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ORIGINAL RESEARCH

Restricted connectivity and population genetic fragility in a globally endangered Hammerhead Shark

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Abstract Vagile, large-bodied marine organisms frequently have wide range dispersion but also dependence on coastal habitats for part of their life history. These characteristics may induce complex population genetic structure patterns, with resulting implications for the management of exploited populations. The scalloped hammerhead, *Sphyrna lewini*, is a cosmopolitan, migratory shark in tropical and warm temperate waters, inhabiting coastal bays during parturition and juvenile development, and the open ocean as adults. Here, we investigated the genetic connectivity and diversity of *S. lewini* in the western Atlantic using large sample coverage (N = 308), and data from whole mitochondrial control region (mtCR) sequences and ten nuclear microsatellite markers We detected significant population genetic structure with both mtCR and microsatellites markers (mtCR: Φ_{ST} = 0.60; $p < 0.001$; microsatellites: D_{est} 0.0794, $p = 0.001$, $F_{ST} = 0.046$, $p < 0.05$), and isolation by distance (mtCR r = 0.363, $p = 0.009$; microsatellites markers r = 0.638, $p = 0.007$). Migration and gene flow patterns were asymmetric and female reproductive philopatry is postulated to explain population subdivisions. The notable population differentiation at

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microsatellites markers indicates low-levels of male-mediated gene flow in the western Atlantic. The overall effective population size was estimated as 299 (215–412 CI), and there was no evidence of strong or recent bottleneck effects. Findings of at least three management units, moderate genetic diversity, and low effective population size in the context of current overfishing calls for intensive management aimed at short and long-term conservation for this endangered species in the western Atlantic Ocean.

Keywords elasmobranch conservation · Endangered species · Genetic connectivity · Genetic diversity

Introduction

Many marine fish species have been commercially harvested for decades. As a consequence many high-level predators, including several shark species, are now endangered or on the edge of extinction (Dulvy et al. 2014; Nachtigall et al. 2017). Given the well-documented worldwide depletion of shark populations (Ferretti et al. 2010; Dulvy et al. 2014), there is an urgent need for increasing knowledge of life history traits and other fundamental aspects of shark biology to support conservation efforts (Simpfendorfer et al. 2011). Sharks are a particularly interesting group for conservation studies given their ecological importance to ecosystems and remarkable features related to their life history, such as their deep ancestry, low genetic diversity and broad geographic distribution (reviewed by Domingues et al. 2018a).

Understanding dispersal patterns is a fundamental step for recognizing structured populations, and for the identification of both Evolutionary Significant Units (ESU) and Management Units (MU) to guide conservation and management decisions (Crandall et al. 2000; Palsbøll et al. 2007; Weersing and Toonen 2009; Funk et al. 2012). Despite advances in mark–recapture and satellite tagging strategies for estimating survival rates and animal long-range movements, these approaches do not reveal if the movements have resulted in reproduction and consequent gene flow (Lowe and Allendorf 2010; Ovenden 2013). Alternatively, molecular techniques are increasingly being used to inform conservation and management (Pinhal et al. 2008, 2009, 2012; Ferrette et al. 2019) and are especially useful for species whose ecology, demography, behaviour or population dynamics are difficult to study using field methods (Ovenden 2013).

Frequently, sharks and other large, highly mobile marine fishes with broad distributions exhibit little population genetic structure (e.g., Graves 1998; Theisen et al. 2008; Domingues et al. 2018b). However, exceptions to this pattern are being observed, and have been attributed to historical and/or contemporary processes such as marine geographic barriers and oceanographic heterogeneity, as well as coastal habits, viviparous reproduction, sedentary behavior, disjunct distributions and reproductive philopatry (e.g., O'Brien et al. 2013; Chapman et al. 2015; Sandoval-Castillo and Beheregaray 2015; Bernard et al. 2016; Domingues et al. 2018a). For example, Bernard et al. (2016) found high levels of intraocean basin matrilineal population structure in the tiger shark *Galeocerdo cuvier*, suggesting female philopatry and sex-biased gene flow. Indeed, reproductive behavior in sharks has important implications for genetic diversity and population structure (Daly-Engel et al. 2010; Domingues et al. 2018a). Philopatric behaviour, in particular, seems to be a common cause for shark population structure (Chapman et al. 2015). Philopatric tendencies can be strong or weak for a given species, and special patterns of this behavior can include natal site philopatry (returning to the natal nursery area) and sex-specific philopatry (where one sex is more philopatric than the other, as in many male birds and female mammals) (Chapman et al. 2015).

The scalloped hammerhead shark, *Sphyrna lewini*, is a large bodied, coastal and semi-oceanic species, found worldwide in tropical seas. Targeted and by-catch fisheries coupled with a valuable price fetched in the shark fin trade have led to severe and widespread population declines for this species. Fisheries surveys in the Northwestern Atlantic have documented severe declines, with the probability of *S. lewini* being overfished estimated as greater than 95% (Hayes et al. 2009). In the Southwestern Atlantic population declines of this shark species have reached up to 80% (Barreto et al. 2016). The recent recognition of a cryptic species, the Carolina hammerhead shark *Sphyrna gilberti*, bearing a very close morphological resemblance to *S. lewini* in the western North and South Atlantic (Pinhal et al. 2012; Quattro et al. 2013), in addition to the occurrence of viable hybrids between

Carolina and scalloped hammerheads inhabiting the US Atlantic coast (Barker et al. 2019) suggests that the population status of *S. lewini* may be of even higher concern. Furthermore, *S. lewini*, like many shark species, possesses intrinsic biological traits similar to marine mammals, such as low reproductive capacity, long gestation, and late age at maturity, making them highly vulnerable to overexploitation (Ebert et al. 2013; Gallagher et al. 2014, Gallagher and Klimley 2018). Unlike most other shark species, *S. lewini* frequently aggregate in large numbers in oceanic or coastal waters, and for this reason are more vulnerable to fishing efforts (Rigby et al. 2019). As a consequence, the IUCN Red List of Threatened and Endangered Species assessed this species as globally Critically Endangered (Rigby et al. 2019).

Population genetic and phylogeographic studies of *S. lewini* have been performed on global (e.g., Duncan et al. 2006; Daly-Engel et al. 2012) and regional scales (e.g., Chapman et al. 2009; Nance et al. 2011; Ovenden et al. 2009, 2011; Quintanilla et al. 2015; Spaet et al. 2015). These studies, utilizing maternally inherited mitochondrial and/or nuclear, bi-parentally inherited markers, have reported the existence of population structure and isolation between ocean basins and in some cases within oceans. These studies also evidenced moderate variability among nursery areas, suggesting connectivity of nursery areas located at short geographic distances (Duncan et al. 2006). Although *S. lewini* population genetics has been studied in different locations, only Chapman et al. (2009) included samples from the Southwest Atlantic, but from limited sampling sites and only using mitochondrial DNA as a marker.

