Regional Studies in Marine Science 55 (2022) 102530

Contents lists available at ScienceDirect

Regional Studies in Marine Science

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

Genetic monitoring of the critically endangered leatherback turtle (*Dermochelys coriacea*) in the South West Atlantic

Sarah Maria Vargas [a,b,*], Ana Carolina Barcelos [a], Rita Gomes Rocha [b,c,d], Paula Guimarães [a], Laís Amorim [a,b], Arturo Martinelli [a], Fabrício Rodrigues Santos [e], José Erickson [a,b], Ana Claudia Jorge Marcondes [f], Sandra Ludwig [a,b]

[a] *Laboratório de Genética e Evolução Molecular, Departamento de Ciências Biológicas, Universidade Federal do Espírito Santo, Av. Fernando Ferrari, 514, Vitória, ES, zip code 29075-910, Brazil*
[b] *Pós-Graduação em Biologia Animal, Universidade Federal do Espírito Santo, Av. Fernando Ferrari, 514, Vitória, ES, zip code 29075-910, Brazil*
[c] *CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, Universidade do Porto, Campus de Vairão, 4485-661 Vairão, Portugal*
[d] *BIOPOLIS Program in Genomics, Biodiversity and Land Planning, CIBIO, Campus de Vairão, 4485-661 Vairão, Portugal*
[e] *Departamento de Genética, Ecologia e Evolução, ICB, Universidade Federal de Minas Gerais, Avenida Antonio Carlos, 6627, 31280-010 Belo Horizonte, MG, Brazil*
[f] *Fundação Projeto Tamar, Rua Principal, s/n, Linhares, ES, zip code 29.914-050, Brazil*

ARTICLE INFO

Article history:
Received 21 January 2022
Received in revised form 31 May 2022
Accepted 2 July 2022
Available online 8 July 2022

Keywords:
Conservation genetics
Marine turtles
Microsatellites
Mitochondrial DNA
Population genetics

ABSTRACT

Although leatherback turtles (*Dermochelys coriacea*) are highly migratory and have a wide oceanic distribution, the South West Atlantic (SWA) subpopulation comprises few reproductive females and is listed as Critically Endangered. Herein, we present temporal genetic assessment of the poorly known subpopulation of the *D. coriacea* from SWA subpopulation. A total of 39 leatherback adult individuals were sampled during seven reproduction seasons along 160 km in the main nesting area in Brazil and analysed using D-loop mtDNA sequences (N = 37) and 25 microsatellite loci (N = 29). We detected genetic differences in temporal mtDNA analysis, possible explained by genetic drift. We also found two different genetic clusters with admixture between them for SWA subpopulation, indicating possible gene flow between different nesting areas. The SWA subpopulation presented low mean number of different alleles, moderate levels of observed and expected heterozygosities and lack of inbreeding. This pattern is possibly the consequence of an important amount of breeding occurrence abroad SWA nesting beach adjacencies, mating behaviours (such as polyandry and/or polygyny), and also some degree of inbreeding avoidance among reproductive individuals. The low remigration rates estimated for SWA subpopulation is possibly related to the high mortality rates at seas. A more comprehensive analysis on the patterns of genetic diversity, operational sex ratios, and inbreeding avoidance could help to inform about population resiliency and better understand the reproductive behaviour to perform recovery strategies and efficiently act to prevent the extinction of this isolated and remarkably threatened leatherback subpopulation from the Atlantic Ocean.

1. Introduction

The leatherback turtles (*Dermochelys coriacea*) are among the most threatened turtle species in the world. Although being highly migratory and presenting a wide oceanic distribution, the South West Atlantic (SWA) subpopulation of *D. coriacea* comprises few reproductive females and is listed as Critically Endangered by IUCN (Tiwari et al., 2013) and by the Brazilian Minister of the Environment (MMA/ICMBio-Brazil) (Almeida et al., 2018). The only regular nesting area for this subpopulation is in south-eastern Brazil around the Doce River mouth. Although an increasing but variable trend in the annual number of nests has been showed, only 143 nesting females have been recorded

* Corresponding author at: Laboratório de Genética e Evolução Molecular, Departamento de Ciências Biológicas, Universidade Federal do Espírito Santo, Av. Fernando Ferrari, 514, Vitória, ES, zip code 29075-910, Brazil.
E-mail addresses: sarah.vargas@ufes.br (S.M. Vargas), carol.barcelos.p@gmail.com (A.C. Barcelos), rgrocha@cibio.up.pt (R.G. Rocha), paularlguimaraes@gmail.com (P. Guimarães), laisamorim20@gmail.com (L. Amorim), martinelliab@gmail.com (A. Martinelli), fsantos@icb.ufmg.br (F.R. Santos), erickson.herpeto@gmail.com (J. Erickson), ana.marcondes@tamar.org.br (A.C.J. Marcondes), sand.ludwig@gmail.com (S. Ludwig).

https://doi.org/10.1016/j.rsma.2022.102530
2352-4855/

for this subpopulation from 1989 to 2017 (Colman et al., 2019). Furthermore, females have high levels of site fidelity to a beach or region (a behaviour known as natal philopatry), and distinct genetic signatures of populations (Dutton et al., 2013; Reid et al., 2019).

Population genetic studies have been characterized the genetic diversity of the SWA leatherback turtle subpopulation and the origin of the individuals found along the Brazilian coast, using the control region of the mitochondrial DNA (mtDNA) (Dutton et al., 1999, 2013; Vargas et al., 2008, 2019). The genetic diversity of the SWA subpopulation was similar to the other larger Atlantic populations (e.g.: Guiana and Trinidad — (Dutton et al., 2013), but the current interpretation associated with the genetic diversity resulted from these studies using only mtDNA are incomplete and may be biased depending if hatchlings were also used to estimate genetic diversity parameters. Thus, several studies have been implementing analysis with biparental inherited molecular markers such as microsatellites (Short Tandem Repeats, STR's) to improve the resolution of the diversity estimates and to produce relevant information about mating systems (Lasala et al., 2018), kinship (Blouin, 2003; Levasseur, 2019), inbreeding (Phillips et al., 2017), the origin of turtles caught as bycatch (Stewart et al., 2016), genetic fingerprinting (genetic-ID; (Roden et al., 2017)) and reproductive success (Yuan et al., 2019). Until now, only one study used STR's to evaluate the genetic structure among subpopulations along the Atlantic Ocean, showing that the SWA subpopulation is genetically isolated from the others Northern, Central, and Eastern Atlantic subpopulations (Dutton et al., 2013). But the genetic diversity of the SWA subpopulation based on STR's was not discussed; neither additional genetic fingerprinting evaluation was performed.

In Brazil, monitoring and capture–mark–recapture programmes throughout the reproductive seasons are based on the identification of individual nesting females during oviposition with external flipper iconel tags (Colman et al., 2019). However, there are high rates of external flipper tag loss in the leatherback turtles (Witt et al., 2011; Garner et al., 2017; Hart et al., 2021), hampering the identification and estimation of the remigration rates for females (6.3% — (Colman et al., 2019)). In fact, there is a chance that a nesting female caught without tags might not be a new verified female (new recruit), but a remigrant that lost her tag (Santidrián Tomillo et al., 2007). Thus, the genetic fingerprinting (genetic-ID) using STR's is a very powerful tool to complement the individual's identification during fieldwork (Roden et al., 2017). This molecular approach allows the identification of remigrant females by finding duplicate genotypes within genetic databases and can also be used to indirectly estimate the age of maturity for marine turtles (Dutton et al., 2005).

