



Are stocks of immature loggerhead sea turtles always mixed?



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ARTICLE INFO

Article history:

Received 18 January 2015

Received in revised form 7 February 2015

Accepted 7 February 2015

Available online xxxx

Keywords:

Caretta caretta

Conservation strategies

mtDNA control region

Sea turtles

ABSTRACT

Sea turtles perform extensive migratory movements between feeding, developmental and nesting areas. Developmental areas for immature loggerhead 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 \pm 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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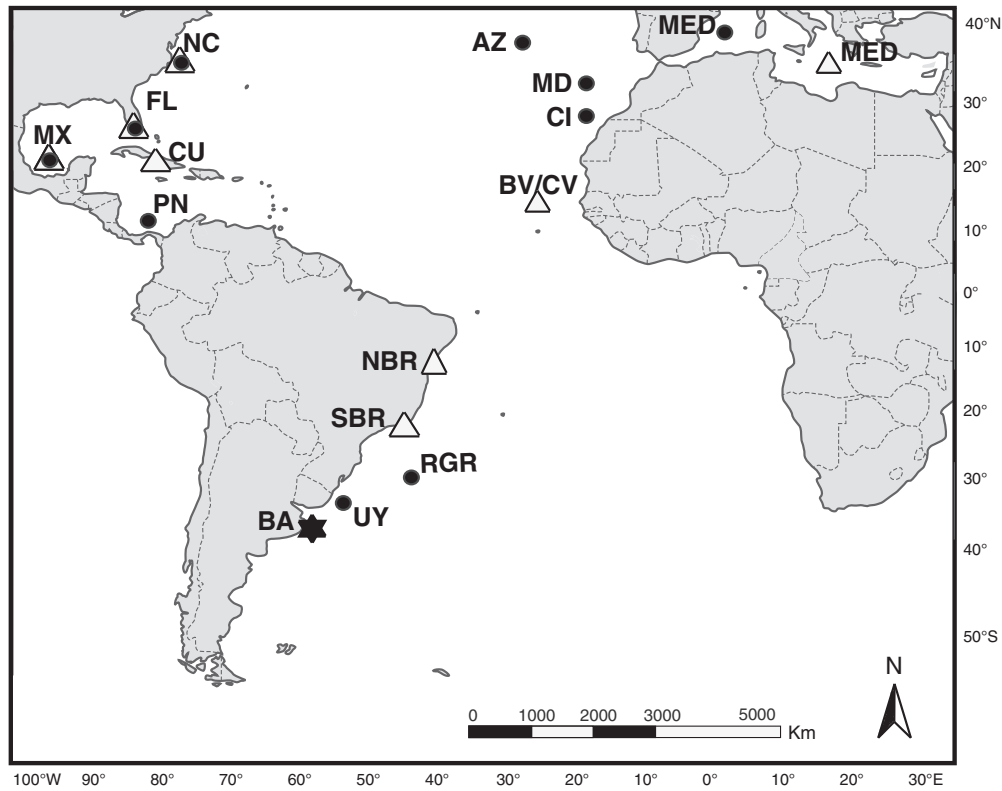


Fig. 1. Nesting colonies and foraging grounds of loggerhead sea turtles (*Caretta caretta*) characterized genetically in eastern and western Atlantic Ocean, and the Mediterranean Sea. The study feeding area in Buenos Aires Province (BA), Argentina, is marked with a star. Foraging grounds from the bibliography 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

Table 1

Frequencies of mtDNA haplotypes of loggerhead sea turtle (*Caretta caretta*) sampled in nesting beaches, as well as foraging grounds, of the Atlantic Ocean and Mediterranean Sea, based on short (~380 bp) and longer (~760 bp, in bold type) sequences of mtDNA (Monzón-Argüello et al., 2010a; Shamblin et al., 2014).

