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Genetic characterization of green turtles (*Chelonia mydas*) from São Tomé and Príncipe: Insights on species recruitment and dispersal in the Gulf of Guinea

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ABSTRACT

Genetic studies on green sea turtles (*Chelonia mydas*) in the Eastern Atlantic have mostly focused on reproductive females, with limited information available regarding juveniles and foraging grounds. Improved understanding of genetic diversity and patterns of connectivity between nesting and foraging grounds is critical to identify management units and delineate suitable conservation strategies. Here we analyzed data from 11 microsatellite markers and sequences of the mitochondrial control region from both juveniles and females sampled in foraging and nesting aggregations around São Tomé and Príncipe islands, in the Gulf of Guinea, West Africa. Both nuclear and mtDNA data were congruent in showing that São Tomé and Príncipe's green turtles population exhibit high levels of genetic diversity, which are similar to those reported for other foraging aggregates in the Atlantic. Although signs of population substructure among foraging and nesting grounds of São Tomé and Príncipe islands were not apparent, our analysis based on mtDNA marker showed that both juvenile and adult turtles were genetically differentiated from other foraging and nesting Atlantic populations. The similar levels of genetic diversity found in both juveniles and females are consistent with the results from mixed stock analyses, which suggested that São Tomé and Príncipe's rookery is the primary source of juveniles to the local foraging aggregation. Taken these aspects in consideration, we argue that São Tomé and Príncipe green turtles show limited dispersal and should be considered an important management unit, and conservation actions in this archipelago must be implemented not only at the level of the rookery but should also include the foraging aggregations.

1. Introduction

For highly migratory species, their ability to disperse affects the connectivity among populations, recruitment patterns, links between foraging and breeding areas and genetic diversity (Bowler and Benton, 2005; Blumenthal et al., 2009; Runge et al., 2014). In marine environments the patterns of dispersal and recruitment result from complex processes, often influenced by species-specific responses to features of both nearshore and pelagic environments. Marine turtles represent well this complexity, and are well suited to study these processes, since they have a complex life cycle that includes multiple phases within neritic and pelagic habitats (Van Buskirk and Crowder, 1994; McClellan and Read, 2007; Arthur et al., 2008) between which both juveniles and adults disperse widely over vast expanses of ocean. Post-hatchling dispersal from natal beaches is an interplay between oriented swimming and passive drift (Scott et al., 2012) and is followed by an epipelagic phase that ranges from five to ten years, after which they recruit to coastal foraging grounds as juveniles (Bolten, 2003). Recruits to a

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foraging ground can be an uneven mix of juveniles originating from rookeries located either in the vicinity or thousands of kilometers away (Bolten et al., 1998; Monzón-Argüello et al., 2010), originating aggregations of mixed genetic origins (Bowen and Karl, 2007). Factors influencing dispersal and settlement of juvenile sea turtles include passive drift in oceanic currents (Carreras et al., 2006; Okuyama et al., 2011), distance to the contributing nesting colonies (Lahanas et al., 1998), as well as water temperature and food availability (Mansfield et al., 2017). Upon reaching sexual maturity, adults periodically migrate between foraging and reproductive grounds showing a remarkable fidelity to specific areas for reproduction (Limpus et al., 1992; Lohmann et al., 2008), typically located in the vicinity of their natal beach (Bowen and Karl, 2007). This so called "natal homing" behavior" leads to distinct breeding populations that, over time, may accumulate genetic differences that can be used as "genetic signatures" to identify groups of individuals back to their population of origin (Allard et al., 1994; Bowen et al., 1992; Encalada et al., 1996). Molecular markers have been an increasingly useful tool to understand patterns of connectivity and dispersal among populations of marine turtles, particularly through estimates of potential contribution of donor (rookeries) to recipient populations (mixed foraging grounds) using mixed-stock analyses (Pella and Masuda, 2001).

Based on some of these studies, the high level of genetic substructure found among marine turtle nesting populations and foraging aggregations, even at a relatively small spatial scale, has led to the emergence of the management unit concept as a novel framework for prioritizing protection at a local/regional level (Wallace et al., 2010). Indeed, the increase in genetic studies have been crucial in improving indirectly our knowledge about behavior, ecology and evolution of these species, providing thus an important support for conservation and management (Bowen and Karl, 2007; Komoroske et al., 2017).

The green turtle (*Chelonia mydas*) is a highly migratory marine organism with a circumglobal distribution, occurring throughout tropical and subtropical regions and, to a lesser extent, temperate waters (Seminoff et al., 2015). This species is considered globally endangered (IUCN, 20018), as like other marine turtle species, it has been facing several threats related to the degradation and loss of nesting and feeding habitats, and especially with their over-exploitation both for food (meat and eggs) and for ornaments (shell) (e.g. Parsons, 1962; Early-Capistrán et al., 2018). This situation together with accumulated evidences for extensive population declines in different areas of the globe, have emphasized the importance of adopting conservation actions to protect marine turtle rookeries and feeding habitats (Wallace et al., 2011). Within the Eastern and South Atlantic basins, some comprehensive studies on green turtle populations using mitochondrial DNA (mtDNA) sequencing have identified several genetically distinct rookeries, including those found in Poilão (Guinea Bissau), São Tomé and Bioko (Gulf of Guinea) and Ascension islands, which may potentially represent independent management units (Formia et al., 2006, 2007; Patrício et al., 2017a). These studies have also expanded the knowledge on migration patterns and connectivity among green turtle populations. For example, a recent genetic study on juvenile dispersal from Guinea Bissau (Patrício et al., 2017a), suggested a high connectivity between rookeries and juvenile aggregations within West African populations. Despite accumulated information on green turtle biology, most genetic studies have been so far based on the analysis of a single type of marker (mtDNA sequences), and focused on rookeries, with limited information regarding juvenile aggregations and males. The São Tomé and Príncipe archipelago is part of a chain of extinct volcanoes called Cameroon Line. Its two main islands, São Tomé and Príncipe, are true oceanic islands separated from the African continent by an ocean approximately 1800 m deep and located about 160 Km apart. These islands' shallow coastal shelf (< 200 m wide) has been recently depicted as holding relatively important aggregations of foraging juvenile green turtles in the Gulf of Guinea (Hancock et al., 2018). The aim of our study is to characterize the genetic diversity of green turtles from São Tomé and Príncipe archipelago using a combination of genetic markers (a mitochondrial fragment and a set of microsatellites). More specifically, we use mtDNA information for i) estimating the contribution of different rookeries in the Atlantic to São Tomé and Príncipe mixed stocks to ascertain their origin (foraging ground-centric approach), and ii) determine the possible dispersal patterns between São Tomé and Príncipe rookeries to foraging areas in the Atlantic (rookery-centric approach). Additionally, we contrast levels of genetic diversity found in São Tomé and Príncipe islands with others previously documented Atlantic populations in order to evaluate the potential importance of these two islands for global green turtle conservation, especially at a regional level through delineation of functional units of management for conservation.

