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Are stocks of immature loggerhead sea turtles always mixed?

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ABSTRACT

Sea turtles perform extensive migratory movements between feeding, developmental and nesting areas. Developmental areas for immature loggerheads turtles (*Caretta caretta*) are usually composed of individuals from multiple distant rookeries. Thus, impacts on such stocks usually affect multiple colonies, which require international efforts for conservation. This study describes the molecular genetic composition of the more austral foraging and developmental grounds of loggerhead sea turtles in the Atlantic Ocean and infers the possible origin and dispersal patterns of the species. Analyses were performed using shorter (380 bp) and larger (760 bp) sequences of the control region of mitochondrial DNA (mtDNA) from 24 samples of bycatch and 37 samples of stranded loggerhead sea turtles on the coast of the province of Buenos Aires, Argentina. All specimens ($N = 61$) were adults and sub-adults (mean ± standard deviation curved carapace length CCL = 68.3 ± 13.4 cm; range: 52.0 to 107.0 cm, $N = 41$ individuals measured). Both shorter and longer mtDNA sequence analysis showed that in the foraging grounds of the Argentinean coast only haplotypes from Brazilian nesting areas (CC-A4 = 98% and CC-A24 = 2% for shorter sequences, and CC A4.2 = 81%, CC A4.1 = 17% and CC A24.1 = 2% for longer sequences) were found. The homogeneous stock located relatively close to the rookery where individuals originated contradicts the paradigm of immature loggerhead sea turtles forming mixed stocks in foraging and developmental areas. The conservation of the stock in coastal areas of Argentina could benefit the nesting population in the nearby Brazilian rookeries, and could be achieved by conservation actions between these two countries, as well as Uruguay in between.

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1. Introduction

The loggerhead sea turtle, *Caretta caretta* (Linnaeus, 1758), is listed as endangered by the International Union for the Conservation of Nature (IUCN, 2014). It is a widely distributed marine vertebrate, inhabiting temperate subtropical and tropical regions of the Atlantic, Pacific and Indian Oceans and the Mediterranean Sea (Conant et al., 2009; Dodd, 1992). After leaving their natal beaches, hatchlings enter into major ocean surface currents and may be transported across entire ocean gyres basins as epipelagic, oceanic juveniles and this likely occurs across the nesting range of sea turtles including the Pacific, Indian, Atlantic and Mediterranean (Bolten et al., 1998; Boyle et al., 2009; Scott et al., 2014).

In the Southwestern Atlantic (SWA) Ocean, major nesting areas are situated in eastern and northeastern Brazil (Marcovaldi and Chaloupka, 2007; Fig. 1). Mark-recapture approaches and satellite tracking studies showed that the north and south Brazilian coasts (Ceará state and Rio Grande Rise), Uruguayan and Argentine coasts are important feeding grounds for this species at different stages of their life cycle (González-Carman et al., 2011; López-Mendilaharsu et al., 2007; Marcovaldi et al., 2010; Reis et al., 2009). On the Argentine coast this species is usually found from the estuary of the Rio de La Plata (Buenos Aires province) to San Matías Gulf (Rio Negro province), frequently during the austral summer, from November to May (González-Carman et al., 2011; Fig. 1).

Previous phylogeographic studies using short mtDNA sequences (380 base pairs — bp) have identified 47 haplotypes in seven nesting areas in the Atlantic Ocean and the Mediterranean Sea (Chaieb et al., 2010; Encalada et al., 1998; Monzón-Argüello et al., 2010a; Reis et al., 2009 and references therein). Recent studies using longer sequences (~800 bp) have described six Regional Management Units (RMUs), and have recognized at least 18 demographically independent management units (MUs), based on female natal homing (Shamblin et al., 2014). Whereas these studies provided substantial information about the genetic population structure in several places, to date there are few genetic studies on loggerhead foraging and development areas in the south Atlantic Ocean (Caraccio et al., 2007; Reis et al., 2009). The studies based on shorter mtDNA sequences had shown that smaller turtles found offshore had origins in a range of rookeries in the south and north Atlantic, Pacific and Mediterranean (Caraccio et al., 2007;

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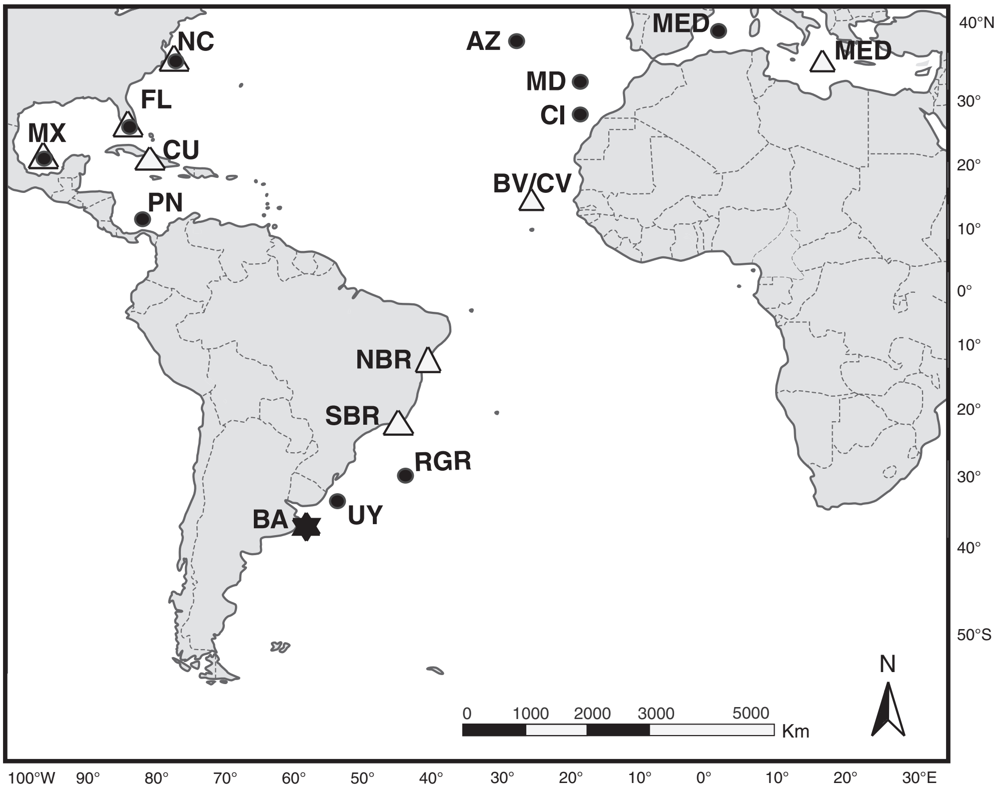

