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Loggerhead turtles (*Caretta caretta*) foraging at Drini Bay in Northern Albania: Genetic characterisation reveals new haplotypes

Can Yilmaz[1], Oguz Turkozan[1,*], Fevzi Bardakci[1], Michael White[2], Esmeralda Kararaj[3]

[1] *Adnan Menderes University, Faculty of science and Arts, Department of Biology, 09010 Aydin, Turkey.* * *Corresponding author.* E-mail: turkozan@adu.edu.tr
[2] *Mediterranean Association to Save the Sea Turtles, 1c Licavitou Street, Athens, Greece.*
[3] *School of Biological Sciences, Tirana University, Tirana, Albania.*

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Abstract. Loggerhead turtles (*Caretta caretta*) was studied over 3 summers in a nearshore habitat, the Patoku area in the southern part of Driniki Bay, Albania. Tissue samples were collected from 40 loggerhead turtles incidentally captured in stavnike fish-traps (a type of pond-net). A fragment of 859 base-pair mt-DNA d-loop region was amplified from these turtles and compared with previously described haplotypes. Haplotype CC-A2.1 (93%) was the dominant haplotype in the region. Two previously unknown haplotypes, CC-A6.1 and CC-A10.4, were described with this study. Furthermore, haplotype CC-A.2.8 was also observed which was previously recorded from Italy. Haplotype and nucleotide diversity were 0.14615 and 0.00017, respectively.

Keywords. *Caretta caretta*, foraging ground, mitochondrial DNA, haplotype, Albania

INTRODUCTION

During their life history, loggerhead turtles (*Caretta caretta*; Linnaeus 1758) generally experience two different ecological stages, oceanic and neritic (Bolten, 2003). The oceanic stage includes the time from when the hatchlings leave the nesting beach until they return to coastal benthic foraging habitats as juveniles (Bolten & Balazs, 1995). According to McClellan and Read (2007), the shift from oceanic to neritic waters is ''complex and reversible" and therefore is not a discrete ontogenetic shift. Although these stages have long been studied across the distributions of loggerhead turtles, in recent decades, molecular tools have become very helpful for the detection of developmental migrations (Bolten et al., 1998) and determination of stock compositions and in foraging and wintering areas.

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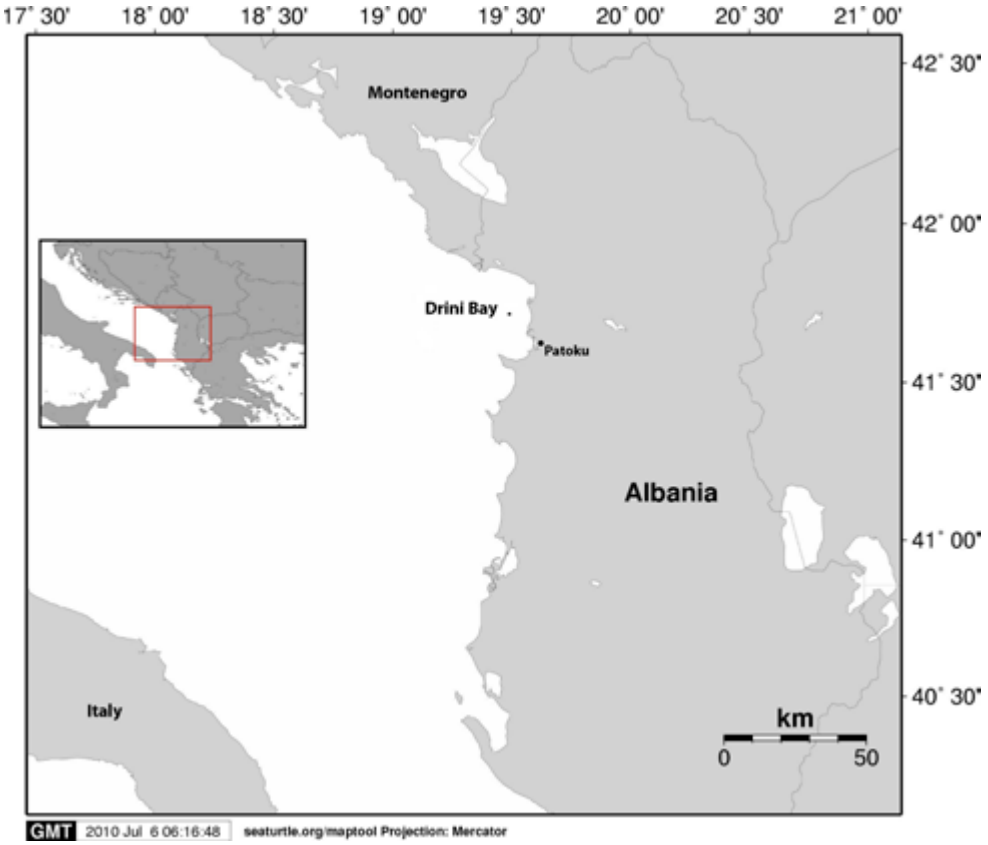

