Journal of Experimental Marine Biology and Ecology 412 (2012) 37–45

Contents lists available at SciVerse ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Genetic composition of green turtle feeding grounds in coastal waters of Argentina based on mitochondrial DNA

Laura Prosdocimi [a,b], Victoria González Carman [c,d], Diego Alejandro Albareda [e,d], Maria Isabel Remis [a,*]

[a] Laboratorio Genética de Poblaciones, Dpto. Ecología, Genética y Evolución, FCEN, Universidad de Buenos Aires, Intendente Güiraldes 2160, C1428EGA Capital Federal, Buenos Aires, Argentina
[b] Fundación Mundo Marino-PRICTMA, Buenos Aires, Argentina
[c] Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP) CONICET, Mar del Plata, Buenos Aires, Argentina
[d] Aquamarina-CECIM-PRICTMA, Buenos Aires, Argentina
[e] Acuario del Jardín Zoológico de Buenos Aires-PRICTMA, Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 12 April 2011
Received in revised form 22 August 2011
Accepted 14 October 2011
Available online 14 November 2011

Keywords:
Chelonia mydas
Endangered species
Feeding ground
Genetic diversity
Mitochondrial DNA
Population structure

ABSTRACT

The green turtle, *Chelonia mydas*, like other species of marine turtles, shows great migratory displacement between its nesting and feeding grounds. In an attempt to characterize the southernmost feeding grounds of this species, mtDNA sequence variation of green turtle aggregations in Argentinean waters was studied to elucidate genetic variation and infer possible origins. The goal of the present study is contemplated within the main purpose of the PRICTMA (Regional Program for Sea Turtle Research in Conservation of Argentina) and the Network ASO-Tortugas (Red Atlántico Sur Occidental-Tortugas) which are dedicated to promoting conservation studies in marine turtles in the region. A 486-bp fragment of the mitochondrial DNA control region was sequenced from 93 samples of incidentally caught juveniles from 2004 to 2007, revealing 9 haplotypes. Nucleotide and haplotype diversity were similar to those detected in other Brazilian feeding grounds (Ubatuba and Atol das Rocas/Fernando de Noronha). Analysis of molecular variance (AMOVA) indicated significant genetic differentiation among 9 western Atlantic feeding grounds for which data is currently available, suggesting variable contributions from different nesting colonies ($F_{ST}=0.29$, $P<10^{-4}$; $\Phi_{ST}=0.55$, $P<10^{-4}$). Mitochondrial DNA haplotype distributions revealed significant heterogeneity among feeding grounds (X^2: 804.84, $P<10^{-4}$). A pairwise analysis revealed that most western Atlantic feeding grounds are genetically differentiated. The weighted and unweighted mixed stock analyses suggests that green turtles at Argentinean feeding grounds originate mainly in the Ascension Island rookery, with less contribution from rookeries in Suriname, Aves Island and Trindade Island.
The present results improve our knowledge of the population structure and migration patterns of the Atlantic green turtle, and inform conservation measures on feeding grounds, which may be thousands of kilometers away from the nesting colonies. This information is required to further government efforts for this endangered species.

1. Introduction

Marine turtles have complex life cycles. During their life span, they use different habitats such as nesting grounds, neritic and oceanic feeding grounds, which are frequently separated by thousands of kilometers (Bolten, 2003a,b; Musick and Limpus, 1997). Adult females are philopatric to their natal beach (Allard et al., 1994; Bass et al., 2006; Bowen et al., 2004; FitzSimmons et al., 1997a; Heppel et al., 2003; Roberts et al., 2004), whereas turtles from multiple breeding populations converge on feeding grounds, habitats where they spend most of their lives (Encalada et al., 1996; Lahanas et al., 1998; Musick and Limpus, 1997; Plotkin, 2003). These features, along with other traits such as differential gene flow patterns between males and females and population overlap during migrations, generate a complex population structure that has profound implications for their management and conservation (Bowen et al., 2005; Encalada et al., 1996). For these reasons, interpreting the patterns of variation based on different molecular markers has been one of the most challenging aspects of research dedicated to understanding the population structure and migratory patterns of marine turtles (Avise, 2004; Bass and Witzell, 2000; Bowen et al., 1992, 2004; FitzSimmons et al., 1997a,b; Karl et al., 1992; Lahanas et al., 1994; Norman et al., 1994; Pella and Milner, 1987).

Structure among Atlantic populations of the green turtle (*Chelonia mydas*) (Linneo, 1758) has been widely studied (see Bjorndal et al., 2006; Bowen et al., 1992; Encalada et al., 1996; Karl et al., 1992). This

* Corresponding author at: Intendente Güiraldes 2160, C1428EGA Capital Federal, Buenos Aires, Argentina. Tel.: +54 11 45763300.
E-mail address: mariar@ege.fcen.uba.ar (M.I. Remis).

0022-0981/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jembe.2011.10.015

species, catalogued as endangered by the International Union for Conservation of Nature (IUCN) (IUCN, 2009; Seminoff and Abreu-Grobois, 2007), is distributed from North Carolina (USA) to Argentina (Epperly et al., 1995; González Carman et al., 2011) in the West Atlantic, and also occurs in the East Atlantic and the Indo-Pacific (Bagley, 2003; Bass et al., 2006; Bowen et al., 1992; Formia et al., 2007; Kamezaki and Matsui, 1995). The main Atlantic rookeries are on Ascension Island, Aves Island, Trindade Island, Costa Rica and Suriname, among others (Bjorndal et al., 2005, 2006; Bowen et al., 1992; Encalada et al., 1996; Formia et al., 2006, 2007; Godley et al., 2001; Kaska, 2000; Schulz, 1975) (see Fig. 1). Phylogeographic analyses indicate the existence of two evolutionarily significant units (ESUs) (sensu Moritz, 1994), one in the Indian Ocean and the other in the Atlantic Ocean, which show high levels of genetic divergence and reciprocal monophyly at the mitochondrial DNA (mtDNA) level (Bourjea et al., 2007; Formia et al., 2006). Nesting colonies in the Atlantic Ocean are genetically differentiated based on mtDNA haplotypes (Bass et al., 2004; Bjorndal et al., 2005; Encalada et al., 1996; Formia et al., 2006, 2007).

Feeding grounds in the western Atlantic have been identified in the United States (Bass and Witzell, 2000; Bass et al., 2006), the Caribbean (Bass et al., 1998; Lahanas et al., 1998; Luke et al., 2004), Brazil (Bjorndal et al., 2006; Naro-Maciel et al., 2007), Uruguay (López-Mendilaharsu et al., 2006) and Argentina (González Carman et al., 2011). The ability to differentiate among nesting colonies based on mtDNA haplotype frequencies has enabled the determination of nesting sources for turtles sampled on feeding grounds. Genetic analyses have only been carried out on some identified feeding grounds from North Carolina (Bass et al., 2006), Bahamas (Lahanas et al., 1998), Nicaragua (Bass et al., 1998), Florida (Bass and Witzell, 2000), Barbados (Luke et al., 2004) and Brazil (Bjorndal et al., 2006; Naro-Maciel et al., 2007). To date, no genetic analyses have been performed on populations inhabiting the southernmost boundary of green turtle distribution in the western Atlantic.

Buenos Aires has two important feeding and development grounds of green turtles: the Rio de la Plata and El Rincón estuaries (González Carman et al., 2011). In both estuaries, economically important activities occur which affect turtle survival, including transportation, tourism, fishing and discharge of domestic and industrial wastes (Mianzán et al., 2001) affecting turtle survival.

