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RESEARCH PAPER

Effects of the Pleistocene on the mitochondrial population genetic structure and demographic history of the silky shark (*Carcharhinus falciformis*) in the western Atlantic Ocean

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Abstract The silky shark, *Carcharhinus falciformis*, is a large-bodied, oceanic-coastal, epipelagic species found worldwide in tropical and subtropical waters. Despite its commercial importance, concerns about overexploitation, and likely ecological significance of this shark as an upper trophic-level predator, understanding of its population dynamics remains unclear for large parts of its distribution. We investigated the genetic diversity, population structure and demographic history of the silky shark along the western Atlantic Ocean based on the use of 707 bp of the mitochondrial DNA control region (mtCR). A total of 211 silky sharks were sampled, originating from five areas along the western Atlantic Ocean. The mitochondrial sequences revealed 40 haplotypes, with overall haplotype and nucleotide diversities of 0.88 (± 0.012) and 0.005 (± 0.003), respectively. The overall population structure was significantly different among the five western Atlantic Ocean regions. Phylogenetic analysis of mtCR sequences from globally sourced silky shark samples revealed two lineages, comprising a western Atlantic lineage and western Atlantic—Indo-Pacific lineage that diverged during the Pleistocene Epoch. In general, tests for the demographic history of silky sharks supported a population expansion for both the global sample set and the two lineages. Although our results showed that silky sharks have high genetic diversity, the current high level of overexploitation of this species

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requires long-term, scientifically informed management efforts. We recommend that fishery management and conservation plans be done separately for the two western Atlantic matrilineal populations revealed here.

Keywords Elasmobranch · Fisheries management · Gene flow · Genetic diversity · Marine connectivity · Phylogeography

Introduction

Determining current spatial patterns of genetic diversity and the degree of isolation or connectivity of populations is fundamental to the study of population dynamics. In mobile species, these patterns may reflect historical events such as vicariance and migrations caused mainly during the Pleistocene Epoch (Avise 2000; Gilman et al. 2010; Grant 2015). In marine systems, climate changes during the last glacial maximum (LGM) are among the most influential factors affecting species distribution and demographic patterns, and the genealogies of most species coalesce during this period (O'Brien et al. 2013; Grant 2015). The understanding of demographic history is profoundly relevant to explain phylogeographic patterns over microevolutionary time scales due to their impact on the structure of gene genealogies (Avise 2000). Thus, elucidating past evolutionary processes facilitates understanding of the mechanisms that shape current genetic differentiation patterns in a given species.

There are relatively few phylogeographic studies on elasmobranchs compared to teleosts (Beheregaray 2008). Encouragingly, the number of phylogeographic studies of sharks has increased in the last decade (Dudgeon et al. 2012; Portnoy and Heist 2012), but there are large gaps in the species examined and the scale of geographic coverage for the many shark species with global distributions. Phylogeography studies are important not only to shed light on evolutionary mechanisms of diversity and population structure, but also for informing management and conservation efforts, particularly of exploited shark species (Rocha et al. 2007).

The silky shark, *Carcharhinus falciformis*, is large-bodied, highly exploited, oceanic and epipelagic species found worldwide in tropical seas. It is most commonly found in waters less than 200 m near continental and insular shelf edges, and also occasionally inshore to 18 m (Ebert et al. 2013). The silky shark has been intensively caught worldwide as bycatch (Bonfil et al. 2008; Ebert et al. 2013) leading to severe population declines (Barreto et al. 2016). Furthermore, silky shark captures are boosted by high shark fin prices, which make this species one of the three most important shark species in the global shark fin trade (Clarke et al. 2006).

Different aspects of silky shark biology have been assessed across its distribution, including reproduction (e.g., Branstetter et al. 1987; Hazin et al. 2007), movements and migration (e.g. Kohler et al. 1998), fisheries (e.g. Beerkircher et al. 2003, Domingues et al. 2016), age and growth (e.g. Branstetter et al. 1987; Sánchez-de Ita et al. 2011) and feeding habits (e.g. Cabrera-Chávez-Costa et al. 2010). Population genetic aspects of this species have been recently reported, but remain in an early state of understanding, particularly with respect to the global geographic distribution of this species (Galván-Tirado et al. 2013). Clarke et al. (2015) examined silky shark phylogeography and population genetics on a global scale, finding strong phylogeographic partitioning with two highly divergent, matrilineal evolutionary lineages corresponding to the western Atlantic and Indo-Pacific Oceans, but panmitic populations along the western Atlantic Ocean. However, since sample representation from the western South Atlantic was limited in their study and silky sharks represent important fishery captures in this region, we investigated if analyses of more geographically widespread samples from along the entire western Atlantic would provide an improved perspective of silky shark population genetic dynamics.

Herein, we expand the phylogeographic and population genetics assessment of silky sharks by Clarke et al. (2015) by substantially increasing the sample sizes and geographic distribution of sharks from the western South Atlantic. We also conduct additional global phylogeographic analyses using an expanded dataset that includes data from Clarke et al. (2015). Specifically, we tested the hypothesis of matrilineal genetic panmixia along the western Atlantic Ocean, and examined the impact of glacial cycles during the Pleistocene on historical population demographic patterns of the silky shark globally. Finally, the possible role of geographic barriers, oceanic currents and ecological behaviors on shaping

silky shark phylogeographic structure are discussed along with their implications for the fisheries management of this highly exploited species.

Materials and methods

Sampling sites and DNA extraction

A total of 211 silky sharks were sampled from five western Atlantic Ocean sites: USA east coast—USA (43), Gulf of Mexico—GM (42), Pará State, Northern Brazil—PA (16); Saint Paul and Saint Peter Archipelago, Northeast Brazil—ASPSP (48) and Santa Catarina State, Southern Brazil—SC [(62) (Fig. 1)].

