Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 4, No. 4, p. 51-57, 2014
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Genetic diversity and phylogeography of hawksbill turtle in the Persian Gulf

M. Tabib[1], F. Frootan[2], M. Askari Hesni[3*]

[1]*Department of Marine Biology, Faculty of Marine Science, Marine Science and Technology University, Khoramshahr, Iran*

[2]*Department Bioengineering, Faculty Chemical and Metallurgical Engineering, Yildiz Technical University, Esenler, Istanbul, Turkey*

[*3]*Department of Biology, Faculty of Science, Shahid Bahonar University, Kerman, Iran*

Article published on April 02, 2014

Key words: Phylogeography, Hawksbill turtle, Mitochondrial DNA, Persian Gulf.

Abstract

Genetic diversity and phylogeography of Hawksbill sea turtle was studied by using sequencing of the mitochondrial DNA (D-loop and tRNA pro region). 45 dead embryos were collected from the Qeshm (25 samples) and Kish Islands (20 samples) in the Persian Gulf. Analysis of sequence diversity showed over 890 bp of the mtDNA D-loop and tRNA pro region revealed 5 polymorphism sites and 7 haplotypes. Two new haplotypes were submitted on NCBI gene bank as Iran3 with GU997696 accession number and Iran7 with JN627023 accession number. Haplotype (*h*) and nucleotide (π) diversity was (*h*= 0.313 and π= 0.0006) for Qeshm Island and (*h*= 0.111 and π=0.0002) for Kish Island, respectively. Total haplotype diversity was calculated as 0.212, which had demonstrated low genetic diversity in this area. The Fst and P-value showed that Hawksbill turtles of Qeshm and Kish Islands are different populations. A comparison of our data with previous studies showed a relationship between the Persian Gulf and Indo-Pacific Hawksbill turtles and also sharp relationship with east Atlantic. Hawksbill turtles of the Persian Gulf have been migrated from the Pacific and the Oman Sea into this area. As a result, evidence of populations migration was not found from the West.

***Corresponding Author:** M. Askari Hesni ✉ mahesni@gmail.com

Introduction

Sea turtles are categorized in two taxonomic families; the Cheloniidae with six species: Loggerhead turtle (*Caretta caretta*); Green turtle (*Chelonia mydas*); Hawksbill turtle (*Eretmochelys imbricata*); Olive ridley turtle (*Lepidochelys olivacea*); Kemp's ridley turtle (*Lepidochelys kempi*) and Flat back turtle (*Natator depressus*) and the Dermochelyiidae with a single highly derived species; the leatherback turtle (*Dermochelys coriacea*) (Bowen and Karl, 2007). Sea turtles of the Persian Gulf belong to two species; the green and Hawksbill turtles (Mobaraki, 2004a). The Hawksbill turtles have a circum global distribution in tropical areas of the Atlantic, Indian and the Pacific Oceans, as well as the Persian Gulf (Groombridge and Luxmoore, 1989; Pritchard and Mortimer, 1999). Hawksbill turtles (*Eretmochelys imbricata*) are a migratory species that travel hundreds or thousands of kilometers to breeding and feeding areas (Troëng *et al.*, 2005; Van Dam *et al.*, 2008).

Forage of Hawksbill turtles in the Atlantic Ocean is sponge (Blumenthal *et al.*, 2009). But the turtles eat alga and other invertebrates and also sponges (Bjorndal, 1997).

Mitochondrial DNA (mt-DNA) carries 37 genes in the vertebrates including 22 tRNA, 2 rRNA and 13 mRNA genes. Furthermore, it containes a non coding region known as D-loop (Saccone *et al.*, 1999). It was affirmed that molecular markers are useful for perceiving migration patterns, feeding ground population composition, natal homing, and the genetic composition and structure of rookeries worldwide (Fitzsimmons *et al.*, 1997a,b; Bolten *et al.*, 1998; Bowen *et al.*, 2005). Genetic studies of nesting and foraging Hawksbill turtles have been executed in the Indo-Pacific and Atlantic basins (Broderick *et al.*, 1994; Broderick and Moritz, 1996; Bass, 1996; Bowen *et al.*, 1996; Koike *et al.*, 1996; Okayama *et al.*, 1996; Tabib *et al.*, 2011; Kazemi Nezhad *et al.*, 2012).