A more detailed genetic evaluation of this endangered shark across a broad Atlantic region is pivotal to identify discrete population structure and assess current levels of genetic diversity. In this study, we utilized two different genetic markers, the complete mitochondrial DNA control region and ten highly polymorphic microsatellite loci, and samples from different regions (including suspected nursery grounds) along the western Atlantic Ocean to (1) assess the genetic diversity, (2) test the null hypothesis of panmixia of *S. lewini* along the western Atlantic Ocean, and (3) estimate the direction and rate of migration. This combined marker approach allowed us to make inferences about population genetic structure and contemporary gene flow, as well as evaluate the potential role of natal philopatry and sex-biased dispersal in the population dynamics of *S. lewini* within the western Atlantic Ocean.

Materials and methods

Sampling, DNA extraction and molecular identification

Tissue samples (muscle and fin clips) of *S. lewini* were collected from 2005 to 2012 in a broad range of the western Atlantic Ocean (Fig. 1). A total of 308 adult individuals (unknown sex) were sampled from eight different collection areas: the Gulf of Mexico (GM; n = 50) and Caribbean Sea (CB; n = 20) in Northwestern Atlantic (NWA); and along the northern Brazilian (NBrz) coast at Pará (n = 9) and Rio Grande do Norte (n = 30), and southern Brazilian (SBrz) coast at Rio de Janeiro (n = 54), São Paulo (n = 53), Santa Catarina (n = 42), and Rio Grande do Sul (n = 50), in the Southwestern Atlantic (SWA). All samples were stored in 95% ethanol at room temperature or at 4 °C for long-term storage. Genomic DNA was extracted from 25 mg of tissue using the QIAGEN Dneasy extraction kit (QIAGEN Inc). Species identification was first conducted by morphological analysis of specimens in the field (rarely available) and subsequently confirmed by means of a Polymerase chain reaction with multiple primers (PCR-multiplex) approach (Abercrombie et al. 2005). The multiplex PCR based identification unequivocally confirmed them as *S. lewini*, thereby excluding cryptic hammerhead sharks from our analysis (Fig. S1). During our large sampling effort, only three cryptic Carolina hammerheads were identified, all in the western South Atlantic Ocean (findings previously reported in Pinhal et al. 2012), This opens a chance, albeit remote, of hybrids between Carolina and scalloped hammerheads, as reported in a narrow range in the USA Atlantic coast (Barker et al. 2019). Putative hybrids were virtually discarded by checking the parental species source of mtCR haplotypes with the BLASTN search tool in the NCBI web page. Previous analysis of mtCR in hybrids indicated Carolina hammerheads are nearly always (95% of cases) the maternal species (Barker et al. 2019). Here, the opposite has been found with all mtCR sequences corresponding to *S. lewini* haplotypes.

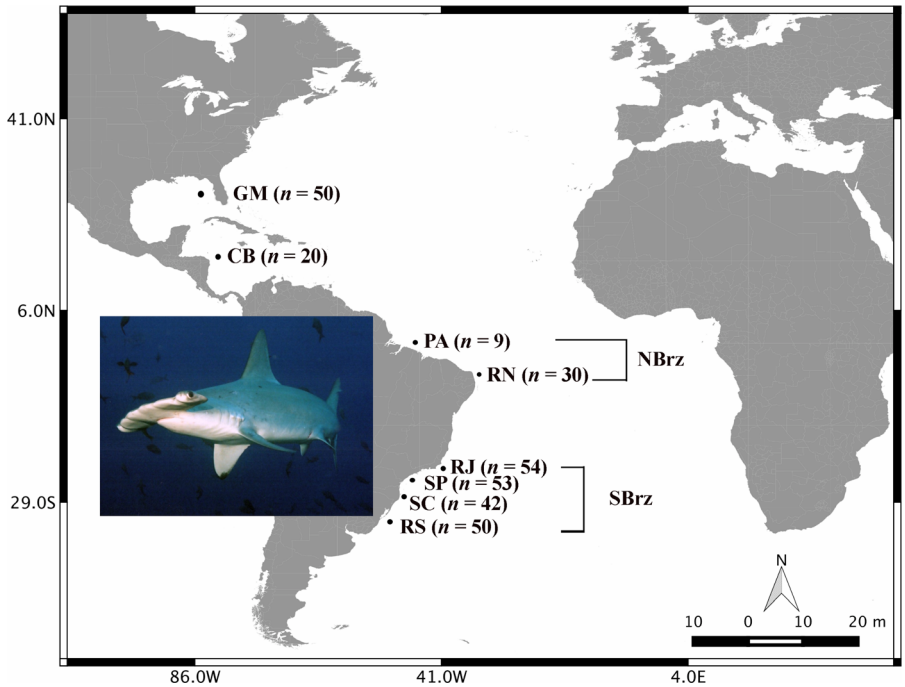

Fig. 1 Location of sampling sites and sample sizes of the *Sphyrna lewini* in the western Atlantic Ocean. Collection areas: (i) western North Atlantic: Gulf of Mexico (GM), Caribbean Sea (CB); and (ii) western South Atlantic: Pará (PA), Rio Grande do Norte (RN), Rio de Janeiro (RJ), São Paulo (SP), Santa Catarina (SC), and Rio Grande do Sul (RS). The scalloped hammerhead shark (*Sphyrna lewini*) image embedded is a copyright of Guy Harvey Ocean Foundation

Mitochondrial DNA sequencing

The entire mitochondrial DNA control region (mtCR) was amplified using the primers DloopF (5′-CTC CCA AAG CCA AGA TTC TG-3′) and DloopR (5′-GGC TTA GCA AGG TGT CTT CTT GG-3′) designed for this study, based on complete mitochondrial genome from *Mustelus manazo* (Cao et al. 1998). PCR reactions of 50µl consisted of 40 µM dNTP's, 10x PCR buffer, 10 pmol/µl of each primer, 10–25 ng extracted DNA, and 1 unit of HotStart Taq DNA polymerase (QIAGEN Inc.). The PCR thermal profile included a denaturation of 15 min at 95 °C, followed by 35 cycles at 94 °C for 1 min, 50 °C for 1 min, 72 °C for 2 min, and a final 5 min extension at 72 °C, and was performed in an iCycler thermocycler (Bio-rad, USA). All PCR reactions were run with a negative control and were visualized on 1.2% agarose gels. PCR purification was performed with the QIAquick PCR purification kit following the manufacturer's protocol (QIAGEN, Inc.). Two internal sequencing primers were designed to achieve double stranded coverage of the entire mtCR: ScHHCRF11 (5′-CCT CTA ATC ATG AGT AAA ATG GCA AT-3′) and ScHHCRR10 (5′-CTT AGA GGA CTG GAA ATC TTG ATC GAG-3′). Cycle sequencing was performed following standard ABI procedure using BigDye Terminator v3.1 (Applied Biosystems, Inc.) and DyeEx 2.0 Spin kits (QIAGEN, Inc.). All sequencing was performed on an ABI 3130 (Applied Biosystems, Inc, USA).

