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Animal Genetics

Multiple haplotypes of *Chelonia mydas* juveniles in a threatened *hotspot* area in Southern Brazil

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Abstract

Mixed stocks are described for *Chelonia mydas* and the frequency of haplotypes in feeding areas can aid understanding of the genetic and ecological diversity, since with this information it is possible to identify the origin of the individuals. The current study aims to characterize and compare genetic diversity along the coast of Paraná with 17 other feeding areas in the Atlantic Ocean. A total of 285 samples from juveniles were DNA sequenced in the control region, resulting in the identification of 12 haplotypes, with a predominance of the CMA8 haplotype (69%) and the first registration of CMA23. For the study subjects, haplotypic and nucleotide diversity were 0.469 ± 0.032 and 0.00189 ± 0.00020, respectively, and comparisons with other feeding areas presented significant values for the majority of FST and ΦST. The results point to the importance of this region and provide evidence that over the years a mixed stock has used the region as a feeding area. This variation could be related to sea currents, climatic changes, and oceanographic characteristics that may alter the availability of food, water temperature, and the presence of turtles. The current results can be considered in conservation plans for *Chelonia mydas*.

Keywords: Threatened species, foraging area, genetic diversity, mitochondrial DNA, mixed stocks.

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Introduction

The green turtle, *Chelonia mydas,* is affected by multiple threats, and thousands of juveniles are found stranded dead along the Brazilian coast annually (Monteiro *et al*., 2016; Tagliolatto *et al*., 2019; Cantor *et al*., 2020). However, although the species has circumglobal distribution and has been classified as 'endangered' by the IUCN (Seminoff, 2004), for the Southwestern Atlantic Ocean, including Brazilian waters, the management unit was recently updated to 'least concern' (IUCN, 2019). The IUCN assessment is based primarily on criteria focusing on genetic population stocks and adult abundance estimates at rookery sites, and juvenile mortality is not considered for classifying the risk assessment. Nevertheless, identifying the geographic origin of juveniles is crucial for understanding connectivity and for planning conservation strategies (Wildermann *et al*., 2018).

The green turtle exhibits migration and habitat use patterns that are associated with different ocean zones, depending on the development phase (stage of the animal's life cycle) and genetic origin. The species uses pelagic and oceanic areas during, approximately, its first 2 or 3 years of life, and then moves to demersal and neritic areas in its juvenile and adult phases (Musick and Limpus, 1997). Individuals from

different nesting areas congregate in feeding areas, and mixed genetic stocks are recorded in these specific areas. The stocks are identified because they feature specific mitochondrial DNA (mtDNA) haplotypes for each nesting site (Naro-Maciel *et al*., 2007; 2008; Shamblin *et al*., 2012); a pattern which is a consequence of the philopatric behavior described for males and females (Jesen *et al*., 2013).

Between 2-4 years of age, juvenile green turtles are recruited from oceanic to coastal zones, and different factors (e.g. ocean currents, genetic origin) influence migratory patterns (Naro-Maciel *et al*., 2014; Andrade *et al*., 2016; Coelho *et al*., 2018). Ontogenetic alterations, including morphological and ecological patterns (Gama *et al*., 2016; Coelho *et al*., 2018), are also associated with diversity in the genetic stocks (Naro-Maciel *et al*., 2016). Studies on the origin of these haplotypes, their presence and frequency in different feeding areas, and the potential new migration routes followed by individuals provide crucial information, both ecological and related to the biogeography of the species. These studies help in establishing and evaluating management and conservation plans aimed at maintaining the genetic and ecological diversity of species (Bowen and Karl, 2007).

Juveniles from different nesting sites are recorded using the Southern and Southeastern coasts of Brazil (Proietti *et al*., 2012; Jordão *et al*., 2015) for feeding and development (Almeida *et al*., 2011; Wallace *et al*., 2011; Andrade *et al*., 2016; Gama *et al*., 2016; Lenz *et al*., 2017). Off the Paraná coast, in Southern Brazil, juvenile green turtles occur annually in waters of the continental shelf, estuaries, and coastal

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islands (Guebert-Bartholo *et al*., 2011; Gama *et al*., 2016). This region is an important feeding area for juveniles, aggregating individuals aged 2–8 years (Andrade *et al*., 2016), migrating from multiple areas (Carman *et al*., 2012; Fuentes *et al*., 2020); however, the area presents high mortality rates and is an anthropogenically threatened *hotspot* (Cantor *et al*., 2020; Fuentes *et al*., 2020).