2. Materials and methods

2.1. Sample collection and DNA extraction

Skin samples from 112 adult females were collected at the primary nesting beaches in the islands of São Tomé (n = 93) and Príncipe (n = 19), between October and February of 2015 and 2016 during night patrols conducted by the staff of Programa Tatô and Príncipe Trust, respectively. Foraging juveniles were hand-captured in both island of São Tomé (n = 34) and Príncipe (n = 7). Samples were taken from the trailing edge of the left hind flippers and stored in 96% ethanol. Sampling locations are detailed in Fig. 1.

Whole-genomic DNA was extracted from all samples collected using QIA Quick DNEasy columns (Qiagen, Inc., Valencia, CA, USA) following standard DNA extraction protocols.

2.2. Microsatellites

We used seven microsatellite loci previously developed for *Caretta caretta* (Cc5H07, CcP2F11, CCP7C06, Ccp7D04, Cc1F01, Cc5C08, Cc1G02, Shamblin et al., 2009), and seven for *Eretmochelys imbricata* (EIM09, EIM40, ERIM25, ERIM03, ERIM19, ERIM21, ERIM22; Miro-Herrans et al., 2008, Shamblin et al., 2013). Microsatellite amplifications were conducted in a Biorad T100 thermocycler using a Multiplex PCR Kit (QIAGEN) following manufacturer's instructions. The microsatellite loci were tested and amplified separately and then combined in two multiplex reactions for the final amplification using the Multiplex Manager 1.2 software (Table S1, Supporting Information). General thermal conditions comprised an initial denaturation for 15 min at 95 °C, followed by an additional step at 95 °C for 30 s., followed by 21 cycles of 1 min 30 s. duration, each at 60 °C with −0.5 °C decrease per cycle (to ensure an optimal annealing temperature for each primer). A second round of equal number of cycles was programmed at a lower, constant temperature (50 °C), set for 1 min each, to exponentially increase the number of amplified fragments. A final extension at 60 °C was programmed for 35 min to promote adenylation and to avoid -A peaks during genotyping. Polymerase chain reaction (PCR) products were separated by capillary electrophoresis on an automatic sequencer ABI3130*xl* Genetic Analyzer (AB Applied Biosystems). Fragments were scored against the GeneScan-500 LIZ Size Standard using the GENEMAPPER 4.1 (Applied Biosystems) and manually checked twice.

2.3. mtDNA

For PCR amplification and sequencing of the CR fragment, we used the primers LCM15382/H950 developed by Abreu-Grobois et al. (2006). Thermal conditions for amplifications consisted of 15 min at 95 °C, followed by 40 cycles of 30 s duration each at 56 °C, 45 s at 72 °C with a final extension at 60 °C for 20 min. Successful amplifications were enzymatically purified, and sequenced following the BigDye Terminator v3.1 Cycle sequencing protocol (Applied Biosystems). Sequencing products were separated in the same automatic sequencer