Recently, the PRICTMA (Regional Program for Sea Turtle Research in Conservation of Argentina) and the Network ASO-Tortugas (Red Atlántico Sur Occidental-Tortugas) have proposed recommendations for conservation of marine turtles in the southwestern Atlantic. Within the scope of these regional programs, the goal of the present study is to use molecular tools to characterize the origin of the green turtles feeding in Buenos Aires (Argentina) the southernmost Atlantic feeding grounds of this species; improving our understanding of population structure and migration on in the Atlantic green turtle. The estimation of the degree to which the different nesting colonies are represented in the studying area is required to conduct conservation

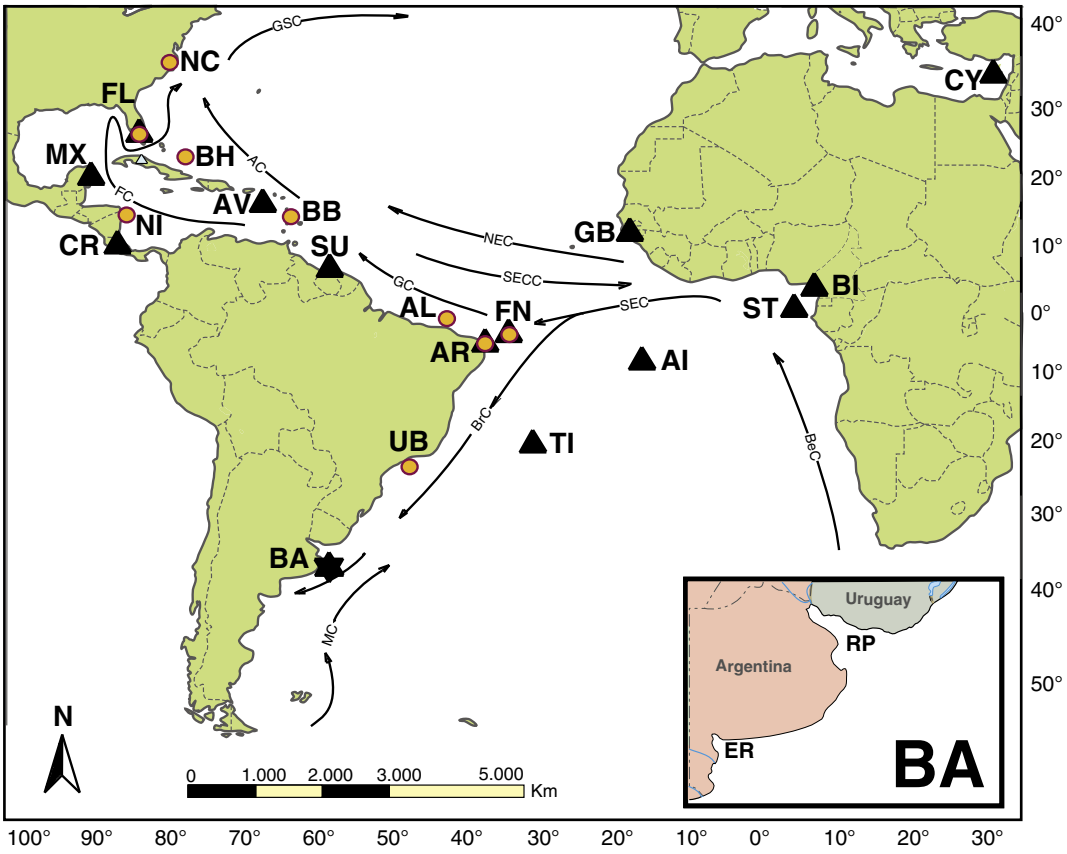

Fig. 1. The map actually shows the nesting populations throughout the Atlantic (east and west) and only the feeding grounds in the western Atlantic. Río de la Plata (RP) and El Rincón (ER) estuaries are located in Buenos Aires Province (BA), Argentina (symbolized by stars). References and abbreviations for other feeding grounds (indicated by circles) and nesting colonies, considered possible sources of turtles feeding at BA (indicated by triangles) are as follows: Bahamas (BH; Lahanas et al., 1998), Nicaragua (NI; Bass et al., 1998), Florida (FL; Bass and Witzell, 2000 and Encalada et al., 1996), Barbados (BB; Luke et al., 2004), North Carolina (NC; Bass et al., 2006), Ubatuba (UB); Almofala (AL) (Naro-Maciel et al., 2007) Fernando de Noronha (FN) and Atol das Rocas (AR) (Bjorndal et al., 2006 and Encalada et al., 1996). Aves Island, Venezuela (AV): Matapica, Suriname (SU): Quintana Roo, Mexico (MX) (Encalada et al., 1996); Lara Bay, Cyprus (CY; Encalada et al., 1996; Kaska, 2000); Tortuguero, Costa Rica (CR; Bjorndal et al., 2005; Encalada et al., 1996); Ascension Island, UK (AI); Poilaõ, Guinea Bissau (GB; Encalada et al., 1996; Formia et al., 2007); Bioko Island, Equatorial Guinea (BI); Saõ Tomé (ST; Formia et al., 2006); Trinidade Island, Brazil (TI; Bjorndal et al., 2006). Arrows represent oceanic currents: Gulf Stream (GSC), Antilles (AC), Florida (FC), North Equatorial Current (NEC), South Equatorial Current (SEC), South Equatorial Counter Current (SECC), Guiana Current (GC), Brazil Current (BrC), Malvinas Current (MC), South Atlantic Current (SAC) and Benguela Current (BeC).

actions in Buenos Aires where substantial anthropogenic pressures will finally have a negative impact on green turtle nesting colonies thousands of kilometers away.

Our specific objectives were: i) to characterize genetic diversity among green turtles on the Buenos Aires feeding grounds ii) to estimate the contribution of nesting colonies on southernmost feeding areas iii) to analyze genetic differentiation among western Atlantic feeding grounds, and iv) to analyze the impact of the present genetic analysis on conservation.

2. Materials and methods

2.1. Study area

The Buenos Aires province contains two important estuaries separated by 630 km (measured as linear distance) where sea turtle feeding takes place. The Rio de la Plata estuary (RP) is located between 34°00′–36°10′S and 55°00′–58°10′W and covers an area of 38,800 km^2 draining a 3,170,000 km^2 basin (Guerrero, 1998; Mianzán et al., 2001).The second estuary is El Rincón (ER), situated in the south of the Buenos Aires province between 38°42′–39°26′S and 62°28′–61°40 W draining a 2300 km^2 basin (Guerrero, 1998) (Fig. 1).

2.2. Sample collection

A total of 93 green turtles were sampled by PRICTMA between 2004 and 2007. Turtles came from by catch in coastal fisheries (76%) and from stranding (24%) occurring in RP (n = 78) and ER (n = 15). All sampled turtles were previously identified as juveniles based on size classification described by Bolten (1999) (González Carman et al., 2011). The mean curved carapace length (CCL) for sampled individuals was 38.1 cm ± 4.45 s.d. (range: 26.6–56 cm). Muscle and skin samples in dead individuals and blood and skin samples in live turtles were collected using standard protocols (Dutton, 1996).

2.3. Laboratory procedures

Genetic techniques — Skin and muscle samples were preserved in 90% ethanol; blood samples were collected in 20% ethylenediaminetetraacetic acid (EDTA). All samples were stored at −4 °C. A DNeasy Tissue Kit was used for DNA extractions, following the manufacturer's instructions for animal tissues (Qiagen Inc.).

A 486 bp mtDNA control region fragment was amplified in both directions using primers LTCM1 and HDCM1 described by Allard et al. (1994). Polymerase chain reaction (PCR) (1.5 mM $MgCL_2$, 1× PCR Buffer, 200 μM each dNTPS, 0.5 μM each primer, 0.6 U Taq, 1 μl template DNA and H_2O to a total volume of 50 μl) was carried out under the following conditions: 3 min at 94 °C, 35 cycles of 45 s at 94 °C, 30 s at 50 °C and 1.5 min at 72 °C, followed by 10 min at 72 °C, using a MGW Biotech Primus PCR System. The samples were sequenced on an ABI 3130XL (Applied Biosystems) using the sequencing service of the Facultad de Ciencias Exactas y Naturales (Universidad de Buenos Aires, Argentina).

2.4. Data analysis

Sequences were edited and aligned using the Bioedit V 7.0 (Hall, 1999) and Clustal programs (Higgins and Sharp, 1988). Polymorphic sites were identified using GENALEX 6 (Peakall and Smouse, 2006). Mitochondrial haplotypes were classified according to the standardized designation provided by the Marine Turtle Sequences website maintained by the Archie Carr Center for Sea Turtle Research at the University of Florida (ACCSTR — http://accstr.ufl.edu/genetics.html).

Haplotype (h) and nucleotide diversity (π) (Nei, 1987) were calculated for each of the feeding ground (RP and ER) and for the entire Buenos Aires coast. Analysis of molecular variance (AMOVA) (Excoffier et al., 2005), incorporating both F_{ST} (using haplotypes frequencies only) and Φ_{ST} (using Kimura 2P genetic distances (Kimura, 1980)), was used to investigate genetic structuring within and among feeding grounds. We reconstructed phylogenetic relationships among haplotypes in the studied feeding grounds using a median-joining network using the program NETWORK v.4.5.0.0 (Bandelt et al., 1999).