Anthropogenic activities interfere in the life cycle stages of sea turtles by causing loss of spawning and feeding areas, increasing interaction with fisheries and mortality, and also contributing to habitat degradation, through discarded pollutants and non-biodegradable waste. Therefore, the Brazilian national plan for sea turtle conservation highlights the importance of increasing scientific and management efforts in regions such as Southeastern and Southern Brazil, where the coastal zone is intensely used for activities that are potentially hazardous to sea turtles and their habitats (Sanches, 1999; Fuentes *et al*., 2020). Improvement in ecological and genetic knowledge will support conservation plans and measures for establishing active initiatives that reduce the threat of exposure and impacts on the species (Mazaris *et al*., 2009; López-Barrera *et al*., 2012). Particularly for the immature life stages of sea turtles, studies on population dynamics and survivorship can contribute information on threats and add support to conservation plans (Wilderman *et al*., 2018).

Evaluating the genetic diversity in feeding areas in Southern Brazil and the variations in different genetic contributions over time is fundamental to understanding and mapping which genetic stocks are most impacted. This knowledge is crucial to assess genetic variability, which includes the potential resilience of a species to continuous degradation of marine ecosystems. Therefore, the present study aimed primarily to characterize green turtles stranded on the Paraná coast from 2007 to 2014 by studying their mtDNA. The secondary objective of this study was to compare the diversity in this area with that recorded in other feeding areas in the Atlantic Ocean.

Material and Methods

Area of the study

The Paraná coast in Southern Brazil (north 25°13' S, 48°1' W; south 25°58' S, 48°35' W), comprises a remnant of Atlantic Forest and a UNESCO Natural World Heritage site (Lana *et al*., 2000). It is the transition zone between the tropical and temperate areas of the Southwestern Atlantic Ocean and is under the influence of the warm-water Brazil Current and cold-water Malvinas Current (Miranda *et al*., 2015). This area is composed of estuarine areas, bays, and a shallow continental shelf which are influenced by the El Niño and La Niña weather phenomena and strongly influenced by rainfall (Vanhoni and Mendonça, 2008).

Estuarine areas are crucial because of their ecological role and the Paraná Estuarine Complex is an important foraging and migratory area for juvenile sea turtles. However, the region presents intensive human activity (marine traffic,

ports, artisanal fisheries, urban areas, and industrial zones), exposing the species to multiple threats (Fuentes *et al*., 2020).

Sampling site

The individuals were found either stranded dead, debilitated, or accidentally captured in fishing nets. Samples of liver, epithelial or muscular tissues from juvenile green turtles were collected along the Paraná coast throughout the year from 2007 to 2014 (Figure 1).

The collected tissues were identified according to the animal's stage of decomposition, adapted from Geraci and Lounsbury (2005), and stored in 100% ethanol until sample preparation for molecular analysis. The curved carapace length (CCL, cm) was measured in all individuals with complete bodies (Wyneken, 2001).

Molecular techniques

Genomic DNA was extracted according to the phenol-chloroform protocol described by Almeida *et al*. (2001). The amplification of the control mtDNA region was performed using primers LCM15382 and H950 (Abreu-Grobois *et al*., 2006), with a final volume of 15 μL (1× PCR buffer, 0.2 mM of each dNTP, 1.5 mM $MgCl_2$, 0.5 μM of each primer, 3 U of Taq DNA polymerase [Invitrogen] and 5–50 ng of the DNA sample). The mixture was incubated at 94°C for 5 min; followed by 35 cycles of 40 s at 94°C, 30 s at 51°C, and 1 min at 72°C; and finally, a further 9 min at 72°C. The samples amplified successfully were read in an ABI PRISM 3500xL automatic sequencer (Applied Biosystems).

Data analysis

The quality of the sequences was checked using the Electropherogram Quality Analysis online software (Togawa and Brigido, 2003). The alignment and manual edition of sequences were performed on MEGA6 (Tamura *et al*., 2013). The haplotypes in this study were identified according to the Archie Carr Center for Sea Turtle Research database (available online from http://accstr.ufl.edu/genetics.html). Haplotype diversity (*h*), nucleotide diversity (π), and haplotype counts were calculated using DnaSP 5.1 software (Librado and Rozas, 2009).

