	4	1													2		1								
Fiji	1	1																							
Maldives	1																		1						
Seychelles	7																		2					3	2
total	136	50	2	1	3	4	12	3	1	2	1	13	1	3	3	1	2	1	20	4	1	2	1	3	2

Pacific origin. Among these substitution sites, only 1 indel was observed at the 24th site from the beginning of the control region. Five transversions were observed at sites 69, 79, 205, 251, and 439. The remaining 34 substitution sites were transitions.

One nesting sample from Okinawa was haplotype 3, and 5 individuals from Ishigaki Island all had haplotype 5 (Table 2). The 9 individuals from the Seribu Islands in Indonesia all had haplotype 14 . Even though sample sizes for these nesting samples were not large, these data suggest that each nesting population may have a specific haplotype as a genetic marker.

A total of 22 haplotypes were detected from 104 foraging individuals from the Yaeyama Archipelago in Okinawa Prefecture (Table 2), indicating that genetic diversity there was high. Almost half of the samples were haplotype 1, and 13.5% were haplotype 3, which was also detected from the nesting individual at Okinawa (Table 2). The third most common haplotype was haplotype 9 which was also detected from the nesting individuals at Ishigaki Island (Table 2). There did not appear to be any differences in haplotype frequencies among the 8 islands sampled in the Yaeyama Archipelago.

The scute samples from the Indo-Pacific region showed that haplotype 1, the most common in the Yaeyama Archipelago, was also detected in samples from the Philippines, Solomon Islands, and Fiji. Haplotype 3, which was the second most common haplotype in the Yaeyama Archipelago, was also detected in the Maldives and the Seychelles. New haplotypes (23 and 24) were detected from scute samples from the Seychelles.

Using the 24 haplotypes detected in the Indo-Pacific samples and 29 haplotypes from Caribbean samples, a phylogenetic tree was generated by the Neighbor-joining method (Fig. 1). The green turtle (*Chelonia mydas*, Allard et al., 1994) was used as an outgroup in forming the phylogenetic tree. The phylogenetic tree was divided into Caribbean and Indo-Pacific haplotypes. Indo-Pacific haplotypes were subdivided into three clusters: Indo-Pacific A, consisting of haplotypes 1–2, 4–6, 9–14, 16, 18, 19–22, and Pac; Indo-Pacific B, consisting of haplotypes 3, 7–8, 15, and 17; and Indo-Pacific C, consisting of haplotypes 23 and 24. Bootstrap analysis indicated that these clusters were supported by more than 98% of replications. Caribbean haplotypes were subdivided into 3 clusters: Caribbean A, consisting of haplotypes CU1, PR4, a, b, f, g, i, and n; and Caribbean B, consisting of haplotypes CU2, CU3, PR1, PR2, PR3, MX1, MX2, v, d, e, h, j, m, o, and p. Haplotype zz, recorded only at Mona Island, Puerto Rico (Díaz-Fernández et al., 1999), was considered to cluster independently.

Network analysis (Fig. 2) represents the evolutionary relationships between haplotypes, and indicates which substitution sites distinguished each haplotype. In this network site 100 was presented as a multiple substitution. Nodes I-P and C, which represent hypothetical ancestors of the Indo-Pacific haplotypes and Caribbean haplotypes, respectively, were separated by 11 substitutions. Three Indo-Pacific clusters had similar distances from the I-P node. Haplotype 1, the most common haplotype in the Indo-Pacific A cluster, had a distance of 13 substitutions from the I-P node; haplotype 3, the most common haplotype in the Indo-Pacific B cluster, was separated by 14 substitutions; and haplotype 24 was separated from the I-P node by 11 substitutions.

In the Indo-Pacific A cluster, 12 haplotypes (including haplotype Pac) differed by 1 substitution from haplotype 1, forming a so-called bush-like tree or fireworks-like tree. All the haplotypes in this cluster were located within 4 substitutions from haplotype 1. Although the Indo-Pacific B cluster was not as complicated as the Indo-Pacific A, haplotypes in this cluster were connected within 3 substitutions. The Indo-Pacific C cluster, which was found only in the Indian Ocean, was composed of 2 haplotypes separated by a single substitution.