Microsatellite genotyping

Ten highly polymorphic microsatellite loci previously isolated by Nance et al. (2009) were utilized in this study (Sle27, Sle28, Sle33, Sle38, Sle45, Sle54, Sle71, Sle77, Sle81 and Sle89). Microsatellite loci were amplified using forward primers with a 5′-M13 tail (Schuelke 2000). Amplification was performed in 25µl reactions consisting of 40 µM dNTP's, 10x PCR buffer, 25 mM MgCl2, 10 pmol/µl forward, reverse and M13 primer, 10–25 ng extracted DNA, and 1 unit of HotStart Taq DNA polymerase (QIAGEN Inc.). PCR thermal profiles consisted of an initial denaturation at 95 °C for 15 min, followed by 35 cycles at

94 °C for 1 min, 57 °C (Sle38, Sle54 e Sle81), 59 °C (Sle28, Sle53 e Sle89), 63 °C (Sle27, Sle33, Sle71 e Sle77) for 1 min, 72 °C for 2 min and a final 5 min extension at 72 °C in an iCycler thermocycler (Biorad, USA). Microsatellite PCR products with different fluorescent dyes were pooled and genotyped with GENESCAN LIZ600 size standard (Applied Biosystems, Inc., USA). All loci were genotyped on an ABI 3130 (Applied Biosystems, Inc., USA) and allele sizes were scored using GENEMAPPER 3.0 (Applied Biosystems, Inc., USA).

Mitochondrial DNA data analysis

Alignment of 308 mtCR sequences was carried out using the algorithm MUSCLE implemented on GENEIOUS v9.1.8 (Biomatters Ltd.). The number of haplotypes (H), polymorphic sites (S), haplotype diversity (h), nucleotide diversity (π), nucleotide composition, and ratio of transitions to transversions were calculated using Arlequin 3.5 (Excoffier and Lischer 2010). Haplotypes were generated from the DNA sequences dataset using the program DNACOLLAPSER, available online in the FaBOX tools (Villesen 2007). A minimum spanning network was drawn in NETWORK v.4.510 based on the full median joining algorithm, at the 95% confidence level, to assess the relationships among haplotypes.

To test the null hypothesis of panmixia based on mtCR, the population genetic structure was estimated among all collection areas using an analogue Wright's pairwise F_{ST} fixation index (Φ_{ST}), with statistical significance tested with 16,000 permutations and $\alpha = 0.05$ (Cockerham and Weir 1993). The estimated probabilities were corrected using Holm-Bonferroni sequential adjustments for multiple tests (Holm 1979). In addition, a global and hierarchical Analysis of Molecular Variance (AMOVA) (Excoffier et al. 1992) was conducted to examine the genetic diversity within and among populations using Arlequin 3.5 (Excoffier and Lischer 2010). The hierarchical AMOVA was conduced by grouping all collection areas into three groups: Gulf of Mexico, Caribbean Sea, and Brazil. The statistical significance of AMOVA was tested with 10,000 permutations and $\alpha = 0.05$ (Cockerham and Weir 1993). A Mantel test (Mantel 1967) was performed to test the hypothesis that genetic differentiation is due to isolation-by-distance by evaluating the correlation between Nei's genetic distance and a matrix of Euclidian geographic distance using the Adegenet package (Jombart 2008) in R v.3.1.2 (R program Core Team 2015). Mantel tests were performed using Pearson correlation with 10,000 permutations.

The number of migrants per generation ($Nm = \Theta.M$) among the identified mtCR *S. lewini* populations was inferred using a coalescent-based program MIGRATE-N v.4.4.3 (Beerli and Palczewski 2010). The software estimates the effective population size as follows: for Θ (mutation-scaled population sizes), defined as $\Theta = Ne\mu$ for mtCR, and M (mutation-scaled immigration rate), defined as $M = m/\mu$ where m is the immigration rate and μ is the mutation rate per site and generation. The models were applied assuming four populations (Gulf of Mexico, Caribbean, North Brazil, and South Brazil) based on pairwise Φ_{ST} results (see results below). Different migration models were set assuming panmixia, asymmetric and symmetric with bidirectional and unidirectional migration, and also with divergence among populations with and without migration on clockwise and anticlockwise pathways (Table S1). All the runs were performed with uniform prior distributions range for Θ of 0–0.1 (Δ 0.01; 1500 bins) and for M 0–12,000,000 (Δ 12,000; 1500 bins). The Markov chain settings were set as one long chain recorded by 100,000 steps incremented every 1000 steps with one replicate of concurrent chains comprising a total of 100,000,000 iterations. A static heating scheme of 4 chains with temperatures 1000.000; 3; 1.5; 1 and a burn-in of 30% per chain was applied to each replicate. The convergence assignment was made in Tracer v1.7.1 (Rambaut et al. 2018) using the output from MIGRATE-N of each model run. After a convergence statement, the best-fit migration model was assigned through Bayes Factor using Bezier scores from MIGRATE-N runs (Beerli and Palczewski 2010), calculated using mtraceR package on R (R core Team 2015). The mutation rate was set at 1.21% Mya for the Sphyrnidae mtCR (Nance et al. 2011) for the calculation of the effective population sizes.

Microsatellite DNA data analysis

Presence of null alleles and genotyping errors was evaluated with MICRO-CHECKER v.2.2.3 (van Oosterhout et al. 2004). For each locus and putative population, deviations from Hardy–Weinberg

equilibrium (HW) and linkage disequilibrium (LD) were calculated in GENEPOP v.4.0 (Raymond and Rousset 1995), using 10,000 steps of dememorization, 1000 batches and 10,000 iterations per batch, and adjusted for multiple tests using the Holm-Bonferroni correction (Holm 1979). Standard microsatellite summary statistics were calculated, including: number of alleles (A), allelic richness (*Ar*), expected heterozygosity (H_E), observed heterozygosity (H_O), and inbreeding coefficient (F_{IS}), using the gstudio and Adegenet packages in R v.3.1.2 (R core Team 2015). To ensure that related individuals (half and full-sibling) were not used in analysis, since they may bias the subsequent estimation of genetic diversity and population structure (Devloo-Delva et al. 2019), we conducted a relatedness analysis based on the allele frequencies among all pairs of *S. lewini* individuals within and among collection areas, using the package Related (Pew et al. 2015) in R v.3.1.2 (R core Team 2015). First, in order to select the best relatedness estimator based on our data, we used a function "compareestimator". The estimator with higher correlation coefficient (r) was chosen. To determine whether individuals within collection areas were more closely related than expected, we compared the observed values for each collection area against deviations from random mating expectations, using 1000 permutations and keeping collection area size constant. If the observed mean relatedness is greater than that of the permuted data, the null hypothesis is rejected.