Muscle and/or fin clips were sampled from sharks landed in fishing ports and by fishery observers while at sea from sharks captured by commercial pelagic tuna long liners. The tissue samples were stored in 95% ethanol at − 20 °C until genomic DNA extraction. Total genomic DNA was extracted from approximately 20 mg of tissue using the DNAQIAmp

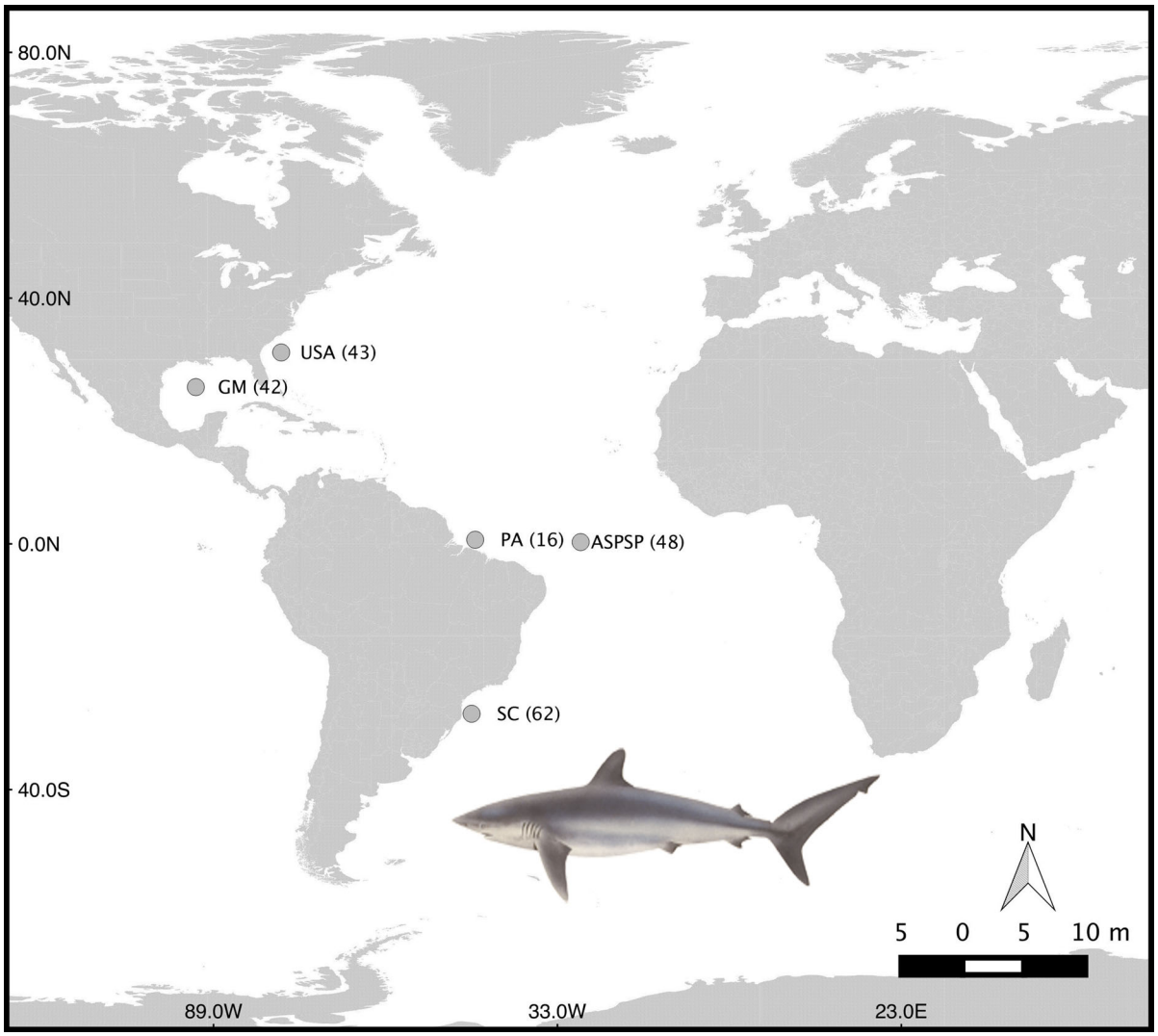

Fig. 1 Location of samples collected and samples sizes in parentheses of the silky shark (*Carcharhinus falciformis*) along the western Atlantic Ocean. Sample site: USA east coast (USA), Gulf of Mexico (GM), Pará State, Northern Brazil (PA), Saint Paul and Saint Peter Archipelago, Northeast Brazil (ASPSP) and Santa Catarina State, Southern Brazil (SC). Samples sizes in parentheses

Tissue kit (Qiagen Inc., Valencia, CA, USA). To prevent morphological species misidentification from causing downstream interpretation errors, species identity of all samples was first confirmed using the multiplex Polymerase Chain Reaction (PCR) method in Domingues et al. (2013).

Amplification and sequencing

The partial mitochondrial DNA control region (mtCR) was amplified by PCR using two external primers: Pro-L (5´-AGGGRAAGGAGGGTCAAACT-´3) (Keeney and Heist 2006) and Prol-Falc2 (5´-CCCGGGGTGAT CCTAATAAT-´3) developed in this study. Amplification was performed in 25µl reactions containing 2 µL of the extracted genomic DNA (concentration unknown), 10.0 mM of each primer, 1X Buffer, 2.5 mM dNTPs, 2.0 units of Taq polymerase (Fermentas, Life Science) and ultrapure water. The PCR thermal cycling profile consisted of 15 min at 95 °C, followed by 35 cycles at: 94 °C for 1 min, 62 °C for 30 s, and 72 °C for 30 s with a final extension step at 72 °C for 7 min. The PCR products were purified using QIAquick PCR purification kit (Qiagen Inc., Valencia, CA, USA) following the manufacturer's instructions. Cycle sequencing was performed using the Applied Biosystems Big Dye Terminator v3.1 kit (Life Technologies, Carlsbad, CA, USA) following the manufacturer's directions. The amplicons were sequenced on an Applied Biosystems 3130 Genetic Analyzer and bases called using Applied Biosystems Sequencing Analysis Software version 5.2. All amplicons were sequenced twice to avoid errors. The sequences were edited manually when necessary using Codon Code Aligner (v. 5.1.5, Codon Code Corporation).