DISCUSSION

Haplotypes 3 and 9 were identified as nesting haplotypes in Okinawa Prefecture, but haplotype 1, which was the most common haplotype in the foraging samples from the Yaeyama

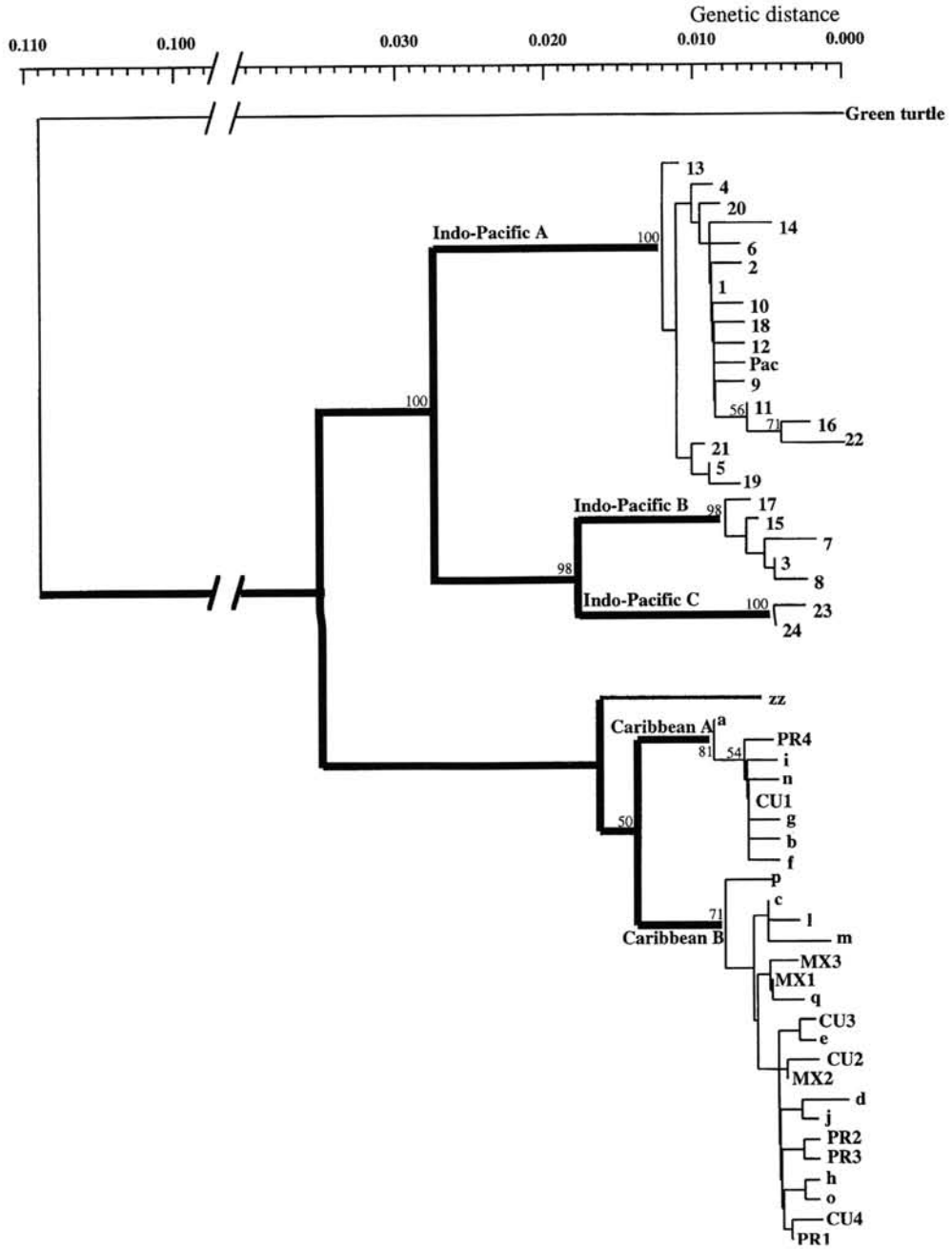

Figure 1. Phylogenetic tree for hawksbill turtle (*Eretmochelys imbricata*) haplotypes based on Neighbor-joining method for the 24 haplotypes found in 136 Indo-Pacific individuals and the 28 haplotypes found in 344 Caribbean individuals. Numbers above the branches indicate bootstrap support for the node.