For comparisons of genetic diversity with other feeding areas of the Southwestern Atlantic Ocean, previously published results were considered, and the 17 included feeding areas were divided into two groups: i) the North Atlantic group, comprising the areas of North Carolina/USA (NC, Bass *et al*., 2006), Florida/USA (FL, Bass and Witzell, 2000), Bahamas (BH, Lahanas *et al*., 1998), Nicaragua (NI, Bass *et al*., 1998), Barbados (BB, Luke *et al*., 2004), and Cape Verde (CV, Monzón-Argüello *et al*., 2010) and ii) the South Atlantic group, comprising the areas of Almofala/BR (AL, Naro-Maciel *et al*., 2007), Fernando de Noronha/BR (FN, Naro-Maciel *et al*., 2012), Rocas Atoll/BR (RA, Naro-Maciel *et al*., 2012), Bahia/BR (BA, Naro-Maciel *et al*., 2012), Espírito Santo/BR (ES, Naro-Maciel *et al*., 2012), Ubatuba/BR (UB, Naro-Maciel *et al*., 2007), Paraná/BR (PR, this study), Arvoredo Island/BR (AI, Proietti *et al*., 2012), Cassino Beach/BR
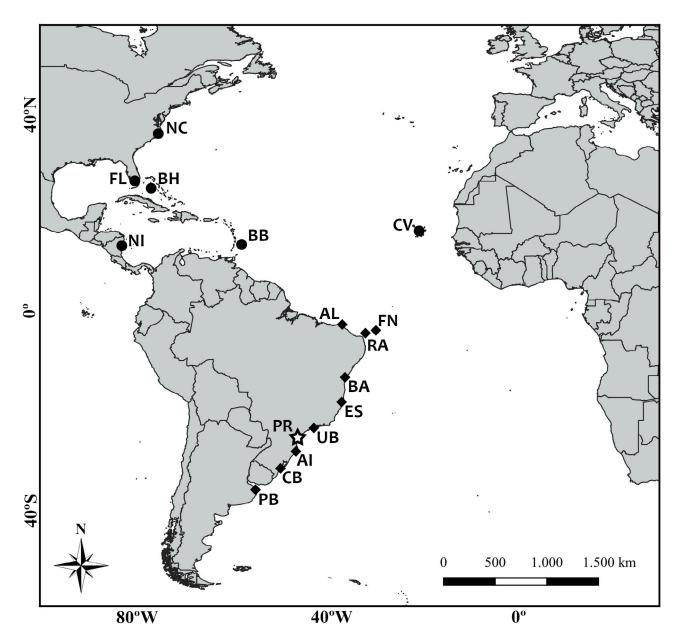
Figure 1 – Map of the sample location on the coast of Paraná (PR), Southern Brazil (symbolized by a star). The other feeding areas of the Atlantic Ocean, the North Atlantic group, including North Carolina/USA (NC), Florida/USA (FL), Bahamas (BH), Nicaragua (NI), Barbados (BB), and Cape Verde (CV) indicated by circles; and the South Atlantic group, including Almofala/BR (AL), Fernando de Noronha/BR (FN), Rocas Atoll/BR (RA), Bahia/BR (BA), Espírito Santo/BR (ES), Ubatuba/BR (UB), Arvoredo Island/BR (AI), Cassino Beach/BR (CB), and Buenos Aires Province/AR (PB) represented by lozenges.

(CB, Proietti *et al.*, 2012), and the Province of Buenos Aires/AR (PB, Prosdoscimi *et al.*, 2012).

The level of total genetic divergence among sub-populations, according to haplotype frequency (F_{ST}) and the Kimura 2P genetic distance parameters (Kimura, 1980) (Φ_{ST}), was calculated using Arlequin 3.5.2.2 software (Excoffier and Lischer, 2010). The same software was used to execute the AMOVA to estimate genetic variance partitioning between the stipulated populations and groups, with significance estimates of 5,000 permutations and $p \leq 0.05$. The AMOVA was executed considering only individuals from the Paraná

coast, separated by year of sample collection, using the same criteria for the estimates.