The Hawksbill sea turtle (*Eretmochelys imbricata*) is listed as Critically Endangered in the IUCN Red Book of Threatened Species (IUCN, 2010), therefor study on its diversity genetic is important. Also there is rare studies on the *Eretmochelys imbricata* genetic diversity and phylogeography, so investigation on this subjects is very important.

Materials and methods

In this research, 45 dead embryos of Hawksbill turtles were collected from Qeshm and Kish islands, and were fixed in 96% ethanol. DNA was extracted from liver and muscle tissues (0.1 g), digested overnight at 56°C in a 1X TE buffer, 0.5 mg/mL proteinase K and 0.01% SDS solution, using standard phenol-chloroform (Hillis and Moritz 1990). An 890-bp portion of the mtDNA D-loop and tRNA Pro was amplified by polymerase chain reaction (PCR) using the H950 (5'-GTCTCGGATTTAGGGGTTTG-3') and LCM15382 (5'-GCTTAACCCTAAAGCATTGG-3') primers (Abreu-Grobois *et al.*, 2006). Amplifications were performed in a total volume of 25 mL containing 5-50 ng whole DNA, 10 mM of dNTP, 10 pmol of each primer, 0.5 U super Taq polymerase and the corresponding reaction buffer (1X). Cycling parameters were 94°C for 5 min, followed by 35 cycles at 94°C for 60 s, 53.5°C for 90 s, and 72°C for 60 s, and a final extension at 72°C for 5 min. All specimence PCR products send for Macrogene Seoul Company.

Sequence alignments were performed with the CLUSTALW (Thompson *et al.*, 1994) software, and haplotype and nucleotide diversity and pairwise FST were calculated using Arlequin v. 2 (Schneider *et al.*, 2000). Phylogenetic tree was performed by the MEGA v 4.0 (Tamura *et al.*, 2007) software.

Results

Analysis of 45 sequences displayed five polymorphic sites and seven haplotypes (Table 1). Most of the haplotypes were identified and recorded before on the NCBI gene bank (Tabib *et al.*, 2011). Two new haplotypes were submitted on NCBI gene bank as Iran3 with GU997696 accession number and Iran7 with JN627023 accession number. Haplotype diversity (*h*) values were 0.313 for Qeshm Island and 0.111 for Kish Island (Table 2). Furthermore nucleotide diversity (π) values were estimated 0.0006

and 0.0002 respectively for Qeshm and Kish Islands (Table 2). Computed population differentiation (FST) value between the populations was 0.999 ± 0.0002 (Permutation 1000) that showed population of 2 Islands are separated. Moreover the estimated gene flow (Nm) value between the Qeshm and Kish islands populations was 0.000. This results demonstrate that two turtle populations are seperate.

Table 1. Polymorphic sites corresponding related to 7 Hawksbill turtle haplotypes detected in the Persian Gulf, a 890-bp fragment of mtDNA D-loop and tRNA Pro sequence.

Base position / Haplotypes	163	165	166	484	644
Iran1	T	C	T	G	N
Iran2	T	C	C	G	T
Iran3	T	C	T	G	T
Iran4	T	C	T	A	T
Iran5	G	C	T	G	T
Iran6	T	T	T	G	T
Iran7	T	C	T	G	C

Table 2. Haplotype (*h*) and nucleotide (π) diversities for two populations of the Hawksbill turtle in the Persian Gulf.

