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ARTICLE OPEN ACCESS

Genetic Assessment of the Spinner Dolphin (*Stenella longirostris*) in the Largest Offshore Sedimentary Basin in Brazil

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Received: 18 June 2024 | **Revised:** 24 March 2025 | **Accepted:** 25 March 2025

Funding: This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (312838/2021-2). Office Français de la Biodiversité. National Science Foundation (1827195) and University of Miami.

Keywords: anthropogenic threats | Atlantic Ocean | genetic diversity | microsatellites | mtDNA | petroleum | population structure

ABSTRACT

Assessing the genetic diversity of a population is critical to evaluate its resilience in the face of anthropogenic impacts. This study aimed to assess the genetic diversity and structure of the spinner dolphins (*Stenella longirostris*) that inhabit the Santos Basin (SB), south and southeast Brazil. Analyses were conducted using seven microsatellite loci and a 686 bp fragment of the mitochondrial DNA (mtDNA) control region. The genetic relationship between SB spinner dolphins with the island-associated spinner dolphins of Fernando de Noronha and spinner dolphins distributed in the western North Atlantic, including the Wider Caribbean region, was also investigated through a 324 bp fragment of the mtDNA control region. Results indicate that the offshore group of spinner dolphins in the SB has high genetic diversity for both mtDNA and nuclear markers. Based on mtDNA, the SB group has moderate genetic connectivity to the western North Atlantic/Wider Caribbean region spinner dolphins, but high genetic differentiation from their closest neighbors (Fernando de Noronha). These findings indicate that although SB spinner dolphins occur in an area of strong anthropogenic impacts, they present a high level of neutral genetic variation, with subtle matrilineally derived structure in the offshore (not island-associated) waters of the western Atlantic.

1 | Introduction

Small cetaceans are vulnerable to an increasing number of anthropogenic impacts such as chemical and noise pollution, fisheries interactions, and climate change, which can result in population declines (Andrews et al. 2013; Nicol et al. 2020; Peterson 2003). Anthropogenic mortality can cause genetic bottlenecks that lead to inbreeding, resulting in the loss of genetic diversity and a consequent reduction in the ability of a population to persist in stressful or changing environments (Hughes et al. 2008; Krützen et al. 2018; Parra et al. 2018). The International Union for Conservation of Nature (IUCN) considers genetic variability essential for biodiversity and conservation (McNeely et al. 1990), and the Convention on Biological Diversity (2021) proposed to preserve 90% of the genetic diversity of all species in its plan objectives. In addition, understanding

the population structure and genetic connectivity is essential for management efforts focusing on populations facing such adversities (Palumbi 2003).

In Brazil, the Santos Basin (SB) is a large sedimentary basin that has the largest oil and natural gas-producing fields in Brazil. Oil and gas activities generate environmental damage in a variety of ways, such as habitat degradation, noise, and chemical pollution. For example, noise pollution can affect communication through masking and have a range of effects on activity budgets and spatiotemporal distribution (Duarte et al. 2021; Nowacek et al. 2007). In addition, these activities can increase the potential risk of oil and gas leakage. For example, oil exposure from the Gulf of Mexico *Deepwater Horizon* oil spill in 2010 caused short- to long-term health issues (e.g., adrenal disease, lung disease, and poor health conditions), increased mortality, and reproductive failure of contaminated common bottlenose dolphins (*Tursiops truncatus*) (Beyer et al. 2016; Venn-Watson et al. 2015). Besides being an area of focus for the oil and gas industry, SB is home to one of South America's largest and most heavily trafficked shipping ports, and the coastline is densely populated in highly urbanized and industrialized cities. Consequently, marine communities of this region, including the 38 species of cetaceans that inhabit these waters, can be impacted by a diversity of anthropogenic threats. To evaluate the potential impacts of human activities, it is necessary to understand species health and genetic variability to guide effective management actions.

Spinner dolphins (*Stenella longirostris*) are distributed in tropical oceanic waters, and some resident populations are also often associated with oceanic islands throughout their range (Leslie et al. 2018; Perrin and Gilpatrick-Jr 1994; Perrin et al. 1999; Perrin 2009). In most documented cases, island-associated spinner dolphins use shallow coastal or reef-associated habitats for resting and socializing; they move to deeper waters for foraging on mesopelagic prey offshore, usually in waters over 1000 m (Norris and Dohl 1980; Norris et al. 1994; Kiszka et al. 2011). In the tropical Atlantic, most information on the ecology, behavior, and population biology of spinner dolphins originates from the island-associated resident population of Fernando de Noronha archipelago (FN), Brazil (3°51′13.71″ S; 32°25′25.63″ W) (e.g., Camargo et al. 2006, Faria et al. 2020; Silva-Jr et al. 2007). In the western Atlantic Ocean, the cold Malvinas/Falkland current (30° S) influences the southern limit in the distribution of spinner dolphins, while their boreal distribution is limited to approximately 40° N (Jefferson et al. 2007; Moreno et al. 2005). In this region, spinner dolphins generally occupy open ocean waters. Around Fernando de Noronha, genetic diversity is low, and this island-associated population seems to be genetically isolated from others (Faria et al. 2020). However, little is known about the genetic variability of spinner dolphins found along the Brazilian coast but not associated with the FN waters (not island-associated offshore spinner dolphins), such as those inhabiting the SB area. The genetic relationship of these offshore spinner dolphins with the island-associated group of FN and other areas of the western Atlantic Ocean is also unknown.

