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Abstract. – Genetic markers are often used to designate population units for management and conservation, but widespread sharing of mitochondrial DNA control-region haplotypes defined from short (< 500 base-pair [bp]) sequences often limits inferences of population connectivity in marine turtles. Haplotype CM-A8, defined from 490-bp sequences, dominated the haplotype profiles of the 3 major green turtle (*Chelonia mydas*) rookeries in Brazil. Previous analyses based on 490-bp haplotypes did not detect differentiation between the northern rookeries of Atol das Rocas and Fernando de Norohna, but did indicate differentiation of the northern rookeries from Trindade Island in the south. We reexamined the stock structure of the Brazilian green turtle rookeries using 817-bp control region and mitochondrial short tandem repeat (mtSTR) sequences. Nine 490-bp haplotypes were subdivided into 41 haplotypes by combining 817-bp and mtSTR sequences. Eight of the 14 CM-A8 turtles from Fernando de Noronha carried mtSTR haplotypes that were not detected in the larger rookeries. Pairwise exact tests indicated that the northern Brazilian green turtle rookeries of the Rocas Atoll and Fernando de Noronha are discrete populations with respect to female natal homing. Moreover, several apparently endemic markers in the 3 Brazilian green turtle nesting populations should improve resolution of future mixed-stock analyses. Comparable data are needed from green turtle rookeries in the central and eastern Atlantic to assess structure and connectivity at the ocean basin scale.

Key Words. – mtSTR; mtVNTR; mitochondrial repeat; stock structure; *Chelonia mydas*

Defining population units is a fundamental aspect of spatial ecology and wildlife conservation but can be hindered by the dispersal capability of many marine organisms. In some highly migratory species, such as cetaceans, marine turtles, and some sharks, natal philopatry to breeding sites can be used to define population boundaries for monitoring and management purposes (Hoelzel 1998; Hueter et al. 2005; Jensen et al. 2013). In marine turtles, the scale of this natal neighborhood may vary across species and even among different populations within a species (Bowen and Karl 2007). Characterizing the site specificity of natal homing is important for contextualizing trend data, understanding demographic processes, and ensuring adequate protection for breeding populations that are discrete with respect to female recruitment. Another fundamental aspect of conservation biology is knowledge of the spatial ecology of individuals representing discrete populations throughout their life cycle, which involves extensive dispersal, developmental and seasonal migrations, and ontogenetic shifts in marine turtles (Bolten 2003).

Resolving population boundaries and migratory connectivity have been highlighted as critical research priorities for marine turtles globally (Hamann et al. 2010). Genetic tools have proven invaluable in defining population structure and exploring the migratory connectivity of breeding and nonbreeding habitats for marine turtles (Jensen et al. 2013). However, despite these seminal contributions from genetic studies in resolving marine turtle natural history, poor marker resolution has limited inference of demographic and migratory connectivity at the regional scale in some cases (Formia et al. 2006). Widespread sharing of haplotypes defined by short control-region sequences is a common pattern in all

marine turtle species with cosmopolitan distributions (Bowen and Karl 2007). For example, green turtle (*Chelonia mydas*) haplotype CM-A8 (defined from 490 base-pair [bp] sequences) accounted for 65%–100% of sampled females from rookeries spanning the South Atlantic from Brazil to western Africa (Encalada et al. 1996; Bjorndal et al. 2006; Formia et al. 2006, 2007) as well as Europa in the Southwest Indian Ocean (Bourjea et al. 2007). Determining whether this haplotype sharing represents historical genetic signature or contemporary connectivity is important for informing management and clarifying demographic processes.