Short sequences	Long sequences	Feeding grounds								Nesting grounds				
		CI	AZ/MD	MED	USA	PN	RGR	UY	BA	BV/CV	USA	NBR	SBR	MED
CC-A1		40	60	107	823	10				127	240			
	CC-A1.1										1287			
	CC-A1.2										26			
	CC-A1.3									139	23			
	CC-A1.4									13	50			
	CC-A1.5									3				
	CC-A1.7									3				
	CC-A1.8									2				
CC-A2		33	50	282	583	26	13	6		2	115			166
	CC-A2.1									4	704			390
	CC-A2.2										1			
	CC-A2.3										12			
	CC-A2.4										12			
	CC-A2.5										15			
	CC-A2.8													4
	CC-A2.9													13
	CC-A2.11										1			
CC-A3		8	7	24	66	3					8			16
	CC-A3.1										116			58
	CC-A3.2													1
CC-A4		1			1		59	32	60			63	113	
	CC-A4.1								10			20	17	
	CC-A4.2								47			34	51	
	CC-A4.3												4	
CC-A5				2	8						1			
	CC-A5.1										2	2		
CC-A6														5
	CC-A6.1													5
CC-A7		2		2	23	1					5			
	CC-A7.1										15			
	CC-A7.2										2			
CC-A8		1	1		4									
	CC-A8.1										8			
CC-A9				4	6	1					3			
	CC-A9.1										9	8		
CC-A10		5	3	2	23	2					7			
	CC-A10.1										64			
	CC-A10.4										1			
CC-A11			1	2	2		19	1		1	1			
	CC-A11.2									1				
	CC-A11.3										9			
	CC-A11.5										1			
	CC-A11.6													
CC-A12			1	1		1								
	CC-A12.1											2		
CC-A13			2	2	10									
	CC-A13.1										4			1
CC-A14		1	3	5	40						2			
	CC-A14.1										29			
CC-A15			1											
CC-A16			1											
CC-A17		1	1	1										
	CC-A17.1										53			
	CC-A17.2										60			
											7			
CC-A18					1									
CC-A19					1	1								
CC-A20					11							1		
	CC-A20.1											10		17
CC-A21				2	1									
	CC-A21.1											2		
CC-A22					1									
CC-A24													13	
	CC-A24.1									1			5	
CC-A25														1
CC-A26				7										
	CC-A26.1													1
CC-A27				1										
	CC-A27.1											1		
CC-A28				1										3
CC-A29				2										
	CC-A29.1													2
CC-A30				1										

(continued on next page)

Table 1 (continued)

Short sequences	Long sequences	Feeding grounds								Nesting grounds				
		CI	AZ/MD	MED	USA	PN	RGR	UY	BA	BV/CV	USA	NBR	SBR	MED
CC-A31				1										
CC-A32	CC-A31.1			2			18							1
CC-A33	CC-A32.1									2				3
CC-A34							1		2					
	CC-A36.1													1
	CC-A36.2													2
	CC-A41.1													1
CC-A42				1										
	CC-A42.1													1
	CC-A43.1													1
CC-A44					1									
CC-A46		1												
	CC-A47.1													1
	CC-A50.1													
	CC-A51.1												2	
	CC-A52.1													2
	CC-A53.1													2
	CC-A59.1													1
	CC-A60.1													1
	CC-A65.1													2
N		93	131	452	1605	45	125	43	61					
Total		10	12	21	18	8	6	5	2					
Haplotype diversity		0.685	0.645	0.552	0.6026	0.622	0.714	0.43	0.0323					
		−0.031	−0.0265	−0.022	−0.0078	−0.068	−0.031	−0.1	−0.031					
Nucleotide diversity		0.036	0.025036	0.027	0.024588	0.026	0.0174	0.01	0.000089					
		−0.018	−0.0128	−0.013	−0.0125	−0.014	−0.002	−0	−0.0003					

h = haplotype diversity, π = nucleotide diversity were calculated only for the short sequences. N represents sample sizes in feeding grounds. References and abbreviations as in Fig. 1. USA includes FL, NC and MX in the foraging grounds and FL, MX and NC in nesting grounds.

et al., 1998; Bowen et al., 2004; Carreras et al., 2006, 2007; Encalada et al., 1998; Monzón-Argüello et al., 2009, 2010a; Reece et al., 2006). The second group includes the foraging grounds of Rio Grande Rise, Brazil (RGR, Reis et al., 2009) and Uruguay (UY, Caraccio et al., 2007) (Fig. 1; Table 1).

