

Assessment of the origin of a Loggerhead Turtle, *Caretta caretta*, found in Kuwaiti waters, using mitochondrial DNA

(Reptilia: Cheloniidae)

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Abstract. 306 base pairs from the control (D-loop) region of the mitochondrial DNA (mtDNA) of a Loggerhead Turtle, *Caretta caretta* Linnaeus, 1758, from Kuwait were sequenced in order to identify the origin of this turtle. Neighbour-joining tree analyses with sequences available in the GenBank showed that it had a close relationship with those of the Atlantic colonies. For adult Loggerhead Turtles of the Atlantic colonies which undertake long distance migrations, the southern extension of Africa might be less formidable as a continental barrier to their passage into the Indian Ocean and subsequently into the Arabian area because of their temperate distribution.

Key words. *Caretta caretta*, mitochondrial DNA, control region haplotype, Kuwait.

Introduction

The Loggerhead Turtle (*Caretta caretta* Linnaeus, 1758) is widely distributed in temperate, subtropical, and tropical waters (DODD 1988). In the Indian Ocean, the largest nesting population occurs on Masirah Island (Oman), with 30,000 nests a year, which is also considered to be the largest aggregation of this species in the world (ROSS & BARWANI 1982). Smaller nesting aggregations in the Indian Ocean occur in Tongaland, South Africa (BALDWIN et al. 2003), Mozambique (BALDWIN et al. 2003), Madagascar (RAKOTONIRINA 2001), the mainland of Oman on the Arabian Sea coast as well as the Halaniyat Islands (Oman) (ROSS 1982) and Socotra Island (Yemen) (PILCHER & SAAD 2000). A small population of Loggerheads also nests in Sri Lanka (KAPURUSINGHE 2006). In the eastern Indian Ocean, Loggerhead nesting is restricted to Western Australia (DODD 1988). The species is also known to nest along the shores of the eastern Mediterranean Sea (BRODRICK & GODLEY 1996, MARGARITOU LIS & REES 2001). Except for Oman, there is no written record of nesting Loggerheads in the eastern Arabian area. However, they have been rarely sighted elsewhere in the region. In the UAE, the presence of Loggerhead Turtles is only known from a few skulls and carapaces of dead animals found on offshore islands west of Abu Dhabi; no confirmed live sightings have been recorded (BALDWIN & GARDENER 2005). AL-MOHANNA & MEAKINS (2000) and MEAKINS & AL-MOHANNA (2000) were the first to report the occurrence of this species in Kuwaiti waters. Although their frequent sightings in Kuwaiti waters have evoked the interest of researchers, no nesting sites have so far been discovered and their presence is probably associated with foraging for food (AL-MOHANNA & GEORGE 2009).

Marine turtles of the family Cheloniidae migrate hundreds or thousands of kilometres between feeding habitats and nesting colonies (CARR 1964), and conservation efforts to date



Fig. 1. Loggerhead Turtle, *Caretta caretta*, rescued after being hit by a boat.

have been hindered by the inability of linking marine turtles at sea with their respective nesting populations (BOWEN 1995). Several marine turtles die every year in migratory corridors and feeding habitats as a result of a variety of direct and indirect threats, and it remains unknown which nesting colonies are affected by such mortalities. Recent developments in the application of molecular genetic markers can help to identify the origin of marine turtles in migratory corridors or at coastal feeding habitats (BOWEN 1995). In this context we conducted a mitochondrial DNA (mtDNA) analysis of a rescued Loggerhead Turtle in Kuwaiti waters.

Material and methods

A Loggerhead Turtle was rescued after being hit by a speeding boat in the southern part of Kuwait Bay, which had caused a serious head injury, and was subsequently released after a period of rehabilitation at the Scientific Centre, Kuwait (Fig. 1). Morphometric measurements of this turtle were taken and skin samples were collected for mtDNA analysis. Genomic DNA was extracted from the skin samples with Puregene[®] DNA Purification Kit. After DNA extraction, a 306 base-pair sequence of the mitochondrial control region was amplified using the turtle-specific primers HDCM-1 (ALLARD et al. 1994) and TCR-5 (NORMAN et al. 1994). The target mitochondrial sequence was amplified by polymerase chain reaction (PCR) using GeneAmp[®] Gold PCR Reagent Kit. The PCR profile comprised an initial denaturation of 10 min at 94° C (to activate the AmpliTaq Gold polymerase), followed by 35 cycles of: 94° C for 45 s, 55° C for 45 s, 72° C for 45 s; and 72° C for 5 min. After that, an agarose check gel was run to confirm amplification, and the

Table 1. A 306 base pairs sequence from the control (D-loop) region of the mitochondrial DNA of the rescued loggerhead.

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1  atttaccact agcatatgat cagtaatggt gtcgattaat ttggctttaa acataaaaat
61  ttattaatntt tacataaaact gttttagtta catgactatt atacaggtaa taagaatgaa
121 atgatatagg acataaaaatt aaaccattat tctcaacccat gaatatcgtc acagtaatag
181 gttattttctt agttcagctc atcacgagaa ataagcaacc cttgttagta agatacaaca
241 ttaccagttt caggccatt aagtcatatc gtacataact gatctattct gcctctggt
301 tgtttt

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PCR product was purified for sequencing using NucleoSpin Extract II Kit. The purified PCR product was labelled with BigDye[®] Terminator v3.1 Cycle Sequencing Kit. The sequencing PCR cycle conditions were: 25 cycles of 95°C for 10 s, 50°C for 5 s and 60°C for 4 min. Extended products were purified by alcohol precipitation followed by washing with 70% ethanol. The DNA pellet was finally resuspended in formamide and sequenced using an automated fluorescent DNA sequencer, ABI 3130xl Genetic Analyzer. The sequenced fragment of mitochondrial control region was then compared to randomly selected published sequences from Atlantic and Pacific colonies in the GenBank database at the National Center for Biotechnology Information (URL of NCBI Home Page: www.ncbi.nlm.nih.gov). A neighbour-joining tree was generated using PHYLIP Ver. 3.66 (TUIMALA 2006). The related taxon, *Lepidochelys kempii* (Garman, 1880), was used as outgroup to root the tree, and input data were randomized with a random seed number and 100 replicates.