The majority of green turtle nesting in the Southwest Atlantic occurs on 3 Brazilian oceanic islands (Fig. 1). Trindade Island (TI), 1200 km off the coast of Espírito Santo, hosts the largest rookery of approximately 1000 females annually (Almeida et al. 2011). The Rocas Atoll (RA) rookery off the coast of Rio Grande do Norte in northeastern Brazil is approximately an order of magnitude smaller than TI (Bellini et al. 2012). The Fernando de Noronha Archipelago (FN), which lies 150 km east of the Rocas Atoll, hosts the smallest remnant nesting population of $<$ 100 nesting females; this population was nearly extirpated by heavy human exploitation prior to legal protection (Bellini and Sanches 1996). Haplotype CM-A8 accounted for 68%, 68%, and 88% of the females sampled at the 3 rookeries, respectively. Analysis based on 490-bp haplotypes indicated differentiation between TI and the northern rookeries but did not detect structure between RA and FN (Bjorndal et al. 2006). Although both RA and FN are United Nations Educational, Scientific and Cultural Organization World Heritage Sites and protected nesting habitats, the primary foraging sites for juveniles and nonbreeding adults from these nesting populations are not known. Fishery bycatch in artisanal gillnets represents a significant anthropogenic threat facing this species in Brazil (López-Barrera et al. 2012). Moreover, marine turtle harvest is still permitted in some territories in the Greater Caribbean region (Humber et al. 2014), so potential threats are not likely to be evenly distributed across foraging habitats in the western Atlantic. Therefore, determining whether RA and FN are discrete nesting populations with respect to female recruitment, and better resolving the foraging distribution of individuals representing these rookeries, are important for contextualizing monitoring and recovery.

Additional genetic markers have increased inference of stock structure in cases of widespread haplotype sharing. In Atlantic leatherback turtles, rookeries that were not differentiated based on mtDNA were distinguished using microsatellites (Dutton et al. 2013). However, sex-biased dispersal and/or migration-mediated gene flow that occurs during periods of population admixture have limited the utility of nuclear markers in defining fine-scale female recruitment patterns in some cases (Bowen and Karl 2007). Microsatellite-based pairwise comparisons indicated significant differentiation among the major western Atlantic green turtle rookeries, including TI and RA (Naro-Maciel et al. 2014). However Bayesian clustering analysis indicated a primary bifurcation between South Atlantic and Caribbean rookeries (Naro-Maciel et al. 2014), suggesting weak structure within each region and limited resolution for assignment tests at fine scales. Deeper sequencing of the mitogenome has revealed population informative variation in green turtles and other marine turtle species (LeRoux et al. 2012; Shamblin et al. 2012, 2014; Dutton et al. 2014). A ubiquitous 490-bp control-region haplotype in Mediterranean green turtles was subdivided into 33 different haplotypes through sequencing of a mitochondrial short tandem repeat (mtSTR) array (Tikochinski et al. 2012). Several haplotypes present among individuals stranded along the Israeli coast were absent in the Israeli rookery, implying that structure occurred among rookeries comprising the Mediterranean green turtle aggregation (Tikochinski et al. 2012). In the present study, we applied the mtSTR marker along with expanded control-region sequences to reassess the genetic diversity and structure in Brazil's green turtle rookeries. The primary objectives were to 1) determine whether RA and FN represent discrete populations with respect to female natal homing, and 2) test whether informative variation occurs among rookeries that could improve resolution of future mixed stock analyses (MSA).

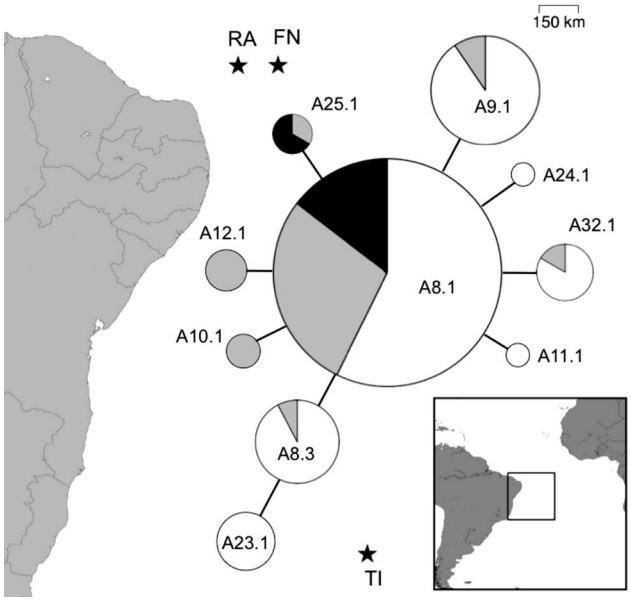

Figure 1. Minimum spanning network depicting control-region haplotypes. Area of the circles is proportional to the number of individuals detected with each haplotype. Stars indicate locations of the 3 major Brazilian green turtle rookeries: TI, Trindade Island (white); RA, Rocas Atoll (gray); FN, Fernando de Noronha (black).