There is a knowledge gap about the temporal distributions of leatherbacks turtles in the SWA, the genetic diversity and the genetic structure of this population are not well understood. Thus, this study aims to: (1) use a molecular identification method (genetic fingerprint) to implement a complementary technique for individual identification (genetic-ID); (2) estimate the genetic diversity and structure using biparental inherited microsatellites (STR's) loci, compare with mtDNA data and with studies from other Atlantic subpopulations; (3) estimate effective population size, and (4) tentatively remigration rates.

2. Material and methods

2.1. Ethic statement

The research was conducted in Brazil and specimens were sampled and manipulated minimizing animal suffering when obtaining a tissue sample for genetic analyses. Sampling was conducted by Fundação Projeto Tamar under SISBIO licences numbers #42760, and #65543-8. The collected tissues were deposited in the Scientific collection of Laboratório de Genética e Evolução Molecular of the Universidade Federal do Espírito Santo under a specific code for each sample (Table S1).

2.2. Study area

The studied area comprises more than 150 km of the northern coast of the state of the Espírito Santo in Brazil (Fig. 1). This area has been recognized as the only regular nesting hotspot of *D. coriacea* in Brazil (Almeida et al., 2018) based on historical monitoring of females and nests by Projeto TAMAR since 1982 (Baptistotte et al., 2003) with a discreet increasing number of females over the years (Colman et al., 2019).

2.3. Sampling

During seven reproductive seasons (2004/05, 2008/09, 2009/10, 2011/12, 2018/19, 2019/20 and 2020/21 - Table S1), occurring annually between October and March, females were spotted during night monitoring after leaving the ocean to nest, especially along the Povoação and Regência beaches, but also northward until Itaúnas, Espírito Santo State (Fig. 1). Once oviposition was complete and while the turtle was covering her nest, we collected tissue samples from the proximal region of the females' anterior flipper with 6 mm punches and stored them in 95% alcohol tubes. Carapace measurements of each female were taken (Bolten, 1999), including curved carapace length (CCL), and flipper tags were newly placed or recorded in the case of remigrant. Occasional samples of animals found dead at the beach were also collected using scalpel and stored in 95% alcohol tubes (Table S1). A total of 39 samples were collected, including four dead males, two dead females, and 33 nesting females. Three out of 35 females were remigrants and all others were identified for the first time, with CCL ranging from 132 to 179.5 cm (Table S1). The number of females sampled for this study ($N = 35$) represents more than 20% of the total females tagged by Fundação Projeto TAMAR since 1999 ($N = 172$ — Fundação Projeto TAMAR personal communication) and our recent set of samples (animals sampled within the last three seasons studied) corresponds to 100% of the females tagged in 2018/19, 2019/20 and 2020/21 seasons.

2.4. Sequencing and genotyping

Genomic DNA (gDNA) was isolated from the tissues using the DNA salt extraction protocol (SDS/NaCl/Proteinase K) described by Bruford et al. (1992). To verify the conditions of the gDNA obtained, a subset was stained with Blue Green® dye (LGC Biotechnology) and visualized on 1% agarose gel under ultraviolet light. The extracted gDNA was further quantified in NanoDropND-100 spectrophotometer (Thermo Scientific) and concentration was standardized in 50 ng/μl.

The amplification of the mitochondrial control region (D-loop) was performed using the primers LCM 15382 and H950 (Abreu-Grobois et al., 2006), and HDCM1 (Allard et al., 1994). Polymerase chain reactions (PCRs) were performed as described by Vargas et al. (2019) and PCR products were processed and sequenced as previously described by Lara-Ruiz et al. (2006). D-loop sequences were checked for quality, then assembled and a consensus was generated using the software Geneious R11.1.5 (Kearse et al., 2012). The consensus sequences were aligned using the MUSCLE algorithm implemented in Geneious.

We also amplified 25 STR's loci: C102, D1 (Dutton and Frey, 2009), DERM05, DERM10, DERM11, DERM15, DERM18, DERM22, DERM32, DERM37, DERM38, DERM39, DERM43, DERM48 (Alstad et al., 2011) and 14-5, LB99, LB110, LB123, LB125, LB128, LB133,

Table 1
Genetic diversity of mitochondrial DNA of *Dermochelys coriacea* of the South West Atlantic subpopulation, and comparison with previous study.

D-loop	N	Haplotypes (f)	H	*h* (SD)	π (SD)	References
1992/93–2003/04	23	Dc1.1 (9) Dc3.1 (14)	2	0.498 (0.053)	0.0032 (0.002)	Dutton et al. (2013)
2004/05–2020/21*	33	Dc1.1 (22) Dc3.1 (9) Dc13.1 (2)	3	0.492 (0.075)	0.0029 (0.0018)	Vargas et al. (2019) and this study
1992/93–2020/21	56	Dc1.1 (31) Dc3.1 (23) Dc13.1 (2)	3	0.533 (0.0317)	0.0039 (0.0023)	Overall

N: sampling number; (f): frequency of haplotypes; H: number of haplotypes; *h*: haplotype diversity; SD: standard deviation; π: nucleotide diversity; * dataset considering only females.