Results

In the sampling period, approximately 1500 sea turtles were recorded along 40 km of the Paraná coast and estuarine areas according to researchers at Laboratório de Ecologia e Conservação (CEM-UFPR). Tissue samples for genetic analysis were collected from 436 green turtles and DNA samples were sequenced in 285 individuals (~65%). From the mtDNA samples analyzed, 12 haplotypes were defined according

to a 485pb region, and 11 of these haplotypes presented 14 polymorphic sites between them, whereas one haplotype (CMA42) had a 4pb insertion/deletion (indel).

Haplotype CMA8 was the most frequently observed haplotype, identified in 195 individuals (69%), followed by CMA5 in 52 individuals (18%). The other 10 haplotypes occurred with a frequency of less than 5%, with only haplotype CMA8 being identified in all years (Table 1).

The CCLs of the analyzed individuals ranged from 27.5 to 47.6 cm (mean±SD = 39.95 ± 7.32 cm), indicating that all individuals are classified as juveniles (<65 cm CCL) (Bressett *et al*., 2010). No variation was observed among the mean CCLs over the years ($p = 0.809$), and no differences were detected among the mean CCLs of individuals identified as having different haplotypes ($p = 0.114$).

However, it was observed that the diversity of haplotypes is affected by the number of samples collected in the same year; 2012 (n = 74) had the greatest number of samples, with seven (7) identified haplotypes, whereas 2007 (n = 4) had the smallest number of samples, with only two (2) identified haplotypes (Table 1). On the other hand, even though the year 2008 had few samples, haplotypes CMA23 and CMA32 were sampled only in that year. The AMOVA revealed that the difference between sampling years was 3.44% and within years was 96.56%. Haplotype (h) and nucleotide (π) diversities were 0.469 ± 0.032 and 0.00189 ± 0.00020, respectively (Table 2).

Table 1 – Number of juveniles green turtle from each haplotype in each sampling year (N), with the CCLs (cm) or mean CCLs (cm) (±SD); number of haplotypes per year (Nº hap); haplotype diversity (h) and nucleotide diversity (π).

	2007	2008	2009	2010	2011	2012	2013	2014	Total
CMA3			1 (49.5)			1 (35.2)			2
CMA5		7 (35.7 ± 8.1)	8 (36.9 ± 2.7)	7 (34.5 ± 1.5)	5 (44.9 ± 12.2)	18 (35.2 ± 3.1)	2 (31.1 ± 5.0)	5 (36.1 ± 5.8)	52
CMA6				1 (37.5)	2 (37.5 ± 4.9)	2 (37.1 ± 3.4)	2 (36.8 ± 1.1)		7
CMA8	3 (42.5 ± 11.1)	8 (40.3 ± 7.6)	24 (38.5 ± 6.0)	11 (37.4 ± 4.4)	32 (41.3 ± 8.4)	46 (42.4 ± 10.7)	25 (40.0 ± 4.4)	46 (41.3 ± 4.8)	195
CMA9		1 (40.5)				1 (39.3)	2 (46 ± 2.4)	2 (43.4 ± 0.2)	6
CMA10	1 (47.0)							4 (41.9 ± 3.9)	5
CMA23		1 (45.5)							1
CMA24			1 (37.5)	1 (38.0)			1 (55.3)	1 (55.5)	4
CMA32		1 (37.0)							1
CMA39					1 (45.0)	2 (41.3 ± 2.5)			3
CMA42						4 (42.4 ± 6.9)	1 (31.8)	1 (40.2)	6
CMA46					1 (38.5)		1 (32.3)	1 (43.0)	3
N	4	18	34	20	41	74	34	60	285
Nº hap	2	5	4	4	5	7	7	7	
h	0.500 (0.265)	0.680 (0.074)	0.458 (0.083)	0.600 (0.077)	0.382 (0.091)	0.489 (0.055)	0.415 (0.104)	0.380 (0.077)	0.469 (0.032)
π	0.00104 (0.00055)	0.00279 (0.00036)	0.00264 (0.00094)	0.00225 (0.00029)	0.00132 (0.00033)	0.00227 (0.00047)	0.00116 (0.00034)	0.00118 (0.00028)	0.00189 (0.00020)