METHODS

We reanalyzed rookery samples previously characterized for 490-bp control-region haplotypes representing

the 3 primary green turtle nesting sites in Brazil: TI ($n = 99$), RA ($n = 37$), and FN ($n = 16$; Bjorndal et al. 2006). We used primers LCM15382 and H950 to amplify an 817-bp fragment of the mitochondrial control region (Abreu-Grobois et al. 2006). PCR reactions were carried out in 20-µl volumes containing 10 mM Tris, pH 8.4; 50 mM KCl, 0.5 µM of each primer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 U of *Taq* DNA polymerase, and approximately 5 ng of genomic DNA. PCR cycling parameters were as follows: 95°C for 3 min; 35 cycles of 95°C for 30 sec, 55°C for 60 sec, 72°C for 30 sec; and a final extension of 72°C for 10 min. PCR products were purified by adding 2 µl of ExoSAP-IT® (USB Corporation) to 7 µl of the PCR reaction and incubated according to the manufacturer's instructions. The mtDNA amplicons were sequenced using ABI BigDye v3.1 (PE Applied Biosystems) with LCM15382 and an internal primer (Cm1820, TCACGAGAATAAGCAAC). We amplified the mtSTR array and sequenced in both directions as previously described (Tikochinski et al. 2012), except that the forward PCR primer was also used as the forward sequencing primer. Negative controls were included in each batch of PCR amplifications and sequencing reactions to detect contamination.

We aligned, edited, and compared control region sequences with previously described haplotypes using the program Sequencher 4.2 (Gene Codes Corporation). Sequences were assigned haplotype designations after nomenclature published on the Archie Carr Center for Sea Turtle Research Web site (http://accstr.ufl.edu/resources/mtdna-sequences/). We scored mtSTR haplotypes by counting the number of "AT" repeats in each of the 4 loci of the array separated by point mutations, such that each individual was represented by a 4-digit numeral code (e.g., 7-12-4-4). In cases of heteroplasmy, the individual was assigned the dominant haplotype based on relative peak heights, as previously described (Tikochinski et al. 2012). The repeatability and robustness of this marker was established through biological and technical replicates in Mediterranean green turtles (Tikochinski et al. 2012). Samples producing novel or ambiguous sequences were subjected to additional rounds of PCR amplification and sequencing as necessary for verification.

We tested for the presence of population structure using genetic distance-based pairwise Φ_{ST} comparisons, pairwise exact tests of population differentiation, and analysis of molecular variance (AMOVA) as implemented in Arlequin version 3.5 (Excoffier and Lischer 2010). The 10-bp indel in the control region and each stepwise change in "AT" repeat-unit number were treated as single mutation events. Because fixation indices were expected to actually decrease through incorporation of the mtSTR data because of increased polymorpshim, we examined overall population structure as well as structure defined only among CM-A8 mtSTR variants to gauge whether these markers actually increased stock resolution. Haplotype diversity was estimated based on Nei (1987). Significance values for AMOVA were obtained from 10,000 permutations. Exact tests of population differentiation were conducted with 100,000 permutations and 10,000 dememorization steps (Raymond and Rousset 1995). *p*-values were corrected for multiple tests using a false discovery rate approach (Benjamini and Yekutieli 2001).

RESULTS

Comparisons between newly generated 817-bp sequences and original 490-bp haplotype data indicated a discrepancy for one sample originally assigned as novel haplotype CM-A33. Duplicate iterations of PCR and sequencing indicated that this individual was actually carrying haplotype CM-A32 (Supplemental Table 1; see supplementary material online at http://dx.doi.org/10.2744/CCB-1152.1.s.1). Alignments of the expanded control-region sequence revealed a novel single nucleotide polymorphism (SNP) that subdivided CM-A8 into two variants, CM-A8.1 and CM-A8.3 (Supplemental Table 2). CM-A8.3 accounted for 18% of CM-A8 TI females but was only detected in a single individual in RA (4% of CM-A8) and was absent in the FN sample. No polymorphism was detected within any of the remaining 490-bp control-region haplotypes, but all CM-A23.1 individuals shared the derived CM-A8.3 transition (Fig. 1; Supplemental Table 2).

Twenty-one mtSTR haplotypes were detected without consideration of control-region SNPs, all but one of which were present among CM-A8 individuals (Table 1). The combination of control-region SNPs and mtSTR variants defined 41 haplotypes, with CM-A8 variants accounting for 24 of these (Table 1). Haplotype diversity defined by the expanded control-region fragment and mtSTR loci was markedly higher than that of 490-bp haplotypes (Table 1). FN had the lowest haplotype diversity when only 490-bp haplotypes were analyzed (0.233) but had the highest value when complete data were considered (0.950).