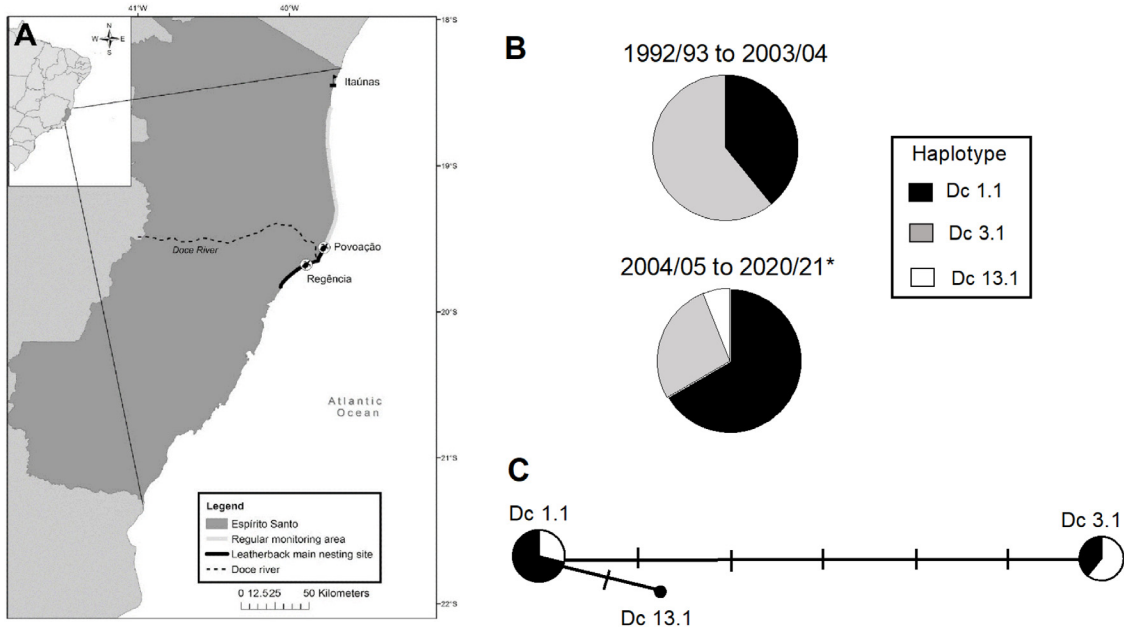

Fig. 1. (A) Locations along the coast of state of Espírito Santo, Brazil, where leatherback marine turtles monitoring was carried out and samples were collected between 2004/05 and 2020/21 nesting seasons, (B) mtDNA D-loop haplotypes frequencies found for two different periods: from 1992/93 to 2003/04 ($N = 23$ - Dutton et al. (2013)) and from 2004/05 to 2020/21 nesting seasons ($N = 33$ — Vargas et al. (2019) and this study) . (C) Haplotype network showing the relationships among the three haplotypes found for the SWA subpopulation and their frequencies reported in the two periods: white for 1992/93 to 2003/04 — Dutton et al. (2013) and black for 2004/05 to 2020/21 nesting seasons — Vargas et al. (2019) and this study. See Table 1 and Table S1 for details. *Only females' samples were considered.

LB141, LB142, LB145, LB157 (Roden and Dutton, 2011) (Supplementary Table S2). These 25 markers have similar fragment sizes, so five different fluorescent markers were used to differentiate between markers during peak analysis (Supplementary Table S2). PCRs were conducted individually for each locus in a total volume of 12.5 μl, including 1x buffer (200 mM Tris HCl (pH 8.4), 500 mM KCl) Invitrogen®, 1.5 mM $MgCl_2$ Invitrogen®, 0.2 mM DNTPs, 0.16 μM for each primer (forward and reverse), 0.16 μM fluorescent marker, 0.5 U of Taq platinum Invitrogen® and 1 μl of DNA (50 ng/μl). The PCR products were genotyped in multiplex reactions (two to five different PCRs products in each multiplex — Supplementary Table S2) using a mix containing 7.0 μl of formamide, 0.5 μl fluorescent molecular Size Standard, and 0.5 μl of the amplified products of each of the five markers were prepared and separated by electrophoresis with ABI Pris 3700 Automatic Sequencer (Thermo Fisher Scientific) using GeneScan ™ 600 LIZ ™ Size Standard v2.0 (Applied Biosystems©). The amplified microsatellite loci were visualized, and their alleles were scored and measured using Geneious.

2.5. Mitochondrial haplotype assignment, genetic diversity and temporal genetic structure

Individual mtDNA haplotypes were assigned using DnaSP v.6 (Rozas et al., 2017), comparing the newly obtained sequences with haplotypes from previous studies (Dutton et al., 2013; Vargas et al., 2019).

We estimated mtDNA haplotype (*h*) and nucleotide (π) diversities using the software Arlequin 3.5.2. (Excoffier and Lischer 2015). Only females were used to estimate genetic diversity for samples collected after 2003/04 nesting season. Pairwise F_{ST} comparisons were conducted to test temporal genetic structure. For this we considered three datasets: samples collected between 1992/93 and 2003/04 nesting seasons (Dutton et al., 2013), female's samples collected between bet 2004/05 and 2020/21 nesting seasons (this study) and the compiled dataset including samples collected along the 28 years' period. A haplotype network was built based on mutation steps to evaluate haplotype relationships, using the Median Joining algorithm implemented in the Network (Bandelt et al., 1999).

The haplotype diversity of the SWA population of *D. coriacea* was compared with previous studies of this species and other sea turtle species (see Supplementary Table S3).

Genetic identification using nuclear data

To avoid pseudoreplication owing to individuals that may have lost their tag, we checked for identical genotypes in GenAlex 6.5 (Peakall and Smouse, 2012). Remigrants, i.e., the same individual captured in different nesting seasons, were only considered once for the subsequent analysis. The probability of identity (PI), i.e., the likelihood that two unrelated random samples will have the exact genotype, and the probability of exclusion (PE), i.e., the proportion of the population that has a genotype that contains at least one allele not present in the mixed profile, were estimated using GenAlex 6.5 (Peakall and Smouse, 2012).

Microsatellite diversity, structure and effective population size

The 25 STR's dataset was evaluated for deviations from Hardy–Weinberg equilibrium (HWE) using GenAlex 6.5 (Peakall and Smouse, 2012), and loci with significant departure from expectations after Bonferroni correction were excluded from the subsequent analysis. The R package PopGenReport was used to analyse the presence of null alleles (Ihaka and Gentleman, 1996), PopGenReport — (Gruber and Adamack, 2015). The linkage disequilibrium (LD) between pairs of loci was calculated using Arlequin 3.5 (Excoffier and Lischer, 2010).

Genetic diversity based on females only, including the observed (H_O) and expected heterozygosity (H_E), mean number of effective alleles (Ne), fixation index (F), and private alleles (P_A) was estimated using the GenAlex 6.5 (Peakall and Smouse, 2012). Inbreeding coefficient (F_{IS}) was estimated using Arlequin 3.5 (Excoffier and Lischer, 2010).

Mean values of genetic diversity of the *D. coriacea* SWA subpopulation, from other subpopulations of this species and other sea turtle species are shown in Supplementary Table S3. As the set of nuclear loci used in each of these studies are different, we suggest caution in direct comparisons among them.

Population structure was evaluated using Bayesian clustering approach implemented in Structure 2.3.4 (Falush et al., 2003). Analyses implemented in Structure were performed independently 10 times for 10^6 iterations after a burn-in period of 5×10^5 iterations, using the admixture model with correlated allele frequencies among populations. We tested 1 to 6 clusters (K) without prior population information. Structure Harvester (Earl and VonHoldt, 2012) was used to summarize the posterior probabilities of each K over all runs (Evanno et al., 2005). CLUMPAK (Kopelman et al., 2015) was used to summarize and graphically represent the results of Structure.

Population structure was also explored using Discriminant Analysis of Principal Components (DAPC). K-means clustering of principal components for $K = 1$ to $K = 6$ and Bayesian Information Criteria (BICs) were used to assess the optimal number of genetic clusters. The value of K with the lowest BIC value was considered optimal. DAPC was applied using the Adegenet package 2.1.6 in R (Jombart, 2008)

Effective female population size was estimated following the Linkage Disequilibrium Method implemented in NeEstimator v2.1 (Do et al., 2014) with a threshold allele frequency of 0.02 for screening out rare alleles, assuming random mating and calculating 95% confidence interval by a Jackknife-across-samples methods (Jones et al., 2016). Only adult females were used in this analysis.

3. Results

3.1. Genetic-ID and remigration

We successfully genotyped STR's for 29 females and 1 male, corresponding to 30 individual genotypes (Supplementary Table S1).

The probability of identity test (PI) showed that the set of microsatellite markers used in this study was highly sensitive for sample individualization. The combined probability of identity for the 22 loci analysed (please see below information on the curated dataset) was extremally low (PI: 2.8×10^{-20}) and probability of exclusion was high (PE = 1.0). We did not identify clones (samples with identical multilocus genotype - MLG) among samples, showing that there are no duplicates among the individuals collected for the studied seasons (Supplementary Table S1).