Fig. 1. The map showing the study area

Drini Bay in northern Albania is one of the important foraging grounds for loggerhead turtles in the Adriatic Sea (White et al., 2011). In this study, we collected tissue samples from loggerhead turtles in a nearshore habitat in the southern part of Drini Bay (Fig. 1) to generate preliminary information about the genetic composition of the foraging population. This is the first genetic characterization study in this area using a fragment of the D-loop mtDNA.

MATERIAL AND METHODS

Sea turtles were studied over three summers (2008-2010) by MEDASSET (Mediterranean Association to Save the Sea Turtles). DNA samples were collected in 2009-2010 from 40 loggerhead turtles in a nearshore habitat, the Patoku area in the southern part of Drini Bay (Fig. 1). Nearly all turtles (99%) were incidentally captured in 'stavnike' fish-traps (a type of pound-net), and subsequently measured and tagged. It was only possible to collect biopsies from a small number of captured turtles due to limited research funds. Preference was given to larger turtles, as there may be a

better chance that their DNA matches existing haplotypic information obtained from nesting beaches (Encalada et al., 1998; Laurent et al., 1998; Carreras et al., 2007; Garafalo et al., 2009; Yılmaz et al., 2011). All haplotypic data on loggerhead and green turtles are housed in the Archie Carr Centre for Sea Turtle Research database (http://accstr.ufl.edu/). In this database there two forms of haplotypic data occurs, shorter (380bp) and longer (over 800bp). The longer haplotypes named according to their similarity to shorter haplotypes till 380 bp. If the longer form is identical to shorter haplotypes until 380 bp and differ after it these longer haplotypes are called as the variants of shorter haplotypes. Bowen et al. (2004) stated that nesting populations contribute more to neighbouring mixed stocks than to more distant mixed stocks. The Mediterranean region, therefore, is the possible source of turtles foraging in Drini Bay. We therefore compared our results with other shorter d-loop sequences available for this species for the Mediterranean (Encalada et al., 1998; Laurent et al., 1998; Carreras et al., 2007; Garafalo et al., 2009; Yılmaz et al., 2011).

Adult and juvenile males were also included in the sampled animals because they represent a significant portion of this foraging population (White et al., 2011). Laparoscopy or hormonal analysis was unavailable, so sex was determined by using CCL (curved carapace length) measurements and tail morphology. Adult males have a greatly extended tail enabling copulation to occur while juvenile males show signs of caudal development the tail widening proximally and extending distally (M. White pers. com. 2012). The minimum CCL for adults was selected as being 70.0 cm although two adult males were slightly smaller than this (White, unpublished data). Short-tailed turtles with a CCL greater than 70.0 cm were assumed to be adult females. Sex of the smaller short-tailed turtles could not be determined, as these could be juveniles of either sex.

Tissue samples were taken from the axillary region near the anterior limbs. The sampling site was wiped with antiseptic (Betadine) prior to excision with a scalpel blade of a small section of skin (1 mm^2). The sample was then placed into a tube with 96% ethanol, which was changed after 10 days.

Morphometric data (CCL and CCW, Curved Carapace Length and Width, respectively) were also collected from all captured turtles following Bolten (1999). Date of capture and the sex of sampled turtles were also recorded.