Different approaches were used to test for population structure between the collection areas. The pairwise population comparisons of D_{est} values (Jost 2008) were estimated using the DEMEtics package in R v.3.1.2 (R core Team 2015). Holm-Bonferroni adjustments of α were used to correct for multiple tests (Holm 1979). STRUCTURE v2.3.4 (Pritchard et al. 2000) was used to identify population mixture and the clustering of groups of individuals. The Markov chain Monte Carlo (MCMC) was run for 1 million generations, with an initial burn-in of 10% steps discarded, and 10 iterations of each *K*, assuming correlated allele frequencies (Falush et al. 2003) and the admixture model. The number of populations *K* was determined based on the ΔK estimation of Evanno et al. (2005). Clumpak (Kopelman et al. 2015) was used to compare all Structure runs into a matrix *Q* of individual membership coefficient and population ancestry components. Additionally, a multivariate approach, Discriminant Analysis of Principal Components—DAPC (Jombart et al. 2010), a multivariate method designed to identify and describe clusters of genetically related individuals, was used to identify discrete populations based on geographic region, first 50 principal components, and 94% of total variation, using the Adegenet package (Jombart 2008; Jombart et al. 2010). DAPC first summarizes genetic data into uncorrelated groups using principal components and then uses discriminant analysis to maximize the among-population variation. Cluster assignments were pre-defined to correspond to a priori defined collect locations. A Mantel test (Mantel 1967) was also performed to test the hypothesis that microsatellites genetic differentiation is due to isolation-by-distance by evaluating the correlation between Nei's genetic distance and a matrix of Euclidian geographic distance using the Adegenet package (Jombart 2008) in R v.3.1.2 (R program Core Team 2015). Mantel tests were performed using Pearson correlation with 10,000 permutations.

Finally, to infer *S. lewini* contemporary migration dynamics among populations previously identified by STRUCTURE and DAPC, the extent and direction of gene flow was estimated by calculating the direction relative migration rate, assuming asymmetric bidirectional gene flow, using the divMigrate function (Sundqvist et al. 2016) of the package diveRsity (Keenan et al. 2013), based on effective number of migrants (*Nm*) (Alcala et al. 2014). The divMigrate function has been used for microsatellite datasets as an alternative to traditional programs because it implements measures of classical and modern genetic differentiation with low computational effort. Moreover, asymmetric bidirectional gene flow is common in marine environments, because physical processes such as oceanic currents can influence migration (Sundqvist et al. 2016). The statistical significance of directional migration was calculated on the basis of 1000 bootstraps and 95% confidence intervals ($\alpha = 0.05$).

Effective population size (N*e*) for each identified population was estimated using single sample methods based on the LD method (Waples and Do 2010), as implemented in NeEstimator2.0 (Do et al. 2014). The rare-allele screening critical value was 0.05. According to Waples and Do (2010), when samples do not have discrete generations (i.e., age-structured species),

the resulting estimate from the LD method can be interpreted as an estimate of the effective number of breeders. The program BOTTLENECK v1.2.02 (Piry et al. 1999) was used to test for recent population size reductions (i.e., bottleneck effect) based on heterozygosity excess. The analysis was performed under a two-phase model (TPM), which is more powerful when fewer than 20 loci are used (Piry et al. 1999). The probability of significant heterozygosity excess was determined using 10,000 replications and a one-tailed Wilcoxon signed-rank tests ($\alpha = 0.05$).

Results

Mitochondrial DNA

The DNA sequence of the complete mtCR from 308 individuals was 1083 to 1085 nucleotides. The nucleotide base composition was 26.75% T, 14.78% C, 45.07% A, and 13.40% G. The sequences had a total of 21 polymorphic sites, including 8 transitions and 15 transversions, comprising 25 haplotypes (GenBank accession number MK636839-MK636863). Overall haplotype (h) and nucleotide (π) diversities were $h = 0.609 \pm 0.0324$ and $\pi = 0.0011 \pm 0.0007$, respectively. Genetic diversity values within collection areas are shown in Table 1. Haplotype 2 was detected in 62% of all *S. lewini* analyzed, and was found in every collection area along the Brazilian coast as well as the Caribbean. From the 17 private haplotypes identified, all 7 haplotypes observed at GM were sampled exclusively in this location. An additional 10 haplotypes were sampled exclusively in CB (3), SP (3), RJ (2), RN (1), and SC (1) collection areas (Fig. 2; Table S2). A total of 17 haplotypes were sampled in two or more individuals. Only three haplotypes were shared among specimens from the western North and South Atlantic. Except for GM haplotypes, no clear phylogeographic grouping of haplotypes was observed.

Global AMOVA analyses of the 308 individuals revealed significant genetic heterogeneity among the 8 collection areas ($\Phi_{ST} = 0.60$; $p < 0.001$). The pairwise Φ_{ST} values between collection areas indicated existence of distinct genetic populations among CB, GM and Brazil (Table 2). Furthermore, population genetic structure was observed between the northern (NBrz) and southern (SBrz) Brazilian collection areas, but not between adjacent northern (PA and RN) and southern (RJ, SP, SC and RS) collection areas (Table 2). The hierarchical AMOVA tests conducted considering three sampled groups (GM, CB and Brazil), showed significant population subdivision (Table 3). Significant levels of genetic diversity were detected within populations and among populations within groups (Table 3). The mtCR Mantel test revealed a low but

Table 1 Genetic diversity indices of the mitochondrial DNA control region and 10 microsatellite loci for *Sphyrna lewini* in the western Atlantic Ocean

Population	mtCR					Microsatellites				
	n	H	S	*h*	π	A	*Ar*	*Ho*	*He*	*Fis*
GM	50	4	3	0.605	0.00070	6.2	4.2	0.53	0.60	0.070
CB	20	6	6	0.705	0.00119	6.3	5.0	0.65	0.68	0.017
PA	09	3	5	0.722	0.00144	4.7	4.3	0.64	0.60	− 0.084
RN	30	5	3	0.720	0.00130	6.2	4.5	0.62	0.61	− 0.016
RJ	54	6	4	0.211	0.00020	7.0	4.5	0.58	0.60	0.028
SP	53	5	4	0.181	0.00017	7.1	4.6	0.56	0.62	0.092
SC	42	3	2	0.180	0.00017	6.9	4.4	0.52	0.59	0.090
RS	50	5	4	0.155	0.00015	7.0	4.7	0.55	0.63	0.115

n: number of individuals analyzed, H: haplotype number, S: polymorphic sites, *h*: haplotype diversity, π: nucleotide diversity, A: Number of alleles, *Ar*: allelic richness, *Ho*: observed heterozygosity, *He*: expected heterozygosity, and *Fis*: inbreeding coefficient

Collection areas: Gulf of Mexico (GM), Caribbean Sea (CB), Pará (PA), Rio Grande do Norte (RN), Rio de Janeiro (RJ), São Paulo (SP), Santa Catarina (SC), and Rio Grande do Sul (RS)