We identified three remigrant females: R0647 firstly detected in 2006 and then in 2008; R0651 detected in 2006 and 2009; and SMV285 detected in 2013 and 2018 (Supplementary Table S1). According to our current genetic database, there is no evidence of more than those three remigrants found by the capture–mark–recapture programme of the Fundação Projeto TAMAR during the seven studied seasons, because no clones (duplicates) were found among the analysed samples using STR's (Supplementary Table S1).

Genetic diversity

We successfully obtained 37 mtDNA sequences (33 from females and 4 from males), corresponding to three D-loop haplotypes previously identified for the *D. coriacea* (Dutton et al., 2013). The haplotype Dc1.1 was the most frequent (24 individuals — 64.86%), followed by haplotypes Dc3.1 (11 individuals — 29.73%) and Dc13.1 (2 individuals — 5.40%) (Supplementary Table S1). Considering only female samples, the haplotype and nucleotide diversities observed were similar to other study of *D. coriacea,* but there was an inversion in the most frequent haplotype found for both studies (Dutton et al., 2013) — Table 1). The Dc1.1 haplotype diverged from Dc3.1 by five steps (Fig. 1. The Dc13.1 diverged by only one mutation step from Dc1.1 and was detected in one remigrant female (R0651) and one female from 2019 (SMV718) (Supplementary Table S1).

Three out of 25 loci showed deviation from HWE after Bonferroni correction (Supplementary Table S2), and were removed from the subsequent analysis. No loci showed excess of null alleles and no significant deviations from LD were observed for any pair of loci. Further analysis were performed with a dataset of 22 loci (Table 2). All STR's loci were polymorphic, with a total of 146 alleles among all 22 loci, with the mean number of alleles per locus of 6.636, ranging from 3 (loci LB141, LB157, LB123, C102 and DERM15) to 14 (locus D1), and maximum of 16% of missing data (2 out of 22 loci).

The SWA leatherback subpopulation showed an observed and expected heterozygosities of 0.661 (standard error, SE, 0.049) and 0.650 (SE = 0.044), respectively, and 12 loci showed an excess of heterozygosity (Ho > He) consequently showing negative values of fixation index (Table S2). But no evidence of inbreeding for the overall subpopulation is record (F_{IS} = 0.001, $p = 0.44$).

Genetic structure and effective population size

Pairwise F_{ST} considering the samples collected between 1992/93 and 2003/04 (Dutton et al., 2013) and female's samples collected between 2004/05 and 2020/21 nesting seasons (Vargas et al. (2019) and this study) showed significant differences (F_{ST} = 0.1361, p = 0.009), but pairwise comparisons between samples collected previously and from Vargas et al. (2019) plus this study, with the compiled dataset showed nonsignificant differences (F_{ST} = 0.0321 and 0.0072; p > 0.05, respectively).

Nuclear DNA supports two genetic clusters ($k = 2$) for the SWA leatherback subpopulation, following multiple approaches (Fig. 2). Twenty-two adult individuals (73.3%) belong to the most common cluster (cluster 1), including the male SMV2698 collected in 2020 (Fig. 2B), six (20%) belong to the other cluster (cluster 2), and two individuals (6.6%) showed admixture between both clusters (Fig. 2B), including one individual (SMV718) bearing the Dc13.1 haplotype exclusively found previously only in

Table 2
Genetic diversity of nuclear DNA of the South West Atlantic *Dermochelys coriacea* subpopulation sampled during the nesting seasons of 2004/05, 2018/19, 2019/20 and 2020/21, considering the curated dataset with 22 microsatellites loci (see Table S2 for details).

	N	Na	Ne	Pa	Ho	He	F
2004/05	2	2.18	2.000	3	0.659	0.415	−0.584
2018/19	7	4.32	3.432	14	0.624	0.601	−0.012
2019/20	6	4.41	3.217	12	0.698	0.605	−0.166
2020/21	14	5.00	3.767	19	0.667	0.634	−0.052
2020/21*	15	5.09	3.783	20	0.636	0.658	−0.028
Overall females	29	6.59	4.001	NA	0.669	0.649	−0.029
Overall adults*	30	6.64	4.035	NA	0.650	0.661	−0.016

* Male data included. N: sampling number; Na: mean number of different alleles; Ne: mean number of effective alleles; Pa: number of private alleles; Ho: mean observed heterozygosity; He: mean expected heterozygosity; F: mean number of fixation index. NA: not available.

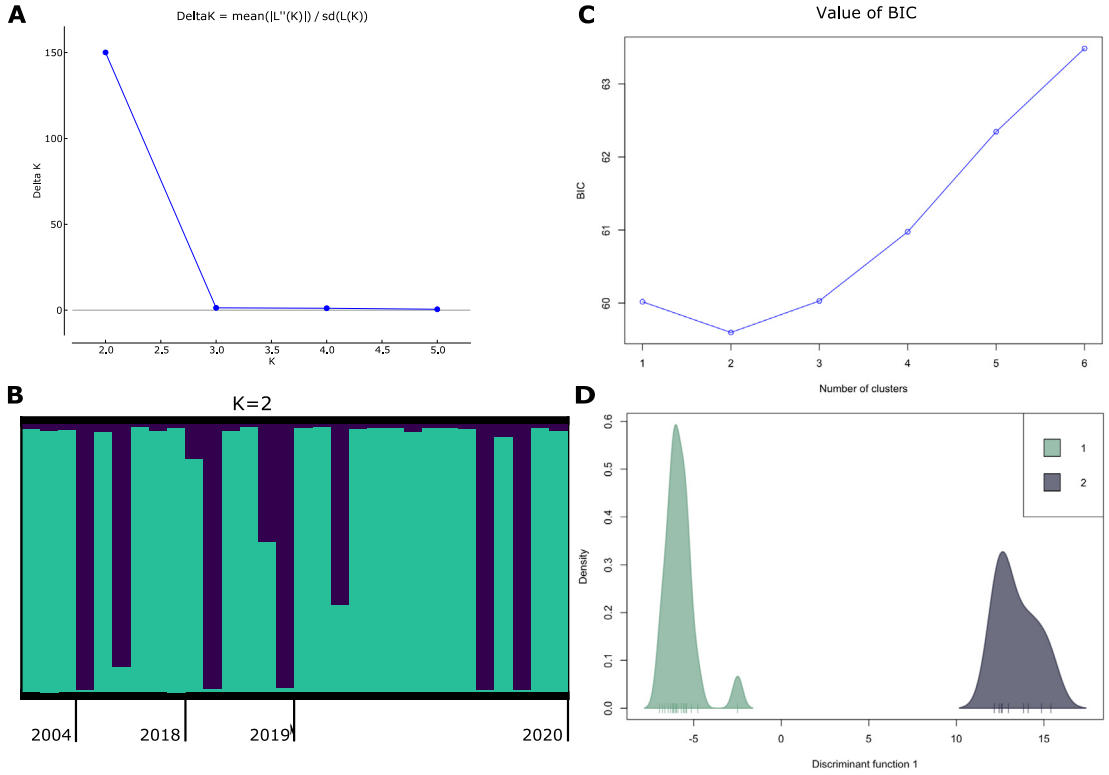

Fig. 2. (A) Plot of Delta K as summarized through Structure Harvester for K ranging from 1 to 6, supporting the model of two genetic clusters as indicated by the maximum value of Delta K. (B) Plot bars showing assignment of adults Southwest Atlantic *Dermochelys coriacea* based on a Bayesian clustering analysis performed using an Admixture Model assuming $K = 2$. Vertical bars represent individuals sampled between 2004/05 and 2020/21 nesting seasons, individuals are grouped according to their sampling date, and colours within each bar correspond to the two genetic clusters. (C) Plot of Bayesian Information Criterion (BIC) for K ranging from 1 to 6 as obtained through adegenet, supporting the model of two genetic clusters as indicated by the lowest BIC value. (D) Density plot of Discriminant analysis of Principal Components (DAPC) highlighting clustering among 30 individuals, assuming $K = 2$ and using the first discriminant function.