Location	Haplotype Diversity(*h*)	Nucleotide Diversity(π)
Qeshm	0.313	0.0006
Kish	0.111	0.0002
Total	0.212	0.00038

Average number of nucleotide differences (Kt) was calculated as 0.222. According to the phylogenetic tree which was drawn by MEGA 4, detected a relationship between the Indo-Pacific and the Persian Gulf Hawksbill turtles (Figure 1).

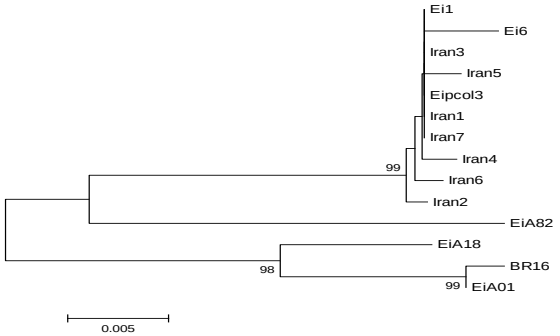

Figure 1. phylogenetic tree- Neighbor-joining tree based on the mtDNA D-loop sequences. Bootstrap values (10000 replicates) are indicated on the branches. Two clades of haplotypes were identified, Haplotypes EiA01(accession nos EF210779), EiA18(accession nos EF210786) (Velaz- Zuazo, 2008) and BR16(accession nos DQ177341)(Lara – Ruiz et al., 2006) are nested in the Atlantic Ocean and EiA82(accession nos GU138123)(Monozon- Argello, 2010) nested in the eastern Atlantic Ocean. Haplotypes Iran1, 2,3, 4, 5,6 and 7 and Ei1(accession nos HM030865), Ei6(accession nosHM030869) (Mohd Arshaad and Syed Abdul Kadir, 2010) and Eipcol3(accession nos HQ890548) (Trujillo- Arias et al., 2011) are nested in the Indo-Pacific Ocean.

Discussion

Hawksbill turtle (*Eretmochelys imbricata*) are known as migratory species. This prompted Archie Carr to postulate that female turtles migrate to their natal nesting beach to reproduce (Carr, 1967). Hendrickson (1958) proposed an alternative explanation for female nestsite fidelity, wherein first-time nesting females follow experienced breeders from the feeding habitat to a nesting beach, and use this site for all subsequent nesting (Owens *et al.*, 1982). This social facilitation would explain the site fidelity of nesting turtles without invoking the extreme behaviour of hatchlings remembering a beach location and finding it decades later for reproduction. If females return faithfully to their rookery of origin, then each nesting population should possess a unique genetic signature in terms of female-transmitted mtDNA. In contrast, social facilitation would allow high rates of female-mediated gene flow between nesting populations that overlap

on feeding grounds. Hence, the most robust tests of natal homing involve populations that overlap on feeding habitats, so that females have a 'choice' between natal homing and social facilitation (Bowen and Karl, 2007) .

Adults immigrate long distance of kilometres just for approaching to the breeding areas (Van Dam *et al.*, 2008). Earlier genetic studies indicated that juvenils of Hawksbill aggregating on foraging areas originate from different nesting beaches (Velez-Zuazo *et al.*, 2008). Despite of the fact, genetic studies of the coastal turtles has shown approximately fixed diversity in mtDNA haplotype frequencies (Maffucci *et al.*, 2006). These results exposed independence of the reproductive units. Except two haplotypes Iran3 with GU997696 accession number and Iran7 with JN627023 accession number haplotype, all of the other haplotypes pointed out in the current study were already defined by Tabib *et al.* (2011). The results show a unique subpopulation of turtle in the Persian Gulf. Genetic diversity among the studied turtles in Qeshm Island is much higher than the ones of Kish Island. these consequences confirms the result explained in the related earlier study (Tabib *et al.*, 2011). It denotes the appropriate condition shore in Qeshm Island, suitable protection, bigger and nearer to the strait of Hormoz than Kish Island So Qeshm Island is more sheltered place for the rookeries. According to the new Hawksbill turtles entrance to Qeshm Island that is coincident on social facilitation so it can be one of the reasons for the genetic diversity in it. Calculated total haplotype diversity value was 0.212 which reveals low genetic diversity in the area of this part of the Persian Gulf. This results is like to Kazemi Nezhad *et al.* (2012) in persian Gulf. On the other hand, high FST and Nm value computed by Alrequin software showes Hawksbill turtle of Qeshm and Kish Islands are separated population that is against result of previous studies (Tabib *et al.*, 2011).