Among the 24 CM-A8 haplotypes, 7 were shared between ≥ 2 rookeries, with 4 of these present in all 3 rookeries. Two variants shared among the 3 rookeries represented the most common haplotypes present in the Brazilian nesting aggregation, accounting for 47% of CM-A8 individuals. Despite this persistence of haplotype sharing with the novel sequences, the remaining 17 haplotypes were unique, with 9, 3, and 5 detected exclusively in TI, RA, and FN, respectively (Table 1). Of 9 mtSTR haplotypes detected in FN CM-A8 females, the 5 unique haplotypes accounted for 57% of sampled individuals.

When only CM-A8 variants were considered, neither genetic-distance-based AMOVA ($\Phi_{ST} = 0.0228$, $p = 0.0782$) nor pairwise Φ_{ST} comparisons indicated differentiation (Table 2). However, frequency-based AMOVA indicated weak structure ($F_{ST} = 0.0238$, $p = 0.0498$), and the RA–FN and TI–FN comparisons were significantly

Table 1. Distribution of mitochondrial control region (CR) and mtSTR haplotypes (repeat) in Brazilian green turtle rookeries. TI = Trindade Island; RA = Rocas Atoll; FN = Fernando de Noronha; HD = haplotype diversity; SE = standard error; bp = base pair.

CR	Repeat	TI	RA	FN
A8.1	6-12-4-4	2		
A8.1	6-13-4-4	6		
A8.1	6-16-4-4		1	
A8.1	7-11-4-4	9	5	1
A8.1	7-11-4-5			2
A8.1	7-12-4-4	22	12	2
A8.1	7-12-4-5			2
A8.1	7-12-5-4			1
A8.1	7-13-4-4	1	3	
A8.1	7-14-4-4	1	1	
A8.1	7-15-4-4	1	1	1
A8.1	7-16-4-4	5	1	2
A8.1	7-17-4-4	3		
A8.1	8-9-4-4	1		
A8.1	8-10-4-4	3		
A8.1	8-12-4-4			1
A8.1	8-13-4-4		2	
A8.1	8-14-4-4		1	
A8.1	8-15-4-4	1		
A8.1	8-16-4-4			2
A8.3	7-11-4-4	3	1	
A8.3	7-11-5-4	1		
A8.3	7-12-4-4	7		
A8.3	7-16-4-4	1		
A9.1	6-12-4-4	3		
A9.1	6-13-4-4	1		
A9.1	7-11-4-4	3		
A9.1	7-12-4-4	11	1	
A9.1	7-13-4-4	1		
A9.1	8-12-4-4		1	
A10.1	7-12-4-4		2	
A11.1	7-17-4-4	1		
A12.1	7-12-4-4		3	
A23.1	7-12-4-4	6		
A24.1	7-13-4-4	1		
A25.1	7-12-4-4		1	2
A32.1	6-12-4-4	1		
A32.1	6-13-4-4		1	
A32.1	7-10-4-4	1		
A32.1	7-11-4-4	2		
A32.1	7-12-4-4	1		
n		99	37	16
HD	490 bp	0.504	0.425	0.233
HD SE		0.052	0.099	0.126
HD	817 bp	0.918	0.874	0.950
HD SE	+ mtSTR	0.016	0.043	0.031

different (Table 2). When all haplotypes were considered, structure was more evident among the 3 rookeries (AMOVA $\Phi_{ST} = 0.0331$, $p = 0.0088$; AMOVA $F_{ST} = 0.0200$, $p = 0.0147$). Pairwise exact tests indicated significant structure among all 3 rookeries (Table 2).

Table 2. Pairwise comparisons for Brazilian green turtle rookeries. Comparisons based only on CM-A8 variants are above the diagonal. Comparisons based on all haplotypes are below the diagonal. Pairwise Φ_{ST} values are without parentheses. Values in parentheses are *p*-values for pairwise exact tests. * indicates significant values after false discovery rate correction for multiple tests. TI = Trindade Island; RA = Rocas Atoll; FN = Fernando de Noronha.