Analysis of genetic variance (AMOVA) based on haplotype frequencies (F_{ST}) and genetic haplotype divergence (ϕ_{ST}) was used to test genetic differentiation among feeding areas of loggerheads (Excoffier et al., 1992). Genetic differentiation between western Atlantic feeding grounds was performed using pairwise comparisons. To analyze differences in migration contributions along western Atlantic coasts, hierarchical AMOVA was also performed using data from the two groups (North and South Atlantic). The statistical significance of F_{ST} and ϕ_{ST} values was tested based on 5000 permutations. All AMOVAs were performed using ARLEQUIN (Excoffier et al., 2005) v. 3.11.

The geographical heterogeneity in mitochondrial haplotype frequency distribution was also evaluated through Monte Carlo simulations, as described by Roff and Bentzen (1989) using the REAP program (McElroy et al., 1991). Comparisons of genetic diversity indices (haplotype and nucleotide diversity) between feeding areas of the Atlantic were carried out by the non-parametric Kruskal–Wallis test.

Relationships among feeding grounds in the Atlantic Ocean were plotted by phenograms based on a per grouping method using unweighted arithmetic means (UPGMA) of F_{ST} and ϕ_{ST} values and through multidimensional scaling (MDS) based on ϕ_{ST} values. These analyses were performed using Statistica® (StatSoft Inc., 1996).

The possible origin of loggerhead turtles in Argentinean coast was inferred using published long mtDNA haplotype sequences for rookeries in the Atlantic Ocean and Mediterranean Sea (Shamblin et al., 2014) (Fig. 1; Table 1).

3. Results

The mean CCL of specimens sampled was 68.3 ± 13.4 cm (range 52.0–107.0 cm, $N = 41$). Most loggerheads (>90%, as some individuals could not be measured due to decomposition) were classified as sub-

adults or late juveniles according to the size of females in nesting areas of Brazil (Lima et al., 2012; Marcovaldi and Chaloupka, 2007) (Fig. 2).

The long fragment of the mitochondrial control region (760 bp) was successfully amplified for 58 loggerheads (Table 1). The short fragment of the mtDNA (380 bp) was amplified for three additional turtles. Two haplotypes based on short sequences were identified among the 61 loggerhead turtles sampled from Buenos Aires coast. These haplotypes (CC-A4 = 98% and CC-A24 = 2%) are widely distributed in nesting colonies of Brazil.

The comparison among feeding grounds based on short mtDNA sequences shows that haplotype ($h = 0.032$) and nucleotide ($\pi = 0.00009$) diversities are relatively lower than those detected in the feeding and development grounds of Brazil and Uruguay (Table 1). No significant differences were found in haplotype diversity (Kruskal–Wallis test $H = 0.55$; $P = 0.46$), but there was higher nucleotide

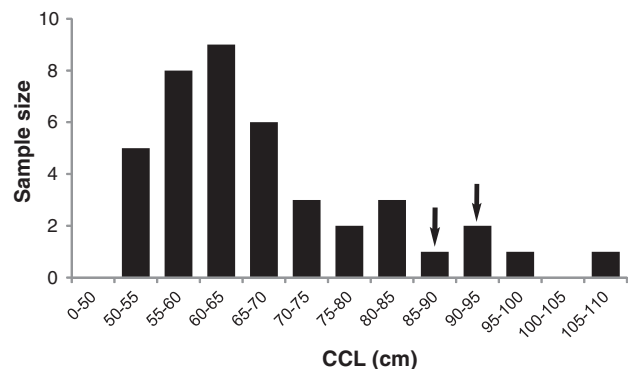


Fig. 2. Curved carapace length (CCL) of loggerhead sea turtles (*Caretta caretta*) sampled for genetic analysis in Argentina from 2003 to 2009. $N = 41$ turtles, differing from the total sample size because some turtles could not be measured. Arrows indicate the minimum CCL of nesting females from the two main Brazilian rookeries, where individuals originated.

diversity in North Atlantic feeding grounds compared to those of the South Atlantic ($H = 5$; $P = 0.025$).