We included the sequence data in a broader analysis of western Atlantic feeding grounds studied at present. We divided all previously described western Atlantic feeding grounds into northwestern Atlantic and southwestern Atlantic groups. The former includes Bahamas (BH); Nicaragua (NI); Florida (FL); Barbados (BB) and North Carolina (NC). The latter group includes the four Brazilian populations (Almofala (AL), Ubatuba (UB), Atol das Rocas (AR) and, Fernando de Noronha (FN)). Because haplotype frequencies at the two nearby feeding grounds of AR and FN (Fig. 1) were not significantly different (Bjorndal et al., 2006), they were combined for further analyses. The RP and ER feeding grounds analyzed here were also combined for additional analyses because they did not exhibit significant heterogeneity at mtDNA level (see results). The complete data set analyzed here, namely Buenos Aires (BA), was classified with the southwestern populations for analysis (Fig. 1 and the references therein).

AMOVA based on F_{ST} and Φ_{ST} was also conducted to analyze the pattern of haplotype distribution among all studied western Atlantic feeding grounds (all published data and the present paper). Genetic differentiation between all western Atlantic feeding grounds was performed using pairwise comparisons. The statistical significance of F_{ST} and Φ_{ST} values was tested by 5000 permutations.

All AMOVAs mentioned above were conducted using ARLEQUIN v3.11 program (Excoffier et al., 2005).

The comparisons of diversity genetic indices (haplotype and nucleotide diversity) among northwestern Atlantic and southwestern Atlantic feeding grounds, were performed through non-parametric tests (Kruskal Wallis ANOVA). Relationships among western Atlantic feeding grounds were represented by unweighted pair grouping method based on arithmetical mean (UPGMA) phenograms on the basis of pairwise F_{ST} and Φ_{ST} values and by multidimensional scaling (MDS) based on Φ_{ST} values. The last two analyses were performed using the STATISTICA program (Statistica Statsoft Inc., 1996).

The extent of geographical heterogeneity in mitochondrial haplotype frequency distribution was further assessed through Monte Carlo simulations, as described by Roff and Bentzen (1989) for mtDNA data. This method compares the extent of heterogeneity (assessed through chi-squared analysis) in the original data matrix to that estimated from repeated randomization on the original matrix. This procedure is designed to minimize the effect of small samples on the validity of the chi-squared test and was conducted in REAP program (McElroy et al., 1991). Chi-squared statistics have been shown to have higher power than sequence-based statistics for detecting population structure (Hudson et al., 1992).

Contributions of nesting colonies to the Buenos Aires (BA) feeding grounds were assessed through Mixed Stock Analysis (MSA) using Bayesian methods implemented in the program BAYES (Pella and Masuda, 2001). Sources used in the MSA belong to 12 Atlantic and Mediterranean nesting colonies described in the literature to date, namely: Lara Bay, Cyprus (Cy); Polião, Guinea Bissau (GB); São Tomé (ST); Bioko Island, Equatorial Guinea (BI); Hutchinson Island, Florida (FL); Quintana Roo, México (MX): Tortuguero, Costa Rica (CR); Aves Island, Venezuela (AV); Matapica, Suriname (SU); Atol das Rocas, Brazil (AR) and Fernando de Noronha, Brazil (FN); Ascension Island, UK (AI); Trindade Island, Brazil (TI) (Table 3; Fig. 1 and the references therein). The AR and FN nesting colonies (Bjorndal et al., 2006) were joined for the MSA analysis, because there were no significant differences of the mtDNA haplotype distribution.

The BAYES program integrates information from the observed data (nesting colonies and feeding grounds) to estimate relative contribution of nesting colonies to foraging grounds. The Bayesian approach

Table 1
Haplotype frequencies, haplotype diversity and nucleotide diversity in green turtle feeding grounds in the western Atlantic.

	Northwestern Atlantic					Southwestern Atlantic				
	BB	NI	BH	NC	FL	UB	AL	AR/FN	BA	
									RP	ER
CM-A1	7		2	34	12					
CM-A2				2	1					
CM-A3	21	54	62	43	43	2	18			
CM-A5	13	6	10	5	3	14	28	5	18	2
CM-A6							3	2	2	
CM-A8	14		1	7		83	53	20	48	11
CM-A9	1					4	3	3	5	
CM-A10	2					3	4	1		1
CM-A15				1						
CM-A16				2			1			
CM-A17	1									
CM-A18				3	2					
CM-A19										
CM-A20			1							
CM-A21			3				1			
CM-A22	1			2	1					
CM-A24						2	1		1	
CM-A26				2						
CM-A27				2						
CM-A28				3						
CM-A32						2	1		2	
CM-A39										1
CM-A42							2		2	
CM-A44						1	1			
CM-A45							1			
CM-A46						1		1		
CM-A55						1				
N	60	60	79	106	62	113	117	32	78	15
No. of haplotypes	8	2	6	12	6	11	13	6	7	4
h	0.773	0.183	0.370	0.729	0.486	0.446	0.716	0.589	0.5691	0.467
	(0.027)	(0.062)	(0.065)	(0.030)	(0.066)	(0.055)	(0.030)	(0.091)	(0.052)	(0.148)
π	0.0104	0.0011	0.0065	0.0053	0.0031	0.0020	0.006	0.002	0.002	0.0018
	(0.005)	(0.001)	(0.003)	(0.003)	(0.002)	(0.001)	(0.003)	(0.001)	(0.0015)	(0.0015)

h = haplotype diversity, π = nucleotide diversity. N = sample size. Standard deviations are indicated in brackets. References and abbreviations correspond with Fig. 1.

using this program is not biased when sample size is small or by the presence of rare haplotypes (Pella and Masuda, 2001). Two MSA were performed, taking into account equal prior probability assigned to each rookery and considering contribution weighted by population size. Size estimates of the nesting populations were taken from references (Bellini and Sanches, 1996; Bellini et al., 1996; Formia et al., 2006; Seminoff, 2002, 2004). The analyses were carried out (about 7500 iterations) till Gelman and Rubin diagnostics confirmed convergence of the chains to the desired posterior density, with most shrink factors near 1.0 and below 1.2. The first halves of the chains were discarded as "burn-in," and estimates were based on the second halves only (Pella and Masuda, 2001). Haplotypes observed on the BA feeding grounds but not previously identified on nesting colonies were excluded from the MSA analysis.

To study whether MSA contributions are affected by the size of the nesting colonies and/or the distance between each nesting colonies and BA feeding grounds, we used a Spearman non-parametric correlation analysis using the STATISTICA program (Statistica Statsoft Inc., 1996).

Table 2
Genetic differentiation among western Atlantic feeding grounds of the green turtles.

	Northwestern Atlantic					Southwestern Atlantic			
	FL	BH	NC	BB	NI	UB	AL	AR/FN	BA
FL		0.050	**0.035**	**0.324**	0.021	**0.846**	**0.640**	**0.836**	**0.853**
BH	0.039		0.051	**0.179**	0.015	**0.728**	**0.515**	**0.672**	**0.728**
NC	**0.065**	**0.168**		**0.228**	**0.079**	**0.750**	**0.559**	**0.712**	**0.754**
BB	**0.131**	**0.182**	**0.055**		**0.294**	**0.413**	**0.168**	**0.322**	**0.413**
NI	**0.098**	0.013	**0.244**	**0.279**		**0.828**	**0.616**	**0.806**	**0.833**
UB	**0.529**	**0.571**	**0.376**	**0.249**	**0.649**		**0.077**	0.007	0.013
AL	**0.305**	**0.341**	**0.193**	**0.054**	**0.418**	**0.081**		0.054	**0.076**
AR/FN	**0.467**	**0.534**	**0.294**	**0.160**	**0.651**	0.003	0.028		0.013
BA	**0.472**	**0.518**	**0.322**	**0.182**	**0.599**	0.011	0.035	0.013	

Pairwise Φ_{ST} values are shown in the upper matrix and pairwise F_{ST} values are indicated in the lower matrix. Statistical significance was assessed using 5000 random permutations. Bold type indicates $P<0.01$. BA includes the samples of ER and RP. Abbreviations correspond with Fig. 1.

3. Results

3.1. Genetic diversity and haplotype distribution

A fragment of 486 bp, of the 5′ end of the mtDNA control region was successfully amplified from 93 green turtles from RP and ER estuaries (Table 1). Analyses of molecular variance (AMOVA), based on haplotype frequencies ($F_{ST} = -0.005$, $P > 0.05$) and on haplotype divergence ($\Phi_{ST} = -0.11$, $P > 0.05$) indicated that there were no significant differences between the aggregations at both BA estuaries. Similarly, the analysis of mtDNA haplotype distribution did not detect significant heterogeneity between both estuaries (X^2: 13.54, $P = 0.13$). Samples from the RP and ER estuaries were combined (Buenos Aires, BA) for further analyses.