Lab work

Isolation of genomic DNA was carried out using the phenol-chloroform method (Hillis et al., 1996). A fragment of 859 base-pair (bp) of the mtDNA d-loop region was amplified by polymerase chain reaction (PCR Mastercycler Personnel, Eppendorf, Germany) using the primer pair LCM15382 and H950 (Abreu-Grobois et al., 2006). The PCR protocol was carried out over 35 cycles at 94 ºC for 30 seconds, 55 ºC for 1 min and 72 ºC for 1 min. PCR products were visualized in agarose gel and purified with the GenElute PCR Clean-Up Kit, (Sigma, Germany). Purified PCR products were sequenced in both forward and reverse directions using a 3730xl capillary system automatic sequencer (Macrogen Inc., S. Korea). Sequences were aligned by eye using the programme BioEdit ver 7.0.9 (Hall, 1999) and compared with previously described haplotypes recorded in the Archie Carr Centre for Sea Turtle Research database (http://accstr.ufl.edu/). In addition, previous data from other sites in the Mediterranean were also included for a general assessment of Mediterranean populations.

RESULTS

The mean CCL of the sampled turtles was 68.8 cm (SD = ± 10.3 cm; range = 32.0-84.5 cm; n = 40). Haplotype CC-A2.1 (93%) was the dominant haplotype obtained in the

Table 1. Data on the loggerhead turtles captured at Patoku region of Albania (novel haplotypes are in bold).

Date	CCL	CCW	Sex	Tag	Haplotype
7/22/09	72.0	67.0	Adult Male	AL0112	CC-A2.1
7/22/09	61.0	56.0	Undetermined	AL0113	CC-A2.1
7/22/09	57.0	53.0	Undetermined	AL0114	CC-A2.1
7/22/09	64.0	58.5	Undetermined	AL0115	CC-A2.1
7/23/09	82.0	72.0	Adult Male	AL0116	CC-A2.1
7/24/09	58.0	53.5	juvenile male	AL0117	CC-A2.1
7/24/09	67.0	64.0	Undetermined	AL0118	CC-A2.1
7/28/09	32.0	30.0	Undetermined	No tag	CC-A2.1
7/29/09	55.5	51.0	Undetermined	AL0119	CC-A2.1
7/29/09	65.0	64.0	juvenile male	AL0120	CC-A2.1
7/29/09	72.0	64.0	juvenile male	AL0121	CC-A2.1
7/29/09	64.5	58.0	Undetermined	AL0122	CC-A2.1
7/30/09	59.0	59.0	Undetermined	AL0123	CC-A2.1
7/31/09	70.0	68.0	Adult Female	AL0124	CC-A2.1
7/31/09	66.5	62.0	juvenile male	AL0125	CC-A2.1
7/31/09	72.0	66.0	Adult Female	AL0126	CC-A2.1
7/31/09	74.0	69.0	Adult Female	AL0127	CC-A2.1
7/31/09	74.5	71.0	juvenile male	AL0128	CC-A2.1
8/1/09	73.0	67.5	Adult Female	AL0129	CC-A2.1
8/1/09	68.0	61.5	juvenile male	AL0130	CC-A2.1
8/1/09	70.0	64.0	juvenile male	AL0131	CC-A2.1
8/2/09	47.5	43.5	Undetermined	AL0132	CC-A2.1
8/2/09	**65.5**	**59.0**	**Undetermined**	**AL0133**	**CC-A6.1**
8/2/09	74.5	70.5	Adult Male	AL0134	CC-A2.1
8/3/09	**72.0**	**69.5**	**juvenile male**	**AL0136**	**CC-A10.4**
8/4/09	76.5	72.0	Adult Female	AL0137	CC-A2.1
8/4/09	68.0	64.0	juvenile male	AL0138	CC-A2.1
8/5/09	73.0	64.5	Adult Male	AL0140	CC-A2.1
6/23/10	84.0	78.0	Adult Female	AL0204	CC-A2.1
6/23/10	84.5	77.0	Adult Male	AL0222	CC-A2.1
6/30/10	83.0	75.0	Adult Female	AL0228	CC-A2.8
6/28/10	73.0	70.0	juvenile male	AL0229	CC-A2.1
6/28/10	70.5	65.0	juvenile male	AL0230	CC-A2.1
6/28/10	66.0	61.0	juvenile male	AL0231	CC-A2.1
6/28/10	73.5	65.0	Adult Male	AL0233	CC-A2.1
7/14/10	76.0	68.0	Adult Female	AL0245	CC-A2.1
7/14/10	82.0	72.0	Adult Male	AL0246	CC-A2.1
7/17/10	55.5	52.0	Undetermined	AL0254	CC-A2.1
7/30/10	70.0	65.0	Adult Female	AL0274	CC-A2.1
7/30/10	78.0	74.5	Adult Female	AL0278	CC-A2.1

region sampled for this study (Table 1). However, two previously unknown haplotypes (according to the Archie Carr database mentioned above) were also found (CC-A6.1 and CC-A10.4; Genbank accession numbers JQ350705 and JQ350706, respectively). The last observed haplotype, CC-A.2.8 was first identified from a juvenile stranded in Puglia (South Adriatic Sea, Italy) in May 2008 (Garafalo, 2010). Haplotype and nucleotide diversity were 0.14615 and 0.00017, respectively.