AMOVA based on the haplotype frequencies ($F_{ST} = 0.01564$, $P < 0.001$) and haplotype divergence ($\Phi_{ST} = 0.326$, $P < 0.001$) demonstrated highly significant differences among feeding grounds in the Atlantic Ocean. Pairwise comparisons between all Atlantic feeding grounds (Table 2) showed that 22 out of the 28 paired comparisons for F_{ST} and Φ_{ST} indices were highly significant after Bonferroni correction (Table 2). Likewise, the analysis of mtDNA haplotype distribution demonstrated significant genetic heterogeneity among all foraging areas ($\chi^2 = 3265.7$, $P < 0.001$).

Multidimensional scaling (MDS) (stress value = 0.00013) and phenograms based on Φ_{ST} genetic distances showed genetic affinities among Atlantic feeding grounds (Fig. 3a). The phenogram obtained by UPGMA showed two main groups, which reflects the geographic distribution of foraging grounds: the first one including feeding areas of the Northern Hemisphere (North Atlantic: CI, AZ/MD, USA, MED, PN) and the second one with feeding grounds in the South Atlantic: UY, RGR, and BA (Argentina). The MDS indicates a clear grouping of feeding areas in the North Atlantic, as well as some dispersion to South Atlantic feeding grounds (Fig. 3b). Similar results were obtained in UPGMA and MDS based on F_{ST} (not shown). To analyze differences in contributions of migration along the Atlantic coasts, hierarchical AMOVAs were conducted considering the variation in the two groups, i.e. North and South Atlantic. AMOVA based on haplotype frequencies showed significant differences between groups ($F_{CT} = 0.184$, $P = 0.034$), between feeding areas within groups ($F_{SC} = 0.125$, $P < 0.001$) and within feeding areas ($F_{ST} = 0.28566$, $P < 0.0001$). The AMOVA based on divergence between haplotypes detected significant variation between foraging grounds within groups ($\Phi_{SC} = 0.149$, $P < 0.001$) and within feeding areas ($\Phi_{ST} = 0.127$, $P < 0.001$).

The analysis of long sequences identified three haplotypes among the 58 loggerhead turtles sampled in Buenos Aires coasts (CC A4.2 = 81%, CC A4.1 = 17% and CC A24.1 = 2%). Thus, loggerhead turtles found in Argentina have their origins exclusively from nesting beaches in Brazil, as all three haplotypes are exclusive of Brazilian rookeries (Table 1). Genetic diversity based on long sequences for Buenos Aires feeding grounds could not be compared with other Atlantic loggerhead feeding areas because these grounds have not been characterized for the long mtDNA fragment up to date.

4. Discussion

All loggerhead sea turtles in Argentinean foraging grounds are from nesting colonies located in Brazil. Based on the short mtDNA sequence, haplotype and nucleotide diversity found in coastal Argentina are relatively lower than in offshore feeding grounds of the Rio Grande Rise—RGR, off the Brazilian coast, and Uruguay—UY. The high haplotype diversity at these feeding grounds could be explained by a higher proportion of small juveniles (Caraccio et al., 2007; Sales et al., 2008), which potentially are originated from rookeries elsewhere. These