Understanding the genetic diversity and connectivity of open ocean spinner dolphins (those that are not island-associated), inhabiting Brazil's largest offshore sedimentary basin (SB), is the first step to having a better understanding of how the animals will respond to future anthropogenic threats. Here, we evaluate the genetic diversity and population structure of SB spinner dolphins (not island-associated) through nuclear (microsatellite) and mitochondrial (control region) markers. The genetic relationship between the SB and FN spinner dolphins and with spinner dolphins of the western North Atlantic Ocean, including the Wider Caribbean region, was analyzed through the mitochondrial marker.

2 | Methods

2.1 | Study Area and Sampling

The primary study area, SB, comprises 272,567 km^2 that extends from Cabo Frio (Rio de Janeiro) to Florianópolis (Santa Catarina), Brazil, including shallow and deep waters (Figure 1). This area is exposed to significant oil exploration and is where the largest oil-producing fields in Brazil are located. In this area, 64 spinner dolphin biopsies were collected using crossbows and floating darts with tips adapted for skin and blubber collection (Lambertsen 1987). Tissue samples were preserved in 70% ethanol. The samples were collected by trained scientists from a research vessel and conducted as part of the Santos Basin Cetacean Monitoring Project (PMC-BS). PMC-BS was conceived at the request of the Brazilian Institute of the Environment and Renewable Natural Resources (IBAMA) as an environmental condition to comply with the Brazilian federal environmental licensing process in order for the production systems to be installed by the oil-state company Petrobras at SB. The objective of the PMC-BS is to evaluate the potential impacts of oil and natural gas exploration, production, and runoff activities on cetaceans in the region.

2.2 | Sample Processing (SB)

Genomic DNA from 64 samples was extracted using the DNeasy Blood and Tissue Kit (Qiagen), according to the manufacturer's protocol. The quality and quantity of the DNA were estimated by electrophoresis gel and with Low Mass DNA Ladder in agarose gel, respectively. Sexing of the samples was determined molecularly by amplifying the SRY and ZFX/ZFY genes, according to Bérubé and Palsbøll (1996). Pearson's chi-square test was performed to verify whether the sex ratio differs significantly from the expected 1:1.

The 64 samples were genotyped using nine microsatellite loci: EV1, EV14, EV37, EV94 (Valsecchi and Amos 1996); KWM12a (Hoelzel et al. 1998); TexVet5, TexVet7 (Rooney et al. 1999); MK6, and D08 (Krützen et al. 2001). Amplification occurred through PCR reactions (10 μL), with concentrations and PCR conditions varying depending on the primer pair used (Tables S1 and S2). Negative and positive controls were used in all PCRs. Genotyping was done on an ABI 3730XL (Applied Biosystems) with molecular weight Genescan 400HD ROX, and the genotype calls were made through Peak Scanner v1.0 (Applied Biosystems).

A 686 base-pair (bp) fragment of the mitochondrial DNA (mtDNA) control region was amplified and sequenced for all