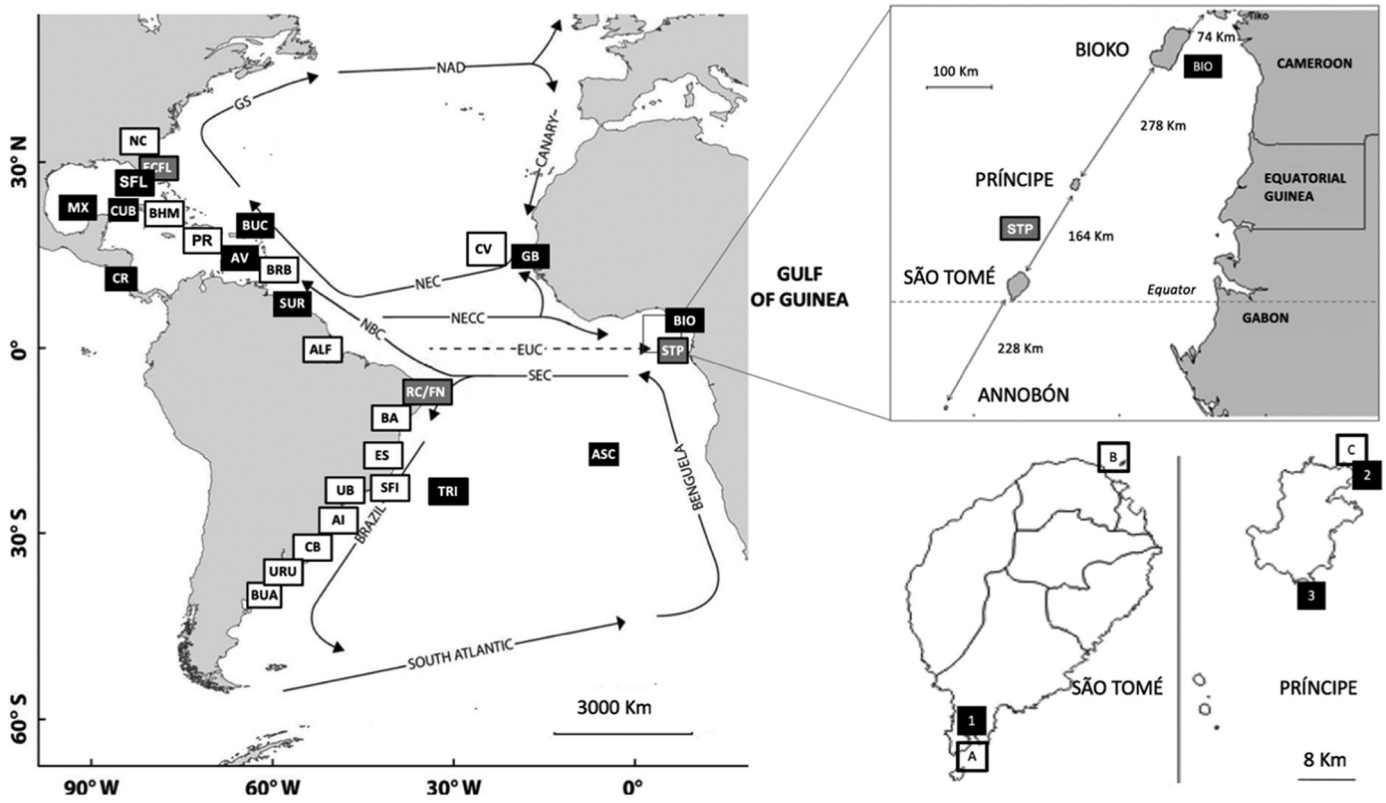

Fig. 1. Location of green turtle (*Chelonia mydas*) rookeries (■), foraging aggregations (□) and locations where rookeries and foraging aggregations co-occur (▩) included in this study and prevailing ocean currents (modified from Patrício et al., 2017a). Detailed location of São Tomé and Príncipe nesting sites and the three foraging areas sampled for this study are depicted in the figure inlet (Nesting areas: 1 – Porto Alegre; 2 – Praia Grande; 3 – Infante. Foraging sites: A – Porto Alegre; B –Cabras islet; C – Mosteiros). Acronym list: EcFL – East Central Florida; SFL – South Florida; MX – Mexico; CR – Costa Rica; CUB – Cuba; BUC – Buck Island; AV – Aves Island; SUR – Suriname; RC/N – Atol das Rocas/Fernando de Noronha; TRI – Trindade Island; ASC – Ascension Island; GB – Guinea Bissau; BIO – Bioko Island; STP – São Tomé and Príncipe Islands; NC – North Carolina; BHM – Bahamas; BRB – Barbados; ALF – Almofala; BA – Bahia; ES Espirito Santo; UB – Ubatuba; AI – Arvoredo Island; BuA – Buenos Aires; CV – Cape Verde; PR – Puerto Rico. References are included in Table S5. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ABI3130*xl* Genetic Analyzer, and were aligned and compared in the software SEQSCAPE 3.0 (Applied Biosystems).

2.4. Genetic diversity and population structure

2.4.1. Microsatellites

The presence/absence of large allele dropouts and null alleles was determined using the software MICROCHECKER 2.2.3 (Van Oosterhout et al., 2004). Departures from Hardy–Weinberg expectations (HWE) and linkage disequilibrium (LD) among the 14 loci were tested in GenALEx 6.503 (Peakall and Smouse, 2012), using the Markov Chain method (Rousset, 2008), and the respective significance was adjusted with sequential Bonferroni correction (Rice, 1989). Mean number of alleles (N_a), allelic richness (AR) and average observed (H_o) and expected (H_e) heterozygosities over loci were estimated using GenALEx. We estimated the level of genetic differentiation (F_{ST}, Weir and Cockerham, 1984) between São Tomé and Príncipe's populations using GenALEx. Statistical significance of F_{ST} values was tested using 1000 iterations, and 95% bootstrapped confidence intervals (CI) were used.

2.4.2. mtDNA

Standard summary statistics, including the number of haplotypes (H), haplotype diversity (h), and nucleotide diversity (π) were calculated in the software DnaSP 5.0 (Librado and Rozas, 2009). All detected haplotypes were aligned and assigned to published haplotypes in the Mitochondrial Sequence Database for the Atlantic Ocean green turtle, hosted by the Archie Carr Center for Sea Turtle Research (University of Florida, Gainsville, Florida, USA) (Table S3). This database together with available data compiled recently by Patrício et al. (2017a), were used to compare levels of genetic diversity and differentiation of São Tomé and Príncipe's population (STP) with other known Atlantic rookeries and foraging aggregations. For this analysis, we used a truncated fragment of 490 bp length, which has been historically used to gather genetic information of other Atlantic populations. We generated a dataset that included haplotype frequency data for São Tomé and Príncipe and existing data for 13 rookeries (n = 1927) and 18 foraging aggregations (n = 1789) in the Atlantic (Table S4a,b, Supporting Information). The genealogical relationships among São Tomé and Príncipe haplotypes and haplotypes from other rookeries were inferred using a median-joining network analysis (Bandelt et al., 1999) implemented in the program POPART (Leigh and Bryant, 2015). Genetic differentiation between rookeries and foraging aggregations in the Atlantic was estimated in ARLEQUIN 3.5 (Excoffier and Lischer, 2010) through pairwise fixation indices (F_{ST}) using haplotype frequencies. A false discovery rate (FDR) correction (Narum, 2006) was applied to calculate the most fitting threshold for the p-value significance considering the number of comparisons involved in the analysis and under an expected threshold of $p < .05$. The interpopulation migration rates were estimated using the formula $F_{ST} = 1/(4N_m + 1)$ (N_m as virtual number of migrants, Wright, 1984).