	TI	RA	FN
TI		−0.0016 (0.0709)	0.0508 (0.0009*)
RA	0.0150 (0.0034*)		0.0586 (0.0058*)
FN	0.0637* (0.0001*)	0.0527* (0.0151*)	

DISCUSSION

Previous research based on short (400–500-bp) mtDNA control-region haplotypes suggested genetic structuring of global green turtle nesting populations on the scale of ≥ 500 km (reviewed in Bowen and Karl 2007). Expanded mtDNA sequencing effort revealed significant structure between RA and FN separated by only 150 km, indicating that these rookeries are discrete populations with respect to female natal homing. Application of these markers in additional nesting sites is required to determine whether the finer scale of population structure detected among Brazilian rookeries is more universal than previously appreciated given limitations of marker resolution.

FN had the highest haplotype diversity of the Brazilian nesting populations despite being the smallest rookery, congruent with mtDNA haplotype diversity patterns reported from western Africa green turtle rookeries (Formia et al. 2006). The São Tome rookery, which hosts < 100 females annually, had much higher haplotype diversity than Ascension or Poilão, which each had a nesting population several orders of magnitude larger. Low haplotype diversity in large rookeries may reflect rapid expansion following a founder event, whereas high genetic diversity in small rookeries could result from admixture via multiple colonization events or retention of ancestral diversity following a bottleneck (Allard et al. 1994; Lahanas et al. 1998). Formia et al. (2006) invoked the latter hypothesis to explain high haplotype diversity in the São Tome rookery. Given the high representation of apparently endemic mtSTR haplotypes in the FN rookery, it may also represent the remnant of a much larger, diverse stock that was severely overexploited prior to protection. Therefore despite the relatively small current size of the FN population, this rookery may harbor important remnant genetic diversity that could be unique among extant nesting populations.

The recognition that RA and FN host discrete green turtle populations has important conservation implications for resolving migratory connectivity. Although both nesting sites are protected, the distribution of juvenile developmental habitats and adult foraging sites for these rookeries remain unresolved. MSA, ocean circulation modeling, and tag return data suggest that the spatial ecology of green turtles is determined by the complex interaction of oceanic dispersal to initial neritic recruitment sites followed by some degree of natal homing as juveniles transition through developmental habitats (Bass et al. 2006; Moncada et al. 2006; Meylan et al. 2011; Naro-Maciel et al. 2012; Putman and Naro-Maciel 2013). MSA resolution is often poor for small rookeries relative to larger ones

because of extensive haplotype sharing (Monzón-Argüello et al. 2010), and RA assignments have been essentially evenly distributed across all western Atlantic foraging aggregations analyzed (Naro-Maciel et al. 2012).

Ocean circulation modeling suggested markedly different trajectories for hatchlings departing from the northern Brazilian rookeries relative to TI, with a large percentage of the former distributed northward into the Greater Caribbean region and the latter southward along the Brazilian coast (Putman and Naro-Maciel 2013). Marine turtle harvest is still permitted in several territories of the Greater Caribbean (Humber et al. 2014), whereas Brazilian law prohibits direct take (Marcovaldi et al. 2005). Under such a scenario, juveniles from the smaller northern Brazilian rookeries would be at higher risk if they remain at Caribbean foraging sites as they reach large juvenile and subadult size classes.

Despite increased resolution of stock structure revealed through use of the mtSTR loci, these markers do have some limitations that should be considered. The high diversity of mtSTR haplotypes present within each of the common control-region haplotypes suggests faster turnover for the mtSTR loci relative to point mutations in the control region. If the mutation rate exceeds the rate of genetic drift, high levels of homoplasy may prevent genetic differentiation even in the absence of gene flow (Lunt et al. 1998). The lack of differentiation between TI and RA CM-A8 mtSTR frequencies in this study may have resulted from haplotype saturation through homoplasy. This presumably high mutation rate also makes it difficult to infer historical processes because of uncertainties in defining ancestral haplotype states and linkages among haplotypes. Given the increased polymorphism uncovered through use of mtSTR markers, larger rookery sample sizes will be required in future studies to produce robust baseline frequencies for mixed stock analyses. Even with demonstration of demographic isolation among the Brazilian rookeries, deeper sampling of the RA and FN rookeries could reduce variance around rookery contribution estimates in mixed stock analyses.