Estimated gene flow is based on the relationship between Nm and the degree of population substracturing as determined by Wright (1951) was 0.000. As twice populations have similar size, Nm describes the average number of individuals per generation migrating between populations (Wolf and Soltis, 1991). In our study gene flow among the populations within each geographic populations of *Eretmochelys imbricata* was low. It is against the data of the previous studies (Tabib *et al.*, 2011).

It was accepted that the Hawskbill turtles of the Persian Gulf are part of the Indian ocean Hawskbill turtles (Kinunen and Walczak, 1971). Result of our study showed a relationship between the Indo-Pacific and the Persian Gulf Hawksbill turtles that it is similar to Nishizawa research (Nishazawa *et al.*, 2012). But there is sharp relationship between Hawksbill turtle in Indo-Pacific and east of Atlantic that is similar Okayama (Okayama *et al.*, 1999).

References

Abreu-Grobois A, Horrocks J, Formia A, Leroux R, Velez-Zuazo X, Dutton PH, Soares L, Meylan A, Browne D. 2006. New mtDNA dloop primers which work for a variety of marine turtle species may increase the resolution capacity of mixed stock analysis. In: Frick MG, Panagopoulou A, Rees A, Williams KL (eds) 26th Annual Symposium on Sea Turtle Biology and Conservation, Crete, Greece, p. 179.

Bass AL. 1996. Application of isolation by distance models to hawksbill turtle (*Eretmochelys imbricata*) nesting sites in the caribbean. In: Proceedings of the International Symposium on Sea Turtle Conservation Genetics (Bowen BW and Witzell WN, eds.). NOAA Technical Memorandum. National Marine Fisheries Service, Southeast Fisheries Science Center, 12-14 September 1995, Miami.

Bjorndal KA. 1997. Foraging Ecology and Nutrition of Sea turtles. In Lutz, P.L.: Musick, J.L. (eds) The Biology of Sea turtles vol 1. CRC Press, Boca Raton, FL, p.199–231.

Blumenthal JM, Austin TJ, Bothwell JB, Broderick AC, Ebanks-Petrie G, Olynik JR, Orr MF, Solomon JL, Witt MJ, Godley BJ. 2009. Diving Behavior and Movements of Juvenile Hawksbill turtles (*Eretmochelys imbricata*) on a Caribbean coral reef. Journal of Coral Reef Studies **28**: 55–65.

Bolten AB, Bjorndal KA, Martins HR, Dellinger T, Biscoito MJ, Encalada SE, Bowen BW. 1998. Transatlantic developmental migrations of loggerhead sea turtles demonstrated by mtDNA sequence analyses. Journal of Ecological Applications. **8:** 1–7.

Bowen BW, Bass AL, Garcia-Rodriguez A, Diez CE, Van Dam R, Bolten A, Bjorndal KA, Miyamoto MM, Ferl RJ. 1996. Origin of hawksbill turtles in a Caribbean feeding area as indicated by genetic markers. Journal of Ecological Applications. **6:** 566-572.

Bowen BW, Bass AL, Soares L, Toonen RJ. 2005. Conservation implications of complex population structure: lessons from the loggerhead turtle (*Caretta caretta*). Journal of Molecular Ecology. **14:** 2389–2402.