DISCUSSION

The haplotype with the highest frequency at the study site was CC-A2.1, which is not surprising since it is a variant of the short haplotype CC-A2; the most common loggerhead haplotype in the Mediterranean (Table 2). The short forms of novel haplotypes CC-A6.1 and CC-A10.4 are variants of CC-A6 and CC-A10; which have only been recorded from Greek islands and Greece (Table 2). Thus, it is probable that the loggerheads in Drini Bay mainly originate from nesting populations in Greece. The female tagged at Patoku (tag # AL0127) that nested at Sekania (Margaritoulis, pers. com.), Zakynthos, in July 2011, supports this possibility. Furthermore, Lazar et al. (2004) reported loggerheads in Croatian waters, in the north of Albania, that had been tagged while nesting at Zakynthos, Greece. In another study, the foraging grounds off the coasts of western Mediterranean have been shown to be inhabited mainly by turtles from the eastern Mediterranean (Carreras et al., 2006). Moreover, that study revealed a difference in genetic structure between northern African and western Mediterranean stocks that could be explained by prevailing ocean currents and water masses.

In conclusion, the new haplotypes of this study are coming from an unsampled nesting site. However, the current findings suggest that the source population of loggerhead turtles foraging in Drini Bay is most likely from Greece based on the information that nesting populations contribute more to neighbouring mixed stocks than to more distant mixed stocks (Bowen et al., 2004), but the sample size must be increased in order to carry out mixed stock analysis and evaluate the contribution of multiple nesting colonies to this foraging ground.

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Table 2. Haplotype frequencies in the Mediterranean that represent possible source populations for the loggerheads foraging in Drini Bay, Albania.

Sampling site	N	Haplotypes (%)													Source
		CC-A2	CC-A3	CC-A6	CC-A10	CC-A13	CC-A20	CC-A29	CC-A31	CC-A32	CC-A43	CC-A52	CC-A53	CC-A3.2	
Zakynthos	20	85	-	5	-	-	-	-	-	10	-	-	-	-	Carreras et al., 2007
Kyparissia	21	90	-	10	-	-	-	-	-	-	-	-	-	-	Encalada et al., 1998
Lakonikos	19	95	-	5	-	-	-	-	-	-	-	-	-	-	Carreras et al., 2007
Greece	10	90	-	-	10	-	-	-	-	-	-	-	-	-	Laurent et al., 1998
Crete	19	100	-	-	-	-	-	-	-	-	-	-	-	-	Carreras et al., 2007
Cyprus	10	100	-	-	-	-	-	-	-	-	-	-	-	-	Carreras et al., 2007
Cyprus	35	100	-	-	-	-	-	-	-	-	-	-	-	-	Encalada et al., 1998
Lebanon	9	100	-	-	-	-	-	-	-	-	-	-	-	-	Carreras et al., 2007
Israel	19	84	-	-	-	-	-	16	-	-	-	-	-	-	Carreras et al., 2007
Western Turkey	16	94	6	-	-	-	-	-	-	-	-	-	-	-	Carreras et al., 2007
Eastern Turkey	32	59	41	-	-	-	-	-	-	-	-	-	-	-	Laurent et al., 1998
Calabria	47	59.6	-	-	-	-	36.2	-	4.2	-	-	-	-	-	Garafalo et al. 2009
Dalyan	40	62.5	37.5	-	-	-	-	-	-	-	-	-	-	-	Yılmaz et al. 2011
Dalaman	20	25	75	-	-	-	-	-	-	-	-	-	-	-	Yılmaz et al. 2011
WTR	76	78.95	21.05	-	-	-	-	-	-	-	-	-	-	-	Yılmaz et al. 2011
MTR	48	95.83	-	-	-	2.083	-	-	-	-	-	-	2.083	-	Yılmaz et al. 2011
ETR	72	83.33	11.11	-	-	-	-	-	-	-	1.39	1.39	1.39	1.39	Yılmaz et al. 2011

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