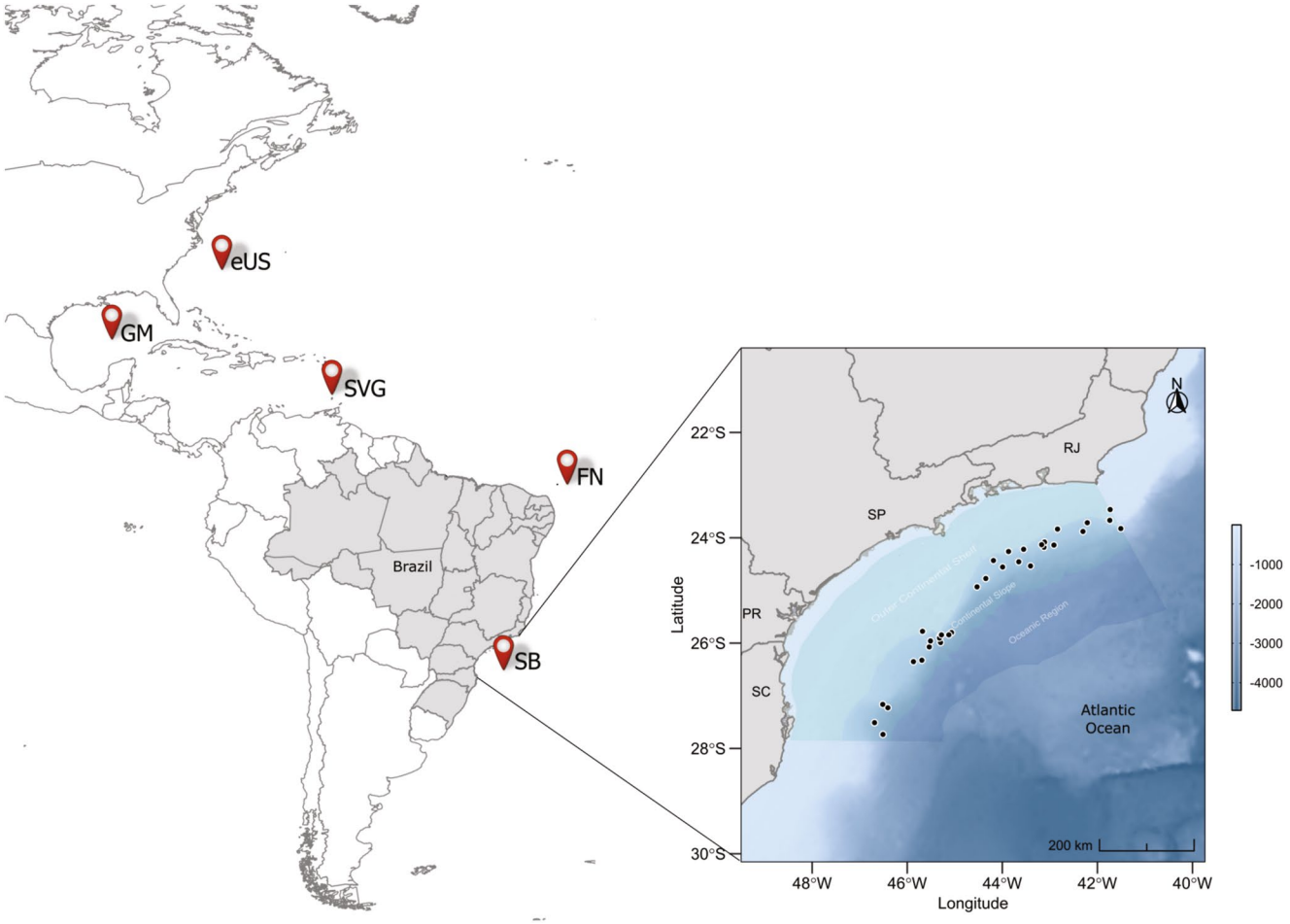

FIGURE 1 | Map of the sampling locations, with focus on the sampling sites in the Santos Basin (Brazil) where 64 spinner dolphin (*Stenella longirostris*) biopsies were obtained between 2016 and 2021 (black circles). The scale shows the depth in meters.

64 samples. MtDNA amplification was performed with primers Dlp1.5 and Dlp8 (Dalebout et al. 1998, 2005). PCR reactions (20 μL) included: 2.5 mM MgCl2, 0.2 mM dNTPs, 0.4 μM of each primer, 0.3 U of the enzyme 10 Platinum Taq DNA Polymerase (Invitrogen), 1X the reaction buffer (Invitrogen), and 1 μL of DNA (approximately 20 ng). The PCR profile was performed with initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 45 s, extension at 72°C for 40 s, and final extension at 72°C for 10 min. All samples were purified using CleanSEQ (Agencourt) and sequenced in the forward and reverse directions using ABI 3730XL (Applied Biosystems). The forward and reverse sequences of the mtDNA control region, generated for each sample, were aligned and manually adjusted, and the variable positions were visually confirmed by chromatograms using SEQUENCHER 5.4.6 (Gene Codes Corporation). Haplotypes were determined using DNAsp v6.12.03 after generating the 686 bp consensus sequence (Rozas et al. 2017). GenBank BLAST nucleotide alignment tool was used to verify species identity (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.3 | Microsatellite Analysis (SB)

The presence of possible duplicate samples was evaluated using the nuclear DNA data in the MSTools program (Park 2001). Congruence with additional sex and mtDNA haplotype information was also considered for potential duplicates. Genotyping errors due to null alleles, allelic dropout, and incorrect calling of stutter peaks were tested in Microchecker v2.2.0.3 (Van Oosterhout et al. 2004) with 10,000 iterations. Each locus was tested for Hardy–Weinberg Equilibrium (HWE) and linkage disequilibrium using Fisher's exact test in GENEPOP v4.6 (Rousset 2008) with 10,000 dememorizations, 1000 batches, and 10,000 batch iterations. A Bonferroni sequential correction was performed to calculate the significance of the results. Loci that showed significance for deviation of HWE, linkage disequilibrium, or potential genotyping errors were discarded from subsequent analyses.