African nesting locations. Assignment probabilities of individuals from cluster 1 averaged 0.98 and from cluster 2 averaged 0.97. The DAPC clustering analysis showed similar results to the Structure analysis, although no overlap between clusters is observed (Fig. 2C and D). Admixed individuals are assigned to cluster 2 in the DAPC analysis.

The effective female population size estimated for SWA leatherback turtles is 27.5 (95% CI = 16.5–57.8).

4. Discussion

The SWA leatherback subpopulation is poorly known, with few studies evaluating their genetic diversity and population structure solely based on mtDNA data (e.g. Dutton et al., 1999, 2013; Vargas et al., 2008, 2013, 2019), and only one with STR's (Dutton et al., 2013). Therefore, our multi-year evaluation using both inheritance data (mtDNA and STR's) for the leatherback females of 2004, 2018, 2019 and 2020 allowed us to determine low diversity, some degree of genetic structure, low remigration rates and a putative male philopatry for this subpopulation.

Our results show that the set of molecular markers chosen was highly effective to identify individuals and monitor the long-term genetic diversity of the SWA critically endangered leatherback subpopulation. The combined probability of identity (2.8×10^{-20}) found for SWA leatherbacks was higher than other studies performed for critically endangered marine turtles (i.e., Levausser 2019) and reflects the power of our genetic data set to be used as auxiliary tools for long-term fieldwork, ecological, and monitoring studies.

For mtDNA, the SWA leatherback subpopulation has one of the highest values of genetic diversity among the subpopulations from the Atlantic Ocean (Dutton et al., 2013; Vargas et al., 2019).

Likewise, the genetic diversity found in this study is similar to the values found for larger Atlantic populations, such as Trinidad and Gabon (Dutton et al., 2013); Supplementary Table S3) and much higher than for other small and isolated populations as Dominica Republic (Carreras et al., 2013); Supplementary Table S3) and South Africa ((Dutton et al., 2013); Supplementary Table S3). The haplotype diversity of leatherback subpopulation is also similar to other endangered species from the Brazilian coast, such as *C. caretta* (Ludwig et al. *submitted*; Supplementary Table S3).

The most frequent haplotype found in the SWA subpopulation was Dc1.1, followed by the Dc3.1 and Dc13.1 (Table 1). The Dc13.1 was previously found only in African nesting sites from Gabon and Ghana and was not found in Brazilian nesting area beforehand (Dutton et al., 2013). Our results contrast with previous study of this subpopulation that did not recovered Dc13.1 haplotype and found different haplotype frequencies (Dutton et al., 2013). In the absence of a minimum number of nesting female samples (about $N = 15$), samples of hatchlings and embryos (randomly collected) were considered to calculate the diversity values (Dutton et al., 2013), which may be reflecting this contrasting result. This practice is very common in studies regarding endangered species like freshwater turtle (Erickson et al., 2015; Viana et al., 2017; Oliveira et al., 2019); consequently, in some cases it could bias the results, especially when the information regarding the relatedness of the individuals is scarce. Another possibility to explain this temporal shift in haplotypes frequencies and consequently genetic differences between the two subsets of samples is the genetic drift. As the populations size for SWA leatherback subpopulation is small and the period sampled was long (28 years), genetic drift might have played a role in the genetic differentiation found when the two periods (from 1992/93 to 2003/04 and from 2004/05 and 2020/21) were compared. This finding gives an important message to the marine biologist in dealing with datasets from multiple years: genetic differences along years can be hidden in compiled datasets, and overall genetic diversity and structure values may not reflect recent scenarios, especially for populations with small population sizes, that are more influenced by genetic drift (Frankham et al., 2010).

We found low mean number of different alleles and moderate levels of observed and expected heterozygosity for the leatherback turtle SWA subpopulation, which is expected for a population with small size (Frankham et al., 2010). Notwithstanding the increasing trend in the annual number of nests between 1990 and 2015 (ranging from 25.6 nests in 1988–1992 to 89.8 in 2013–2017) and the increasing numbers of females nesting per year (between 15 and 18) (Colman et al., 2019), the SWA subpopulation has the lowest number of nests and females when comparing with others subpopulations from the North Atlantic (Colman et al., 2019; Dutton et al., 2005).

No inbreeding was detected for the leatherbacks females from SWA subpopulation, which might be associated with broad migratory behaviour of the species (Fossette et al., 2014), enabling finding a reproductive partner from different population sources and also with the small effective populations size (as shown by our results of effective female population size and by (Colman et al., 2019), reducing the chances of encounters among males and females from the same SWA source subpopulation. Mating strategies like polyandry (Lasala et al., 2018), and/or inbreeding avoidance already reported for the endangered gopher tortoise (Yuan et al., 2019) can also be accounted to explain negative inbreeding coefficient values.

Although we detected population structure using D-loop mtDNA only when comparing the two subsets of samples collected along the 28-year period, nuclear data supports two clusters with admixture between them within the more recent subset of samples (collected from 2004/05 to 2020/21). While tentatively, this structure may reflect different family lineages and/or different genetic pools contributing to the differentiation of the SWA leatherback subpopulation. In one side, our dataset is possibly comprised by related individuals and this may inflate k values (Pritchard et al., 2000). But on the other side, behaviour patterns may also explain the observed genetic structure. Tag, telemetry and genetic studies have been showing migration patterns of individuals from African nesting locations to the Brazilian waters, or contrariwise (Almeida et al., 2014; Billes et al., 2006; Fossette et al., 2014; Vargas et al., 2019). This behaviour agrees with the evidence of two genetic clusters among SWA leatherbacks, including the presence of two admixed females, one bearing the Dc13.1 haplotype (SMV718), previously found only in Gabon and Ghana, Africa (Dutton et al., 2013). Samples from African nesting sites and more samples of SWA females are needed to further investigate these hypotheses.

Furthermore, the low rate of remigrants observed in the SWA leatherback turtles may be associated with high levels of fisheries pressures and bycatch, both in Brazilian (Fiedler et al., 2012) and international waters (Shamblin et al., 2014; Sales et al., 2008). There is some evidence showing that leatherbacks migrate from Brazilian nesting beaches to Africa, especially towards some areas near the coastline of Namíbia and Angola (Almeida et al., 2011, 2014). During these migrations the leatherback turtles are potentially exposed to areas with strong longline fishing pressure, as highlighted for some authors (Honig et al., 2008; Fossette et al., 2014), increasing the probability to be caught. Thus, the SWA subpopulation is categorized as highly bycatch by global studies (Wallace et al., 2010, 2013), demanding urgent conservation actions, mainly towards the reduction of fishing bycatch. In addition, at the Brazilian coast, particularly at the Espírito Santo coast, anthropic actions such as interaction with fisheries, photopollution, and habitat loss have also been threatened this population (Magris et al., 2019). Furthermore, the extreme habitat degradation caused by the Fundão mining dam, which occurred in November 2015 in the city of Mariana, Minas Gerais (Marta-Almeida et al., 2016), could also affect the leatherback turtles in those areas, and consequently impact the population size of this threatened species. Thus, the loss of genetic health and consequently reduction of population size could prompt the species to an extinction vortex (Frankham et al., 2010).