Fig. 1. Nesting colonies and foraging grounds of loggerhead sea turtles (*Caretta caretta*) characterized genetically in eastern and western Atlantic Ocean, and the Mediterranean Sea. The study feeding area in Buenos Aires Province (BA), Argentina, is marked with a star. Foraging grounds from the bibliography are indicated by circles: North and South Florida and Dry Tortugas (FL), Georgia, North and South Carolina (NC), Gulf of México (MX), Panama (PN), Canary Islands (CI), Rio Grande Rise (RGR), Uruguay (UY), Azores and Madeira Archipelagos (AZ/MD), Mediterranean at Lampedusa, Gimenesies, Pituses, north Spain, east and west Italy (MED). Nesting rookeries are indicated by triangles: Quintana Roo and Gulf of Mexico (MX), North and South Florida, Georgia and Dry Tortugas (FL), North and South Carolina (NC), Cuba (CU), Tunes, Calabria, Kyparissia and east Turkey (MED); Boa Vista/Cabo Verde (BV/CV), Sergipe and Bahia states in northeastern Brazil (NBR), Rio de Janeiro and Espírito Santo states, in southeastern Brazil (SBR).

Reis et al., 2009), while larger and more coastal individuals off Uruguay came only from Brazilian rookeries.

Herein, long mtDNA sequences from loggerhead foraging grounds in Argentina were analyzed, aiming to improve our understanding about migration patterns of this threatened species. The specific objectives are: i) estimate genetic diversity in coastal areas of Argentina; ii) assess the contribution of different nesting colonies to Argentinean feeding areas; iii) compare contribution of rookeries to different foraging grounds in SWA; and iv) discuss the implications of these results for the conservation of the species in its southernmost distribution.

2. Materials and methods

2.1. Sampling

Skin and muscle samples of sixty-one loggerhead sea turtles were collected by the Regional Program for Sea Turtle Research and Conservation of Argentina (PRICTMA), from November 2004 to March 2010. Sampling along the coast of the Buenos Aires province (34°00′–36°10′S and 55°00′–58°10′W) was performed according to Dutton (1996). Twenty-four samples were from incidental bycatch in coastal fisheries and 37 samples were obtained from strandings. Curved carapace length (CCL) was measured with flexible tape according to Bolten (1999), and used to infer the life-stage by comparing with data on the minimum size of nesting females in rookeries of origin.

2.2. Laboratory analysis

DNA extractions were performed using a DNeasy Kit following manufacturer's instructions (QIAGEN Inc.). A 760 bp fragment from the control region was amplified using primers LCM15382 (5′-GCT TAA CCC TAA AGC ATT GG-3′) and H950 (5′-GTC TCG GAT TTA GGG GTT TG-3′) (Abreu-Grobois et al., 2006) according to the conditions described in Shanker et al. (2004). Amplified fragments were purified and sequenced in MACROGEN INC. (Seoul, South Korea).

2.3. Data analysis

Sequences were aligned using BioEdit v. 7.0 (Hall, 1999) and Clustal (Higgins and Sharp, 1988) and polymorphic sites were identified with the program Genalex 6 (Peakall and Smouse, 2006). Mitochondrial haplotypes for loggerhead sea turtles were classified according to haplotype designations of 380 bp and 760 bp sequences deposited in the DNA database at the Archie Carr Center for Sea Turtle Research (ACCSTR-http://accstr.ufl.edu/genetics.html).

Arlequin v. 3.11 (Excoffier et al., 2005) was employed to estimate haplotype (h) and nucleotide (π) diversities (Nei, 1987) for the Buenos Aires coast and for each of the feeding areas in the Mediterranean and Atlantic Ocean. Because only shorter mtDNA sequences are available currently from most feeding grounds in the Atlantic and Mediterranean, the short sequence database (380 bp) was used to analyze genetic differentiation among feeding areas. Feeding grounds were divided into two main geographical groups: the North Atlantic (including Mediterranean) and the South Atlantic groups. The first group contains feeding grounds from north and south Florida, Dry Tortugas (FL), North and South Carolina (NC), Gulf of Mexico (MX) (Bass et al., 2004; Bowen et al., 2004; Rankin-Baransky et al., 2001; Reece et al., 2006), Panamá (PN, Engstrom et al., 2002), Azores and Madeira (AZ/MD, Bolten et al., 1998), Canary Island (CI, Monzón-Argüello et al., 2009) and Lampedusa, Gimenesies, Pituses, northern Spain, east and west Italy (MED) (Bolten

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