Results and discussion

Morphometric measurements of the rescued Loggerhead Turtle were recorded before its release. The weight of the turtle was estimated as 110-120 kg; other measurements: curved carapace length 100 cm, curved carapace width 87 cm; plastron length 69 cm; plastron width 62 cm; head length 25 cm; head width 23 cm. A 306 base-pair sequence was named haplotype C and submitted to the GenBank (accession number DQ924967) (Table 1). Fig. 2 shows a neighbour-joining tree generated after randomly selecting sequences from Atlantic and Pacific colonies from a search for similar sequences in the GenBank database with Basic Local Alignment and Search Tool (BLAST). The neighbour-joining tree shows that it has a close relationship with those of the Atlantic colonies. Genetic studies on Loggerhead Turtles in the Indian Ocean are scanty. BOWEN et al. (1994) assayed Loggerheads using restriction site analysis of mitochondrial DNA, which revealed unique haplotypes in Oman and South Africa. However, in order to elucidate their relationship to each other and to populations in the other oceanic basins, sequencing analysis needs to be carried out. BLAST result also showed that it had 100% identity with haplotype CcA11.1, reported present in a bycatch of the North Atlantic.

Since the Loggerhead Turtle has a temperate distribution, the southern extension of Africa might be less formidable as a continental barrier to its contemporary dispersal. It is also well known that adult Loggerheads undertake reproductive migrations that range from tens to thousands of kilometres (MEYLAN et al. 1983, HUGHES 1989, LIMPUS et al. 1992), and the presence of Loggerheads belonging to the Atlantic colonies in the Indian Ocean/Arabian Gulf is therefore possible, a hypothesis that can be tested with molecular genetic data as of

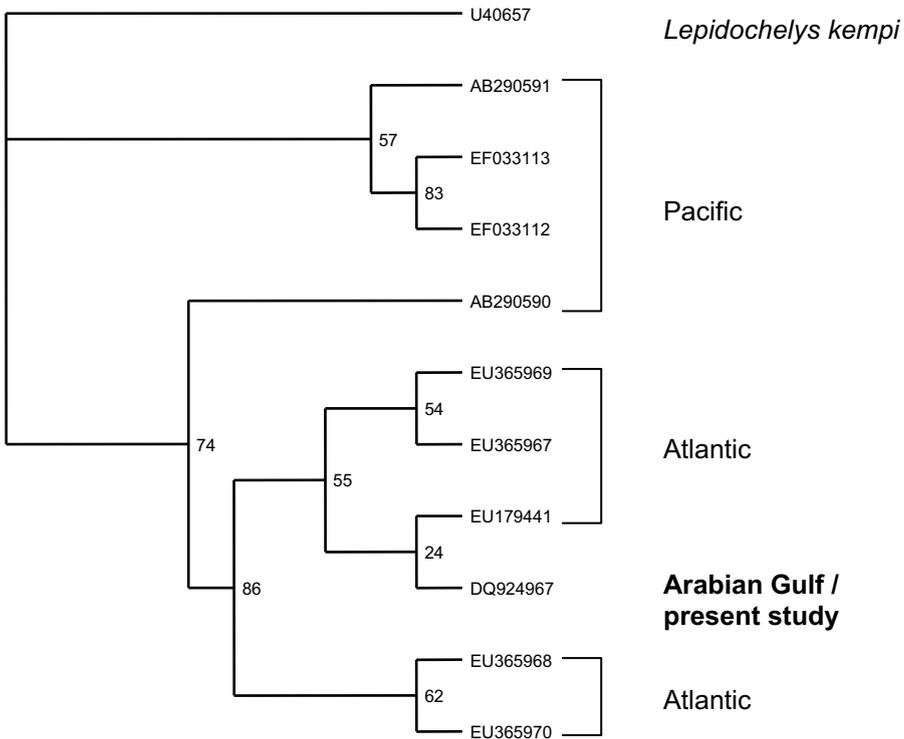


Fig. 2. Neighbour-joining tree for *Caretta caretta* from different oceanic basins, based on mtDNA control region sequences and rooted using *Lepidochelys kempii* as an outgroup. The numbers at specific nodes represent the percentage recovery of the particular node in 100 bootstrap replications.

the present study (Fig. 2). In many cases researchers know the locations of feeding grounds and the locations of nesting populations, but they do not know which nesting populations use which feeding areas (BOWEN 1995). An emerging conservation concern has brought into focus this fundamental gap in the scientific knowledge of marine turtles.

When the genetic distinctiveness of marine turtle nesting populations was discovered, it became apparent that mtDNA polymorphisms could be applied as natural genetic tags to identify feeding cohorts far from the nesting colony (BOWEN 1995). Compared to the externally applied tags, this technique helps identify the source of turtles captured far from their nesting beach with relative expediency and accuracy. Consequently, as marine turtle populations continue to decline, these data have immediate conservation applications.

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