Finally, the occurrence of one dead male in the beginning of the 2020/21 nesting season belonging to the same genetic cluster of the SWA females from 2020, raise important questions: Is the phylopatric behaviour a rule or an exception for SWA leatherback males? Did this male contribute to this reproductive season? But additional male and hatchling samples are need to answer these questions.

5. Conclusions

Overall, this genetic dataset is useful to better understand the current remigration rates and the origin of individuals from feeding areas or stranded and to improve conservation actions for the SWA subpopulation. This will aid the continuous monitoring programme and conservation actions running since 1980's (the Projeto TAMAR — The Brazilian Sea Turtle Conservation Programme) along leatherback turtles main nesting area in the Brazilian coast (Thomé et al., 2007; Colman et al., 2019). With continuing sampling and genotyping efforts to update this molecular database, in the near future, even if one female were found without flipper tags, we will be able to know if she is a remigrant or a recruit. This methodology is a great asset to better understand the remigration rates because flipper tag loss is common among leatherbacks (Garner et al., 2017), and particularly in the study area this loss is sometimes reported.

A more comprehensive analysis about the patterns of genetic diversity for both genders (indirect for breeder's males and direct for females), operational sex ratios, relatedness, inbreeding avoidance and reproductive fitness will help us to better understand the population resiliency, their reproductive behaviour and the offspring quality to effectively act to prevent the extinction of this threatened and isolated subpopulation from the Atlantic Ocean. Hence, the assessment of genetic diversity patterns becomes essential to guide biodiversity conservation policies, particularly for threatened species. For the SWA leatherback subpopulation, the implementation of in water conservation and the following surveillance of the on-going management actions must be strengthened, otherwise, more than four decades of females and nests protection, environmental education, and other local community conservation actions might be counterweighted by the deceased of adult individuals in open seas.

CRediT authorship contribution statement

Sarah Maria Vargas: Conceived the study, Wrote the manuscript, Obtained funding, Conducted bioinformatics, Data analyses. **Ana Carolina Barcelos:** Laboratory work. **Rita Gomes Rocha:** Conducted bioinformatics, Data analyses, Wrote the manuscript. **Paula Guimarães:** Provided tissue samples, Logistic support in the field. **Laís Amorim:** Laboratory work. **Arturo Martinelli:** Laboratory work. **Fabrício Rodrigues Santos:** Obtained funding, Provided tissue samples. **José Erickson:** Laboratory work. **Ana Claudia Jorge Marcondes:** Obtained funding, Provided tissue samples, Logistic support in the field. **Sandra Ludwig:** Conducted bioinformatics, Data analyses.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The present study was carried out as part of the Aquatic Biodiversity Monitoring Programme – Ambiental Area I – established by the Technical-Scientific Agreement (DOU number 30/2018) between FEST and Renova Foundation. We thank FEST for the scholarships of Sarah Vargas, Ana Carolina Barcelos, Arturo Martineli, José Erickson, Sandra Ludwig. Rita Gomes Rocha was supported by research contract (CEECIND/01087/2018) from the Portuguese Foundation for Science and Technology (FCT) - Portugal. This study was supported in part by the IBAMA/ICMBio - Brazil, the Projeto TAMAR Foundation - Brazil, CAPES - Brazil, ANA - Brazil, FAPES - Brazil, CNPq - Brazil, FAPEMIG - Brazil (APQ03625-17) and the Federal University of Espírito Santo - Brazil. We thank Juliana Justino, Monique Nascimento, Angelica Bolzan, and the Núcleo de Genética aplicada à Conservação da Biodiversidade for technical support. We thank the two reviewers for valuable comments on the previous version of this manuscript. We thank Tommy Magalhães, Roberto Garcia and Projeto TAMAR fieldwork team for samples collection. We also thank Bruno Giffoni, Luciana Medeiros, Alexandro Santos and Cecilia Baptistotte for helpful comments on the manuscript.

Funding sources

This research was financially supported by Renova Foundation - Brazil via its Technical-Scientific Cooperation Agreement n° 30/2018 with FEST - Brazil and to CAPES-FAPEMIG-FAPES-CNPq-ANA grant APQ03625-17 referred to in the Acknowledgements.

Appendix A. Supplementary data

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.rsma.2022.102530.

References

Abreu-Grobois, A., Horrocks, J., Formia, A., Dutton, P., LeRoux, R., Vélez-Zuazo, X., Soares, L., Meylan, P., 2006. New mtDNA Dloop primers which work for a variety of marine turtle species may increase the resolution of mixed stock analysis. In: Proceedings of the 26th Annual Symposium on Sea Turtle Biology. ISTS, Island of Crete, Greece, p. 179.

Allard, M.W., Miyamoto, M.M., Bjorndal, K.A., Bolten, A.B., Bowen, B.W., 1994. Support for natal homing in green turtles from mitochondrial DNA sequence. Copeia 1, 34–41.

Almeida, A.P., Eckert, S.A., Bruno, S.C., Scalfoni, J.T., Giffoni, B., López-Mendilaharsu, M., Thomé, J.C., 2011. Satellite-tracked movements of female leatherbacks, *Dermochelys coriacea* (Testudines, Dermochelyidae), from an endangered nesting population in Southeastern Brazil. Endanger. Species Res. 15, 77–86.

Almeida, A.D.P., Filgueiras, H., Braby, R., Tiwari, M., 2014. Increasing evidence of leatherback migrations from brazilian beaches to the West African Coastline. Afr. Sea Turt. News 1, 9–14.

Almeida, A.P.L.S., Thomé, J.C.A., Baptistotte, C., Marcovaldi, M.A., dos Santos, A.S., Lopez-Mendilaharsu, M., 2018. *Dermochelys coriacea* (Vandeli, 1761). In: Instituto Chico Mendes de Conservação da Biodiversidade (Org.). Livro Vermelho da Fauna Brasileira Ameaçada de Extinção: Volume IV - Répteis. ICMBio, Brasília, pp. 42–46.

Alstad, T.I., Shamblin, B.M., Bagley, D.A., Ehrhart, L.M., Nairn, C.J., 2011. Isolation and characterization of tetranucleotide microsatellites from the leatherback turtle (*Dermochelys coriacea*). Conservation Genet Resour 3, 457–460.

Bandelt, H.J., Forster, P., Röhl, A., 1999. Median-joining networks for inferring intraspecific phylogenies. Mol. Biol. Evol. 16, 37–48.

Baptistotte, C., Thomé, J.C.A., Bjorndal, K.A., 2003. Reproductive biology and conservation status of the loggerhead sea turtle (*Caretta caretta*) in Espírito Santo State, Brazil. Chelonian Conserv. Biol. 4, 523–529.