As the degree of kinship between the samples can influence the results of population structure, we investigated the mean value of kinship (r) between pairs of individuals from the SB group using the R package *related* (Pew et al. 2015) in R v4.2.1 (R Core Team 2022). By using observed allele frequencies, simulations of 500 pairs for four kinship types (parent-offspring, full-sibling, half-sibling, and unrelated) were conducted for seven different kinship indices available in the package (dyadml, lynchli, lynchrd, quellergt, ritland, trioml, and wang) to define the most appropriate index for the data in question. The kinship index with the highest Pearson correlation coefficient was used to estimate r between the samples.

Genetic structuring was investigated using a Bayesian clustering approach with the program STRUCTURE v2.3.4 (Pritchard et al. 2010). Two ancestry models available in this program were used to estimate the number of populations (K) that best explain the observed genetic variability. As recommended, the Admixture model was used as a starting point for analyses because it considers the possibility of mixed ancestry for each individual since it is a common feature in natural populations (Pritchard et al. 2010). On the other hand, the No Admixture model would be more appropriate for discrete populations and more potent for detecting subtle structures (Pritchard et al. 2010). We employed ten independent runs for K ranging from one to five for both models, using 1000,000 iterations and a burn-in of 200,000. We compared the ΔK method (Evanno et al. 2005) and the mean log-likelihood of the data [LnP(D)] (Pritchard et al. 2010) using the program CLUMPAK web version (Kopelman et al. 2015) and the membership proportions of each individual. The following analyses considered only one genetic cluster in this region since no population structure was found in the SB (see Results).

The inbreeding coefficient (F_{IS}) and the expected (He) and observed (Ho) heterozygosities for each microsatellite locus and their averages were determined using the program ARLEQUIN v3.5.2.2 (Excoffier and Lischer 2010). The total number of alleles per locus and the mean and per locus allelic richness were estimated in Fstat v2.9.3.2 (Goudet 2001). The contemporary effective population size (Ne) was calculated using the linkage disequilibrium method with random mating and exclusion of single alleles, implemented in NeEstimator v2.0 to understand the number of individuals contributing to genetic diversity (Do et al. 2014). This method allows us to exclude alleles below a specific frequency (P_{crit}: 0.05, 0.02, and 0.01) before estimating the Ne since rare alleles can distort this estimate (Waples and Do 2010). The confidence interval was estimated using parametric (chi-square) and non-parametric (jackKnife) estimation, but only parametric analysis was considered for the interpretation of the results due to the number of loci ($n = 7$) used being lower than one hundred, as instructed by the manual (Do et al. 2014).

2.4 | mtDNA Sequence Analysis (SB)

The 64 sequences of the 686 bp fragment of the mtDNA control region were aligned in MUSCLE implemented in MEGA v6.06 (Tamura et al. 2013). All sequences were used in the mtDNA statistical analyses described below since no duplicates were identified by the nuclear DNA analysis (see Results). The total number of haplotypes was identified in DNAsp v6.12.03 (Rozas et al. 2017). Nucleotide (Nei 1987) and haplotypic (Nei and Tajima 1981) diversities were inferred in ARLEQUIN v3.5.2.2 (Excoffier and Lischer 2010). The genetic relationship among SB spinner dolphins was investigated by constructing a haplotypic network using the median-joining network method in PopART v1.7 (Leigh and Bryant 2015).

2.5 | mtDNA Sequence Analysis (Western Atlantic Ocean)

We included 20 mtDNA control region haplotypes of spinner dolphins sampled outside the SB and available in GenBank (Table S3). Additionally, we included 39 samples of spinner dolphins collected off Saint Vincent and the Grenadines (SVG), which corresponded to 23 haplotype sequences of the mtDNA control region (see Supporting Information for more details on these SVG samples). We aimed to examine the genetic diversity, genetic relationship, and level of differentiation between SB spinner dolphins and those sampled along the western Atlantic Ocean. In summary, we used the following number of haplotypes from: not island-associated individuals of SB ($n = 30$) (this study), island-associated individuals of Fernando de Noronha (FN; $n = 11$) (Faria et al. 2020), individuals from SVG ($n = 23$; this study), offshore spinner dolphins from the Gulf of Mexico (GM; $n = 9$) (Andrews et al. 2013; Kingston et al. 2009) and off the east coast of the United States (eUS; $n = 3$; Andrews et al. 2013) (Table S3). Due to the differences in length between the haplotypes, the final alignment of the mtDNA, created using MUSCLE, included fragments of 324 bp. The shortened sequences led to the merging of some previous haplotypes and to a reduced number of haplotype sequences used in subsequent analyses (see Results). Genetic diversity (nucleotide and haplotypic) was inferred in ARLEQUIN v3.5.2.2 (Excoffier and Lischer 2010). The haplotypic network was created using the median-joining network method in PopART. The level of genetic differentiation (F_{ST} and Φ_{ST}) among the different spinner dolphin geographic groups of the western Atlantic was estimated in ARLEQUIN, with 10,000 permutations. The number of individuals for each geographic area was obtained from this study (SB and SVG), literature (GM and eUS: Andrews et al. 2013; FN: Faria et al. 2020), or personal communication of unpublished data (GM and eUS; Lynsey Wilcox—NOAA/SEFSC) (Table S3). The best model of evolution to estimate differentiation based on Φ_{ST} was identified using the function *ModelTest* in the R package *phangorn* v2.11.1 (Schliep 2011), with the Bayesian Information Criterion (BIC) algorithm: Tamura-Nei (Tamura and Nei 1993) with invariant sites. The genetic differentiation analyses were conducted following two scenarios: (1) grouping the eUS, GM, and SVG samples (here renamed as western North Atlantic, wNA); and (2) considering each location separately, with the exception of eUS, due to its small sample size.