Table 2 – Number of green turtles per haplotype, haplotype diversity (*h*) and nucleotide diversity (π) of the mtDNA control region of green sea turtles present in different feeding areas in the Atlantic Ocean. NC = North Carolina/USA; FL = Florida/USA; BH = Bahamas; NI = Nicaragua; BB = Barbados; CV = Cape Verde; AL = Almofala; FN = Fernando de Noronha/BR; RA = Rocas Atoll/BR; BA = Bahia/BR; ES = Espírito Santo/BR; UB = Ubatuba/BR; PR = Paraná/BR; AI = Arvoredo Island/BR; CB = Cassino Beach/BR; PB = Province of Buenos Aires/AR; N = Number of samples; Nº hap = Number of haplotypes; *h* = Haplotype diversity; π = Nucleotide diversity. Standard deviations are presented in parentheses.

	North Atlantic							South Atlantic										
	NC	FL	BH	NI	BB	CV	Total	AL	FN	RA	BA	ES	UB	PR	AI	CB	PB	Total
CMA1	34	12	2		7	1	56		2	1								3
CMA2	2	1					3											
CMA3	43	43	62	54	21	2	225	18	4	8		2	2	2	1			37
CMA5	5	3	10	6	13	23	60	28	52	21	14	47	14	52	25	20	20	293
CMA6						1	1	3	4	2	2	6		7	2	2	2	30
CMA8	7		1		14	17	39	53	46	36	23	87	83	195	70	62	59	714
CMA9					1		1	3		7	3	6	4	6	5	3	5	42
CMA10					2		2	4	4	3		3	3	5	2	1	1	26
CMA15	1						1											
CMA16	2						2	1										1
CMA17					1		1		1									1
CMA18	3	2					5											
CMA20			1				1											
CMA21			3		1		4	1										1
CMA22	2	1					3											
CMA23								1			2	2		1	3	2		11
CMA24									2		1	2	2	4	3	2	1	17
CMA25																1		1
CMA26	2						2											
CMA27	2						2											
CMA28	3						3											
CMA32								1	1			2	2	1	1	3	2	13
CMA36																2		2
CMA39														3	1		1	5
CMA42								2	1					6	1	1	2	13
CMA44								1					1					2
CMA45								1							1	2		4
CMA46													1	3				4
CMA55													1					1
N	106	62	79	60	60	44		117	117	78	45	157	113	285	115	101	93	
Nº hap	12	6	6	2	8	5		13	11	7	6	9	10	12	12	12	9	
h	0.729	0.485	0.370	0.183	0.773	0.588		0.701	0.642	0.703	0.647	0.603	0.446	0.469	0.572	0.561	0.525	
	(0.030)	(0.067)	(0.065)	(0.062)	(0.028)	(0.045)		(0.032)	(0.027)	(0.037)	(0.053)	(0.00095)	(0.056)	(0.032)	(0.045)	(0.051)	(0.052)	
π	0.00530	0.00331	0.00642	0.00381	0.01027	0.00419		0.00655	0.00415	0.00551	0.00242	0.00262	0.00205	0.00189	0.00234	0.00199	0.00193	
	(0.00078)	(0.00103)	(0.00113)	(0.00129)	(0.00036)	(0.00107)		(0.00073)	(0.00027)	(0.00086)	(0.00023)	(0.00030)	(0.00044)	(0.00020)	(0.00033)	(0.00021)	(0.00021)	

Concerning haplotype occurrence in the 17 Southwestern Atlantic Ocean feeding areas and their classification into two major groups (North and South Atlantic), the AMOVA results indicated that the greatest difference is actually between the groups, whereas the smallest difference is between feeding areas within the groups (Table 3). Comparing all the feeding areas, 81 of 120 F_{ST} comparisons and 73 of 120 Φ_{ST} comparisons were statistically significant, considering $p \leq 0.05$ (Table 4). Particularly for the Paraná coast feeding area, F_{ST} comparisons presented non-significant values for BA, ES, UB, AI, CB, and PB, whereas Φ_{ST} presented non-significant values for FN, RA, BA, ES, UB, AI, CB, and PB (Table 4).

Table 3 – AMOVA values according to the feeding areas described for green sea turtles in the Atlantic Ocean, considering differences between the groups (North and South Atlantic), between feeding areas within the groups and between feeding areas overall.