A total of nine haplotypes, defined by seven variable sites and a 4 bp insertion/deletion (indel), were detected at the BA feeding grounds (Table 1, Fig. 2). Haplotype relationships were illustrated in a median-joining network (Fig. 2). Around the central sequence CM-A8 emerge branches to other haplotypes most of them separated from CM-A8 by one or two nucleotide substitutions. The CM-A42 haplotype, which

Table 3
Green turtle mtDNA haplotype frequencies from nesting colonies in the western Atlantic.

	Cy	GB	ST	BI	FL	MX	CR	AV	SU	AR/FN	AI	TI
CM-A1					11	7						
CM-A2					1							
CM-A3					12	5	395	3				
CM-A4							1					
CM-A5			1			1	32	27	13			
CM-A6			1	5					1		11	
CM-A7									1			
CM-A8		70	13	45						50	204	67
CM-A9										7	9	19
CM-A10										2	5	
CM-A11										1		1
CM-A12										5		
CM-A13	25											
CM-A14	1											
CM-A15						1						
CM-A16						1						
CM-A17						2						
CM-A18						3						
CM-A20							2					
CM-A21							3					
CM-A23											1	6
CM-A24											7	1
CM-A25										3	1	
CM-A32										1	1	4
CM-A33												1
CM-A35			1									
CM-A36			1									
CM-A37			1									
CM-A38			2									
CM-A39											1	
CM-A44											1	
CM-A45											1	
CM-A46											2	
CM-A50											1	
N	26	70	20	50	24	20	433	30	15	69	245	99
No. of haplotypes	2	1	7	2	3	7	5	2	3	7	13	7
Distance to BA (km)	12,186	6890	7651	7971	7208	7248	5917	5874	4790	4296	2387	3220
Nesting female	100	2523	90	407	779	1587	26,535	267	1814	129	3709	3000

N represents the sample size. References and abbreviations correspond with Fig. 1. The sizes of the nesting colonies are according to Naro-Maciel et al. (2007).

has not been described in any nesting ground to date, is separated by a 4 bp indel from CM-A8 and was found in two individuals from the BA feeding grounds.

The most common haplotype among BA feeding grounds samples was CM-A8, occurring in 55% of samples. CMA-5 was the second-most common haplotype and was found in 18.6% of the samples. Among nesting colonies, CM-A5 was found most frequently in CR, AV and SU, whereas CM-A8 was found most frequently in African and Brazilian rookeries (GB, ST, BI, TI, and AR/FN and AI) (Table 3). The remaining BA haplotypes were observed at low frequencies (≤5%), and were also encountered in samples from rookeries in TI, AR/FN, AI, GB, ST, BI, MX, CR, and SU (Table 3).

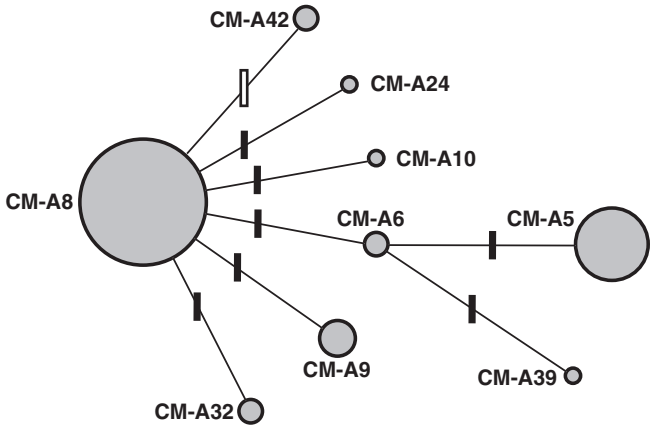

Fig. 2. Inferred genealogical relationship among green turtle mtDNA haplotypes from the BA feeding grounds. The diameter of each circle is proportional to the number of individuals found for each haplotype. Solid bars indicate inferred single nucleotide substitutions; the open bar represents a 4 bp insertion/deletion.

Haplotype diversity among BA samples was similar to that found in other southwestern Atlantic feeding grounds (UB and AR/FN), but lower than that observed in some northwestern Atlantic feeding grounds (BB and NC) (Table 1). Nucleotide diversity was generally higher in northwestern Atlantic feeding grounds. The Kruskal–Wallis test showed that there were significant differences in nucleotide diversity between northwestern Atlantic and southwestern Atlantic feeding grounds ($H = 6$; $P = 0.01$).

3.2. Genetic differentiation among western Atlantic feeding grounds

Haplotype frequencies were significantly different among all western Atlantic feeding grounds. AMOVA based on haplotype frequencies alone ($F_{ST} = 0.29$, $P < 10^{-4}$) and considering divergence between haplotypes ($\Phi_{ST} = 0.55$, $P < 10^{-4}$) revealed significant genetic differences among feeding grounds. Mitochondrial DNA analyses based on haplotype frequencies and genetic distances showed that 29% and 55% (respectively) of the variance could be accounted for by differences among feeding grounds. Similarly, the analyses of mtDNA haplotype distributions revealed significant heterogeneity among locations (X^2: 804.84, $P < 10^{-4}$). The pairwise comparisons among all western Atlantic feeding grounds after applied Bonferroni correction revealed that 29 out of 36 F_{ST} pairwise comparisons and

28 out of 36 Φ_{ST} comparisons were statistically significant after sequential Bonferroni correction (Table 2). Additionally, feeding grounds in northwestern Atlantic appear to be more different than feeding grounds in southwestern region. Pairwise analysis between northwestern feeding grounds demonstrated that almost comparisons except BH-FL and BH-NI exhibited significant differences. Pairwise comparisons between southwestern feeding grounds showed that only comparisons between AL with respect to BA and UB feeding grounds revealed significant genetic heterogeneity.

MDS (stress value = 0.00013) and phenogram based on Φ_{ST} genetic distances show the genetic affinities among western Atlantic feeding grounds (Fig. 3). In the UPGMA phenogram two main clusters are evident reflecting the relative major geographic distribution of feeding grounds. One group clustered feeding areas belonging to southwestern distribution and the other principal cluster assembled northwestern feeding grounds. The multidimensional scaling analysis showed again the strongest division between northwestern and southwestern coasts and demonstrated that northwestern feeding grounds seem to be more heterogeneous than feeding areas in southwestern Atlantic.

To analyze differences in migration contributions along western Atlantic coasts, analyses of molecular variance were performed using data from the two principal clusters in the tree. The AMOVA in southwestern Atlantic indicated significant differences among feeding grounds (6.10% and 3.9%, respectively), both in cases when genetic distances were included in the model (Φ_{ST}: 0.061, $P<10^{-4}$) and when genetic distances were excluded from the model (F_{ST}: 0.039, $P<10^{-4}$). Genetic differentiation among southwestern Atlantic feeding ground showed large differentiation between AL and BA as well as between AL and UB (Table 2).

In the same way, AMOVA based on haplotype frequencies and using genetic distances showed significant differences among five northwestern feeding grounds (F_{ST}: 0.137, $P<10^{-4}$, Φ_{ST}: 0.146, $P<10^{-4}$). As evidenced in MDS analysis, feeding grounds in northwestern distributions exhibited more heterogeneity (14% based on F_{ST} and 15% considering Φ_{ST}) than feeding grounds in southwestern Atlantic.