Archipelago, was not identified anywhere as a nesting haplotype. This is probably due to the limited number of nesting samples, especially at Kuro Island, which is thought to be the largest nesting colony in the Yaeyama Archipelago.

The less common Cuban nesting haplotypes CU2, CU3, and CU4 did not cluster in Caribbean A with haplotype CU1, but did in Caribbean B, indicating that a single nesting population does not always include only haplotypes from the same cluster. Although the haplotype frequencies for three Caribbean rookeries from Cuba, Mexico, and Puerto Rico (Díaz-Fernández et al., 1999) indicated that each nesting population has specific haplotypes as genetic markers, the one exception was PR1, the main haplotype for the Puerto Rican nesting population, which was also detected in one individual from the Cuban nesting population. This may suggest that nesting females are not completely fixed at a specific rookery, and there is a possibility of dispersal of nesting individuals from the main rookery to other rookeries. Consequently, geographical origin and current location of the haplotype are not completely homologous - a nesting population may exhibit several

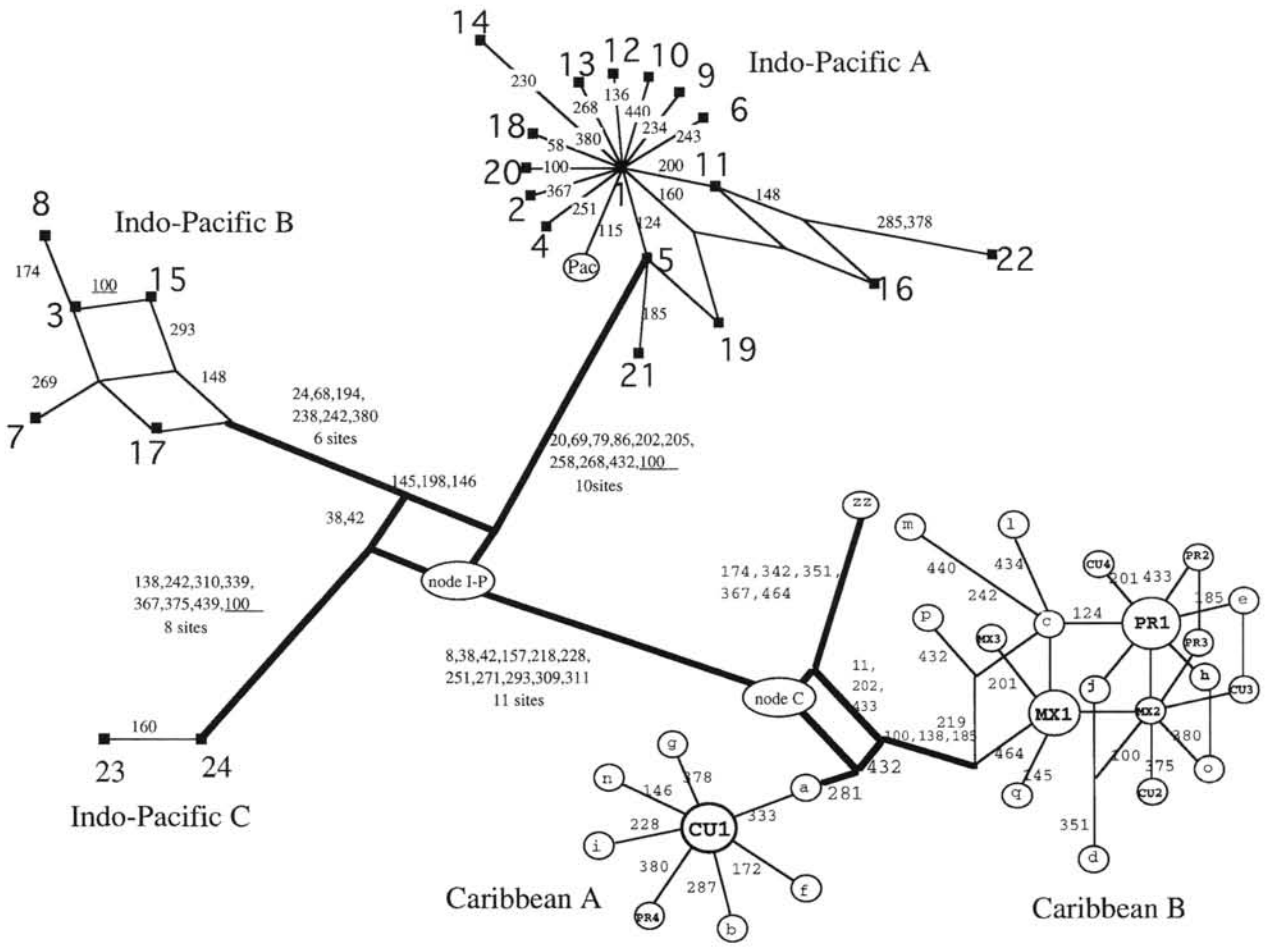

Figure 2. Network analysis of hawksbill turtle haplotypes based on 24 haplotypes from the Indo-Pacific region and 28 haplotypes from the Caribbean region for 481 bp of the mitochondrial control region sequence. Numbers refer to polymorphic sites; site 100 is not illustrated as a network but represented as a multiple substitution; parallel substitutions between clusters are not illustrated as a network in this figure.

haplotypes of different origins, indicating occasional haplotype crossovers between nesting colonies during the long evolutionary history of the species.

Due to the low number of nesting samples, contribution rates could not be calculated, so haplotype diversity (h) (Nei and Tajima, 1981) for the 104 individuals from the Yaeyama Archipelago were compared with those of the 115 individuals from Isla de Pinos, Cuba, and the 106 individuals from Mona Island, Puerto Rico (Díaz-Fernández et al., 1999). Numbers