Billes, A., Fretey, J., Verhage, B., Huijbregts, B., Giffoni, B., Prosdocimi, L., Albareda, D., Georges, J., Tiwaru, M., 2006. First evidence of leatherback movement from Africa to South America. Mar. Turt. Newsl. 111, 13–14.

Blouin, M.S., 2003. DNA-based methods for pedigree reconstruction and kinship analysis in natural populations. Trends Ecol. Evol. 18, 503–511.

Bolten, A.B., 1999. Techniques for measuring sea turtles. In: Eckert, K.L., Bjorndal, K.A., Abreu-Grobois, F.A., Donnelly, M. (Eds.), Research and Management Techniques for the Conservation of Sea Turtles. IUCN/SSC Marine Turtle Specialist Group Publication No. 4.

Bruford, M.W., Hanotte, O., Brookfield, J.F.Y., Burke, T., 1992. Single-locus and multilocus DNA fingerprinting. In: Hoelzel, A.R. (Ed.), Molecular Genetic Analysis of Populations: A Practical Approach. pp. 225–269.

Carreras, C., Godley, B.J., Leon, Y.M., Hawkes, L.A., Revuelta, O., Raga, J.A., Tomas, J., 2013. Contextualising the last survivors: population structure of marine turtles in the Dominican Republic. PLoS One 8, e66037.

Colman, L.P., Themé, J.C.A., Almeida, P., Baptistotte, C., Barata, P.C.R., Brederick, A.C., Ribeiro, F.A., Vila-Verde, L., Godley, B.J., 2019. Thirty years of leatherback turtle dermochelys coriacea nesting in Espírito Santo, Brazil, 1988–2017: reproductive biology and conservation. Endanger. Species Res. 39, 147–158.

Do, C., Waples, R.S., Peel, D., Macbeth, G.M., Tillett, B.J., Ovenden, J.R., 2014. NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. Mol. Ecol. Resour. 14, 209–214.

Dutton, P.H., Bowen, B.W., Owens, D.W., Barragan, A., Davis, S.K., 1999. Global phylogeography of the leatherback turtle (*Dermochelys coriacea*). J. Zool. 48, 397–409.

Dutton, D.L., Dutton, P.H., Chaloupka, M., Boulon, R.H., 2005. Increase of a Caribbean leatherback turtle *Dermochelys coriacea* nesting population linked to long-term nest protection. Biol. Cons. 126, 186–194.

Dutton, P.H., Frey, A., 2009. Characterization of polymorphic microsatellite markers for the green turtle (*Chelonia mydas*). Mol. Ecol. Resour. 9, 354–356.

Dutton, P.H., Roden, S.E., Stewart, K.R., LaCasella, E., Tiwari, M., Formia, A., Thomé, J.C., Livingstone, S.R., Eckert, E., Chacon-Chaverri, D., Rivalan, P., Allman, P., 2013. Population stock structure of leatherback turtles (*Dermochelys coriacea*) in the Atlantic revealed using mtDNA and microsatellite markers. Conserv. Genet. 14, 625–636.

Earl, D.A., VonHoldt, B.M., 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv. Genet. Resour. 4, 359–361.

Erickson, J., Oliveira, D.P., Marioni, B., Farias, I.P., 2015. Analysis of the mating system of *Podocnemis sextuberculata* in the lower Purus River of the Brazilian Amazon: another record of multiple paternity in chelonians. Salamandra 51, 215–220.

Evanno, G., Regnaut, S., Goudet, J., 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. 14, 2611–2620.

Excoffier, L., Lischer, H.E., 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol. Ecol. Resour. 10, 564–567.

Falush, D., Stephens, M., Pritchard, J.K., 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164, 1567–1587. http://dx.doi.org/10.3410/f.1015548.197423.

Fiedler, F.N., Sales, G., Giffoni, B.B., Monteiro-Filho, E.L., Secchi, E.R., Bugoni, L., 2012. Driftnet fishery threats sea turtles in the Atlantic Ocean. Biodivers. Conserv. 21, 915–931.

Fossette, S., Witt, M.J., Miller, P., Nalovic, M.A., Albareda, D., Almeida, A.P., Broderick, A.C., Chacón-Chaverri, D., Coyne, M.S., Domingo, A., Eckert, S., Evans, D., Fallabrino, A., Ferraroli, S., Formia, A., Giffoni, B., Hays, G.C., Hughes, G., Kelle, L., Leslie, A., López-Mendilaharsu, M., Luschi, P., Prosdocimi, L., Rodriguez-Heredia, S., Turny, A., Verhage, S., Godley, B.J., 2014. Pan-Atlantic analysis of the overlap of a highly migratory species, the leatherback turtle, with pelagic longline fisheries. Proc. R. Soc. B 281, 20133065. http://dx.doi.org/10.1098/rspb.2013.3065.

Frankham, R., Ballou, J.D., Briscoe, D.A., 2010. Introduction to Conservation Genetics, second ed. Cambridge University Press, Cambridge, p. 618.

Garner, J.A., MacKenzie, D.S., Gatlin, D., 2017. Reproductive biology of Atlantic leatherback sea turtles at Sandy Point, St. Croix: the first 30 years. Chelonian Conserv. Biol. 16, 29–43.

Gruber, B., Adamack, A.T., 2015. Landgenreport: a new R function to simplify landscape genetic analysis using resistance surface layers. Mol. Ecol. Resour. 15, 1172–1178.

Hart, K.M., Guzy, J.C., Smith, B.J., 2021. Drivers of realized satellite tracking duration in marine turtles. Mov. Ecol. 9, 1–14.

Honig, M.B., Petersen, S.L., Duarte, A., 2008. Turtle bycatch in longline fisheries operating within the Benguela current large marine ecosystem. Collect. Sci. Pap. ICCAT 62, 1757–1769.

Ihaka, R., Gentleman, R., 1996. R: a language for data analysis and graphics. J. Comput. Graph. Statist. 5, 299–314.

Jombart, T., 2008. adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24, 1403–1405. http://dx.doi.org/10.1093/bioinformatics/btn129.

Jones, A.T., Ovenden, J.R., Wang, Y.G., 2016. Improved confidence intervals for the linkage disequilibrium method for estimating effective population size. Heredity (Edinb) 117, 217–223.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., Drummond, A., 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28, 1647–1649.

Kopelman, N.M., Mayzel, J., Jakobsson, M., Rosenberg, N.A., Mayrose, I., 2015. CLUMPAK: a program for identifying clustering modes and packaging population structure inferences across K. Mol. Ecol. Resour. 15 (5), 1178–1191. http://dx.doi.org/10.1111/1755-0998.12387.

Lara-Ruiz, P., Lopez, G.G., Santos, F.R., Soares, L.S., 2006. Extensive hybridization in hawksbill turtles (*Eretmochelys imbricata*) nesting in Brazil revealed by mtDNA analyses. Conserv. Genet. 7, 773–781.

Lasala, J.A., Hughes, C.R., Wyneken, J., 2018. Breeding sex ratio and population size of loggerhead turtles from Southwestern Florida. PLoS One 13, e0191615.

Levasseur, K.E., 2019. Population and Kin Structure of Hawksbill Turtles: Insights on Natal Homing Precision, Time to Maturity and the Male Component of the Breeding Population (Doctoral dissertation). Retrieved from https://scholarcommons.sc.edu/etd/5587.