3 | Results

3.1 | Sex Identification, Genetic Diversity, and Population Structure of the SB Spinner Dolphins

Among the 64 samples used in this study, 42 were females and 22 were males, indicating a significantly higher proportion of biopsied females (Chi-squared = 2.595; df = 1; p-value = 0.1072). Most individuals were successfully genotyped for all nine microsatellite loci except for one who was genotyped for eight loci. No duplicates were found among the samples. Two loci (TexVet5 and D08) demonstrated the presence of null alleles and HWE deviation, even after Bonferroni sequential correction, and TexVet5 also presented a genotyping error. No loci showed linkage disequilibrium. We therefore excluded TexVet5 and D08 loci from subsequent analyses (see Table 1 for a reference on the diversity of the remaining loci). The 686 bp fragment of the mtDNA control region was successfully amplified and sequenced for all 64 samples. The alignment of the 64 mtDNA control region sequences revealed the presence of

30 different haplotypes (Figure 2), with 40 polymorphic sites and the absence of indels.

Spinner dolphins from the SB showed high genetic diversity for nuclear and mtDNA markers. A mean allelic richness (Ra) of 13.265 (S.D.: 3.984) and mean expected (He) and observed (Ho) heterozygosities of 0.8 (S.D.: 0.235) and 0.772 (S.D.: 0.218), respectively, were observed (Table 1). For the mtDNA, the haplotypic (H) and nucleotide (π) diversities were 0.897 (S.D.: 0.02) and 0.015 (S.D.: 0.008), respectively.

TABLE 1 | Genetic diversity of the Santos Basin spinner dolphins for seven microsatellite loci.

Locus	Na	Ra	He	Ho	F_{IS}
EV1	14	13.984	0.902	0.813	0.099
EV14	17	16.937	0.901	0.891	0.011
EV37	15	14.968	0.867	0.922	−0.064
EV94	16	15.984	0.925	0.906	0.02
KWM12a	10	10.000	0.822	0.746	0.094
TexVet7	6	5.984	0.271	0.297	−0.095
MK6	15	15.000	0.909	0.828	0.09
Mean	13	13.265	0.8	0.772	0.02

Abbreviations: F_{IS}, Inbreeding coefficient; He, Expected heterozygosity; Ho, Observed heterozygosity; Na, Number of alleles; Ra, Allelic richness.

High kinship ($r > 0.45$) values were observed among five pairs of SB individuals that were sampled independently. Thus, the population structure analysis was performed twice, one using the total number of samples ($n = 64$) and another excluding one individual from each related pair ($n = 59$). However, all 64 samples were considered in the subsequent analyses since there were no significant differences in the results of population structure regardless of the presence or absence of related individuals (results not presented).

The best K to explain the population structure of SB spinner dolphins based on the observed genetic variability was identified as $K = 1$ for both models tested based on the mean log-likelihood of the data [LnP(D)] and the membership proportions of each individual (Figure 3; Figures S1 and S2). Although ΔK estimated values of $K > 1$ for both models (Figure S1), informal pointers of population structure need to corroborate the results of best K. Thus, the expectation would be to find asymmetric coefficients for assigning individuals to clusters, which were not found. Furthermore, ΔK cannot estimate $K = 1$, even when there is no population structure (see Evanno et al. 2005).