AMOVA	
Between groups	64.53
Between feeding areas within the groups	7.97
Between individuals within the feeding areas	27.47

Discussion

A total of 12 haplotypes were identified among the 285 juvenile green turtles sampled along the Paraná coast, Southern Brazil. A predominance of the haplotype CMA8, concerning both number of individuals and continuous presence over the years, reinforces the genetic characteristics recorded in different regions over the Southwestern Atlantic Ocean. The number of haplotypes and the genetic diversity values found for the individuals sampled between 2007 and 2014 highlight the relevance of this region as a feeding and development ground for multiple genetic stocks of juvenile green turtles from the South Atlantic Ocean.

Considering the individuals from the Paraná coast by sampling year, the second most frequent haplotype (CM-A5) was observed for all years except 2007, and the other 10 haplotypes occurred with a relatively low frequency ($\leq$ 5%). Three haplotypes occurred more frequently on the Paraná coast than in the other previously described Atlantic feeding areas; haplotype CMA42 was identified in six individuals, whereas haplotypes CMA39 and CMA46 were identified only in Brazil, once in Arvoredo Island (Santa Catarina state) and once in Ubatuba (São Paulo state), respectively (Naro-Maciel *et al*., 2007; Proietti *et al*., 2012). These differences in haplotype occurrence may be associated with biogeographic and migratory behavior, affected by climate and oceanographic conditions. The Paraná coast is humid mesothermal (subtropical) and is influenced by polar air masses in winter and by tropical Atlantic masses in summer (Vanhoni and Mendonça, 2008). These variations, including the food availability and coastal water mass and currents, might influence the presence of animals from different reproductive stocks in feeding areas (Proietti *et al*., 2009; Prosdocimi *et al*., 2012).

The haplotypes analyzed in this study are identified mainly from nesting areas in the South Atlantic (coast of Brazil and Africa) (Formia *et al*., 2006; Naro-Maciel *et al*., 2007; Proietti *et al*., 2009; Proietti *et al*., 2012) and only three of them (CM-A3, CM-A5 and CM-A6) are described for nesting areas in the North Atlantic and the Caribbean (Encalada *et al*., 1996; Naro-Maciel *et al*., 2007; Proietti *et al*., 2009). Future studies addressing biogeographic issues should correlate genetic diversity and oceanographic and climatic factors. This approach would make an important contribution to understanding the potential effects of climate change on mixed stocks using specific foraging grounds.

Overall, 10 haplotypes were identified with low frequency in the present study; this finding is consistent with other studies conducted along the Brazilian coast (Naro-Maciel *et al*., 2007; Naro-Maciel *et al*., 2012; Proietti *et al*., 2012; Jordão *et al*., 2015). Considering *h* and π values, this study presents results similar to those presented for other feeding areas across the whole Atlantic Ocean (Bass *et al*., 1998; Lahanas *et al*., 1998; Bass and Witzell, 2000; Luke *et al*., 2004; Bass *et al*., 2006; Naro-Maciel *et al*., 2007, 2012; Monzón-Argüello *et al*., 2010; Proietti *et al*., 2012; Prosdoscimi *et al*., 2012). Comparing the results of the present study with those of a previous study conducted on the Paraná coast between 2005 and 2008 (Jordão *et al*., 2015), the diversity of haplotypes increased: six haplotypes were identified in both studies, six are new for this region, and only one was not sampled again (CM-A1). These results reinforce the importance of this feeding area for multiple stocks of green turtles, and also that maintaining management and conservation actions is crucial to guarantee the genetic variability along the Southwestern Atlantic Ocean.

The difference between the North and South Atlantic groups (AMOVA between groups of 64.56%) may be associated with the geographical distances between the feeding areas and the influence of sea currents; the relatively warm currents that flow off the Brazilian coast (Equatorial Atlantic and Central South Atlantic) form a direct front against the cold-water Malvinas Current (Tomczak and Godfrey, 2003). These different currents interfere with the presence of individuals in the feeding areas because, reportedly, individuals remain on the coast of Argentina and Uruguay during summer and autumn and migrate to Southern Brazil where waters are warmer in winter (Carman *et al*., 2012). The values found for genetic differences could also result from colonization events that influence the distribution of animals among nesting areas and the location of the feeding areas (Bass *et al*., 2006).