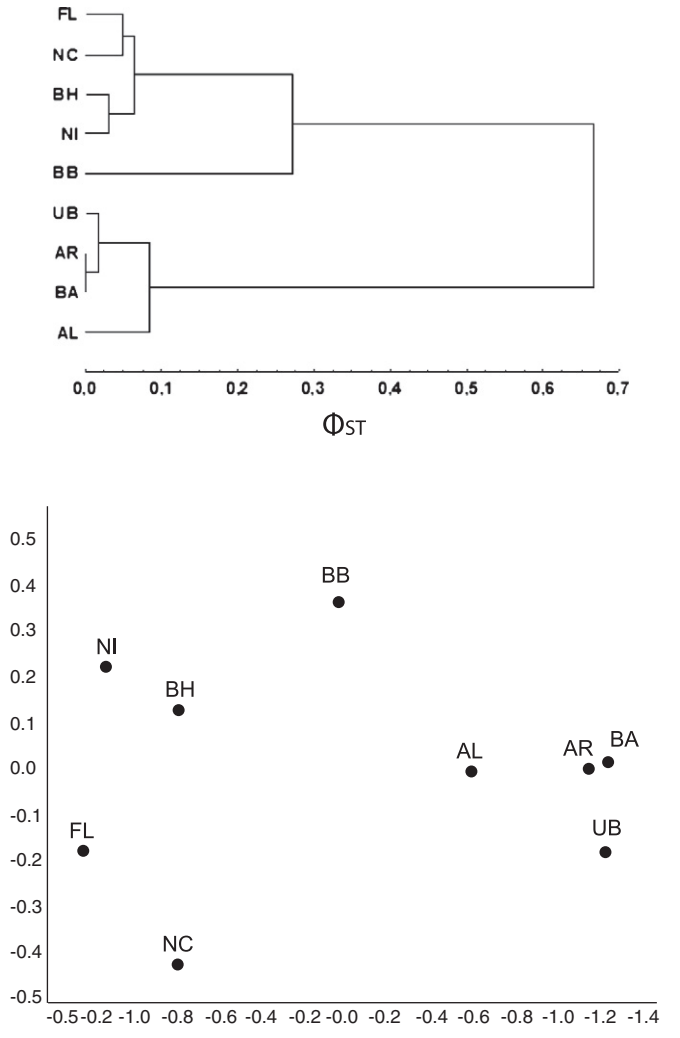

Fig. 3. UPGMA tree (A) and Multidimensional Scaling Analysis (MDS) (B) showing relationships among 9 western Atlantic feeding grounds of green turtle on the basis of pairwise Φ_{ST} values (0.014 was added to each original Φ_{ST} value in order to avoid negative numbers).

3.3. Mixed stock analyses

Mixed stock analysis based on equal prior probability to each source and taking into account contribution weighted by population size demonstrated that AI is the main source of the BA feeding grounds (61% and 60% respectively), with lesser contributions from SU, AV and TI rookeries (Table 4). The confidence intervals for AI were narrower and did not encompass zero for both MSA with prior weighted and non-weighted.

No significant relationships were detected between MSA results and the size of the nesting colonies as well as the geographic distance from nesting colonies ($r = -0.082$ $P = 0.80$; $r = 0.52$ $P = 0.09$ respectively).

4. Discussion

To protect a migratory species, it is critical to study its rangewide population structure (Frankham et al., 2002; Heppel et al., 2003). Several recent studies addressing the genetic composition in green turtle feeding grounds (mixed-stock) provide useful information about the spatial pattern of movements and distribution (Bass and Witzell, 2000; Bass et al., 2006; Bolker et al., 2007; Luke et al., 2004; Naro-Maciel et al., 2007). Understanding the genetic composition of feeding grounds allows researchers to track the status of

Table 4
Mixed stock analysis (MSA) of Buenos Aires green turtle control region haplotypes considering equal priors (1) and contribution weighted by population size (2).

Stock	MSA	Mean	Standard deviation	2.5%	Median	97.5%
TI	1	0.0743	0.1067	0.0000	0.0105	0.3667
	2	0.0922	0.1214	0.0000	0.0276	0.4052
AI	1	0.6140	0.1616	0.2235	0.6562	0.8189
	2	0.5839	0.1638	0.1798	0.6174	0.8070
AR	1	0.0226	0.0660	0.0000	0.0001	0.2252
	2	0.0214	0.0658	0.0000	0.0000	0.2212
AT	1	0.0047	0.0195	0.0000	0.0000	0.0450
	2	0.0044	0.0153	0.0000	0.0000	0.0457
BI	1	0.0172	0.0521	0.0000	0.0000	0.1946
	2	0.0315	0.0739	0.0000	0.0000	0.2782
GB	1	0.0357	0.0758	0.0000	0.0002	0.2770
	2	0.0184	0.0515	0.0000	0.0000	0.1877
SU	1	0.1293	0.1130	0.0000	0.1599	0.3117
	2	0.1630	0.1198	0.0000	0.1992	0.3517
AV	1	0.0983	0.1061	0.0000	0.0327	0.2868
	2	0.0813	0.1056	0.0000	0.0037	0.2927
CR	1	0.0010	0.0033	0.0000	0.0000	0.0101
	2	0.0010	0.0035	0.0000	0.0000	0.0103
MX	1	0.0010	0.0034	0.0000	0.0000	0.0096
	2	0.0011	0.0038	0.0000	0.0000	0.0110
FL	1	0.0010	0.0037	0.0000	0.0000	0.0109
	2	0.0010	0.0037	0.0000	0.0000	0.0104
CY	1	0.0010	0.0033	0.0000	0.0000	0.0101
	2	0.0009	0.0032	0.0000	0.0000	0.0097

Mean values are shown with standard deviations. The 2.5% and 97.5% values indicate the upper and lower bounds of the 95% confidence interval. Abbreviations correspond with Fig. 1.

populations, obtain data to support stock assessment analyses, and provide life cycle information.

This study represents the first attempt to understand the origins of green turtle feeding grounds in their southernmost Atlantic distribution. We studied green turtles from two estuaries in the Buenos Aires province (RP and ER). All genetic differentiation approaches (AMOVAs through F_{ST} and Φ_{ST} and chi-squared test) demonstrated homogeneity in haplotype distribution between the two estuaries allowing us to consider them a single group for the purposes of subsequent analyses. However, the absence of genetic differentiation between estuaries may be an artifact of small sample size (ER) and should be interpreted carefully.

We analyzed genetic diversity using mitochondrial control region sequences, assessing our results in a framework of existing data from other western Atlantic feeding grounds. As a general factor, nucleotide diversity was significantly greater in northwestern feeding grounds than in southwestern feeding aggregations. This result may be explained by the fact that northwestern feeding ground haplotypes are more diverse with respect to southwestern feeding areas. In an early phylogeographic analysis of the Atlantic and Mediterranean nesting grounds of green turtle Encalada et al. (1996) distinguished two haplotype groups. In northwestern feeding grounds exhibited haplotypes belonging to two different haplotype clades whereas in southwestern feeding grounds showed most of the haplotypes belonging to one clade.

Analysis of molecular variation showed that a large proportion of the variance (29% with F_{ST} and 55% with Φ_{ST}) in western Atlantic feeding grounds could be accounted for by differences among them. Several studies (including the present paper) have demonstrated through different statistical approaches (Chi-square comparisons; exact-test; F_{ST} and Φ_{ST} pairwise comparisons) that most western Atlantic feeding grounds are genetically differentiated (Bass and Witzell, 2000; Bass et al., 2006; Luke et al., 2004; Naro-Maciel et al., 2007).

Phenogram and MDS analyses demonstrated the existence of two differentiated feeding ground groups: northwestern and southwestern. Partial AMOVAs revealed higher differences among northwestern feeding grounds than among southwestern feeding grounds.

Among northwestern feeding grounds, Barbados and North Carolina, with high indices of genetic diversity and a larger number of contributing nesting colonies, displayed genetic differentiation with almost pairwise comparisons (Tables 1 and 2) (Bass et al., 2006; Luke et al., 2004). Phenogram and MDS analysis also evidenced that BB is the most differentiated Atlantic feeding ground. Nicaragua and Bahamas feeding grounds are drawn mainly from the Costa Rica nesting colony (90% and 80% respectively) and exhibit comparatively lower levels of haplotype diversity (Table 1) (Bass et al., 1998; Lahanas et al., 1998). Consequently, pairwise comparisons did not reveal genetic differentiation between these feeding grounds (Table 2). In agreement, Φ_{ST} phenogram clustered these feeding areas together. The Florida feeding grounds contained main contributions from Costa Rica (48%) and from the adjacent Florida nesting colony (37%) and the haplotype diversity was comparatively intermediate with respect to other northwestern feeding grounds (Table 1) (Bass and Witzell, 2000). Juvenile green turtles from Florida feeding grounds did not differ from a similar juvenile foraging population from Bahamas in pairwise comparisons based on F_{ST} and $\Phi_{ST,}$ but differ significantly in F_{ST} pairwise comparison with respect to the adult foraging group from Nicaragua (Table 2). The last result may be attributed to the fact that Florida and Nicaragua foraging grounds represent different development stages of green turtle life span. The detected differences between haplotype compositions indicate a nonrandom distribution of green turtles among northwestern feeding grounds.