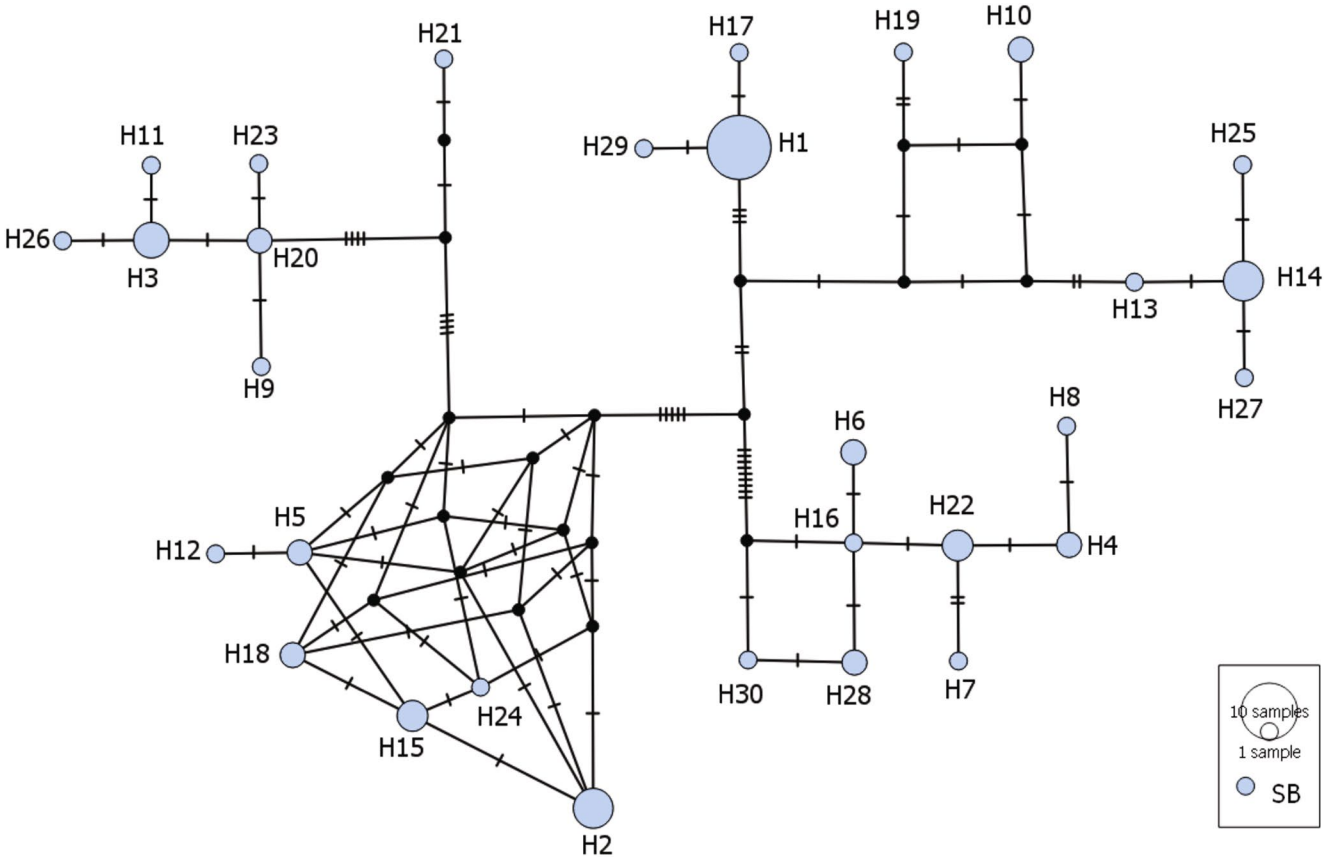

FIGURE 2 | Haplotypic network using median-joining network method for the 30 haplotypes of the Santos Basin spinner dolphins. The size of the circles is proportional to the frequency of each haplotype. Black circles indicate possible unsampled or extinct intermediate haplotypes. Small black dashes on the lines connecting the haplotypes are equivalent to the number of mutations. See Table S4 for more information on the haplotypes. Fragment size: 686 bp.