In studies on South Atlantic feeding areas, non-significant values were observed when comparing the data with other areas geographically close to the Paraná coast, which was expected because these regions have similar conditions in terms of the influence of sea currents and other environmental factors. Similar results were observed for feeding areas of the Province of Buenos Aires on the coast of Argentina, which presented significant differences when compared with the five feeding areas in the Northeastern Atlantic, but also with one northern area in the Southwestern Atlantic Ocean (Prosdoscimi *et al*., 2012). Moreover, on the Brazilian coast, two feeding areas in Southern Brazil (Rio Grande do Sul state) exhibited genetic differences when compared with the North Atlantic areas, but no differences from Ubatuba and Rocas Atoll in Southeastern and Northeastern Brazil (Proietti *et al*., 2012). The composition of feeding areas might depend on the movement of individuals

Table 4 – F_{ST} according to haplotype frequency and Φ_{ST} using the Kimura 2P genetic distance parameters between the feeding areas described for green sea turtles in the Atlantic Ocean. F_{ST} is in the lower part of the table and Φ_{ST} in the upper part. Values in boldface and marked with an asterisk (*) indicate significant values ($p \leq 0.05$). NC = North Carolina; FL = Florida; BH = Bahamas; NI = Nicaragua; BB = Barbados; CV = Cape Verde; AL = Almofala; FN = Fernando de Noronha; RA = Rocas Atoll; BA = Bahia; ES = Espírito Santo; UB = Ubatuba; PR = Paraná; AI = Arvoredo Island; CB = Cassino Beach; PB = Province of Buenos Aires.

	NC	FL	BH	NI	BB	CV	AL	FN	RA	BA	ES	UB	PR	AI	CB	PB
NC	-	0.04832	-0.00590	0.03620	**0.23020***	**0.73514***	**0.57064***	**0.73953***	**0.64395***	**0.76481***	**0.79865***	**0.77715***	**0.83629***	**0.78544***	**0.78754***	**0.77191***
FL	**0.06507***	-	0.02679	-0.00617	**0.35212***	**0.81922***	**0.66072***	**0.81375***	**0.73165***	**0.86415***	**0.87251***	**0.86993***	**0.90090***	**0.87438***	**0.88456***	**0.86395***
BH	**0.16787***	0.03877	-	0.00809	**0.20367***	**0.67557***	**0.52953***	**0.70266***	**0.59373***	**0.70080***	**0.76703***	**0.73244***	**0.81499***	**0.74232***	**0.73876***	**0.72378***
NI	**0.24401***	**0.09810***	0.01343	-	**0.30436***	**0.76501***	**0.61871***	**0.77808***	**0.68374***	**0.80259***	**0.83977***	**0.82430***	**0.87639***	**0.83088***	**0.83545***	**0.81623***
BB	**0.05503***	**0.13103***	**0.18186***	**0.27902***	-	**0.32753***	**0.14645***	**0.37235***	**0.21585***	**0.33626***	**0.45238***	**0.36539***	**0.51753***	**0.38581***	**0.36448***	**0.36292***
CV	**0.27769***	**0.43270***	**0.48096***	**0.59643***	**0.12231***	-	**0.09570***	-0.01396	**0.05324***	-0.03035	0.00683	**0.10847***	**0.07009***	0.01205	-0.02966	0.03239
AL	**0.19327***	**0.30512***	**0.34087***	**0.41797***	**0.05419***	**0.05428***	-	**0.09228***	0.00343	**0.08005***	**0.11288***	**0.05175***	**0.10606***	**0.05942***	0.03622	**0.06051***
FN	**0.26233***	**0.39279***	**0.42752***	**0.50732***	**0.11051***	-0.00940	0.03520	-	0.04527	-0.01836	0.00637	**0.07376***	0.04556	0.00570	-0.02287	0.02126
RA	**0.21361***	**0.34459***	**0.39034***	**0.47796***	**0.07083***	0.04290	-0.00464	0.02490	-	0.01993	0.04248	-0.00866	0.01138	-0.01232	-0.04492	-0.00304
BA	**0.27135***	**0.43090***	**0.48707***	**0.59264***	**0.12351***	0.03327	0.01187	0.01563	-0.00308	-	-0.01482	**0.09478***	0.03998	0.01252	0.00529	0.01732
ES	**0.29830***	**0.43096***	**0.46541***	**0.53585***	**0.14773***	**0.05119***	0.01908	0.03136	0.00717	-0.01064	-	**0.05569***	0.01473	-0.00353	-0.02433	0.00538
UB	**0.37569***	**0.52883***	**0.57126***	**0.64876***	**0.24937***	**0.21737***	0.08079	**0.16262***	**0.07996***	**0.07014***	**0.05047***	-	0.01306	0.02195	0.01856	0.01225
PR	**0.37084***	**0.49934***	**0.52877***	**0.58776***	**0.23065***	**0.16037***	**0.06056***	**0.12100***	**0.05546***	0.03577	0.02429	0.00199	-	-0.00061	-0.00974	-0.00406
AI	**0.30735***	**0.44978***	**0.49191***	**0.56738***	**0.16606***	**0.10014***	0.02736	**0.06917***	0.01869	0.00168	0.00152	0.01657	0.00203	-	-0.01648	-0.00978
CB	**0.30733***	**0.45348***	**0.49871***	**0.57670***	**0.16984***	**0.10955***	0.03076	**0.07657***	0.02421	0.00696	0.00514	0.01275	0.00060	-0.00770	-	-0.02359
PB	**0.32169***	**0.47217***	**0.51756***	**0.59893***	**0.18197***	**0.11329***	0.03551	**0.08002***	0.02583	0.00778	0.00471	0.01146	-0.00170	-0.00749	-0.00759	-