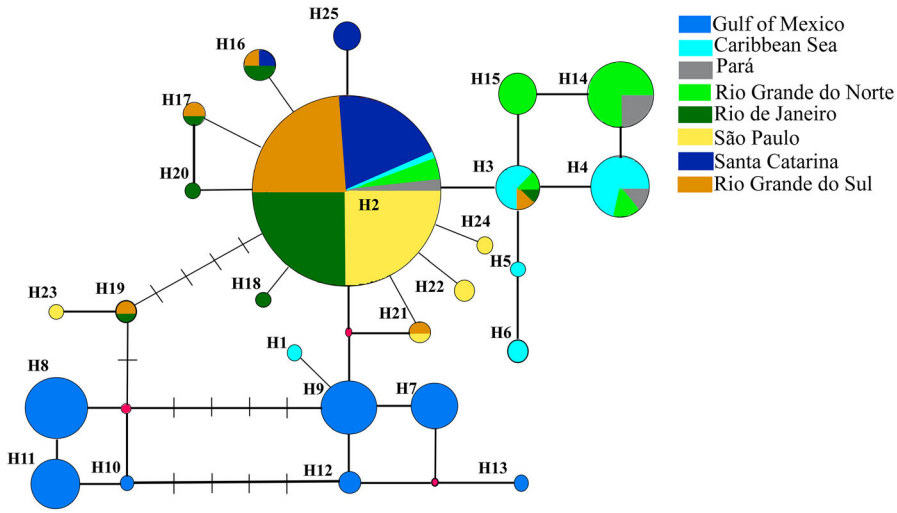

Fig. 2 A median-joining haplotype network for the entire mitochondrial DNA control region of the scalloped hammerhead shark (*Sphyrna lewini*). The circle sizes are proportional to the haplotype frequency. Cross marks on branches represent mutational steps. Red dots represent hypothetical haplotypes not sampled or extinct in the populations

Table 2 Pairwise Φ_{ST} *Sphyrna lewini* from the western Atlantic Ocean based on mitochondrial DNA control region

Population	GM	CB	PA	RN	RJ	SP	SC	RS
GM	–							
CB	**0.59**	–						
PA	**0.55**	**0.15**	–					
RN	**0.62**	**0.24**	− 0.03	–				
RJ	**0.65**	**0.64**	**0.65**	**0.60**	–			
SP	**0.64**	**0.65**	**0.66**	**0.61**	0.00	–		
SC	**0.64**	**0.69**	**0.72**	**0.63**	0.01	0.01	–	
RS	**0.64**	**0.65**	**0.66**	**0.60**	− 0.02	− 0.01	0.01	

Bold values indicate significance ($p < 0.05$) after Holm–Bonferroni correction

Collection areas: Gulf of Mexico (GM), Caribbean Sea (CB), Pará (PA), Rio Grande do Norte (RN), Rio de Janeiro (RJ), São Paulo (SP), Santa Catarina (SC), and Rio Grande do Sul (RS)

Table 3 Hierarchical Analysis of Molecular Variance (AMOVA) based on mitochondrial DNA control region sequences of *Sphyrna lewini* in the western Atlantic Ocean

mtCR		DF	SSD	VC	%V	Φ	*P* Value
Among groups	Φ_{CT}	2	174.413	1.39669	65.47	0.25112	0.0346
Among populations within groups	Φ_{SC}	5	38.253	0.18496	8.67	0.74143	< 0.0001
Among populations among groups	Φ_{ST}	300	165.477	0.55159	25.86	0.65473	< 0.0001

Three groups defined: Gulf of Mexico, Caribbean Sea, Brazil

DF degrees of freedom, *SSD* sum of squares, *VC* variance component, *%V* percent of variance

significant correlation between geographic and genetic distance (r = 0.363, p = 0.009) (Fig. S2A).

After testing sixteen different migration models (Table S1), the best-fit convergent model was set as the full asymmetric migration between the populations (Table S3). The coalescent-based estimate of mtCR gene flow among populations was asymmetric, and indicated a higher magnitude of migration from CB to NBrz (PA and RN) with a Nm = 13,073 (2369–35,976 CI), followed by GM to SBrz (RJ, SP, SC, RS) with Nm=9.763 (58.64–26,920 CI) (Fig. 3a; Table S4).

Microsatellites

A total of 308 *S. lewini* individuals were genotyped at ten microsatellite loci. No evidence of null alleles was found at these loci using MICRO-CHECKER. A few genotypes at distinct loci in several collection areas deviated significantly from the expectations of Hardy–Weinberg equilibrium owing to an excess of homozygotes (Table S5). There were no missing data in the *S. lewini* microsatellite dataset. All pairwise comparisons showed that loci were not in linkage disequilibrium after global and population-specific analyses. The genetic relatedness estimator of Wang (Wang 2002) showed the higher correlation coefficient (r = 0.796). Overall, individuals of *S. lewini* within and between each collection area did not deviate from that expected under random mating (Fig. S3). Therefore, all individuals were considered unrelated and did not bias further analysis. All loci presented a uniform average allelic richness (mean 4.67 ± 0.28). The allelic richness per collection area samples ranged from 4.2 (CB) to 5.0 (RS), respectively. The observed and expected heterozygosity per locus ranged from 0.08 (Sle54) to 0.76 (Sle77, Sle89), and from 0.08 (Sle54) to 0.78 (Sle77), respectively, whereas the per collection area values ranged from 0.65 (CB) to 0.52 (SC), and 0.68 (CB) to 0.59 (SC), respectively. The inbreeding coefficients per locus ranged from 0.00 (Sle89) to 0.28 (Sle53), and for each collection area from − 0.016 (RN) to 0.115 (RS) (Table 1; Table S5). Also, 17 private alleles were identified in most of the collection areas, with exception of PA and RN, which had no private alleles (Table S6).

Tests for microsatellite-based genetic structure, global D_{est} and F_{ST} indexes revealed significant values for *S. lewini* along the western Atlantic Ocean (D_{est} = 0.0794, p = 0.001; F_{ST} 0.046, $p < 0.05$). Similar to the mtCR Φ_{ST} results, the microsatellite F_{ST} index showed low but significant values of genetic differentiation when GM and CB individuals were compared to each other and to any of the sampling locations in Brazil (Table 4). The D_{est} index values were slightly higher, but concordant in revealing significant genetic differentiation among GM, CB, and

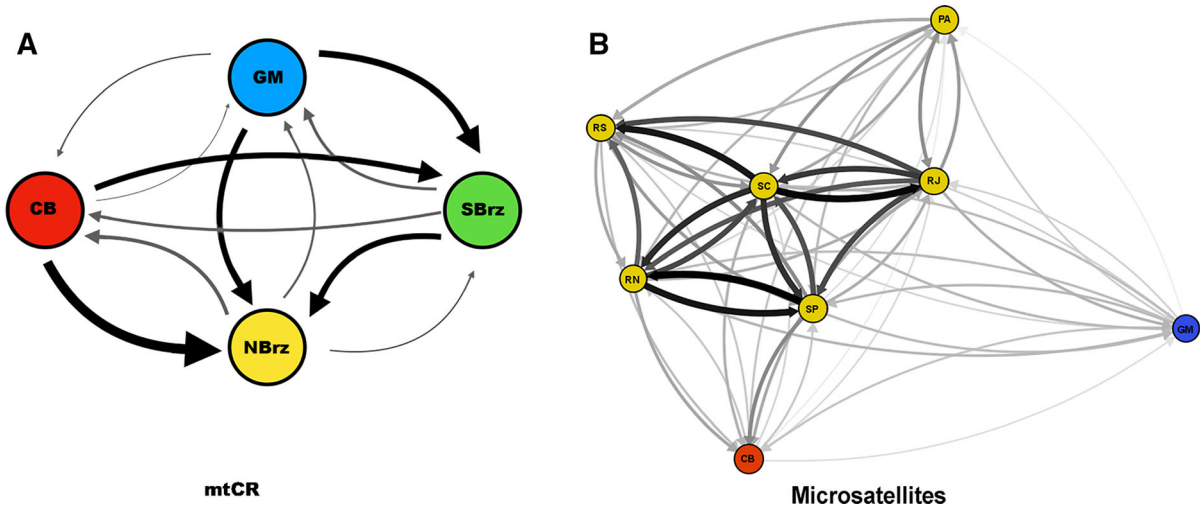

Fig. 3 Migration rates among *Sphyrna lewini* populations. **a** Historical gene flow (mtDNA) estimated by migrate-n, and **b** contemporary gene flow (microsatellites) estimated by divMigrate package. For both A and B arrows were weighted according to number of migrant values presented in Tables S2 and S5, and arrowheads show the estimated direction of gene flow. Collection area: Gulf of Mexico (GM), Caribbean Sea (CB), Pará (PA), Rio Grande do Norte (RN), Rio de Janeiro (RJ), São Paulo (SP), Santa Catarina (SC), and Rio Grande do Sul (RS)