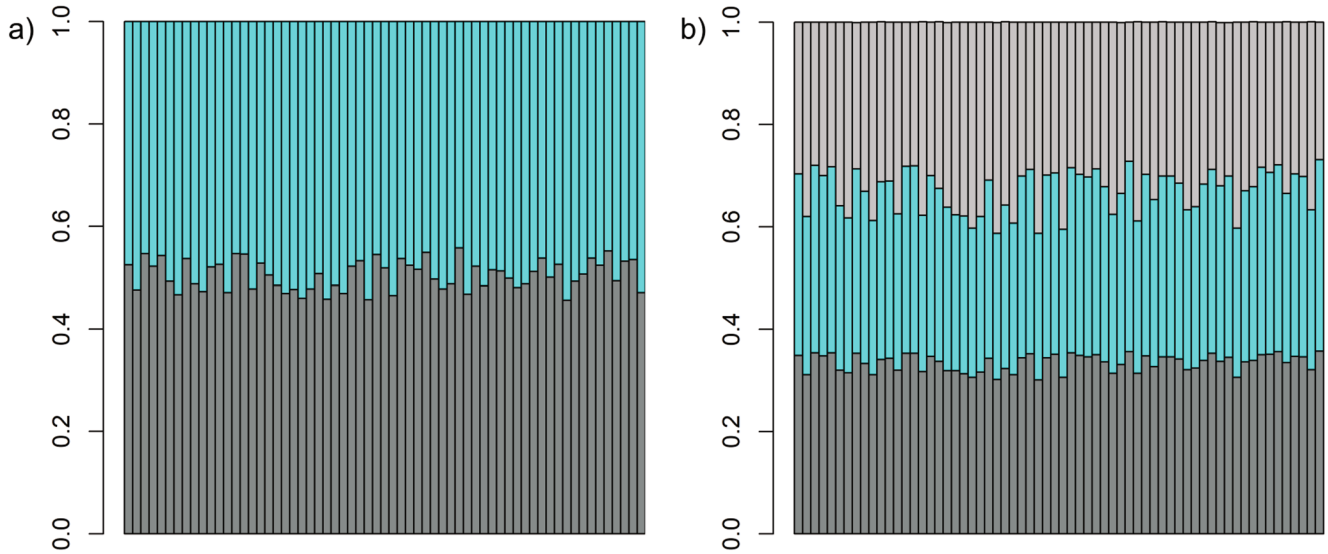

FIGURE 3 | STRUCTURE bar plot of the proportional membership of each individual of Santos Basin spinner dolphin in each cluster for (a) K = 2 and (b) K = 3 for the No Admixture model. Each column represents an individual, and the colors represent the membership proportions of the individuals in each genetic cluster.

The estimated contemporary effective population size (Ne) for the SB spinner dolphins was 540 individuals (95% Cis = 197 – ∞) for the $P_{crit} = 0.02$, which is indicated to provide better accuracy when the number of individuals sampled for genetic analysis is higher than 25 (see Waples 2006).

3.2 | Genetic Population Structure and Diversity of Spinner Dolphins in the Western Atlantic Ocean

A total of 46 distinct haplotypes of 324 bp were obtained for the western Atlantic spinner dolphins (Figure 4). The reduction of the mtDNA fragment from 686 bp to 324 bp led to a decrease in the number of haplotypes found among the SB samples: from 30 distinct haplotype sequences of 686 bp to 17 distinct haplotypes of 324 bp (Table S4). Of these 17 SB haplotypes, 12 were unique to this region, whereas the other five haplotypes were shared with spinner dolphins of the wNA (H1, H3, H15, H21) or with FN spinner dolphins (H13; Figure 4). For FN, the initial 11 haplotypes retrieved from GenBank were reduced to eight distinct haplotypes of 324 bp, of which seven are unique to FN. For the GM, of the nine distinct haplotype sequences retrieved from GenBank, two haplotypes merged into haplotype H1 and another two into haplotype H39 after the concatenation of the sequences to 324 bp (Table S4). This resulted in seven distinct GM haplotypes of 324 bp, of which three (H41, H42, H44) were unique to the GM. The other four GM haplotypes were shared with SVG (H1, H39, H40, H43), SB (H1), or eUS (H43) (Figure 4). With the reduction of the mtDNA fragment, there was only one unique haplotype (H38) among the eUS samples. The other two eUS haplotype sequences were shared with SB (H15), GM (H43), and SVG (H15, H43; Figure 4). The concatenation to 324 bp led to a decrease in the number of haplotypes found among the SVG samples: from 23 to 22 distinct haplotypes of 324 bp (Table S4). Of these, 15 are unique to SVG and seven were shared with eUS, GM, and/or SB. There was no clear geographic structure in the haplotype network, although most of the FN haplotypes grouped in a unique clade. The SB, GM, and SVG haplotypes distributed along the haplotype network, and spinner dolphins of these areas shared a great number of haplotypes (Figure 4).

Genetic differentiation based on the mtDNA control region was estimated between three major areas: SB, FN, and wNA (GM + SVG + eUS), and when considering each geographic area (with $n > 10$ individuals; see Table S3) separately: SB, FN, GM, and SVG. The results indicated significant moderate genetic differentiation between the offshore individuals of SB and wNA spinner dolphins and a significant high differentiation between these and the island-associated individuals from FN (Table 2-A). The results for each locality showed low to moderate genetic divergence between SB, SVG, and GM, all of which were highly differentiated from FN, consistent with matrilineally derived isolation of this group (Table 2-B). The results indicated a greater degree of connectivity between SB spinner dolphins and those from SVG than with FN dolphins. Further, there was only moderate differentiation between SB and GM spinner dolphins, despite the great geographic distance between these areas. When focusing on the wNA geographic areas, we observed moderate differentiation between SVG and GM.

The mitochondrial genetic diversity of spinner dolphins in the western Atlantic Ocean is high. However, when considering the different locations separately, FN is the only area that does not maintain this high genetic diversity pattern (see Table 3).