between feeding areas, the abundance of nesting sites, the distances between the nesting and feeding areas, philopatry, and sea currents (Prosdoscimi *et al.*, 2012).

According to Wright's scale (1978), F_{ST} and Φ_{ST} values are significant when considering the comparisons between the Paraná coast and North Atlantic areas, and the comparisons with genetic difference values are either high or very high. Comparing the Paraná coast with other South Atlantic areas, F_{ST} had significant values for AL, FN, and RA, and Φ_{ST} had significant values for AL, but genetic difference values were either moderate or small. The genetic difference values obtained between the Paraná coast and other regions could be the result of oceanographic parameters, considering that the sea currents may favor individuals following the direction of the south coast of Brazil or move them away from Brazil with other predominant currents (Naro-Maciel *et al.*, 2007). Phylogeographic analysis in nesting grounds of green turtles distinguished two haplotype groups (Northwestern and Southwestern), and the pattern of distribution is non-random among feeding areas and could be influenced by ocean currents (Prosdocimi *et al.*, 2012).

Concerning the samples in the present study according to the sampling year, on average, five haplotypes were identified per year, but the occurrence of haplotype CMA23 was novel. This haplotype is exclusively described for South Atlantic nesting areas (Ascension Island and Rocas Atoll/Fernando de Noronha) (Proietti *et al.*, 2012). The occurrence of this haplotype brings new information on this feeding area and for the entire Regional Management Unit (RMU). By updating information about the species in the RMU, it is possible to improve the protection of the nesting populations, since this considers the overlapping of areas of nesting and feeding, regarding reproduction and threats (Wallace *et al.*, 2011). The identification of the occurrence of *Chelonia mydas* haplotypes in the foraging areas enables identification of the presence of mixed stocks, which makes the foraging areas important in terms of conservation, since their preservation ensures that a suitable place is maintained for the occurrence of individuals from different nesting areas. This survey can be considered in the elaboration of plans for monitoring water quality, food availability, vessel traffic, installations of new projects, and other modifications that may change the conditions of the Paraná coast in the future.

Collectively, the results of the included samples from 2007 to 2014 along the Paraná coast, support the use of this specific foraging ground by juvenile *Chelonia mydas* from multiple origins, and evidence its considerable genetic variability. The study also highlights the presence of different haplotypes in different years and the importance of developing systematic and continuous monitoring of feeding areas to understand connectivity and migratory patterns. This variation over the years may be related to the sea currents, climatic issues, or other oceanographic characteristics; these and other factors should also be investigated more carefully in the future. Furthermore, future studies should include analysis using different molecular markers to improve knowledge on turtle origins and biogeography. In general, the results contribute crucial information to improve management and conservation plans for *Chelonia mydas* and its habitats.

Conflict of Interest

The authors have no conflicts of interest to declare.

Author Contributions

FSA and CSS conceived and designed the study; CD collected the samples; CSS performed the analysis; CSS, LP, CD, and FSA wrote the manuscript and approved the final version.

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