Application of the markers identified in the present study in Atlantic foraging aggregations should better resolve the distribution of juveniles from the northern rookeries, particularly the smaller, remnant FN rookery. Comparable data are needed from green turtle nesting populations in the central and eastern Atlantic, as well as the Southwest Indian Ocean, to fully realize the potential for increased stock resolution and provide more complete baseline data for MSA. Resolving population structure and migratory connectivity have been identified as critical research priorities for marine turtle conservation globally (Hamann et al. 2010), and this study has demonstrated the efficacy of using the mtSTR array to improve resolution of population structure in Southwest Atlantic green turtles. Sequencing of the mtSTR array in other green turtle nesting populations and foraging aggregations is needed to determine its utility for increased resolution of stock structure and mixed stock analysis.

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LITERATURE CITED

ABREU-GROBOIS, F.A., HORROCKS, J., FORMIA, A., LEROUX, R., VELEZ-ZUAZO, X., DUTTON, P., SOARES, L., MEYLAN, P., AND BROWNE, D. 2006. New mtDNA d-loop primers which work for a variety of marine turtle species may increase the resolution of mixed stock analyses. In: Frick, M., Panagopoulou, A., Rees, A., and Williams, K.L. (Eds.). Book of Abstracts, Twenty-sixth Annual Symposium on Sea Turtle Biology and Conservation. Athens, Greece: International Sea Turtle Society, p. 179.

ALLARD, M.W., MIYAMOTO, M.M., BJORNDAL, K.A., BOLTEN, A.B., AND BOWEN, B.W. 1994. Support for natal homing in green turtles from mitochondrial DNA sequences. Copeia 1994:34–41.

ALMEIDA, A.P., MOREIRA, L.M.P., BRUNO, S.C., THOMÉ, J.C.A., MARTINS, A.S., BOLTEN, A.B., AND BJORNDAL, K.A. 2011. Green turtle nesting on Trindade Island, Brazil: abundance, trends, and biometrics. Endangered Species Research 14:193–201.

BASS, A.L., EPPERLY, S.P., AND BRAUN-MCNEILL, J. 2006. Green turtle (*Chelonia mydas*) foraging and nesting aggregations in the Caribbean and Atlantic: impact of currents and behavior on dispersal. Journal of Heredity 97:346–354.

BELLINI, C. AND SANCHES, T. 1996. Reproduction and feeding of marine turtles in the Fernando de Noronha Archipelago, Brazil. Marine Turtle Newsletter 74:12–13.

BELLINI, C., SANTOS, A.J.B., GROSSMAN, A., MARCOVALDI, M.Â., AND BARATA, P.C.R. 2012. Green turtle (*Chelonia mydas*) nesting on Atol das Rocas, north-eastern Brazil, 1990–2008. Journal of the Marine Biological Association of the United Kingdom 93:1117–1132.

BENJAMINI, Y. AND YEKUTIELI, D. 2001. The control of the false discovery rate in multiple testing under dependency. Annals of Statistics 29:1165–1188.

BJORNDAL, K.A., BOLTEN, A.B., MOREIRA, L., BELLINI, C., AND MARCOVALDI, M.Â. 2006. Population structure and diversity of Brazilian green turtle rookeries based on mitochondrial DNA sequences. Chelonian Conservation and Biology 5:262–268.

BOLTEN, A.B. 2003. Variation in sea turtle life history patterns: neritic vs. oceanic developmental stages. In: Lutz, P.L., Musick, J.A., and Wyneken J. (Eds.). The Biology of Sea Turtles. Volume II. Boca Raton, FL: CRC Press, 455 pp.

BOURJEA, J., LAPÈGUE, S., GAGNEVIN, L., BRODERICK, D., MORTIMER, J.A., CICCIONE, S., ROOS, D., TAQUET, C., AND GRISEL, H. 2007. Phylogeography of the green turtle, *Chelonia mydas*, in the Southwest Indian Ocean. Molecular Ecology 16:175–186.

BOWEN, B.W. AND KARL, S.A. 2007. Population genetics and phylogeography of sea turtles. Molecular Ecology 16:4886–4907.

DUTTON, P.H., JENSEN, M.P., FRUTCHEY, K., FREY, A., LACASELLA, E., BALAZS, G.H., ZÁRATE, P., CHASSIN-NORIA, O., SARTI-MARTINEZ, A.L., AND VELEZ, E. 2014. Genetic stock structure of green turtle (*Chelonia mydas*) nesting populations across the Pacific islands. Pacific Science 68:451–464.