Among southwestern feeding grounds, the Buenos Aires feeding grounds exhibited indices of genetic diversity comparable to those detected in Ubatuba and Atol das Rocas/Fernando de Noronha. The MSA of joint stock presented here suggests that green turtles at Argentinean feeding grounds originate mainly in the Ascension Island rookery, with lesser contributions from more distant nesting colonies, Suriname, Aves Island and Trindade Island (Table 4). Ubatuba and Atol das Rocas/Fernando de Noronha exhibited relative mixed stock contributions comparable to those in the Buenos Aires feeding grounds (Bjorndal et al., 2006; Naro-Maciel et al., 2007). As a result, pairwise comparisons did not show genetic significant differences between Buenos Aires with respect to Ubatuba and Atol das Rocas/Fernando de Noronha (Table 2). However, the limited sample size of Atol das Rocas/Fernando de Noronha might have affected comparisons considering these latter feeding grounds. Within southwestern feeding grounds, Almofala showed the largest number of contributing nesting colonies (Ascension Island (43%), Aves Island (18%), Costa Rica (15%), Suriname (8%) and Trindade (6%)) (Naro-Maciel et al., 2007) displayed the highest values of haplotype and nucleotide diversity (Table 1) and exhibited genetic differentiation with respect to Buenos Aires and Ubatuba. In agreement phenogram and MDS plot tended to cluster UB-AR/FN-BA whereas AL shows high differentiation. The observed variation pattern denotes, as detected in the northwestern Atlantic, a nonrandom distribution of green turtles among southwestern feeding grounds.

Several factors, such as the movements of individuals among feeding grounds, the sizes of nesting colonies, the distance between breeding and feeding grounds, juvenile natal homing and ocean currents, have been suggested to explain the composition of feeding areas (Bass and Witzell, 2000; Bass et al., 2006; Bjorndal and Bolten, 2008; Bolten, 2003b; Broderick et al., 2001; Lahanas et al., 1998; Luke et al., 2004).

The movements of juvenile green turtles might be invoked to explain differences between neritic feeding grounds (Bass et al., 2006; Bjorndal and Bolten, 2008; Witham, 1998). At present, we do not have data about green turtle movements in Buenos Aires estuaries. Future home range satellite telemetry studies will improve our understanding of green turtles in Argentina.

Some studies have analyzed the importance of rookery size or the distances from feeding ground to candidate nesting colony in the proportional contribution to feeding grounds and the results have differed. Bass and Witzell (2000) demonstrated that the distance between nesting and feeding grounds was an important factor in the Florida feeding ground composition. Nesting ground population size has been suggested to show an important role shaping the contribution in both Bahamas and Nicaragua feeding grounds (Bass et al., 1998, 2006; Lahanas et al., 1998). Contrariwise, in Barbados, Almofala, Ubatuba and Buenos Aires there was no significant influence of either size or distance of the nesting colonies from the feeding grounds on the estimated contributions of the different nesting colonies to feeding areas (Luke et al., 2004; Naro-Maciel et al., 2007; the present paper).

It has been suggested that ocean currents have great influence in shaping green turtle feeding aggregation composition (Bass and Witzell, 2000; Bass et al., 2006; Luke et al., 2004; Naro Maciel et al., 2007).

Some papers proposed the influence of the several ocean currents (Gulf Stream (GSC), Antilles (AC), Florida (FC) and Equatorial (EC) Currents) modeling northwestern feeding areas (Bass et al., 2006 and Luke et al., 2004) (see Fig. 1). It have been invoked that the high diversity in Barbados and North Carolina with respect to east-central Florida feeding grounds may be caused by their position relative to ocean currents (Bass et al., 2006). In particular Barbados feeding ground composition may be the result of its location with respect to the coalescence of the North Equatorial and South Equatorial Currents (NEC and SEC respectively) (Luke et al., 2004) leading to high differentiation from each of the other Atlantic northwestern feeding grounds.

Ocean currents may also be important factors molding the genetic composition of juveniles in the Buenos Aires feeding grounds. The main current flowing toward this area is the Brazilian Current, which runs north to south along the South American coastline to Argentina. In turn, the Brazilian Current (BrC) represents the southern branch of

the South Equatorial Current, whose northern branch flows into the Caribbean Guiana Current (GC) and toward northern South America and the Caribbean. The South Equatorial Counter Current (SECC) flows counter to the SEC, as its name implies, but its effect on the foraging ground composition may have a seasonal component (Boltovskoy, 1981; Piola and Matano, 2001; Tomczak and Godfrey, 2003) (Fig. 1).

Ascension Island, the main contributor to the BA feeding ground (61%), is located along the path of the SEC. It is therefore likely that this current (and its bifurcating branches) plays a major role in dispersal of Ascension individuals toward Argentina and other foraging grounds along the South American coastline. On the other hand, Trinidade though geographically closer to Argentina (ca. 3220 km), is far enough offshore that it is not touched by the SEC or BrC, which may explain its lower contribution to the BA foraging grounds (7%). The contribution from Suriname (13%) and Aves Island (10%) may be mainly influenced by the SECC.

The similarity between the Buenos Aires and Ubatuba foraging grounds, and their differentiation with respect to the other Brasilian green turtle foraging area in Almofala might also be explained by the influence of ocean currents on hatchling dispersal. Almofala, for instance, located in northern Brazil is likely to be influenced by the NEC and the GC, both of which do not touch southern Brazil and Argentina (Bass et al., 2006; Naro-Maciel et al., 2007).

The results presented here suggest that ocean currents may constitute the deciding factor molding the genetic composition of foraging aggregations in Argentina. The Buenos Aires estuaries may be of particular importance to sea turtles since they reach these feeding grounds after extensive developmental migrations (which, in the case of nesting colonies in the southwestern Atlantic, range from 3000 to 9000 km).

Temporal variation could have a significant effect on estimates of feeding ground composition (Bass et al., 2006; Bjorndal and Bolten, 2008). However, most analyses conducted on feeding grounds have only considered samples at one time or samples collected over several years pooled together for analysis (Bjorndal and Bolten, 2008). Among the four southwest Atlantic feeding grounds analyzed to date, only the approach of Naro-Maciel et al. (2007) evaluated temporal variation. These authors concluded that there was no significant variation among years in Almofala (AL) and Ubatuba (UB). However, long term temporal studies in feeding grounds would be useful in analyzing the dynamics of development and feeding areas of the green turtle.

Despite the number of studies dedicated to detection and characterization of both nesting and feeding areas, important questions remain. Several feeding ground studies have detected previously unidentified haplotypes, suggesting either the presence of unsampled nesting colonies or the existence of these new haplotypes at low frequencies in previously studied nesting areas (Bass et al., 2006; Luke et al., 2004; Naro-Maciel et al., 2007). In the present study we detected the CM-A42 haplotype, which has not been previously described from any nesting beach. This haplotype is separated by a 4 bp indel from the most common haplotype CM-A8 in Buenos Aires and has also been detected in feeding grounds of Africa (Corisco Bay in Gabon and the Gulf of Guinea) and Brazil (Almofala) in relatively low frequencies (0.004, 0.006 and 0.017 respectively) (Formia, 2002; Naro-Maciel et al., 2007).

Interestingly, feeding grounds reporting the CM-42 haplotype (Buenos Aires as well as Almofala and west Africa) all exhibited major contributions from Ascension Island with smaller contributions from Bioko and Guinea Bissau (Formia, 2002; Naro-Maciel et al., 2007; this paper). Other haplotypes which may also have been contributed to these feeding grounds by the same rookeries are CM 26 and CM 32. Therefore, one can hypothesize that the detected distributions of these haplotypes and that of CM-42 are related to the influence of the same oceanic currents, flowing between the rookeries of origin and the feeding grounds. In other words, the South Atlantic gyre including the Benguela Current (BeG), the dominant current in the eastern South Atlantic (Tomczak and Godfrey, 2003), the SEC and the BrC, may sweep hatchlings originating from rookeries along their path, such as Ascension and Bioko Islands. Following this developmental migration, hatchlings might then recruit to foraging grounds also located along the same circular current system, thus effecting dispersal of haplotypes (Fig. 1). This hypothesis would also suggest putative areas of haplotype origin (Fig. 1). However, further genetic studies increasing the sample size of identified nesting colonies as well as detecting new nesting areas are necessary and can improve our understanding of the genetic structure of this species in the South Atlantic.

Successful management of migratory species requires an understanding of population structure throughout their ranges. Due to the wide-ranging migrations of the green turtle, long-term international cooperation in the analysis of both nesting and feeding grounds is essential for protection efforts. Our results represent a considerable contribution to the study of possible migration routes, and can be utilized to implement mitigation measures and plans for conservation, not only in nesting colonies but also in development and feeding areas of this severely threatened species.