Table 4 Population pairwise F_{ST} (above) and D_{est} (below) values from 10 microsatellite loci for *Sphyrna lewini*

	GM	CB	PA	RN	RJ	SP	SC	RS
GM	–	**0.067**	**0.078**	**0.034**	**0.058**	**0.041**	**0.049**	**0.063**
CB	**0.193**	–	**0.061**	**0.034**	**0.052**	**0.034**	**0.054**	**0.047**
PA	**0.170**	**0.153**	–	**0.026**	0.005	**0.037**	0.004	0.015
RN	**0.091**	**0.106**	0.044	–	**0.015**	0.004	0.007	**0.022**
RJ	**0.131**	**0.146**	0.011	**0.033**	–	**0.017**	0.006	**0.021**
SP	**0.097**	**0.107**	**0.068**	0.006	**0.043**	–	**0.012**	**0.029**
SC	**0.105**	**0.155**	0.012	0.014	0.020	**0.026**	–	**0.017**
RS	**0.155**	**0.144**	0.042	**0.051**	**0.053**	**0.072**	**0.045**	–

Bold values indicate significance $p < 0.05$ after Holm–Bonferroni correction. Collection areas: Gulf of Mexico (GM), Caribbean Sea (CB), Pará (PA), Rio Grande do Norte (RN), Rio de Janeiro (RJ), São Paulo (SP), Santa Catarina (SC), and Rio Grande do Sul (RS)

the Brazilian collection areas. The pairwise F_{ST} and D_{est} indexes demonstrated no consistent concordance in genetic structuring among the collection areas within Brazil (Table 4). DAPC multivariate approach based on eight geographic regions run in an attempt to maximize variation between localities, separated all collection areas in three groups: GM, CB and overlapped all collection areas from Brazil into a same group (Fig. 4a). Likewise STRUCTURE cluster analyses supported the existence of 3 populations ($K = 3$) (Fig. 4b; Fig. S4). Comparison of the proportional membership (Q) inferred by STRUCTURE showed high level of admixture. Some individuals sampled in SP have a different allele composition, somewhat similar to the allelic composition of individuals sampled in the GM. However, the other collection areas in Brazil shared a large number of alleles and do not constitute structured populations. The microsatellite Mantel test indicated a high and significant IBD ($r = 0.638$, $p = 0.007$) (Fig. S2B). Although no significant asymmetries were detected, relative pairwise migration and gene flow demonstrated a strong and directional connectivity pattern among southwest Atlantic populations, except PA samples probably because of their low sample size. On the other hand, lowest genetic exchange occurred between southwest Atlantic, CB, and GM, with nearly identical patterns of genetic population structure demonstrated by cluster analyses (STRUCTURE and DAPC), and pairwise fixation indexes (D_{est} and F_{ST}) (Fig. 3b; Table S7). The directional migration, between South and North Brazil (yellow) was found to be strongest, whereas directional migration from GM (blue) and CB (red) to other locations was considerably lower (Fig. 3b; Table S7).

The recent population size reduction (bottlenecks effect) test did not show any significant excesses of heterozygosity under the TPM ($p < 0.05$), indicating that *S. lewini* populations from western Atlantic Ocean have not experienced a recent or strong bottleneck. The overall N*e* for *S. lewini* in the western Atlantic Ocean was 299 (215–412 CI). The N*e* values ranged from 129 (Caribbean Sea) to 425 (Brazil) (Table 5).

Discussion

Understanding connectivity and migration patterns is a fundamental step in guiding proper conservation and management decisions for endangered species. In this study we carried out an assessment of genetic diversity and population genetic structure of *S. lewini* in the western Atlantic Ocean. This is the first study to examine the fine-scale population genetic structure of this endangered hammerhead shark in this region, based on both mtCR and nuclear microsatellites markers. Except for the Pará (PA) collection area, our sample sizes are robust and equivalent to, or even larger, than those in previous reports on the genetic dynamics of this species in this region. The *Sphyrna lewini* shows moderate to low genetic diversity at mtCR, and moderate diversity at ten microsatellites markers. Significant genetic structure was detected in the western Atlantic Ocean, demonstrating the presence of at least three regional management units: Gulf of Mexico, Caribbean Sea, and Brazil. The effective