Genetic diversity and population structure

Genetic diversity parameters such as nucleotide composition, number of transitions and transversions, haplotype (h) and nucleotide (π) diversities, number of haplotypes (H), variable sites (S) and polymorphism distribution were estimated using Arlequin 3.5 (Excoffier and Lischer 2010). To test the null hypothesis of western Atlantic panmixia, the genetic structure of the populations was estimated among all localities using an analogue Wright's F_{ST} (Φ_{ST}) with the 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). The global population structure and proportional variance distributed among sampling locations was estimated using an analysis of molecular variance—AMOVA (Arlequin 3.5; Excoffier et al. 1992) in a hierarchical framework of different population scenarios. A Mantel test was performed to determine if there was significant isolation by distance (IBD) among populations by testing for a correlation between genetic and geographic distance (the center of each sampling locality was used to estimate the minimal nautical distance between them) using R v.3.1.2 (R Core Team 2014). The statistical significance of the result was tested with 10,000 permutations.

Phylogenetics and demographic history

Phylogenetic relationships among the silky shark mtCR haplotypes were estimated using Maximum-Likelihood (ML) and Bayesian approaches. Assessment of global scale relationships among Atlantic and Indo-Pacific Ocean sharks was conducted by combining haplotypes from our samples with those from Clarke et al. (2015) (GenBank Accession Numbers KM267565–267626). Both ML and Bayesian trees were built under the HKY + I model based on the Akaike Information Criterion (AIC) as implemented in jModelTest v. 2.1.6 (Darriba et al. 2012). The program Mega 6.0 (Tamura et al. 2013) was used to generate the ML tree with 1000 bootstrap replicates. Mitochondrial control region sequences from the congener sharks *Carcharhinus limbatus* (GenBank accession number: AY766146.1) and *Carcharhinus plumbeus* (GenBank accession number: GU724583) were used as outgroups.

To estimate the divergence time between silky shark lineages identified in the ML tree, we used the program BEAST v 1.8.2 (Drummond et al. 2012) under a constant size prior and strict clock model. According to Drummond and Bouckaert (2015) a strict clock model is usually preferred over the relaxed clock model for assessing intraspecific phylogeography. Due to lack of a species-specific mutation rate for silky sharks, we used the mutation rate estimated for the congeneric species *Carcharhinus limbatus* (0.43% Myr^{-1}, Keeney and Heist 2006). The posterior distribution of parameters was estimated using MCMC with 10 million generations (first

10% discarded as burn-in). The convergence of the model and the effective sample size (ESS) was checked using TRACER v. 1.6 (Rambaut et al. 2014). TREEANNOTATOR 1.8.2 (Drummond et al. 2012) was used to summarize the information of the tree produced by BEAST, and FIGTREE v 1.4.2 (Rambaut 2008) was used to view the phylogenies. The relationships among haplotypes and their geographic distribution was assessed using the Median-joining algorithm in NETWORK 4.6.1.1 (www.fluxus-engineering.com).

Different approaches were applied to infer population demographic history for western Atlantic silky sharks. The mismatch analysis was tested using two models: sudden demographic expansion (Rogers and Harpending 1992) and spatial expansion (Ray et al. 2003). Theoretical studies have shown that populations at demographic equilibrium have a multimodal distribution, whereas the unimodal distribution is interpreted as demographic expansion (Slatkin and Hudson 1991, Rogers and Harpending 1992). The goodness of fit between the observed and expected mismatch distribution for both models was used to test the null hypothesis of demographic expansion by calculating the Harpending's raggedness index—H*ri* (Harpending 1994) and the sum of squared deviations (SSD), as implemented in Arlequin 3.5. In addition, two other neutrality tests, Ramos-Onsis and Rozas's R_2 (Ramos-Onsis and Rozas 2002) and Fu's F*s* (Fu 1997) were run, and statistical significance of each index tested with 10,000 simulations under coalescent process as implemented in DNAsp v.5 (Librado and Rozas 2009). Both R_2 and Fu's F*s* tests are more powerful at detecting events of demographic expansion and deviations from neutrality, with R_2 being more robust for small samples sizes, whereas Fu's *Fs* is better for large samples sizes (Ramos-Onsis and Rozas 2002).

Bayesian skyline plots (BSP) were used to infer female effective population sizes and its changes over time using the software BEAST v 1.8.2 (Drummond et al. 2012). This model generates a posterior probability for female effective population size using Markov Chain Monte Carlo (MCMC). The MCMC was run for 10 million generations and the first 10% were discarded as the burn-in times. We assumed the substitution model HKY-I, strict molecular clock, a piecewise-constant Bayesian skyline tree prior and a mutation rate of 0.43% Myr^{-1} as mentioned above. We assumed a generation time of 8 years for Atlantic silky sharks (Branstetter 1987). The Bayesian skyline plot reconstruction was conducted in TRACER v. 1.6 (Rambaut et al. 2014).

Results

Genetic diversity and population structure

A total of 707 bp of the mtCR were resolved from 211 silky sharks. The DNA fragment sequenced begins at 119 bp and ends at 825 bp relative to the entire mtCR sequence from the silky shark (GenBank: KM267626.1). The final sequences resolved 40 haplotypes (GenBank Accession Numbers KU497450–KU497489), 23 variable sites, and 23 mutations (17 transitions, 5 transversions, 1 indel). The nucleotide composition was 30.73% A, 20.38% C, 14.06% G, and 34.83% T. Overall haplotype and nucleotide genetic diversities were $h = 0.885$ ($\pm$ 0.012) and $\pi = 0.005$ ($\pm$ 0.003), respectively. The haplotype values ranged between a low of 0.789 $\pm$ 0.035 (SC) and a high of 0.925 $\pm$ 0.021 (USA); nucleotide values ranged between a low of 0.003 $\pm$ 0.002 (SC and PA) and a high of 0.007 $\pm$ 0.004 (USA) (Table 1). The haplotypes H8 (n = 50), H9 (n = 37) and H6 (n = 26) were the most common, occurring in all localities (Fig. 2). A total of 22 haplotypes were singletons and occurred mainly in the ASPSP site (Table S1).