Magris, R.A., Marta-Almeida, M., Monteiro, J.A.F., Ban, N.C., 2019. A modeling approach to assess the impact of land mining on marine biodiversity: assessment in coastal catchments experiencing catastrophic events (SW Brazil). Sci. Total Environ. 659, 828–840. http://dx.doi.org/10.1016/j.scitotenv.2018.12.238.

Marta-Almeida, M., Mendes, R., Amorim, F.N., Cirano, M., Dias, J.M., 2016. Fundão Dam collapse: Oceanic dispersion of River Doce after the greatest Brazilian environmental accident. Mar. Pollut. Bull. 112, 359–364.

Oliveira, J.A., Farias, I.P., Costa, G.C., Werneck, F.P., 2019. Model-based riverscape genetics: disentangling the roles of local and connectivity factors in shaping spatial genetic patterns of two Amazonian turtles with different dispersal abilities. Evol. Ecol. 33, 273–298.

Peakall, R., Smouse, P.E., 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research - an update. Bioinformatics 28, 2537–2539.

Phillips, K.P., Jorgensen, T.H., Jolliffe, K.G., Richardson, D.S., 2017. Evidence of opposing fitness effects of parental heterozygosity and relatedness in a critically endangered marine turtle? J. Evol. Biol. 30, 1953–1965.

Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics 155, 945–959.

Reid, B.N., Naro-Maciel, E., Hahn, A.T., FitzSimmons, N.N., Gehara, M., 2019. Geography best explains global patterns of genetic diversity and postglacial co-expansion in marine turtles. Mol. Ecol. 00, 1–13.

Roden, S.E., Dutton, P.H., 2011. Isolation and characterization of 14 polymorphic microsatellite loci in the leatherback turtle (*Dermochelys coriacea*) and cross species amplification. Conserv. Genet. Resour. 3, 49–52.

Roden, S.E., Stewart, K.R., James, M.C., Dodge, K.L., Dell'Amico, F., Dutton, P.H., 2017. Genetic fingerprinting reveals natal origins of male leatherback turtles encountered in the Atlantic Ocean and Mediterranean Sea. Mar. Biol. 164 (181).

Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J.C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S.E., Sánchez-Gracia, A., 2017. DnaSP 6: DNA sequence polymorphism analysis of large data sets. Mol. Biol. Evol. 34, 3299–3302.

Sales, G., Giffoni, B., Barata, P.C.R., 2008. Incidental catch of sea turtles by the Brazilian pelagic longline fishery. J. Mar. Biol. Assoc. United Kingdom 88, 853–864.

Santidrián Tomillo, P., Vélez, E., Reina, R.D., Piedra, R., Paladino, F.V., Spotila, J.R., 2007. Reassessment of the leatherback turtle (*Dermochelys coriacea*) nesting popilaton at Parque Nacional Marino Las Baulas, Costa Rica: Effects of conservation efforts. Chelonian Conserv. Biol. 6, 54–62.

Shamblin, B.M., Bolten, A.B., Abreu-Grobois, F.A., Bjorndal, K.A., Cardona, L., Carreras, C., Nel, R., 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., 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.

Stewart, K.R., LaCasella, E.L., Roden, S.E., Jensen, M.P., Stokes, L.W., Epperly, S.P., Dutton, P.H., 2016. Nesting population origins of leatherback turtles caught as bycatch in the U.S. pelagic longline fishery. Ecosphere 7, e01272.

Thomé, J.C., Baptistotte, C., Moreira, L.M.P., Scalfoni, J.T., Almeida, A.P., Rieth, D.B., Barata, P.C., 2007. Nesting biology and conservation of the leatherback sea turtle (*Dermochelys coriacea*) in the state of Espírito Santo, Brazil, 1988–1989 to 2003–2004. Chelonian Conserv. Biol. 6, 15–27.

Tiwari, M., Wallace, B.P., Girondot, M., 2013. Dermochelys coriacea (Southwest Atlantic Ocean subpopulation). http://dx.doi.org/10.2305/IUCN.UK.2013-2.RLTS.T46967838A46967842.en, The IUCN Red List of Threatened Species 2013: e. T46967838A46967842. Downloaded on 09 February 2021.

Vargas, S.M., Araújo, F.C.F., Monteiro, D.S., Estima, S.C., Almeida, A.P., Soares, L.S., Santos, F.R., 2008. Genetic diversity and origin of Leatherback Turtles (*Dermochelys coriacea*) from the Brazilian coast. J. Hered. 99, 215–220.

Vargas, S.M., Lins, L.S.F., É, Molfetti., Ho, S.Y.W., Monteiro, D., Barreto, J., Colman, L., Vila-Verde, L., Baptistotte, C., Thomé, .J.C.A., Santos, F.R., 2019. Revisiting the genetic diversity and population structure of the critically endangered leatherback turtles in the South-west Atlantic Ocean: insights for species conservation. J. Mar. Biol. Assoc. United Kingdom 99, 31–41.

Vargas, S.M., Molfetti, E., Vilaça, S.T., Monteiro, D.S., Estima, S.C., Soares, L.S., Almeida, A.P., Thoisy, B., Naro-Maciel, E., Santos, F.R., 2013. Mixed stock analysis of leatherback turtles feeding in Brazil: records over four years. In: Proceedings of the 33rd Symposium on Sea Turtle Biology and Conservation. ISTS, Baltimore, MD, p. 246.

Viana, M.N.S., Oliveira, J.A., Agostini, M.A.P., Erickson, J., Morais, G.M., Monjeló, L.A.S., Andrade, P.C.M., Félix-Silva, D., Oliveira-Júnior, W.P., Sites, J.W., Herbk, T., Farias, I.P., 2017. Population genetic structure of the threatened amazon river turtle, *Podocnemis sextuberculata* (Testudines, Podocnemididae). Chelonian Conserv. Biol. 6, 128–138.

Wallace, B.P., Kot, C.Y., DiMatteo, A.D., Lee, T., Crowder, L.B., Lewison, R.L., 2013. Impacts of fisheries bycatch on marine turtle populations worldwide: toward conservation and research priorities. Ecosphere 4, 1–49.

Wallace, B.P., Lewison, R.L., McDonald, S.L., McDonald, R.K., Kot, C.Y., Kelez, S., Bjordan, R.K., Finkbeiner, E.M., Helmbrecht, S., Crowder, L.B., 2010. Global patterns of marine turtle bycatch. Conserv. Lett. 3, 131–142.

Witt, M.J., Bonguno, E.A., Broderick, A.C., Coyne, M.S., Formia, A., Gibudi, A., Mounguengui, G.A.M., Moussounda, C., NSafou, M., Nougessono, S., Parnel, R.J., Sounguet, G., Verhage, S., Godley, B.J., 2011. Tracking leatherback turtles from the world's largest rookery: assessing threats across the south atlantic. Proc. R. Soc. Lond. [Biol] 278, 2338–2347.

Yuan, M.L., White, K.N., Rothermel, B.B., Zamudio, K.R., Tuberville, T.D., 2019. Close-kin mationg, but not inbred parents, reduces hatching rates and offspring quality in threatened tortoise. J. Evol. Biol. 32, 1152–1162.