of haplotypes detected were 22, 14, and 16, respectively, and the haplotype diversities were calculated as 0.776, 0.777, and 0.791, respectively (Table 3). This suggests that these 3 foraging populations exhibit comparable haplotype diversities, consisting of 40–50% of the most common haplotype, 15–30% of the second and third most common haplotypes, and accompanied by several less common haplotypes.

Another similar characteristic was recognized in the genetic structure between the Indo-Pacific and Caribbean hawksbills (Fig. 2). In the Indo-Pacific A cluster there were 12 haplotypes with 1-substitution differences radiating from haplotype 1, and in the Caribbean A cluster, 7 haplotypes radiated from CU1 with 1-substitution differences. The Caribbean B cluster had a complicated network consisting of multiple 1-substitution differences. These 1-substitution differences suggest a recent common ancestor for each cluster.

In the phylogenetic tree (Fig. 1), the three Indo-Pacific clusters were supported by high bootstrap values and may represent a geographic cline from the Pacific to the Indian Ocean. Indo-Pacific A consisted of nesting samples from Japan and Indonesia, most of the foraging samples from Japan, and scute samples from the Philippines and Solomon Islands. Indo-Pacific B consisted of nesting and some foraging samples from Japan and scute samples from the Maldives and the Seychelles. Indo-Pacific C was composed only of scute samples from the Seychelles.

Table 3. Haplotype diversity (h) (Nei and Tajima, 1981) for three foraging populations of hawksbills. Data from Cuba and Puerto Rico from Díaz-Fernández et al. (1999).

	Number of Samples	Haplotypes	h
Yaeyama Islands, Japan	104	22	0.776
Isla de Pinos, Cuba	115	14	0.777
Mona Island, Puerto Rico	106	16	0.791

Network analysis (Fig. 2) showed that substitution differences between these three clusters and node I-P were similar: 12 substitutions for haplotype 5 in Indo-Pacific A; 12 substitutions for haplotype 17 in Indo-Pacific B; and 11 substitutions for haplotype 24 in Indo-Pacific C. These data indicate that these three clusters may have separated contemporaneously.