Bowen BW, Karl SA. 2007. Population genetics and phylogeography of sea turtles. Journal of Molecular Ecology. **16:** 4886-4907.

Broderick D, Moritz C, Miller JD, Guine M. 1994. Genetic studies of the Hawksbill turtle (*Eretmochelys imbricate*): evidence for multiple stocks in Australian waters. Pacific Conservation Biology. **1:** 123-131.

Broderick D, Moritz C. 1996. Hawksbill Breeding and Foraging Populations in the Indo-Pacific Region. In: Proceedings of the International Symposium on Sea Turtle Conservation Genetics (Bowen BW and Witzell WN, eds.). NOAA Technical Memorandum. National Marine Fisheries Service, Southeast Fisheries Science Center, 12-14 September 1995, Miami.

Carr AF. 1967. So Excellent a Fish: A Natural History of Sea Turtles. Scribner, New York.

Fitzsimmons NN, Limpus CJ, Norman JA, Goldizen AR, Miller JD, Moritz C. 1997a. Philopatry of male marine turtles inferred from mitochondrial DNA markers. Proceedings of the National Academy of Sciences, USA, **94:** 8912–8917.

Fitzsimmons NN, Moritz C, Limpus CJ, Pope L, Prince R. 1997b. Geographic structure of mitochondrial and nuclear gene polymorphisms in Australian green turtle populations and male-biased gene flow. Journal of Genetics **147:** 1843–1854.

Groombridge B, Luxmoore R. 1989. The Green Turtle and Hawksbill (Reptilia: Cheloniidae): World Status, Exploitation, and Trade. CITES Secretatiat, Lausanne.

Hendrickson JR. 1958. The green sea turtle, *Chelonia mydas* (Linn.) in Malaya and Sarawak. Proceedings of the Zoological Society of London, **130:** 455–535.

Hillis DM, Moritz C. 1990. Molecular Taxonomy. Sinauer Associates Inc. Publishers, Sanderland.

IUCN (International Union for Conservation of Nature). 2010. The IUCN red list of threatened species, Version 2010.1. Available:http: //www.iucnredlist.org.

Kazemi Nezhad SR, Modheji E, Zolgharnein H. 2012. Polymorphism analysis of Mitochondrial Dna control region of Hawksbill turtles (*Eretmochelys imbricata*) in the Persian Gulf. Journal of Fisheries and Aquatic Science. 7(5): 339- 345.

Kinunen W, Walczak PS. 1971. Persian Gulf Sea Turtle Nesting Surveys. Job Completion Rept., Division of Research Development, F7-F50.

Koike H, Okayama T, Baba Y, Diaz R. 1996. Conservation Genetics for the CITES-Listed Animals - Mitochondrial DNA Analysis Using the Scutes of Hawksbill Turtles. International Symposium on Network and Evolution of Molecular Information, 20-22 April 1996, Tokyo.

Lara-Ruiz P, Lopez GG, Santos FR, Soares LS. 2006. Extensive hybridization in hawksbill turtles (*Eretmochelys imbricata*) nesting in Brazil revealed by mtDNA analyses. Conservation Genetics. **7**: 773-781.

Maffucci F, Kooistra WHCF, Bentivegna F. 2006. Natal origin of loggerhead turtles, *Caretta caretta*, in the neritic habitat off the Italian coasts, Central Mediterranean. Biological Conservation. **127**: 183-189.

Mobaraki A. 2004a. Nesting of the hawksbill turtle in Shidvar Island, Hormozgan Province, Iran. Mar. Marine Turtle Newsletter. **103**: p. 13.

Mohd Arshaad W, Syed Abdul Kadir SA. 2010. Identification of the stock population of green and hawksbill turtles in the southeast asian region. (unpublished).

Monozon- Argello C, Rico C, Marco A, Lopez P, Lopez- Jurado LF. 2010. Genetic characterization of eastern Atlantic hawksbill turtles at a foraging group indicates major undiscovered nesting populations in the region. Journal of Experimental Marine Biology and Ecology . **387:** 9-14.