Historical demography of the adult (females) population was examined by the neutrality tests of Tajima's D (Tajima, 1989), Fu's F_s (Fu, 1997), and R_2 (Ramos-Onsins and Rozas, 2002), which evaluate whether the polymorphism conforms to a neutral model of evolution. Statistical significance was determined by comparing estimated values against a distribution generated from 10,000 random samples under the

Table 1
Levels of genetic diversity found in *Chelonia mydas* in São Tomé and Príncipe islands (rookeries and foraging grounds) based on 11 microsatellite loci.

Population	N	Na (± se)	AR (± se)	Ho (± se)	He (± se)
Rookery					
São Tomé	72	12.9 (± 1.36)	10.97 (± 4.01)	0.803 (± 0.035)	0.799 (± 0.037)
Príncipe	19	10.0 (± 1.07)	10.26 (± 4.01)	0.799 (± 0.042)	0.797 (± 0.037)
São Tomé and Príncipe	**91**	**11.4 (± 0.90)**	**13.40 (± 5.49)**	**0.801 (± 0.027)**	**0.798 (± 0.025)**
Foraging aggregation					
São Tomé	23	11.43 (± 1.07)	5.63 (± 1.30)	0.778 (± 0.040)	0.811 (± 0.028)
Príncipe	7	7.00 (± 0.59)	5.20 (± 1.26)	0.788 (± 0.044)	0.770 (± 0.026)
São Tomé and Príncipe	**30**	**9.21 (± 0.74)**	**11.58 (± 3.96)**	**0.783 (± 0.029)**	**0.790 (± 0.019)**

Key: *N* number of samples; *Na* number of alleles; *AR* allelic richness; *H0* observed heterozygosity; *He* expected heterozygosity
Values for the São Tomé and Príncipe rookery and foraging aggregation are highlighted in bold

hypothesis of selective neutrality and population equilibrium, with no recombination (Hudson, 1990), using the coalescent simulator in DnaSP. Historical demographic changes were also examined by estimating fluctuations in the effective population size over time using the Bayesian Skyline Plot (BSP) method (Drummond et al., 2005), as implemented in BEAST 2.5.0 (Bouckaert et al., 2014). For this analysis, we used a strict clock model and a substitution rate of 0.0015×10^{-9} mutations/site/year (Lahanas et al., 1994). A *Markov Chain Monte Carlo* (*MCMC*) sampling algorithm was used in the HKY model estimated by JMODELTEST 0.1.1 (Posada, 2008). The MCMC chains were run 200 million generations, sampled every 10,000 generations with a 10% burn-in. All results were examined using TRACER 1.6 (Rambaut et al., 2014). Convergence was assessed with ESS (effective sample size) > 200.

2.5. Mixed-stock analysis

A many-to-many Mixed-Stock Analysis (MSA) was performed to estimate the contributions of 14 green turtle stocks of the Atlantic to the São Tomé foraging grounds (foraging ground-centric MSA), as well as the dispersal of hatchlings originating from the São Tomé and Príncipe rookery (rookery-centric MSA). We used the "mixstock" package (Bolker et al., 2007) in R (R Core Team, 2011), a Bayesian algorithm that uses the MCMC method and a hierarchical model (Bolker et al., 2007), and WinBugs (Lunn et al., 2000) using rookery size as prior (Prosdocimi et al., 2012). São Tomé and Príncipe rookeries were grouped due to the lack of genetic differentiation (see results section) and geographic proximity (160 Km). Haplotypes observed by Formia et al. (2006) for the São Tomé rookery and their frequencies were added to our sample for this island (n = 26 females added), for a total sample size of 138 adult females used for this study. Because many of the previous studies used shorter sequence fragments (~490 bp), for comparative purposes, we used a mixed stock analysis (MSA) using cropped sequences. Seven chains were run using 20,000 MCMC steps with a burn-in of 10,000 to calculate the posterior distribution. Convergence of MCMC estimates to a desired posterior probability was assessed using the Gelman–Rubin shrink factor (Gelman and Rubin, 1992), increasing the MCMC steps until all values obtained were < 1.2. This diagnostic compares the variation of a single chain to the total variation among chains, and convergence is achieved if the shrink factor is < 1.2 for each chain (Pella and Masuda, 2001). The final MSA was run twice, once with uniform priors (each rookery was equally likely to contribute with individuals to the São Tomé and Príncipe's foraging aggregations), and then using weighed priors (rookery size entered as a prior under the assumption that larger rookeries provide larger contributions to foraging grounds). The estimated sizes of each rookery were taken from Seminoff et al. (2015). Individuals with orphan haplotypes (i.e., not observed in any of the nesting rookeries) were removed from the analysis (Pella and Masuda, 2001). After we obtained our results from the MSA, we added these individuals back into the analysis and calculated the contribution of the 'unknown' rookeries to the stock mixture.