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LITERATURE CITED

ALLARD, M.W., MIYAMOTO, M.M., BJORNDAL, K.A., BOLTEN, A.B., AND BOWEN, B.W. 1994. Support for natal homing in green turtles from mitochondrial DNA sequences. Copeia 1994:34-41.

AVISE, J.C. 1994. Molecular Markers, Natural History and Evolution. New York: Chapman and Hall.

BRODERICK, D., MORITZ, C., MILLER, J.D., GUINEA, M., PRINCE, R.I.T., AND LIMPUS, C.J. 1994. Genetic studies of the hawksbill turtle *Eretmochelys imbricata*: evidence for multiple stocks in Australian waters. Pacific Conserv. Biol. 1:123-131.

BASS, A.L., GOOD, D.A., BJORNDAL, K.A., RICHARDSON, J.I., HILLIS, Z.M., HORROCKS, J.A., AND BOWEN, B.W. 1996. Testing models of female reproductive migratory behavior and population structure in the Caribbean hawksbill turtle, *Eretmochelys imbricata*, with mtDNA sequences. Mol. Ecol. 5:321-328.

BOWEN, B.W., AND AVISE, J.C. 1996. Conservation genetics of marine turtles. In: Avise, J.C., and Hamrick, J.L. (Eds.). Conservation Genetics — Case Histories from Nature. New York: Chapman and Hall , pp. 190-237.

BOWEN, B.W., BASS, A.L., GARCIA-RODRIGUEZ, A., DIEZ, C.E., VAN DAM, R., BOLTEN, A., BJORNDAL, K.A., MIYAMOTO, M.M., AND FERL, R.J. 1996. Origin of hawksbill turtles in a Caribbean feeding area as indicated by genetic markers. Ecol. Applications 6(2):566-572.

DÍAZ-FERNÁNDEZ, R.; OKAYAMA, T.; UCHIYAMA, T.; CARRILLO, E.; ESPINOSA, G.; MARQUEZ, R.; DIEZ, C.; AND KOIKE, H. 1999. Genetic sourcing for the hawksbill turtle, *Eretmochelys imbricata*, in the northern Caribbean region. Chelonian Conservation and Biology 3(2):296-300.

HIGGINS, D.G., BLEASBY, A.J., AND FUCHS, R. 1992. CLUSTAL V: improved software for multiple sequence alignment. Computer Applications in the Biosciences 8(2):189-191.

HUSON, S.H. 1997. SplitsTree: A Program for Analyzing and Visualizing Evolutionary Data. Math. Classification: 92-04, 92A10

KOCHER, T.D., THOMAS, W.K., MEYER, A., EDWARDS, S.V., PÄÄBO, S., VILLABLANCA, F.X., AND WILSON, A.C. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc. Natl. Acad. Sci. USA 86:6196-6200.

KOIKE, H., OKAYAMA, T., AND BABA, Y. 1998. Conservation and genetics of CITES animal species. In: Erdelen, W. (Ed.). Conservation, Trade and Sustainable Use of Lizards and Snakes in Indonesia. Mertensiella 9:37-44.

MILLER, J.D. 1997. Reproduction in sea turtles. In: Lutz, P.L., and Musick, J.A. (Eds.). The Biology of Sea Turtles. Boca Raton, FL: CRC Press, pp. 51-81.

NEI, M., AND TAJIMA, F. 1981. DNA polymorphism detected by restriction endonucleases. Genetics 97:145-163.

NORMAN, J.A., MORITZ, C., AND LIMPUS, C.J. 1994. Mitochondrial DNA control region polymorphisms: genetic markers for ecological studies of marine turtles. Mol. Ecol. 3:363-373.

SAITOU, N., AND IMANISHI, T. 1989. Relative efficiencies of the Fitch-Margoliash, maximum-parsimony, maximum-likelihood, minimum-evolution, and neighbor-joining methods of phylogenetic tree construction in obtaining the correct tree. Mol. Biol. Evol. 6:514-525.

SAITOU, N., AND NEI, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4:406-425.

THOMPSON, J.D., HIGGINS, D.G., AND GIBSON, T.J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Reserch 22:4673-4680.

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