Acknowledgments

Sampling was possible thanks to all members of the Programa Regional de Investigación y Conservación de Tortugas Marinas de la Argentina (PRICTMA, www.prictma.com.ar) (Buenos Aires Zoo; Aquamarina — CECIM; Proyecto Peyu; Fundación Mundo Marino; Reserva de Usos Múltiples Bahía Blanca, Bahía Falsa y Bahía Verde). We would like to thank Dr. Angela Formia for her valuable comments and discussions. We are also grateful to Lic. Javier H. Otero for his helpful assistance in figure edition. Funding was provided by the Field Veterinary Program of Wildlife Conservation Society — WCS; National Fish Wildlife Foundation — NFWF (no. 2006-0091-006) and Buenos Aires University (X-186). This is INIDEP contribution no. 1691. This study adhered to the legal requirements of the countries in which the work was carried out, and to all institutional guidelines. **[RH]**

References

Allard, M.W., Miyamoto, M.M., Bjorndal, K.A., Bolten, A.B., Bowen, B.W., 1994. Support for natal homing in green turtles from mitochondrial DNA sequences. Copeia 1, 34–41.
Avise, J.C., 2004. Molecular Markers Natural History and Evolution, Second edition. Sinauer Associates, Inc., Sunderland M.A., USA.
Bagley, D.A., 2003. Characterizing juvenile green turtles (*Chelonia mydas*) from three east central Florida Developmental habitats. MS Thesis. University of Central Florida, Orlando, FL.
Bandelt, H.J., Forster, P., Röhl, A., 1999. Median-joining networks for inferring intraspecific phylogenies. Mol. Biol. Evol. 16, 37–48.
Bass, A.L., Witzell, W.N., 2000. Demographic composition of immature green turtles (*Chelonia mydas*) from the east central Florida coast, evidence from mtDNA markers. Herpetologica 56, 357–367.
Bass, A.L., Lagueux, C.J., Bowen, B.W., 1998. Origin of green turtles. *Chelonia mydas* at "sleeping rocks" of the northeast of Nicaragua. Copeia 4, 1064–1069.
Bass, A.L., Epperly, S.P., Braun-McNeill, J., 2004. Multi-year analysis of stock composition of loggerhead turtle (*Caretta caretta*) foraging habitat using maximum likelihood and Bayesian methods. Conserv. Genet. 5, 783–796.
Bass, A.L., Epperly, S.P., Braun-McNeill, J., 2006. Green turtle (*Chelonia mydas*) foraging and nesting aggregations in the Caribbean and Atlantic, impact of currents and behavior on dispersal. J. Hered. 97, 346–354.
Bellini, C., Sanches, T.M., 1996. Reproduction and feeding of marine turtles in the Fernando de Noronha Archipelago, Brazil. Mar. Turt. Newsl. 74, 12–13.
Bellini, C., Marcovaldi, M.Â., Sanches, T.M., Grossman, A., Sales, G., 1996. Atol Das Rocas Biological Reserve: second largest *Chelonia* rookery in Brazil. Mar. Turt. Newsl. 72, 1–2.
Bjorndal, K.A., Bolten, A.B., 2008. Annual variation in source contributions to mixed stock, implications for quantifying connectivity. Mol. Ecol. 17, 2185–2193.
Bjorndal, K.A., Bolten, A.B., Troeng, S., 2005. Population structure and genetic diversity in green turtles nesting at Tortuguero, Costa Rica, based on mitochondrial DNA control region sequences. Mar. Biol. 147, 1449–1457.
Bjorndal, K.A., Bolten, A.B., Moreira, L., Bellini, C., Marcovaldi, M.A., 2006. Population structure and diversity of Brazilian green turtle rookeries based on mitochondrial DNA sequences. Chelonia Conserv. Biol. 5 (2), 262–268.
Bolker, B.M., Okuyama, T., Bjorndal, K.A., Bolten, A.B., 2007. Incorporating multiple mixed stocks in mixed stock analysis, 'many-to-many' analyses. Mol. Ecol. 16, 685–695.