3. Results

3.1. Genetic diversity

3.1.1. Microsatellites

All females and 30 of the 43 juveniles sampled were successfully genotyped at 14 microsatellite loci. Evidence of potential allele dropouts or null alleles was found for the loci CCP7C06, ERIM19 and ERIM22, and for this reason these were eliminated from further analysis. Allele frequencies at all remaining 11 loci were within expectations of Hardy-Weinberg equilibrium, and no pairs of loci showed significant linkage disequilibrium after sequential Bonferroni correction. In the adult population, the number of alleles per locus ranged from 5 to 21, with an average of 11.5. The mean number of alleles ranged from 6.3 (São Tomé) to 6.8 (Príncipe). Levels of allelic diversity adjusted for sample size (allelic richness) ranged from 12.9 (São Tomé) to 10.0 (Príncipe), whereas observed and expected heterozygosity ranged from 0.804 ± 0.035 (São Tomé) to 0.799 ± 0.042 (Príncipe) and from 0.799 ± 0.037 (São Tomé) to 0.797 ± 0.037 (Príncipe), respectively. Regarding foraging grounds (juveniles), the mean number of alleles ranged from 6.5 (São Tomé) to 5.2 (Príncipe). Observed and expected heterozygosity ranged from 0.778 ± 0.040 (São Tomé) to 0.783 ± 0.029 (Príncipe) and from 0.811 ± 0.028 (São Tomé) to 0.790 ± 0.019 (Príncipe), respectively. Genetic diversity at the microsatellite level for females and juveniles sampled in São Tomé and Príncipe are presented in Table 1; comparisons with published data from other populations in the Atlantic are summarized in Table S2 (Supporting Information).

3.1.2. Mitochondrial DNA

Sequencing alignment revealed 8 haplotypes in the sampled adult population, totalizing in combination with data from Formia et al. (2006), 9 distinct haplotypes (Table 2), and 7 haplotypes in the juveniles sampled at the foraging aggregations; summary statistics for

Table 2
Genetic diversity for mtDNA Control Region of São Tomé and Príncipe *Chelonia mydas* rookeries and foraging aggregations.

Population	N	H	Hd (± sd)	Π (± sd)
Rookery				
São Tomé	119	9	0.607 (± 0.047)	0.0020 (± 0.002)
Príncipe	19	3	0.433 (± 0.117)	0.0001 (± 0.009)
São Tomé and Príncipe	**138**	**9**	**0.590 (± 0.044)**	**0.0019 (± 0.001)**
Foraging aggregation				
São Tomé	36	6	0.602 (± 0.052)	0.0020 (± 0.002)
Príncipe	7	3	0.524 (± 0.209)	0.0011 (± 0.001)
São Tomé and Príncipe	**43**	**7**	**0.547 (± 0.080)**	**0.0019 (± 0.003)**

Key: *N* number of samples; *H* number of haplotypes; *Hd* haplotype diversity; *Π* nucleotide diversity; *sd* standard deviation.
Values for the São Tomé and Príncipe rookery and foraging aggregation are highlighted in bold

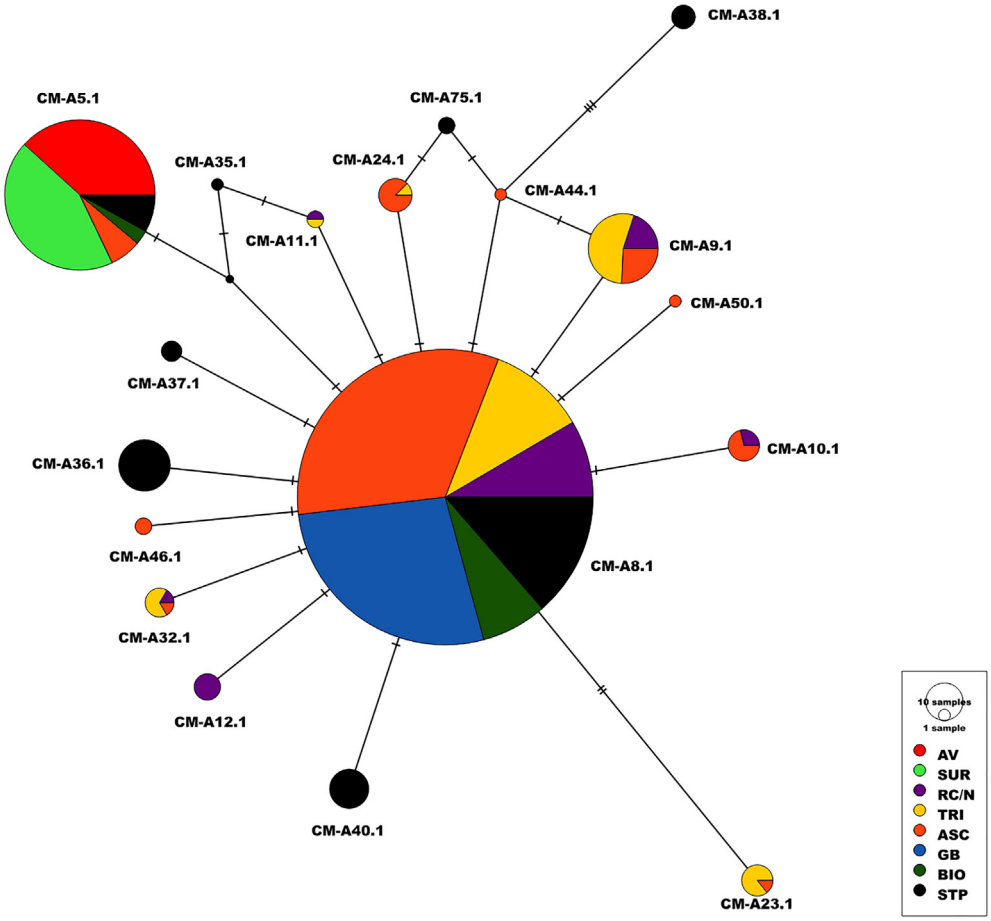

Fig. 2. The genealogical relationships between São Tomé and Príncipe's female haplotypes and other *C. mydas* rookeries, as indicated by the median-joining network of mitochondrial control region haplotypes found in the Atlantic. Acronyms are listed in Fig.1 and Table S5.