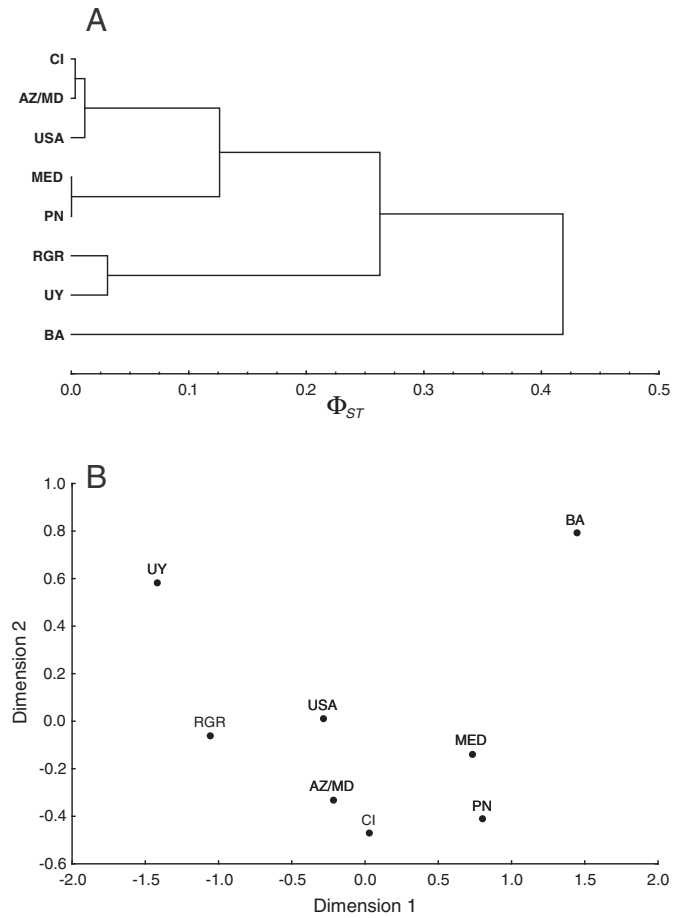


Fig. 3. Phenogram (UPGMA) (A) and multidimensional scaling – MDS (B) showing the relationship between nine feeding grounds of loggerhead sea turtles (*Caretta caretta*) in western Atlantic Ocean based on genetic distances Φ_{ST} (0.009 was added to each value to avoid negative rates). USA includes FL and NC. Codes of locations as in Fig. 1.

results also show that when turtles recruit to coastal areas, stocks switch from mixed to single origin stocks.

North Atlantic feeding grounds show higher genetic heterogeneity in comparison to South Atlantic feeding grounds, probably due to a larger number of contributing rookeries. Graphical representations of the relationships between Atlantic feeding grounds showed two main clusters, corresponding to the North and South Atlantic. The MSD in particular showed that feeding grounds in the South Atlantic appear to be more distinct from each other than feeding areas in the North Atlantic. This result may be related to the high genetic variability recorded in foraging grounds, i.e. offshore southern Brazil at Rio Grande Rise (RGR) and Uruguay (UY) (Caraccio et al., 2007; Reis et al., 2009). When the different contributions along western Atlantic coasts were analyzed through a hierarchical AMOVA, significant differences were

Table 2

Genetic distinctiveness among foraging zones of loggerhead sea turtles (*Caretta caretta*) in the Atlantic Ocean and Mediterranean Sea.

	CI	AZ/MD	MED	USA	PN	RGR	UY	BA
CI		−0.0057	0.0822	0.0064	0.0904	0.1368	0.2320	0.3767
AZ/MD	−0.0056		0.1165	−0.0014	0.1247	0.1145	0.1979	0.3931
MED	0.0852	0.0827		0.1361	−0.0088	0.3272	0.4319	0.1404
USA	0.0036	−0.0006	0.1081		0.1532	0.0981	0.1655	0.3558
PN	0.0524	0.0578	−0.0069	0.0907		0.3414	0.4909	0.1968
RGR	0.2693	0.2919	0.3390	0.3323	0.2822		0.0218	0.5942
UY	0.3870	0.4067	0.4361	0.4180	0.4256	0.0718		0.8085
BA	0.4060	0.3867	0.1553	0.3554	0.2752	0.5162	0.7640	

Φ_{ST} values are shown in the upper triangular matrix and the F_{ST} in the lower part. Statistical significance was assessed using 5000 random permutations. Numbers in bold indicate $P < 0.01$. Abbreviations as in Fig. 1. USA includes FL and NC.

detected between feeding areas of the North and South Atlantic based on haplotype frequencies. These results indicate that both feeding areas include similar haplotypes, but differ in their frequencies.