The hypothesis of panmixia of silky sharks along the sampled region of the western Atlantic Ocean was rejected based on a statistically significant overall $\Phi_{ST} = 0.058$ ($P < 0.0001$), revealing population genetic structure. The AMOVA including all locations showed that higher genetic variation was found within locations (94.2%), whereas hierarchical AMOVA that tested different scenarios showed no population structure (Table 2). On the other hand, the pairwise Φ_{ST} values revealed significant matrilineal genetic differentiation mainly between locations from the Northwest Atlantic and the Southwest Atlantic. However, after sequential Holm-Bonferroni correction for multiple tests, only SC versus USA and SC versus GM were statistically significant (Table 3). The greatest difference was observed between SC and USA ($\Phi_{ST} = 0.160$, $P < 0.00$). The Mantel test of pooled samples did not reveal correlation between genetic distance and geographic distance ($r^2 = 0.155$,

Table 1 Genetic diversity indices and neutrality test results for western Atlantic silky sharks (*Carcharhinus falciformis*)

Sample site	*n*	S	H	*h*	π
USA	43	15	18	0.925 ± 0.021	0.007 ± 0.004
GM	42	17	19	0.918 ± 0.025	0.006 ± 0.003
PA	16	10	6	0.800 ± 0.069	0.003 ± 0.002
ASPSP	48	16	20	0.904 ± 0.025	0.005 ± 0.003
SC	62	12	11	0.789 ± 0.035	0.003 ± 0.002
Overall	211	23	40	0.885 ± 0.012	0.005 ± 0.003
L1	29	16	28	0.998 ± 0.012	0.004 ± 0.005
L2	11	13	11	0.999 ± 0.039	0.005 ± 0.001

n number of individuals, *S* polymorphic sites, *H* haplotype number, *h*: haplotype diversity and standard deviation, *π* nucleotide diversity and standard deviation

Sample site: USA east coast (USA), Gulf of Mexico (GM), Pará State, Northern Brazil (PA), Saint Paul and Saint Peter Archipelago, Northeast Brazil (ASPSP) and Santa Catarina State, Southern Brazil (SC)

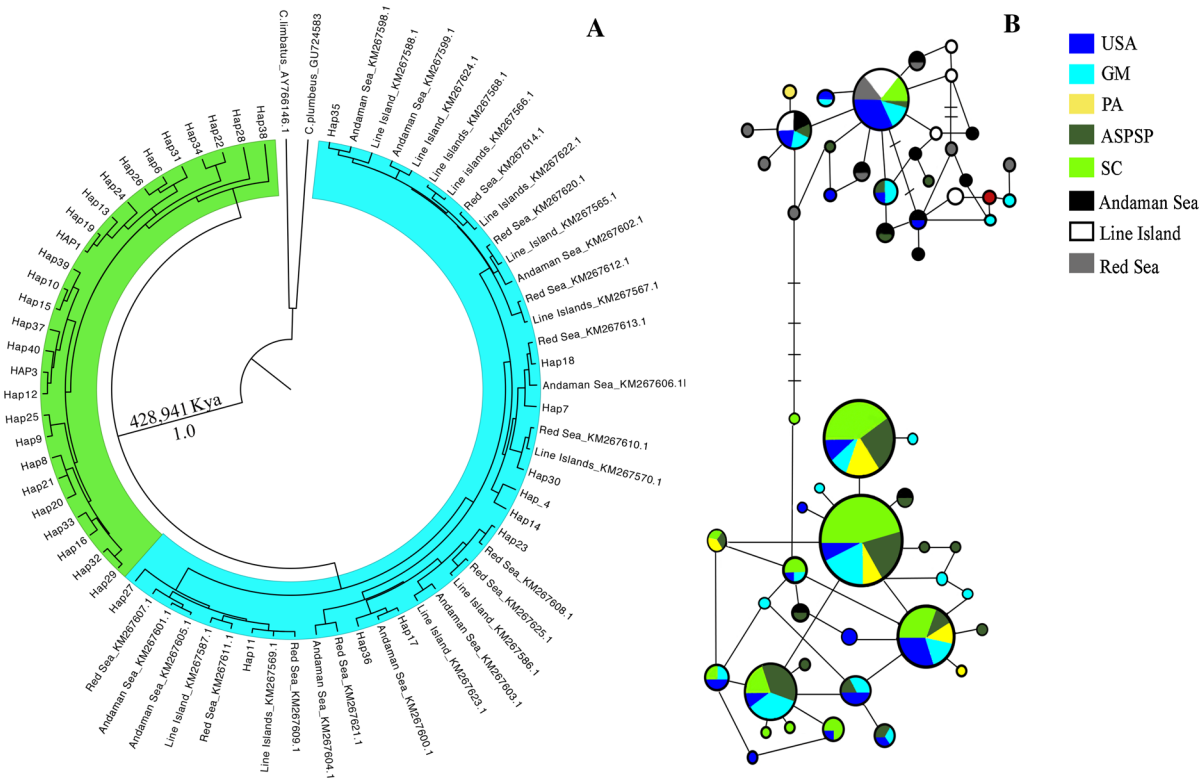

Fig. 2 Phylogenetic relationships inferred from 707 bp of mitochondrial DNA control region sequences from globally distributed silky sharks (*Carcharhinus falciformis*). **a** Bayesian phylogenetic tree with age shown above the branch for selected node over thousands of years (Kya). Color codes: blue: Lineage 1, and green: Lineage 2. **b** A median-joining haplotype network for the mitochondrial DNA control region. The circle sizes are proportional to the haplotype frequency. Connecting branches correspond to a mutational step between haplotypes; cross marks on branches represent individual mutational steps. Geographic color codes represent geographic sampling locations (red is median vector). Sample site: USA east coast (USA), Gulf of Mexico (GM), Pará State, Northern Brazil (PA), Saint Paul and Saint Peter Archipelago, Northeast Brazil (ASPSP) and Santa Catarina State, Southern Brazil (SC)