resulting genetic diversity for each population are included in Table 2. The genealogical relationships among São Tomé and Príncipe female haplotypes together with available published data from other rookeries are depicted in Fig. 2. The haplotypes CM-A10, CM-A40 and CM-A75 are reported in these rookery for the first time, while all other haplotypes observed in females and/or juveniles (CM-A5, CM-A6, CM-A8, CM-A35, CM-A36, CM-A37, and CM-A38) were already reported in Formia et al., (2006) (exact frequencies for each haplotype are depicted in Table S3, Supporting Information). The haplotype CM-A35, previously observed by Formia et al., (2006) in the nesting population, was not reported in this study, but was found in our sample of juveniles. The haplotype CM-A10 was found exclusively in the foraging aggregation, although it had been previously described for Ascension Island (Encalada et al., 1996; Formia et al., 2007) and Brazilian rookeries (Encalada et al., 1996; Bjorndal et al., 2006). There was one predominant haplotype, CM-A8, found in 57.6% and 65.1% of the samples (rookery and foraging aggregations, respectively). Overall, the haplotype diversity found in females was high (0.610 ± 0.046) when compared to the mtDNA diversity found in green turtle rookeries from the Eastern Atlantic and Ascension Island (Table S5, Supporting Information).

3.2. Population structure and demography

There was no significant genetic differentiation between the rookery of São Tomé and that of Príncipe using either mitochondrial ($F_{ST} = -0.008$, $p = .693$ or nuclear markers ($F_{ST} = 0.009$, $p = .318$). Based on this overall lack of genetic differentiation between the two islands at both rookery and foraging aggregation levels, we treated each as representing either as a single population, or a single foraging aggregation, respectively (São Tomé and Príncipe, STP) for downstream analyses.

Considering the combined data set, we found that mtDNA frequencies at the STP rookery were significantly different from the African rookeries of Ascension and Bioko islands, and Poilão (Guinea-Bissau), as well as all other rookeries included in this study for comparative purposes. Estimates of interpopulation migration (number of virtual migrants, Wright, 1978) between STP and other rookeries were low in most cases ($Nm < 0.1$), being only relatively high between STP and the Brazilian rookeries of Atol das Rocas/Noronha ($Nm = 4.5$), Trindade Island ($Nm = 3.54$), and the Eastern Atlantic rookeries of Bioko and Ascension ($Nm = 2.18$ and $Nm = 2.30$ respectively). In addition, the STP foraging aggregation showed no significant differences in haplotype frequencies with the STP rookery ($F_{ST} = -0.00895$, $p = .6944$), and was found to be significantly differentiated from all foraging aggregations sampled in the Atlantic, with F_{ST} values ranging from 0.032–0.841, $p < .001$ (Table S5, Supporting Information).

Neutrality tests applied to the mtDNA dataset revealed non statistically significant values of Tajima's D ($D = -2.60206$), Fu's Fs ($F = 0.34723$) and Ramos-Onsins and Rozas' R_2 statistic ($R_2 = 0.047$), which were in agreement with the relatively stability of *C. mydas* populations of São Tomé and Príncipe inferred by the coalescent BSP analysis (Fig. 3).

3.3. Mixed stock analysis

A strong link between São Tomé and Príncipe's (STP) foraging aggregations and rookery is shown by the MSA results (Fig. 4 and Table

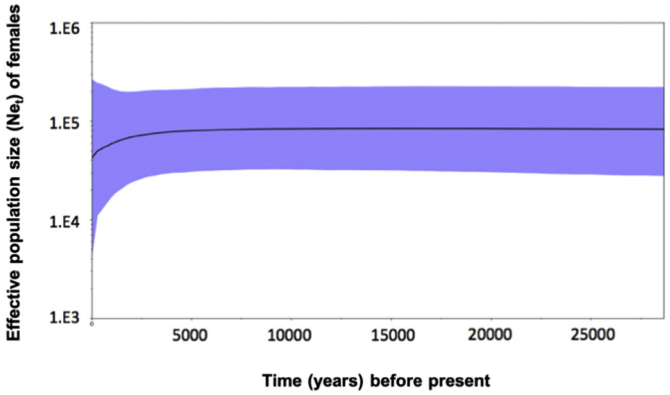

Fig. 3. Bayesian skyline plot showing the effective population size fluctuation throughout time of *Chelonia mydas* from São Tomé and Príncipe, West Africa. Solid line represents median estimations; purple area indicates confidence interval. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

S6, Supporting Information), indicating that São Tomé and Príncipe rookery was the most likely contributor to the local foraging grounds, with an estimated mean contribution of 57%, and maximum contribution up to 72%. However, when we consider the size of the source population as a weighing prior, Guinea Bissau stands out as the highest contributor to STP foraging aggregation, with São Tomé and Príncipe and Ascension islands with similar contributions. In neither case the nearby island of Bioko has any relevant contribution.

4. Discussion

Our results provide the most comprehensive analysis to date of genetic diversity of São Tomé and Príncipe's (STP) green turtle population. We have expanded previous analyses on green turtle genetic diversity in the region by increasing the number of mtDNA sequences for the adult population, and adding for the first time nuclear data for both rookery, and foraging aggregations, resulting from the analysis of 11 microsatellite loci. Our results showed that São Tomé and Príncipe islands were not genetically differentiated at either mtDNA or nuclear level, suggesting that females nesting on these two islands should be considered as belonging to a single population or rookery. Both our nuclear and mtDNA data confirm previous findings of Formia et al. (2006), who based solely on mtDNA data showed that São Tomé and Príncipe's rookery exhibits high genetic diversity when compared with values reported for other Atlantic rookeries, and being especially high when compared to other Eastern Atlantic populations (Tables S2 and S5). Based on mtDNA analysis, the STP rookery (when pooling both islands) was significantly differentiated from the others in the Atlantic, including the nearby island of Bioko (approximately 270 Km distant to Príncipe Island). Interestingly, this result contradicts the lack of differentiation reported by Patrício et al. (2017a) between these two nearby rookeries, which is likely related with the larger sample size used in the present study (five-fold larger) compared to that of Patrício et al. (2017a). Indeed, while the highest frequency (≈ 60%) of the CM-A8 haplotype in both rookery and foraging aggregation was similar to previous results obtained by Formia et al. (2006) for São Tomé's rookery and also for other Atlantic rookeries and foraging aggregations (Bjorndal et al., 2006; Formia et al., 2007; Naro-Maciel et al., 2006; Proietti et al., 2012), the increase of sample size allowed the identification of another relatively highly frequent (20%) haplotype (CM-A36), which had been previously observed at very low frequency in São Tomé island (Formia et al., 2006) and in foraging areas in Brazil (Proietti et al., 2009). Additionally, we detected for the first time in a rookery the haplotypes CM-A40 which was only previously found in other West Africa foraging grounds (CM-A40, A. Formia, *unpublished data*), and CM-A75, reported in Brazil (Naro-Maciel et al., 2012).