DUTTON, P.H., RODEN, S.E., STEWART, K.R., LACASELLA, E.L., TIWARI, M., FORMIA, A., THOMÉ, J.C., LIVINGSTONE, S.R., ECKERT, S.A., CHACON-CHAVERRI, D., RIVALAN, P., AND ALLMAN, P. 2013. Population stock structure of leatherback turtles (*Dermochelys coriacea*) in the Atlantic revealed using mtDNA and microsatellite markers. Conservation Genetics 14:625–636.

ENCALADA, S.E., LAHANAS, P.N., BJORNDAL, K.A., BOLKER, B., MIYAMOTO, M.M., AND BOWEN, B.W. 1996. Phylogeography and population structure of the Atlantic and Mediterranean green turtle *Chelonia mydas*: a mitochondrial DNA control region sequence assessment. Molecular Ecology 5:473–483.

EXCOFFIER, L. AND LISCHER, H.E.L. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10:564–567.

FORMIA, A., BRODERICK, A.C., GLEN, F., GODLEY, B.J., HAYS, G.C., AND BRUFORD, M.W. 2007. Genetic composition of the Ascension Island green turtle rookery based on mitochondrial DNA: implications for sampling and diversity. Endangered Species Research 3:145–158.

FORMIA, A., GODLEY, B.J., DONTAINE, J.-F., AND BRUFORD, M.W. 2006. Mitochondrial DNA diversity and phylogeography of endangered green turtle (*Chelonia mydas*) populations in Africa. Conservation Genetics 7:353–369.

HAMANN, M., GODFREY, M.H., SEMINOFF, J.A., ARTHUR, K.E., BARATA, P.C.R., BJORNDAL, K.A., BOLTEN, A.B., BRODERICK, A.C., CAMPBELL, L.M., CARRERAS, C., CASALE, P., CHALOUPKA, M., CHAN, S.K.F., COYNE, M.S., CROWDER, L.B., DIEZ, C.E., DUTTON, P.H., EPPERLY, S.P., FITZSIMMONS, N.N., FORMIA, A., GIRONDOT, M., HAYS, G.C., CHENG, I.J., KASKA, Y., LEWISON, R., MORTIMER, J.A., NICHOLS, W.J., REINA, R.D., SHANKER, K., SPOTILA, J.R., TOMÁS, J., WALLACE, B.P., WORK, T.M., ZBINDEN, J., AND GODLEY, B.J. 2010. Global research priorities for sea turtles: informing management and conservation in the 21st century. Endangered Species Research 11:245–269.

HOELZEL, A.R. 1998. Genetic structure of cetacean populations in sympatry, parapatry, and mixed assemblages: implications for conservation policy. Journal of Heredity 89:451–458.

HUETER, R.E., HEUPEL, M.R., HEIST, E.J., AND KEENEY, D.B. 2005. Evidence of philopatry in sharks and implications for the management of shark fisheries. Journal of Northwest Atlantic Fishery Science 37:239–247. doi:10.2960/J.v35.m493.

HUMBER, F., GODLEY, B.J., AND BRODERICK, A.C. 2014. So excellent a fishe: a global overview of legal marine turtle fisheries. Diversity and Distributions 20:579–590.

JENSEN, M.P., FITZSIMMONS, N.N., AND DUTTON, P.H. 2013. Molecular genetics of sea turtles. In: Wyneken J., Lohmann, K.J., and Musick, J.A. (Eds.). The Biology of Sea Turtles. Volume III. Boca Raton, FL: CRC Press, pp. 135–154.

LAHANAS, P.N., BJORNDAL, K.A., BOLTEN, A.B., ENCALADA, S.E., MIYAMOTO, M.M., VALVERDE, R.A., AND BOWEN, B.W. 1998. Genetic composition of a green turtle (*Chelonia mydas*) feeding ground population: evidence for multiple origins. Marine Biology 130:345–352.

LEROUX, R.A., DUTTON, P.H., ABREU-GROBOIS, F.A., LAGUEUX, C.J., CAMPBELL, C.L., DELCROIX, E., CHEVALIER, J., HORROCKS, J.A., HILLIS-STARR, Z.M., TROËNG, S., HARRISON, E., AND STAPLETON, S. 2012. Re-examination of population structure and phylogeography of hawksbill turtles in the Wider Caribbean using longer mtDNA sequences. The Journal of Heredity 103:806–20.

LÓPEZ-BARRERA, E.A., LONGO, G.O., AND MONTEIRO-FILHO, E.L.A. 2012. Incidental capture of green turtle (*Chelonia mydas*) in gillnets of small-scale fisheries in the Paranaguá Bay, southern Brazil. Ocean and Coastal Management 60:11–18.