Bolten, A.B., 1999. Techniques for measuring sea turtles. In: Eckert, K.L., Bjorndal, K.A., Abreu-Grobois, F.A., Donnelly, M. (Eds.), Research and Management Techniques for the Conservation of Sea Turtles. IUCN/SSC Marine Turtle Specialist Group Publication, pp. 110–114.
Bolten, A.B., 2003a. Active swimmers-passive drifters: The oceanic juvenile stage of loggerheads in the Atlantic Ocean. In: Bolten, A.B., Witherington, B.E. (Eds.), Loggerhead Sea Turtles. Smithsonian Books, Washington, DC, USA, pp. 63–78.
Bolten, A.B., 2003b. Variation in sea turtle life history patterns: neritic versus oceanic developmental stages. In: Lutz, P.L., Musick, J.A., Wyneken, J. (Eds.), The Biology of Sea Turtles, vol 2. CRC Press, Boca Raton, FL, pp. 243–457. 257.
Boltovskoy, D., 1981. Atlas del zooplancton del Atlántico Sudoccidental y métodos de trabajo con el zooplancton marino. Publ. Especial INIDEP, Mar del Plata (Argentina). 936 pp.
Bourjea, J., Lapégue, S., Gagnevin, L., Broderick, D., Mortimer, J., Ciccione, S., Roos, D., Taquet, C., Grizel, H., 2007. Phylogeography of the green turtle, *Chelonia mydas*, in the Southwest Indian Ocean. Mol. Ecol. 16, 175–186.
Bowen, B.W., Meylan, A.B., Ross, J.P., Limpus, C.J., Balazs, G.H., Avise, J.C., 1992. Global population structure and natural history of the green turtle (*Chelonia mydas*) in terms of matriarchal phylogeny. Evolution 46, 865–881.
Bowen, B.W., Bass, A.L., Chow, S.M., Bostrom, M., Bjorndal, K.A., Bolten, A.B., Okuyama, T., Bolker, B.M., Epperly, S., Lacasella, E., 2004. Natal homing in juvenile loggerhead turtles (*Caretta caretta*). Mol. Ecol. 13, 3797–3808.
Bowen, B.W., Bass, A.L., Soares, L., Toonen, R.J., 2005. Conservation implications of complex population structure, lessons from the loggerhead turtle (*Caretta caretta*). Mol. Ecol. 14, 2389–2402.
Broderick, A.C., Godley, B.J., Hays, G.C., 2001. Trophic status drives inter-annual variability in nesting numbers of marine turtles. Proc. R. Soc. Lond. B 268, 1481–1487.
Dutton, P.H., 1996. Methods for collection and preservation of samples for sea turtle genetic studies. In: Bowen, B.W., Witzell, W.N. (Eds.), Proceedings of the International Symposium on Sea Turtles Conservation Genetics, NOAA Technical Memorandum NMFS-SEFSC, Miami (FL), 396, pp. 17–24.
Encalada, S.E., Lahanas, P.N., Bjorndal, K.A., Bolten, A.B., Miyamoto, M.M., Bowen, B.W., 1996. Phylogeography and population structure of the Atlantic and Mediterranean green turtle *Chelonia mydas*, a mitochondrial DNA control region sequence assessment. Mol. Ecol. 4, 473–483.
Epperly, S.P., Braun, J., Veishlow, A., 1995. Sea turtles in North Carolina waters. Conserv. Biol. 9, 384–394.
Excoffier, L., Laval, G., Schneider, S., 2005. Arlequin version 3.0, an integrated software package for population genetics data analysis. Evol. Bioinform. Online 1, 47–50.
FitzSimmons, N.N., Limpus, C.J., Norman, J.A., Goldizen, A.R., Miller, J.D., Moritz, C., 1997a. Philopatry of male marine turtle inferred from mitochondrial DNA markers. Proc. Natl. Acad. Sci. U. S. A. 94, 8912–8917.
FitzSimmons, N.N., Moritz, C., Limpus, C.J., Pope, L., Prince, R., 1997b. Geographic structure of mitochondrial and nuclear gene polymorphisms in Australian green turtle populations and male-biased gene flow. Genetics 147, 1843–1854.
Formia, A., 2002. Population and genetic structure of the green turtle (*Chelonia mydas*) in West and Central Africa; implications for management and Conservation (dissertation). Cardiff (UK) — Cardiff University.
Formia, A., Godley, B.J., Dontaine, J.F., Bruford, M.W., 2006. Mitochondrial DNA diversity and phylogeography of endangered green turtle (*Chelonia mydas*) populations in Africa. Conserv. Genet. 7, 353–369.
Formia, A., Broderick, A.C., Glen, F., Godley, B.J., Hays, G.C., Bruford, W., 2007. Genetic composition of the Ascension Island green turtle rookery based on mitochondrial DNA, implications for sampling and diversity. Endanger. Species Res. 3, 145–158.
Frankham, R., Balou, J.D., Briscoe, D.A., 2002. Introduction to Conservation Genetics. Cambridge University Press, UK.
Godley, B.J., Broderick, A.C., Hays, G.C., 2001. Nesting of green turtles (*Chelonia mydas*) at Ascension Island, South Atlantic. Biol. Conserv. 97, 151–158.
González Carman, V., Álvarez, K., Prosdocimi, L., Inchaurraga, M.C., Dellacasa, R., Faiella, A., Echenique, C., González, R., Andrejuk, J., Mianzan, H., et al., 2011. Temperate SW Atlantic: a feeding and developmental habitat for endangered sea turtles. Mar. Biol. Res. 7, 500–508.
Guerrero, R.A., 1998. Oceanografía física del estuario de Río de la Plata y el sistema costero de El Rincón. In: Lasta, C. (Ed.), Resultados de una campaña de evaluación de recursos demersales costeros de la Provincia de Buenos Aires y del litoral uruguayo: INIDEP Informe Técnico, Mar del Plata, Argentina, 21, pp. 29–54.
Hall, T.A., 1999. BioEdit, a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series, pp. 95–98.
Heppel, S.S., Snover, M.L., Crowder, L.B., 2003. Sea turtle population ecology. In: Lutz, P.L., Musick, J.A., Wyneken, J. (Eds.), The Biology of Sea Turtle. CRC Press, Boca Ratón (FL), pp. 275–306.
Higgins, D.G., Sharp, P.M., 1988. CLUSTAL, A package for performing multiple sequence alignment on a microcomputer. Gene 73, 237–244.
Hudson, R.R., Boos, D.D., Kaplan, N.L., 1992. A statistical test for detecting geographic subdivision. Mol. Biol. Evol. 9, 138–151.
IUCN, 2009. IUCN 2009: IUCN Red List of Threatened Species. Version 2009.2. www.iucnredlist.org.
Kamezaki, N., Matsui, M., 1995. Geographic variation in skull morphology of the green turtle, *Chelonia mydas*, with a taxonomic discussion. J. Herpetol. 29, 51–60.
Karl, S.A., Bowen, B.W., Avise, J.C., 1992. Global population structure and male mediated gene flow in the green turtle (*Chelonia mydas*), RFLP analyses of anonymous nuclear DNA regions. Genetics 31, 163–173.
Kaska, Y., 2000. Genetic structure of Mediterranean sea turtle populations. Turk. J. Zool. 24, 191–197.
Kimura, M., 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16, 111–120.
Lahanas, P.N., Miyamoto, M.M., Bjorndal, K.A., Bolten, A.B., 1994. Molecular evolution and population genetics of Greater Caribbean green turtles (*Chelonia mydas*) as inferred from mitochondrial DNA control region sequences. Genetica 94, 57–67.
Lahanas, P.N., Bjorndal, K.A., Bolten, A.B., Encalada, S.E., Miyamoto, M.M., Valverde, R.A., Bowen, B.W., 1998. Genetic composition of green turtle (*Chelonia mydas*) feeding grounds population, evidence for multiple origins. Mar. Biol. 130, 345–352.
López-Mendilaharsu, M., Estrades, A., Caraccio, M.N., Calvo, V., Hernández, M., Quirici, V., 2006. Biología, ecología y etología de las tortugas marinas en la zona costera Uruguaya. In: Menafra, R., Rodríguez-Gallego, L., Scarabino, F., Conde, D. (Eds.), Bases para la conservación y el manejo de la costa Uruguaya, Montevideo, pp. 247–258.
Luke, K., Horrockas, J.A., Le Roux, R.A., Dutton, P.H., 2004. Origins of green turtle (*Chelonia mydas*) feeding aggregations around Barbados, West Indies. Mar. Biol. 144, 799–805.
McElroy, D., Moran, P., Bermingham, E., Kornfield, I., 1991. REAP. The Restriction Enzyme Analyses Package. Version 4.0. Department of Zoology. Migratory Fish Research Institute and Center for Marine Studies, University of Maine, Orono, USA.
Mianzán, H.W., Lasta, C.A., Acha, E.M., Guerrero, R.A., Macchi, G.J., Bremec, C., 2001. The Río de la Plata estuary Argentina — Uruguay. In: Seeliger, U., De Lacerda, L.D., Kjerfve, B. (Eds.), Ecological Studies: Coastal Marine Ecosystems of Latin America. Springer-Verlag, Berlin, pp. 186–204.
Moritz, C., 1994. Defining "Evolutionarily Significant Units" for conservation. Trends Ecol. Evol. 9, 373–375.
Musick, J.A., Limpus, C.J., 1997. Habitat utilization and migration in juvenile sea turtles. In: Lutz, P., Musick, J.A. (Eds.), The Biology of Sea Turtles. CRC Press, Boca Raton, Florida, pp. 137–163.
Naro-Maciel, E., Becker, J.H., Lima, E.H.S.M., Marcov, M.A., Desalle, R., 2007. Testing dispersal hypotheses in foraging green sea turtles (*Chelonia mydas*) of Brazil. J. Hered. 98, 29–39.
Nei, M., 1987. Molecular Evolutionary Genetics. Columbia University Press, New York (NY).
Norman, J.A., Moritz, C., Limpus, C.J., 1994. Mitochondrial DNA control region polymorphisms, genetic markers for ecological studies of marine turtles. Mol. Ecol. 3, 363–373.
Peakall, R., Smouse, P.E., 2006. GENALEX 6, genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Notes 6, 288–295.
Pella, J.J., Masuda, M., 2001. Bayesian methods for analysis of stock mixtures from genetic characters. Fish. Bull. 9, 151–167.
Pella, J.J., Milner, G.B., 1987. Use of genetic markers in stock composition analysis. In: Ryman, N., Utter, F. (Eds.), Population genetics and fishery management. University of Washington Press, Seattle (WA), pp. 247–276.
Piola, A.R., Matano, R.P., 2001. Brazil and Falklands (Malvinas) Currents. In: Steele, J.H., Thorpe, S.A., Turekian, K.K. (Eds.), Encyclopedia of Ocean Sciences. Academic Press, Londres, pp. 340–349.
Plotkin, P., 2003. Adult migrations and habitat use. In: Lutz, P.L., Musick, J.A., Wyneken, J. (Eds.), The Biology of Sea Turtles. CRC Press, Boca Raton, FL, pp. 225–242.
Roberts, M.A., Schwartz, T.S., Karl, S.A., 2004. Global population genetic structure and male-mediated gene flow in the green sea turtle (*Chelonia mydas*), analysis of microsatellite loci. Genetics 166, 1857–1870.
Roff, D.A., Bentzen, P., 1989. The statistical analysis of mitochondrial DNA polymorphisms, X^2 and the problem of small samples. Mol. Biol. Evol. 6 (5), 539–545.
Schulz, J.P., 1975. Sea turtles nesting in Suriname. Zool. Verh. (Leiden) 143, 3–172.
Seminoff, J.A., 2002. IUCN Red List Status Assessment Green Turtle (*Chelonia mydas*). Marine Turtle Specialist Group, The World Conservation Union IUCN, Gland (Switzerland).
Seminoff, J.A., 2004. Global Status Assessment Green Turtle (*Chelonia mydas*). Marine Turtle Specialist Group, The World Conservation Union IUCN, Gland (Switzerland).
Seminoff, J.A., Abreu-Grobois, F.A., 2007. Assessing the global status of sea turtles under the IUCN globally distributed species criteria: assumptions, caveats, limitations, and a call for more demographic data. In: Mast, R.B., Hutchinson, B.J., Hutchinson, A.H. (Eds.), Proceedings of the Twenty-fourth Annual Symposium on Sea Turtle Biology and Conservation, 567. NMFS-SEFSC, San Jose, Costa Rica, pp. 34–35.
Statistica Statsoft Inc., 1996. Statistica 5 for Windows (Computer Program Manual). Tulsa OK.
Tomczak, M., Godfrey, J.S., 2003. Regional Oceanography: An Introduction, 2nd edn. Daya Publishing House, Delhi, pp. 229–252. Chapter 14.
Witham, R.M., 1998. The "Lost Years" question in young sea turtles. Am. Zool. 20, 525–530.