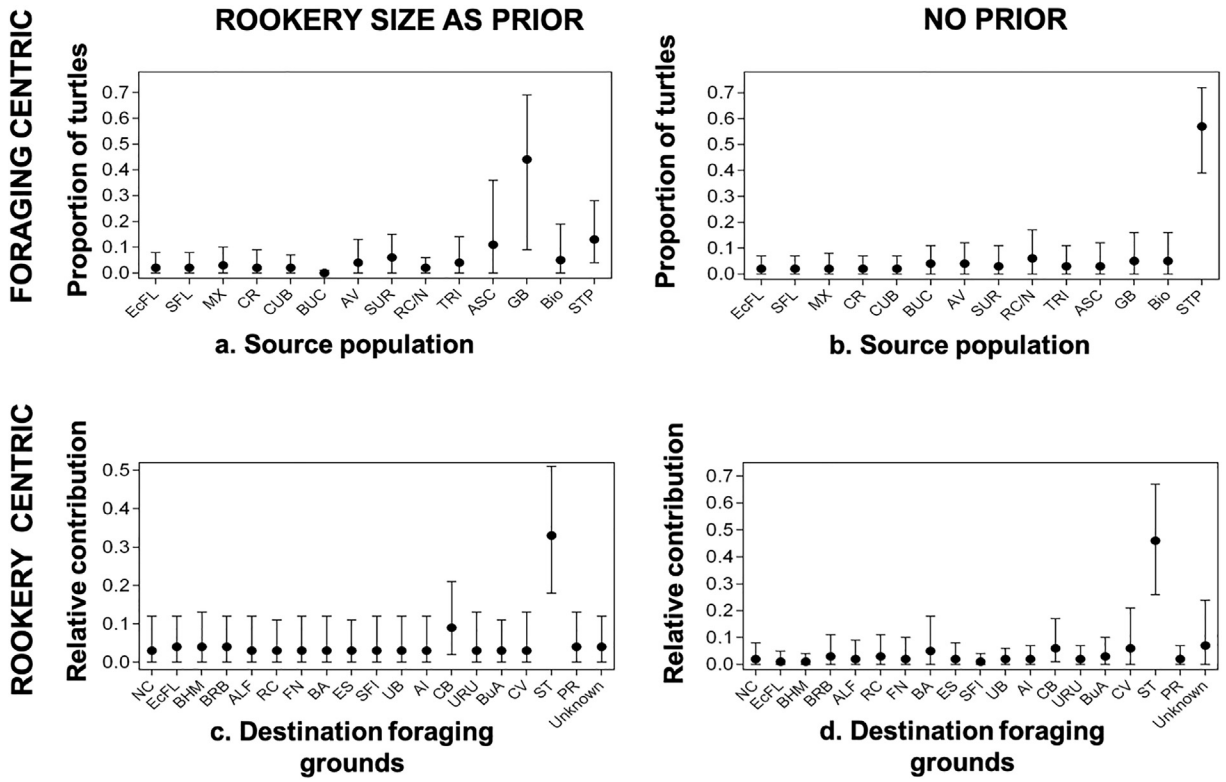

Fig. 4. a and b Estimated source contributions of Atlantic rookeries to the foraging aggregations of São Tomé and Príncipe with and without priors, respectively; c and d. Estimated contribution of São Tomé and Príncipe rookery to Atlantic foraging grounds, with and without priors, respectively. Bars represent 95% confidence intervals. Acronym list and references are included in Tables S5.

Analysis of historical population demographic changes using neutrality tests and the Bayesian Skyline Plot (BSP) suggested that São Tomé and Príncipe's rookery has been historically stable, with only subtle fluctuations in effective population size. This result is somewhat intriguing in light of what was previously found by Formia et al. (2006) for the neighboring island of Bioko, where they found much lower levels of genetic diversity and signals compatible with a unimodal distribution model of a rapidly expanding population. According to these authors, the higher diversity found in São Tomé and Príncipe may have resulted from the impact of a relatively high influx of immigrants and subsequent admixture but also hypothesized that this archipelago corresponds to a remnant of a larger ancestral population in the region. While our results do not allow us to make assumptions about the impact of migratory influx on levels of genetic diversity, the relatively stable demographic history of São Tomé and Príncipe rookery as deduced from mtDNA information, suggests a long-term persistence of the environmental conditions of the hydroclimatic zone where these islands are located, that likely functioned as a Pleistocene marine refuge area (Le Loeuff and Von Cosel, 1998).

It is well documented that during the Pleistocene, the cyclical climate changes (glacial and interglacial periods) caused contractions and expansions of the tropical zone in the eastern Atlantic with profound modifications on sea-level and concomitantly on ecological conditions of the region (Le Loeuff and Von Cosel, 1998). These modifications were especially noticeable in Bioko Island, which during the last glaciation period was repeatedly connected to the continent (Rohling et al., 1998). By contrast, the oceanic islands of São Tomé and Príncipe have never been connected to the continent and probably have only suffered very slight alterations on ecological conditions compared to Bioko Island. While based on these available evidences it is possible to deduct that processes of population extinction-recolonization versus persistence have shaped the current patterns of genetic variability at Bioko and São Tomé and Príncipe's rookeries, respectively, further studies combining paleoecological and genetic data with species distribution models (SDMs) will be crucial to illuminate this hypothesis.