Table 2 Analysis of molecular variance (AMOVA) based on mitochondrial control region sequences from western Atlantic silky sharks (*Carcharhinus falciformis*)

	Structuring hypothesis	Φ_{CT}	*P*	% of variation
2 populations				
1	USA + GM × ASPSP + PA + SC	0.08	> 0.05	8.0
2	USA + GM × PA + SC	0.11	> 0.05	10.9
3	USA + GM + PA + SC × ASPSP	− 0.05	> 0.05	− 5.6
3 populations				
4	USA + GM × PA + SC x ASPSP	0.06	> 0.05	6.6
All populations				
5	USA + GM + PA + SC + ASPSP	0.058	< 0.05	5.8

Sample sites: USA east coast (USA), Gulf of Mexico (GM), Pará State, Northern Brazil (PA), Saint Paul and Saint Peter Archipelago, Northeast Brazil (ASPSP) and Santa Catarina State, Southern Brazil (SC)

Table 3 Pairwise Φ_{ST} values (below diagonal) and *P* values (above diagonal) for western Atlantic silky sharks (*Carcharhinus falciformis*)

Sample site	USA	GM	PA	ASPSP	SC
USA	–	0.270	**0.012**	**0.011**	**0.000***
GM	0.004	–	**0.047**	0.291	**0.004***
PA	**0.126**	**0.067**	–	0.192	0.380
ASPSP	**0.065**	0.003	0.021	–	0.122
SC	**0.160***	**0.072***	0.001	0.013	–

Analysis based on mitochondrial DNA control region sequences. Significant values ($P < 0.05$) before Holm-Bonferroni correction are bolded. Values marked by asterisks remain significant after sequential Holm-Bonferroni correction for multiple tests

Sample sites: USA east coast (USA), Gulf of Mexico (GM), Pará State, Northern Brazil (PA), Saint Paul and Saint Peter Archipelago, Northeast Brazil (ASPSP) and Santa Catarina State, Southern Brazil (SC)

$P > 0.05$), failing to reject the null hypothesis of no IBD.

Phylogenetic and demographic history

The Bayesian (Fig. 2a) and ML (Figure S2) trees for the combined western Atlantic and Indo-Pacific (from Clarke et al. (2015) (GenBank Accession Numbers KM267565–267626) concordantly showed two deeply divergent lineages, lineage 1 (L1) and lineage 2 (L2). The L1 was composed of 29 haplotypes from the western Atlantic and the Indo-Pacific Oceans, whereas L2 exhibited 11 haplotypes exclusively from the western Atlantic Ocean. The divergence time between the two lineages was estimated as 428,941 thousand years ago (Kya) (95% highest posterior density—HPD = 257, 422–589,578 Kya; Fig. 2a). The median joining haplotype network also agreed with the results of the phylogenetic trees, resulting in two main clades separated by four mutational steps (Fig. 2b). The L1 was composed of haplotypes sampled from both the western Atlantic and Indo-Pacific, and had less frequent haplotypes with relatively more singleton haplotypes. The L2 was composed of silky sharks sampled exclusively from the western Atlantic, and included the most common haplotypes (H6, H8, and H9), which were linked to many other less frequent haplotypes forming a faint star phylogeny.

The demographic parameters showed contrasting results for different tests. The mismatch distribution for the overall global sample was bimodal, whereas it was unimodal for L1 and L2 (Fig. 3b, Figure S1B, D). The H*ri* index for both demographic models resulted