LUNT, D.H., WHIPPLE, L.E., AND HYMAN, B.C. 1998. Mitochondrial DNA variable number tandem repeats (VNTRs): utility and problems in molecular ecology. Molecular Ecology 7: 1441–1455.

MARCOVALDI, M.Â., PATIRI, V., AND THOMÉ, J.C. 2005. Twenty-five years protecting Brazilian sea turtles through a community-based conservation-programme. MAST 3/4: 39–62.

MEYLAN, P.A., MEYLAN, A.B., AND GRAY, J.A. 2011. The ecology and migrations of sea turtles 8. Tests of the developmental habitat hypothesis. Bulletin of the American Museum of Natural History 357:1–77.

MONCADA, F.G., ABREU-GROBOIS, F.A., MUHLIA-MELO, A., BELL, C.D., TROËNG, S., BJORNDAL, K.A., BOLTEN, A.B., MEYLAN, A.B., ZURITA, J.C., ESPINOSA, G., NODARSE, G., MÁRQUEZ-MILLÁN, R., FOLEY, A., AND EHRHART, L. 2006. Movement patterns of green turtles (*Chelonia mydas*) in Cuba and adjacent Caribbean waters inferred from flipper tag recaptures. Journal of Herpetology 40:22–34.

MONZÓN-ARGÜELLO, C., LOPEZ-JURADO, L.F., RICO, C., MARCO, A., LOPEZ, P., HAYS, G.C., AND LEE, P.L.M. 2010. Evidence from genetic and Lagrangian drifter data for transatlantic transport of small juvenile green turtles. Journal of Biogeography 37: 1752–1766.

NARO-MACIEL, E., BONDIOLI, A.C.V., MARTIN, M., ALMEIDA, A.P., BAPTISTOTTE, C., BELLINI, C., MARCOVALDI, M.Â., SANTOS, A.J.B., AND AMATO, G. 2012. The interplay of homing and dispersal in green turtles: a focus on the Southwestern Atlantic. Journal of Heredity 103:792–805.

NARO-MACIEL, E., REID, B.N., ALTER, S.E., AMATO, G., BJORNDAL, K.A., BOLTEN, A.B., MARTIN, M., NAIRN, C.J., SHAMBLIN, B.M., AND PINEDA-CATALAN, O. 2014. From refugia to rookeries: phylogeography of Atlantic green turtles. Journal of Experimental Marine Biology and Ecology 461:306–316.

NEI, M. 1987. Molecular Evolutionary Genetics. New York: Columbia University Press.

PUTMAN, N.F. AND NARO-MACIEL, E. 2013. Finding the 'lost years' in green turtles: insights from ocean circulation models and genetic analysis. Proceedings of the Royal Society B: Biological Sciences 280:20131468.

RAYMOND, M. AND ROUSSET, F. 1995. An exact test of population differentiation. Evolution 49:1280–1283.

SHAMBLIN, B.M., BJORNDAL, K.A., BOLTEN, A.B., HILLIS-STARR, Z.M., LUNDGREN, I., NARO-MACIEL, E., AND NAIRN, C.J. 2012. Mitogenomic sequences better resolve stock structure of southern Greater Caribbean green turtle rookeries. Molecular Ecology 21:2330–2340.

SHAMBLIN, B.M., BOLTEN, A.B., ABREU-GROBOIS, F.A., BJORNDAL, K.A., CARDONA, L., CARRERAS, C., CLUSA, M., MONZÓN-ARGÜELLO, C., NAIRN, C.J., NIELSEN, J.T., NEL, R., SOARES, L.S., STEWART, K.R., VILAÇA, S.T., TÜRKOZAN, O., YILMAZ, C., AND DUTTON, P.H. 2014. Geographic patterns of genetic variation in a broadly distributed marine vertebrate: new insights into loggerhead turtle stock structure from expanded mitochondrial DNA sequences. PLoS ONE 9:e85956.

TIKOCHINSKI, Y., BENDELAC, R., BARASH, A., DAYA, A., LEVY, Y., AND FRIEDMANN, A. 2012. Mitochondrial DNA STR analysis as a tool for studying the green sea turtle (*Chelonia mydas*) populations: the Mediterranean Sea case study. Marine Genomics 6:17–24.

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