The juvenile green turtles at the São Tomé and Príncipe's foraging aggregations exhibit high levels of genetic diversity, which are similar to those reported for other aggregates (Bass and Witzell, 2000; Prosdocimi et al., 2012). This result was somewhat expected as these aggregations are typically composed of individuals from mixed stocks. In this study, we found significant differentiation (as revealed by F_{ST}) between São Tomé's foraging aggregation and others sampled in the Atlantic, but similarity between both the rookery and the foraging aggregation. This relative genetic homogeneity found in this foraging aggregation could be explained by the high contribution from the São Tomé and Príncipe's rookeries compared with the negligible contribution from outside rookeries, as evidenced in the foraging-centric Mixed Stock Analysis (MSA) estimates (without rookery size as a prior). The exception to this pattern is the relatively high contribution of the Eastern Atlantic's largest rookery, Guinea Bissau, when rookery size was included in the estimates. A previous study performed by Godley et al. (2010), which used unpublished sequences from 75 juveniles from São Tomé island found that the Poilão rookery would contribute a maximum of 10% to São Tomé foraging aggregation, which contrasts with our maximum contribution of 40%. Without the knowledge of haplotype frequencies used in that study, it is not possible to hypothesize about the contrasting results, however using rookery size as an informative prior in our MSA, when the largest rookery is virtually fixed to the highly frequent and widespread haplotype CM-A8 in eastern Atlantic populations, including São Tomé and Príncipe's foraging aggregation (Patrício et al., 2017a) is likely to bias estimates.

The rookery-centric analysis of our data further suggests the position of the São Tomé and Príncipe's rookery as the primary source of juveniles foraging in São Tomé, which is consistent with the lack of genetic differentiation between the rookery and the foraging aggregation and the similar levels of genetic diversity found in both juveniles and females. Although these results seem to run parallel to the natal homing behavior and the "closest to home" hypothesis, according to which the immature turtles tend to settle in foraging aggregations closest to their natal home (Bowen and Karl, 2007), given the lack of comparisons outside the rookery and foraging aggregation at nuclear level, further work assessing nuclear gene flow via male dispersal, migration-mediated gene flow, or overlapping foraging grounds will be crucial to clarify this hypothesis. Indeed, evidence from our mtDNA analysis suggests that connectivity between São Tomé and Príncipe and South Western Atlantic foraging grounds is occurring. For example, the rare haplotype CM-A75 is shared between the foraging ground of Fernando de Noronha (Brazil) and both foraging aggregation and rookery of São Tomé and Príncipe. Moreover, the MSA also suggests that São Tomé and Príncipe rookery contributes, albeit only moderately, as a source population for Cassino Beach (Brazil). These evidences for transoceanic connectivity, namely between the Eastern and the South Western Atlantic, are consistent with results from previous studies based on mark-recapture and telemetry analysis (Pritchard, 1973; Luschi et al., 1998; Marcovaldi et al., 2000; Grossman et al., 2007), as well as other MSA studies (Naro-Maciel et al., 2006; Proietti et al., 2009; Monzón-Argüello et al., 2010; Patrício et al., 2017a). Furthermore, according to a study by Scott et al. (2017), dynamic oceanic conditions in the Gulf of Guinea result in seasonal dispersion variability driven by wind changes arising from the yearly north/southward migration of the intertropical convergence zone. This results in varying degrees of hatchling retention, with increasing westerly dispersion of hatchlings throughout the hatching season, with the majority of simulated hatchlings dispersing west into the South Atlantic Ocean with the South Equatorial Current. This pattern of dispersal in the Gulf of Guinea seems to gain additional support by the higher migration rates between São Tomé and Príncipe rookery and Brazil than with the Eastern Atlantic rookeries, as suggested by our analyses.

5. Conclusions and conservation implications

Understanding patterns of connectivity and dispersal among species with complex life cycles and exposure to multiple threats such as marine turtles, is crucial for prioritizing conservation and management measures. Assessing the genetic diversity of green turtles from the São Tomé and Príncipe archipelago through a combination of genetic markers, we showed that nesting and foraging turtles found on these islands exhibit relatively high levels of genetic diversity, representing an important genetic pool in the region. Moreover, the high genetic differentiation found between this archipelago's turtle population (both foraging and nesting) and others from the Atlantic suggests that this archipelago should be defined in the future an important conservation management unit. Although the use of a different type of genetic markers provided additional insight into our knowledge about the genetic structure of green turtle rookery and foraging aggregations in São Tomé and Príncipe, assessing small-scale patterns of connectivity at the regional level (e.g. Gulf of Guinea), and between ocean basis would benefit greatly by an increased effort in sampling other East African rookeries, and the use of nuclear markers, such as microsatellites.

Although important gaps persist in our knowledge about sea turtle ecology, it is well documented that ocean currents strongly affect the migratory behavior and dispersal pathways of this organisms (Luschi et al., 2003; Mansfield et al., 2017). While it is likely that the strong relationship found here between the rookery and foraging aggregation is linked to the effects of major oceanic currents, namely the Gulf and the South Equatorial currents (Luschi et al., 1998; Scott et al., 2017), further analyses based on satellite technologies and novel numerical simulation models (e.g. Briscoe et al., 2018) could be crucial to test whether the "closest to home" hypothesis (Bolker et al., 2007) fits the population dynamics of São Tomé and Príncipe.

Improving our knowledge on patterns of connectivity and demography of the species in this region, will ultimately lead to an

integrative and effective conservation and management plan.

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Declarations of Competing Interests

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jembe.2019.151181.

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