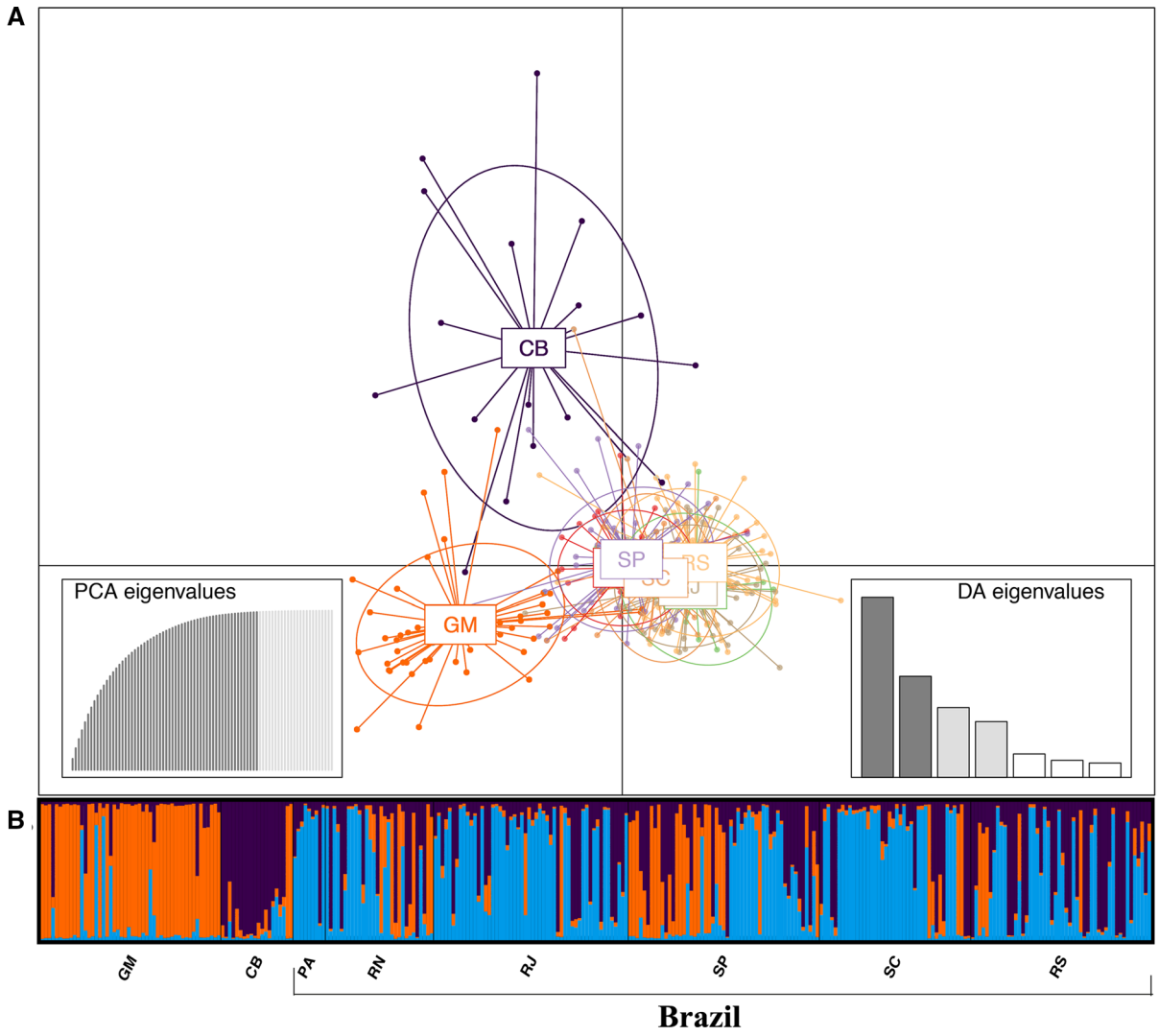

Fig. 4 **a** DAPC plot from the first and second components from nuclear microsatellite genotypes of the *Sphyrna lewini* in the western Atlantic Ocean. **b** Proportional membership (*Q*) of each *Sphyrna lewini* in the genetic cluster inferred by STRUCTURE with K = 3. A vertical bar represents each individual, and the length of each bar indicates the probability of membership in each cluster

population size was low for all sampled areas, although there was no evidence of recent or strong bottleneck events.

Genetic diversity

In general, mtCR and nuclear microsatellite genetic diversity showed levels similar to other shark species in the western Atlantic, such as the semi-coastal/oceanic tiger shark *G. cuvier* (h = 0.65, π = 0.0014, Ho = 0.71, Bernard et al. 2016), and the coastal nurse shark *Ginglymostoma cirratum* (h = 0.48, π = 0.008, Ho = 0.58, Karl et al. 2012). On the other hand, the mtCR and microsatellite variability in *S. lewini* was lower than reported for primarily pelagic shark species in the Atlantic Ocean, such as the silky shark *Carcharhinus falciformis* (h = 0.88, π = 0.005, Domingues et al. 2018b) and the blue shark *Prionace glauca* (h = 0.89, π = 0.003, Ho = 0.63, Veríssimo et al. 2017). These findings are consistent with the idea that pelagic sharks have greater genetic diversity than

Table 5 Effective population size for *Sphyrna lewini* within the three management units identified in the western Atlantic Ocean

Population	n	N*e*	Upper	Lower
GM	50	203	∞	74
CB	20	129	∞	49
Brz	229	307	553	203
Total	308	299	452	215

Brazilian populations subdivided into Northwest and Southwest Brazil for comparative visualization only. n: number of individuals; N*e*: effective population size, Upper and Lower: 95% Confidence Intervals. Collection areas: Gulf of Mexico (GM), Caribbean Sea (CB), Brz (Brazil)

coastal and semi-oceanic shark species (Karl et al. 2011).

The genetic diversity at mtCR of *S. lewini* was higher than previously reported for this species in western Atlantic Ocean ($h = 0.375$, $\pi = 0.0013$, Chapman et al. 2009). This was probably due to the increased sequence dataset utilized in our study (complete mtCR) compared to the partial mtCR dataset in Chapman et al. (2009), and the larger number of samples we evaluated. In addition, for microsatellites markers observed heterozygosity (Ho) showed similar values to *S. lewini* from South Carolina (Ho = 0.537) and lower values than Pacific (Ho = 0.739) and Indian (Ho = 0.729) Oceans (Daly-Engel et al. 2012), revealing lower genetic diversity for *S. lewini* in the Atlantic Ocean than others oceans. Among hammerhead shark species *S. lewini* has by far undergone the highest population decline worldwide (Ferretti et al. 2010, Barreto et al. 2016). In the Atlantic Ocean, *S. lewini* abundance has decreased approximately 75% in the North Atlantic (Hayes et al. 2009) and 80% in the South Atlantic (Cortés et al. 2010; Barreto et al. 2016). Although some studies have related overfishing to erosion of genetic diversity (e.g., Hauser et al. 2002, Pinsky and Palumbi 2014), it is difficult to ascertain the role of overfishing in the low to moderate genetic diversity we detected in *S. lewini*. In addition to overfishing, other factors could also be affecting the genetic diversity of *S. lewini* in the western South Atlantic, such as reproductive behavior (e.g. multiple paternity and philopatry), and even their broad geographic distribution (Chapman et al. 2009; Domingues et al. 2018a, b).

Compared to other endangered shark species, overall N*e* for *S. lewini* was modest, similar to that found in the zebra shark *Stegostoma fasciatum* from Australia (Dudgeon and Ovenden 2015) and in the great white shark *Carcharodon carcharias* from South Africa (Andreotti et al. 2016). In particular, the Gulf of Mexico population of *S. lewini* presented a very similar N*e* to the critically endangered smalltooth sawfish *Pristis pectinata* in the same location (Chapman et al. 2011). Although a recent bottleneck effect was not detectable for *S. lewini* in this study, probably because only few generations have passed since the beginning of overfishing, these results appear to corroborate the assumption that shark populations from the Gulf of Mexico region have suffered a N*e* reduction (Hayes et al. 2009). Estimating N*e* of endangered populations and species is important because genetic drift can erode genetic variation through fixation of deleterious alleles (Hare et al. 2011). Empirical studies suggest that a $Ne \geq 100$ is necessary to prevent short-term genetic erosion and loss of fitness over five generations, whereas a $Ne \geq 1000$ is required to retain long-term evolutionary potential (Frankham et al. 2014). Therefore, the estimated N*e* of *S. lewini* is sufficient for short-term conservation efforts, but may be too low to ensure long-term health of the species, even when considering upper values from the estimated N*e* confidence intervals. However, these results should be considered carefully because sample size at some location were low and could affect these estimates.