Ocean currents likely have a strong impact on the distribution of sea turtle hatchlings, both for loggerheads and other sea turtle species, and influence the location of foraging grounds (e.g. [Monzón-Argüello et al., 2010b](#)) and may also impact adults ([Luschi et al., 2003](#)). However, currents are not the only factor influencing sea turtle dispersion with sub-adult and adult sea turtles being able to move independently of the ocean flows ([Casale et al., 2002](#); [Chaloupka and Limpus, 2002](#); [Chapman et al., 2011](#)). The composition of stocks may be explained by movements between foraging and nesting areas, as well as by changes in oceanographic conditions ([Bolten and Witherington, 2003](#); [Bowen et al., 2005](#); [Boyle et al., 2009](#); [Hatase et al., 2002](#)). The potential role of currents driving the pattern found in the current study, e.g. by limiting the occurrence of individuals from distant rookeries, deserves further investigation, as outlined by [Fossette et al. \(2012\)](#).

Genetic studies based on mtDNA indicate that the contribution of sub-adult individuals to the composition of feeding ground populations is influenced by the size of regional nesting populations ([Bass et al., 2004](#); [Bowen et al., 2005](#); [Engstrom et al., 2002](#); [Norrsgard and Graves, 1996](#); [Rankin-Baransky et al., 2001](#)).

Nesting areas for loggerhead turtles in Brazil are considered to be an evolutionarily significant unit (ESU, *sensu* [Moritz, 1994](#)), or Regional Management Units (RMU) as it was recently defined ([Shamblin et al., 2014](#)). Since 1980, it has been recognized that management actions in foraging areas in Argentina are essential for the conservation of nesting areas in Brazil ([Marcovaldi and Chaloupka, 2007](#); [Shamblin et al., 2014](#)). The current study provides new evidence regarding migration patterns of loggerhead turtles in the SWA Ocean, offering key information to support the implementation of mitigation measures on Argentinean coasts to complement existing conservation actions in Brazil ([Marcovaldi and Chaloupka, 2007](#)). Threats to loggerhead turtles in the region include those resulting from the ingestion of anthropogenic debris ([Bugoni et al., 2001](#)), contamination by heavy metals ([Silva et al., 2014](#)) and fishery bycatch ([Fiedler et al., 2012](#); [Sales et al., 2008](#)). Our data highlight the importance of developing management plans and regional strategies in Argentina to protect the Brazilian RMU. In addition, our results support the importance of Marine Protected Areas (MPAs) aiming to preserve not only local species but also migratory species such as sea turtles ([Norse and Crowder, 2004](#); [Sobel and Dahlgren, 2004](#)). The perspective from the current study of a single-origin stock could allow a more direct inference on the relationship between foraging and nesting grounds, i.e. problems affecting loggerhead sea turtles in their foraging ground in Argentina could result in effects in populations nesting in Brazil, instead of an assumed effect dissipated among a range of potential rookeries of origin. Different from more offshore aggregations of loggerhead and other sea turtle species, loggerheads form a homogeneous stock in their southernmost developmental and foraging ground in the Atlantic. This pattern might be found elsewhere and requires further investigation.

Acknowledgments

Access to study animals was kindly facilitated by the Regional Program for Sea Turtle Research and Conservation of Argentina (PRICTMA, which includes Acuario de Buenos Aires, Aquamarina - CECIM, Fundación Mundo Marino and Reserva Natural de Usos Múltiples Bahía Blanca, Bahía Falsa y Bahía Verde). The wildlife agencies of Buenos Aires provinces and the National Wildlife Agency of Argentina issued permits and supported our research. We would like to thank Lic. Sergio Rodríguez-Heredia, Lic. Karina Álvarez and Dr. Victoria González-Carman for their assistance during fieldwork and special thanks to MSc. Javier H. Otero for his constant support. We are also grateful to the fishermen from San Clemente del Tuyú, Ingeniero White, Puerto Rosales and Villa del Mar who provided information and collaborated

with the program. We thank the English revision and suggestions generously provided by Dr. David Cundall. Funding was provided by the Buenos Aires Zoo to DA, the Wildlife Conservation Society, Fondo para la Conservación Ambiental from Banco Galicia, and Universidad de Buenos Aires (0358BA). This study complies with legal requirements of the countries in which the work was carried out, and to all institutional guidelines. LB received a fellowship from the Brazilian Council for Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico, CNPq, Brazil; Proc. #308697/2012-0). [SS]

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