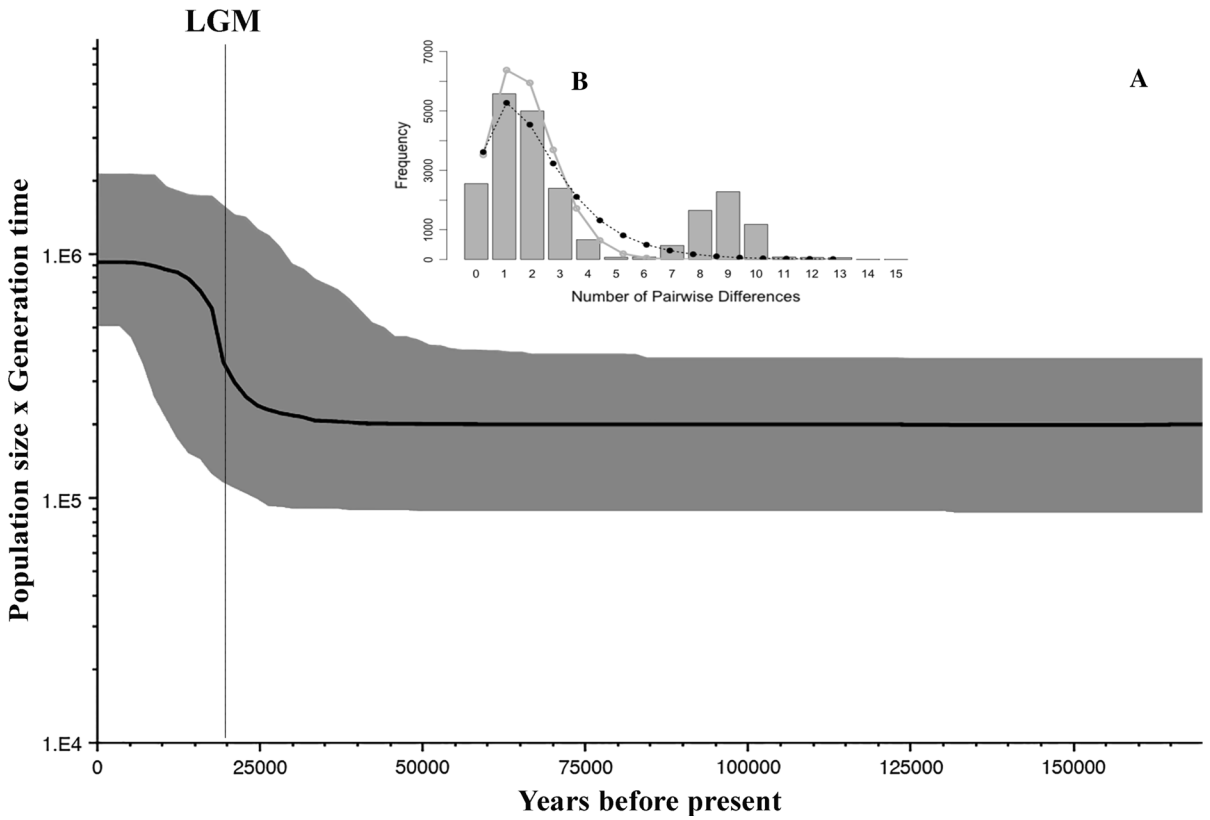

Fig. 3 Bayesian skyline plot and mismatch distribution based on mitochondrial DNA control region of western Atlantic samples of the silky shark (*Carcharhinus falciformis*). **a** Bayesian skyline plot showing the historical trends of female effective population sizes. Solid lines represent median estimates, and shaded areas represent the 95% HPD limits. **b** Mismatch distribution: observed values distribution represented by vertical bars. Solid line represents demographic expansion, and dashed line represents spatial expansion

in non-significant, low values for all locations. This result did not reject the null hypothesis of population expansion, whereas the SSD tests showed significant low values for GM and overall samples under a sudden expansion model and only for the USA population under the spatial expansion model (Table 4). The R_2 values were not significant at any location, whereas Fu's *Fs* test showed negative values in all locations. Only GM, ASPSP, and the overall data gave significant values ($P < 0.02$). Moreover, the mismatch distribution parameters showed zero values to θ_0, while θ_1 values tended to be infinite (Table 4). The demographic analysis for L1 also presented contrasting results with significant deviation from the mismatch distribution for both sudden and spatial expansion models (SSD and H*ri*); however Fu's *Fs* supports the population expansion hypothesis. In contrast, L2 showed a unimodal mismatch distribution and non-significant values of SSD and H*ri* for both models, and a significant value for the R_2 test. This does not reject the hypothesis of population expansion (Table 4). The Bayesian skyline plots to track historical fluctuation of female effective population size for the western Atlantic sample set of mtCR sequences showed a demographic increase starting about 30,000 years ago until about 10,000 years ago (Fig. 3a). The historical female effective population size was estimated to be 116,000 females. Both lineages (L1 and L2) had a population expansion that started about 90,000 years ago (Figure S1A, C).

Table 4 Mismatch distribution parameters and neutrality test results of western Atlantic silky sharks (*Carcharhinus falciformis*)

Sudden-expansion model							Spatial-expansion model				Neutrality tests			
Sample site	θ_0	θ_1	SSD	P SSD	*Hri*	*P Hri*	SSD	*P* SSD	H*ri*	*P* H*ri*	Fu's *Fs*	*P Fs*	R_2	*P* R_2
USA	0.000	7.523	0.046	0.151	0.046	0.408	**0.047**	**0.018**	0.046	0.230	− 4.446	0.058	0.156	0.921
GM	0.000	∞	**0.240**	**0.001**	0.052	0.960	0.035	0.094	0.052	0.242	− **6.606**	**0.011**	0.128	0.695
PA	0.000	∞	0.016	0.296	0.089	0.422	0.016	0.220	0.089	0.412	− 0.569	0.371	0.158	0.591
ASPSP	1.760	∞	0.027	0.227	0.054	0.249	0.027	0.061	0.054	0.253	− **8.673**	**0.001**	0.108	0.554
SC	0.007	∞	0.006	0.172	0.067	0.254	0.006	0.103	0.067	0.281	− 2.283	0.168	0.083	0.316
Overall	0.000	79.375	**0.031**	**0.013**	0.049	0.325	0.027	0.060	0.049	0.244	− **22.404**	**0.000**	0.079	0.514
L1	0.000	3.414	**0.030**	**0.000**	**0.099**	**0.002**	**0.030**	**0.001**	**0.099**	**0.005**	− **0.019**	**0.000**	0.124	0.080
L2	0.050	3.414	0.005	0.654	0.042	0.456	0.027	0.363	0.042	0.698	0.251	0.000	**0.166**	**0.026**

Fu's *Fs* test significant values ($P < 0.02$) are bolded

Other tests significant values ($P < 0.05$) are bolded

Sample sites: USA east coast (USA), Gulf of Mexico (GM), Pará State, Northern Brazil (PA), Saint Paul and Saint Peter Archipelago, Northeast Brazil (ASPSP) and Santa Catarina State, Southern Brazil (SC)

θ_0 theta at time zero, θ_1 theta at present time, *SSD* sum of squared deviations, *P SSD p* value of SSD, *Hri* Harpending's index, *P Hri* *p*-value of H*ri*, *Fu's Fs* Fu's neutrality test, *P Fs* *p*-value of Fu's test, R_2 Ramos-Onsis and Roza's neutrality test, *P* R_2 *p*-value of R_2 test

Discussion

The present results provide new insights into patterns of genetic differentiation and demographic history of the highly migratory, apex predator, silky shark in the western Atlantic Ocean. In summary, silky shark exhibited high mitochondrial control region genetic diversity, and statically significant population structure between the Northwest and Southwest Atlantic that was not detected in previous studies. We also found two globally divergent lineages originating during the Pleistocene, comprising a combined western Atlantic/Indo-Pacific lineage (L1) and an exclusively western Atlantic lineage (L2). Furthermore, demographic analyses supported a population expansion of both these lineages starting during the Pleistocene.