Population enetic structure and migration

Genetic heterogeneity was detected among *S. lewini* collection locations in the western Atlantic Ocean. Overall levels of genetic differentiation observed for mtCR were comparable to those reported for other coastal and pelagic shark species with similar life history strategies in this region, *i.e.* the lemon shark *Negaprion brevirostris* (Ashe et al. 2015), the Caribbean reef shark *Carcharhinus perezi* (Bernard et al. 2017), the silky shark *C. falciformis* (Domingues et al. 2018b), the night shark *C. signatus* (Domingues et al. 2019), and the tiger shark *G. cuvier* (Carmo et al. 2019). Analysis of mtCR sequences and nuclear microsatellites both showed population genetic structuring among Gulf of Mexico, Caribbean Sea, and Brazil populations, albeit with some level of admixture. Chapman et al. (2009) also observed such mtCR population structure for *S. lewini* in the western

Atlantic Ocean. Additionally, mtCR population structure appears to be present within Brazil, with significant genetic differentiation observed between the Northwest (PA and RN) and Southwest (RJ, SP, SC, RS) Brazilian populations. Findings of mtCR genetic population structure for various shark species along the western Atlantic Ocean are congruent with philopatric reproductive behaviour or coastal-dependency of each species. For example, pelagic shark species with high capacity of locomotion such as *Pseudocarcharias kamoharai* (Ferrette et al. 2015), *P. glauca* (Veríssimo et al. 2017), *C. falciformis* (Domingues et al. 2018b), and *Alopias superciliosus* (Morales et al. 2018) showed little or absent genetic population structure in the Atlantic Ocean. On the other hand, coastal or reef-associated shark species, such as *N. brevisrostris* (Ashe et al. 2015), *C. perezi* (Bernard et al. 2017), and *G. cuvier* (Carmo et al. 2019), showed higher levels of mtCR population subdivision, similar to our findings. Such mtCR population genetic structure observed in coastal-dependency shark species, indicates that individuals of *S. lewini* can remain close to, or home back to, their natal region for parturition rather than expected based on the potential mobility of the shark species. Indeed, reproductive philopatric behavior has been used to explain population structure for several shark species as an alternative to the presence of physical barriers (Chapman et al. 2015).

Although *S. lewini* was previously reported as displaying global male-mediated gene flow (Daly-Engel et al. 2012), the microsatellite population-level analyses outcomes observed by us suggest there could be ecological, oceanographic, or behavior barriers to male dispersion strong enough to restrict connectivity (Bernard et al. 2016). For instance, it can be hypothesized that Amazon river's outflow, which extends thousands of kilometers in the Atlantic Ocean, might constitute a soft barrier between Caribbean and Brazil (Floeter et al. 2008). Moreover, Gulf of Mexico and Caribbean are recognized as distinct ecoregions from one another on the basis of faunal composition and oceanographic landscapes (Spalding et al. 2007; Kulbicki et al. 2013). However, given *S. lewini* high mobile capacity and based on microsatellite results, it is possible that male homing may also be occurring. Despite this microsatellite population-level based differentiation, the STRUCTURE output showed high level of admixture among identified clusters, but few ancestry membership proportions for individuals, suggesting some level of migration with little reproductive exchange. Indeed, the link between the movements of *S. lewini* individuals and the structure of their population are related to reproductive philopatric behavior, where male and female probably migrate to different sites in the Atlantic Ocean, but returning to same area for parturition. Therefore, individuals of *S. lewini* with high coefficient of ancestry belonging to Gulf of Mexico and Caribbean and found in the Brazil cluster probably are migrating between these regions, but they will return to population home to mating and parturition. However, this interpretation should be considered carefully, because high level of admixture also can be caused by low F_{ST} values or use of a small number of microsatellites, factors recognized to potentially bias admixture analysis (Putman and Carbone 2014); also, genetic drift could play a role in shaping such similar allele frequencies between populations purely by chance. Nevertheless, individual-level assignment tests (i.e., STRUCTURE and DAPC analysis) and assessment of migration patterns showed that there is different degree of migration and consequent gene flow among these locations. Indeed, assessment of migration patterns from mtCR and microsatellites demonstrated that the populations previously identified have less genetic exchange among them. Furthermore, analysis of microsatellites data using divMigrate networks showed that the magnitude and direction of gene flow was nearly identical to patterns of population genetic structure found by STRUCTURE and DAPC. The connectivity within Brazil sampling regions was substantially higher compared with connectivity to Gulf of Mexico and Caribbean.

In addition to restricted genetic connectivity among populations of *S. lewini* in the Atlantic Ocean, IBD test results from both molecular markers also suggest that individuals of *S. lewini* do not disperse long-distances. Previous shark tagging studies also support these results (Wells et al., 2018). For example, Kohler and Turner (2001) reported that the average distance travelled by conventionally tagged *S. lewini* was approximately only 100 km, with one individual travelling a maximum distance of 1671 km. Such genetic isolation as a result of geographic distance between populations offers evidence that male and female individuals of *S. lewini* are most likely moving between nearby regions (Chapman et al. 2009, 2015).

This statement was previously observed for others shark species in the Atlantic Ocean such as the bonnethead *Sphyrna tiburo* (Portnoy et al. 2015), and the lemon shark *N. brevirostris* (Ashe et al. 2015). In both studies the reproductive philopatric behavior is used to explain IBD. Therefore, rather than oceanographic or geophysical barrier, reproductive philopatric behavior is the main factor driving population subdivision in *S. lewini* in the Atlantic Ocean. Finally, considering the rarity of observed long-distance migrations by *S. lewini* and the extent and geographic level of genetic structure observed, we hypothesize that *S. lewini* has at least three populations in the western Atlantic Ocean, and each population with its own nursery ground and distinct ontogenetic and annual cycles for migration from nursery grounds to offshore habitats.

Implications for conservation and management

Sphyrna lewini were among the most frequently landed sharks during the 20th Century by artisanal and industrial fisheries worldwide, with consequent severe declines in populations putatively impacting their evolutionary potential (Hayes et al. 2009; Gallagher and Klimley 2018). Our results offer new insights into the genetic connectivity and diversity dynamics of *S. lewini* populations in the western Atlantic, through large sampling efforts in several locations known to correspond to coastal nursery grounds in the Southwest Atlantic Ocean, and by including the analysis of bi-parentally inherited, nuclear microsatellite loci. Our fine-scale population genetics analysis revealed a low level of population structure in Brazil and the existence of at least three management units within the western Atlantic Ocean, comprising *S. lewini* inhabiting the Gulf of Mexico, Caribbean Sea and Brazil. Identification of these genetic populations can guide the implementation of international measures aimed at *S. lewini* management and conservation by considering the intrinsic demographic characteristics of each population. Finally, the low effective population size of *S. lewini* in the western Atlantic highlights the need for urgent conservation measures to safeguard the demographic and evolutionary potential of this endangered species.

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Authors contribution DP, CM, MS designed the study; DP, OBFG, MS collected data; DP and CB performed lab work; RRD, DP, BF analyzed the data; RRD and DP wrote the manuscript; all authors edited and revised the manuscript.

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Data accessibility The sequencing data and microsatellite dataset of *Sphyrna lewini* in this study are archived in GenBank (accession numbers: MK636839-MK636863) and Dryad database (https://doi.org/10.5061/dryad.pp8js50), respectively.

Compliance with ethical standards

Conflict of interest The authors have no conflict of interests.

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