Nishizawa H, Okuyama J, Abe O, Kobayashi M, Arai N. 2012. Mitochondrial DNA Variation in Hawksbill Turtles (*Eretmochelys imbricata*) Nesting on Ishigaki Island, Japan. Marine Turtle Newsletter. **132**: 1-2.

Okayama T, Diaz R, Koike H, Diez CE. 1996. Mitochondrial DNA analysis of the Hawksbill turtle. I. Haplotype detection among samples in the Pacific and Atlantic Oceans. International Symposium on Network and Evolution of Molecular Information, 20-22 April 1996, Tokyo.

Okayama T, Diaz-Fernandez R, Baba Y, Halim M. 1999. Genetic diversity of the hawksbill turtle in the Indo- Pacific and Caribbean regions. Chelonian Conservation and Biology. **3**: 362-367.

Owens DW, Grassman MA, Hendrickson JR. 1982. The imprinting hypothesis and sea turtle reproduction. Journal of Herpetologica, **38:** 124–135.

Pritchard PCH, Mortimer JA. 1999. Taxonomy, external morphology, and species identification. In: K.L.Eckert etal, (eds). Research and management techniques for the conversation of sea turtles. IUCN/SSC Marine Turtle Specialist Group Publication **4:** 21-38.

Saccone C, Giorgi CO, Gissi C, Pesole G, Reyes A. 1999. Evolutionary genomics in metazoa: the mitochondrial DNA as a model system. Journal of Genetics **238:** 195-209.

Schneider S, Roessli D, Escoffier L. 2000. Arlequin: a software for population genetics data analysis, version 2.000. Genetic and Biometry Laboratory, Departement of Anthropology, University of Geneva, Switzerland.

Tabib M, Zolgharnein H, Mohammadi M, Salari-Aliabadi MA, Qasemi A, Roshani S, Rajabi Maham H, Frootan F. 2011. mtDNA variation of the critically endangered hawksbill turtle (*Eretmochelys imbricata*) nesting on Iranian islands of the Persian Gulf. Genetics and Molecular Research **10** (3): 1499-1503. Available:http://www.funpecrp.com.br.

Tamura K, Dudley J, Nei M, Kumar S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4. Molecular Biology and Evolution. **24**: 1596-1599.

Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research. **22:** 4673-4680.

Troëng S, Rankin E. 2005. Long-term conservation efforts contribute to positive green turtle *Chelonia mydas* nesting trends at Tortuguero, Costa Rica. Biological Conservation **121**: 111–116.

Trujillo-Arias N, Amorocho D, Lopez DC, Mejia-Ladino LM. 2011. Caracterizacion genetica de la Tortuga carey (Eretmochelys imbricata) en Colombia, basada en la region control de and mt. CONGRESO: III Congreso Colombiano de Zoología LUGAR DE CELEBRACIÓN: Colombia. AÑO:. 21-26 novomber 2010. Colombia.

Van dam RP, Diez CE, Balazs GH, Colon LAC, McMillan WO, Schroeder B. 2008. Sex-specific migration patterns of hawksbill turtles breeding at Mona Island, Puerto Rico. Journal of Endangered Species Research. **4:** 85–94.

Velez-Zuazo X, RamosWD, Van Dam RP, Diez CE, Serrano X, Abreu- Grobois A, McMillan WO. 2008. Dispersal, recruitment and migratory behavior in a hawksbill sea turtle population. Journal of Molecular Ecology **17:** 839- 853.

Wolf PG, Soltis PS. 1991. Genetic divergence and relationships in the *Ipomopsis aggregata* complex and related species (Polemoniaceae). American Journal of Botany **72**: 232-244.

Wright S. 1951. The genetical structure of populations. Annals of Eugenics Journal **15:** 323-354.