Genetic diversity and population structure

The overall genetic diversity detected in our study in the mtCR of silky sharks across much of the species' western Atlantic distribution ($h = 0.885 \pm 0.012$, $\pi = 0.005 \pm 0.003$) is very similar to that reported by Clarke et al. (2015) ($h = 0.93 \pm 0.01$, $\pi = 0.005 \pm 0.003$), despite their assessment of a comparatively much smaller sample size from the Southwestern Atlantic (Clarke et al. 2015, n = 39; our study n = 126). Our results are also consistent with that of Clarke et al. (2015) in showing that much higher matrilineal genetic diversity exists in Atlantic silky sharks compared to those from the Pacific ($h = 0.48 \pm 0.03$, $\pi = 0.0009 \pm 0.0001$; Galván-Tirado et al. 2013). This notable difference in genetic diversity of silky sharks from the two ocean basins is probably related to their different evolutionary histories in the different ocean basins, rather than overfishing in the Pacific, since both Pacific and Atlantic populations have suffered intense fishing pressure over the last few decades (Clarke et al. 2006; Baum et al. 2003; Barreto et al. 2016). Greater overfishing is also unlikely to be the cause of the lower genetic diversity observed in Pacific silky sharks, as the genetic signature of a recent abrupt demographic decrease may not be detected in the short term in species with long generation times and high connectivity (Allendorf et al. 2008; Rodríguez-Zárate et al. 2013; Pinsky and Palumbi 2014).

Overall, silky sharks generally showed higher genetic diversity compared to most congener species (Table S2), possibly because of the pelagic nature of the silky shark. The congeners with primarily coastal habitats such as *C. limbatus*, *C. melanopterus*, *C. leucas* and *C. sorrah*, showed generally lower genetic

diversity. In contrast, pelagic congeners (*C. obscurus*) and pelagic non-conger species (Table S2) overall showed higher genetic diversities, consistent with the observations of Karl et al. (2011) that pelagic sharks have higher genetic diversity compared to coastal species.

The AMOVA (overall Φ_{ST} 0.058, $P < 0.0001$) and overall pairwise Φ_{ST} values (Table 3) both revealed significant matrilineal genetic differentiation in western Atlantic silky sharks. These results are different from previous findings that did not reveal divergence among different areas along the western Atlantic Ocean in this species (Clarke et al. 2015). The different results between these two studies are probably due to inclusion of sampling sites further along the Southwestern Atlantic in our study. These outcomes could indicate the presence of at least two populations in the western Atlantic: Northwest (USA, GM) and Southwest Atlantic (SC). Indeed, this population subdivision has already been used for stock assessment of many pelagic shark species (ICCAT 2015), but is based solely on an assumed north versus south hemispheric division and not on scientific evidence (Cortés et al. 2015).

These population structure findings also raise the following question: What could be the source of such genetic differentiation patterns in a species with high dispersal capacity? Two likely explanations, or a combination of them, could explain this: oceanic currents and reproductive philopatry. Several authors have suggested that oceanic currents may affect the population structure of marine species (Rocha 2003; Rocha et al. 2005; White et al. 2010; Han et al. 2012; Mendonça et al. 2011, 2013). According to Ovenden et al. (2013) ocean currents can acts as a permeable barrier to gene flow causing population subdivision. Indeed, the pattern of genetic connectivity of the silky sharks observed here matches oceanic current patterns in the Atlantic Ocean. The main oceanic current that passes through the ASPSP is the strong South Equatorial Current (SEC) localized between 20° S to 2°–3° N. This current flows westward toward the Brazilian shelf and bifurcates near 16°S with one branch heading northwards as the North Brazil Current (NBC). The other is a weaker southward branch including the Brazil Current (BC) connecting the ASPSP to the SC and PA populations. Conversely, the SEC meets the NBC flowing northwestward to form the Guiana Current, which reaches the Gulf of Mexico and connects ASPSP with GM and USA populations.

This oceanic current pattern has been used to explain the strong population structure of the two coastal shark species along the western Atlantic Ocean, *Rhizoprionodon porosus* (Mendonça et al. 2011) and *Rhizoprionodon lalandii* (Mendonça et al. 2013) and the bony fish *Macrodon ancylodon* (Santos et al. 2006). However, it is difficult to confirm this interpretation based only on genetic data; studies based on mark-recapture and tagging data methods could help elucidate this aspect.

Although oceanic currents could be influencing the matrilineal population structure of silky sharks, other factors should be considered, including reproductive philopatry, behavior and life history. We found no evidence of a relationship between genetic distance and geographic distance ($r^2 = 0.155$, $P > 0.05$) across our large sampling range. The matrilineal population structure of the silky shark could potentially be interpreted as a signature of female reproductive philopatry. Reproductive studies strongly support this hypothesis in the silky shark as specific locations such as the Gulf of Mexico (Branstetter et al. 1987; Bonfil et al. 1993) and Southern Brazil (Amorim et al. 1998) have been reported as reproductive grounds in the western Atlantic Ocean. Indeed, philopatry can generate population structure in sharks and can precluded reproductive mixing despite extensive movements (Benavides et al. 2011; Chapman et al. 2015). Female philopatry has been inferred indirectly through genetic data in many *Carcharhinus* species (e.g., Keeney et al. 2005; Portnoy et al. 2010; Benavides et al. 2011; Karl et al. 2011). However, we recognize the need to use biparentally inherited markers to confirm if the observed population structure in silky sharks reflects both sexes or only females.

Phylogenetic and demographic history

The relatively recent, deep phylogenetic isolation of two silky shark matrilines comprising a large clade (L1) with haplotypes from both the western Atlantic and Indo-Pacific, and a second clade (L2) with haplotypes restricted to the western Atlantic (Fig. 2A) hints at an allopatric divergence between Indo-Pacific and western Atlantic silky sharks, potentially originated by vicariant events followed by secondary

contact (Avise 2000). Our results based on additional collection sites in the Southwestern Atlantic and increased sample sizes of silky sharks confirm the phylogeographic patterns reported by Clarke et al. (2015). Notably, this intraspecific phylogenetic divergence pattern between Atlantic and Indo-Pacific oceans has been observed in many species with high dispersal potential such as *Istiophorus platypterus* (Graves and McDowell 2003), *Thunnus obesus* (Martinez et al. 2006), *Lepidocybium flavobrunneum* (Brendtro et al. 2008), and *Prionace glauca* (Veríssimo et al. 2017).

The divergence times estimated for the two silky sharks global lineages (428,941 Kya; 257, 422–589,578 Kya 95%—HPD) corresponds to the major sea level and temperature cycles (100–800 Kya) during the Pleistocene (Lambeck et al. 2002). In this period, the cold Benguela Current Barrier around the southern tip of Africa is posited to have reduced or temporarily ceased connectivity between tropical species in the Indo-Pacific and Atlantic oceans. However, the Benguela current barrier also is known to be transiently semi-permeable, with disrupting flows of warmer water from the Indian Ocean's south-westward Agulhas Current (Briggs and Bowen 2013; Peeters et al. 2004; Ovenden 2013). Such permeability events may have allowed asymmetric westward dispersal event(s) from the Indian Ocean into the Atlantic, causing secondary contact of silky shark allopatric populations and resulting in the present haplotype distribution observed in lineage L1. Such ocean basin divergence followed by secondary contact around the tip of southern Africa has been documented now for several marine species (Peeters et al. 2004; Bernard et al. 2016; Bowen et al. 2016; Gaither et al. 2016).

Although different approaches to test the hypothesis of silky shark demographic expansion yielded conflicting results, the combination of results mainly from more powerful tests (i.e. Fu's *Fs*, R_2 and BSP) suggest a sudden demographic and spatial expansion for the overall silky shark samples as well as L1 and L2 lineages. Although there are several approaches to determinate the evolutionary drivers acting in a specific gene region, approaches based on the coalescent theory (Kingman 1982) are more powerful (Ramos-Onsins and Rozas 2002; Grant 2015). According to Ramos-Onsins and Rozas (2002), Fu's *Fs* and R_2 are the most powerful tests; the R_2 test is the most powerful tool for testing small samples sizes (~ n = 10), whereas the Fu's *Fs* is better for large samples (~ n = 50). Although a bimodal distribution is generally interpreted as a signature of population stability, we infer here that the bimodality of the overall sample set is a result of the population subdivision and presence of two sympatric lineages, rather than demographic stability (Rogers and Harpending 1992; Maltagliati et al. 2010). In addition, insignificant Tajima's D values (not shown) associated with multimodal mismatch distributions are often possible indicators of a population bottleneck. The high negative and significant values of Fu's *Fs* tests suggest that western Atlantic silky shark populations experienced a bottleneck followed by a sudden expansion, either spatial or demographic. Moreover, the zero value of θ_0 suggests a small population before expansion, and θ_1 values going to infinity hint at a large and sudden population expansion (Rogers 1995).

The demographic history of the silky shark along the western Atlantic Ocean could be attributed to the differences in climate and habitat condition during the Pleistocene. It is consistent with previous findings of some large pelagic fishes (Graves 1998; Martinez et al. 2006; Theisen et al. 2008; Bernard et al. 2014), and this explanation is likely applicable to the silky shark as well. During this period, coastal regions were severely affected by fluctuations in temperature and sea levels were up to 130 m below present levels (Ludt and Rocha 2015). Even though silky sharks are mainly oceanic, their coastal dependence during some part of their life-history results from a need for nursery area and habitats for juveniles (inshore to 18 m). This coastal habitat dependency may have resulted in the bottleneck effect followed by an abrupt population expansion (spatial and demographic) started before the LGM when warm water created routes of migrations between the Indian and South Atlantic Oceans (Peeters et al. 2004; Bowen et al. 2016). The BSP revealed a historical female effective population size of 160,000. This value is very similar to other shark species such as *Sphyrna lewini* (140,000; Duncan et al. 2006), *Galeorhinus galeus* (198,296; Chabot and Allen 2009) and *C. leucas* (193,000; Karl et al. 2011).

Implications for conservation

Our silky shark genetic assessment adds an important contribution for future conservation of this species in the Atlantic Ocean. Our results offer new insights about western Atlantic silky shark population structure and history, indicating at least two populations comprising the Northwest (USA and GM) and Southwest Atlantic (SC), with both having complex evolutionary histories linked with past environmental changes.

These findings also have direct implications for fisheries management of the silky shark, one of most vulnerable fishery species due to their low productivity and high susceptibility to pelagic longline gear (Cortés et al. 2010). Silky sharks are overexploited in the entire Atlantic Ocean and listed globally as Near Threatened by IUCN (Baum et al. 2003, 2004; Bonfil et al. 2009; Barreto et al. 2016). Recently, the International Commission for the Conservation of Atlantic Tunas (ICCAT) prohibited silky shark catch and commercialization in the Atlantic Ocean (ICCAT 2011). Thus, considering their high vulnerability, current level of population declines (Cortés et al. 2010; Barreto et al. 2016), and the present results, we recommend that western Atlantic silk sharks be considered minimally as two distinct populations for conservation and management purposes to ensure survival of their evolutionary potential. Finally, we recognize the need to add nuclear markers (e.g., microsatellites) to this and past shark genetic studies to gain further insight into population dynamics of this species of conservation concern.

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