4 | Discussion

This study aimed to investigate the genetic connectivity of the spinner dolphins distributed in the SB, Brazil's largest oil exploration area, with those from surrounding areas to gain insight into the vulnerability of these dolphins to future localized environmental impacts. Our results showed that the SB spinner dolphins exhibit high genetic diversity. When compared to spinner dolphins from the western North Atlantic (wNA), the SB dolphins display moderate genetic differentiation. However, there

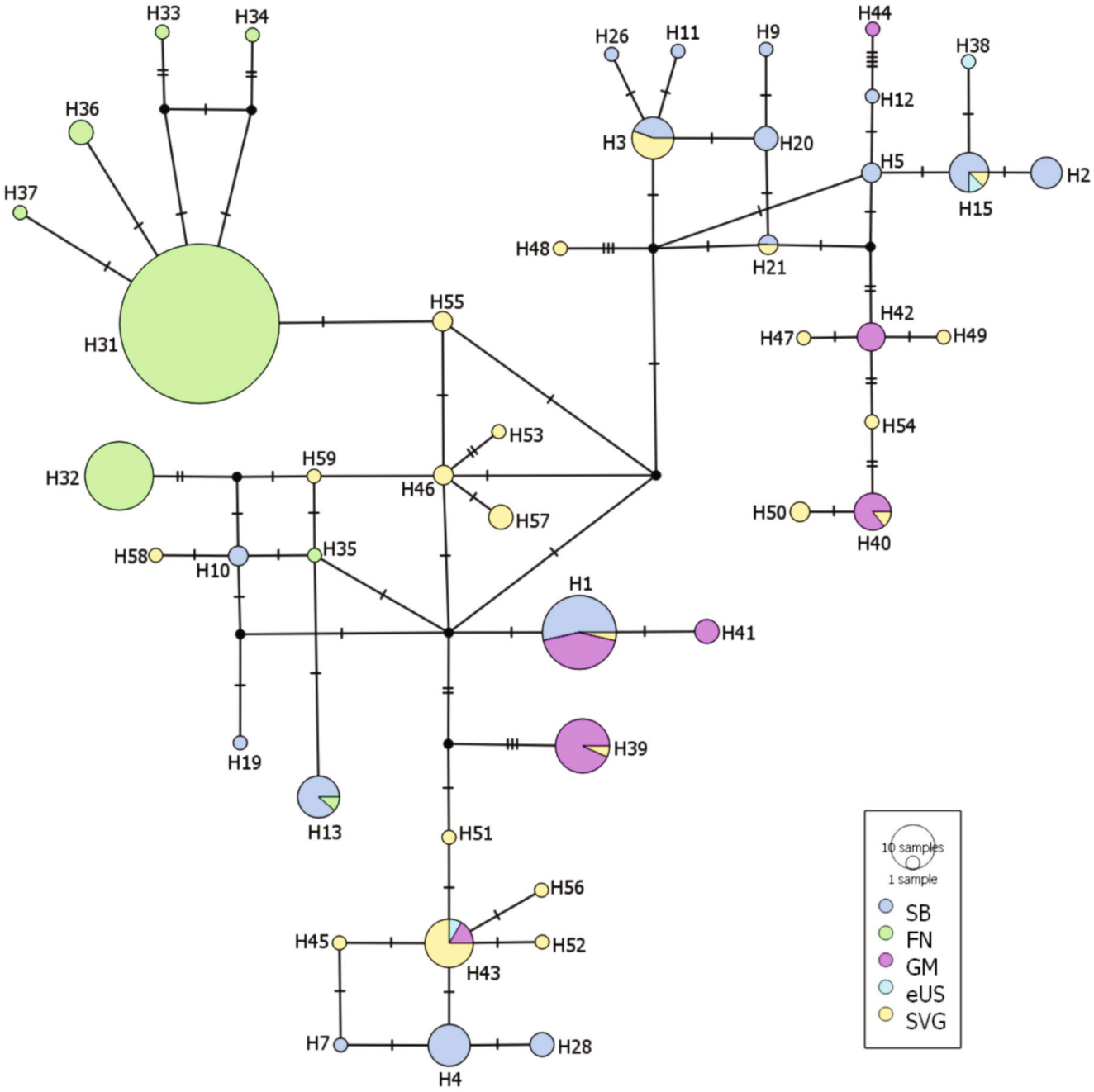

FIGURE 4 | Haplotypic network using the median-joining network method for the 46 haplotypes of western Atlantic spinner dolphins. The size of the circles is proportional to the frequency of each haplotype. Black circles indicate possible unsampled or extinct haplotypes. Small black dashes on the lines connecting the haplotypes are equivalent to the number of mutations. Table S4 shows the original names of each haplotype obtained from GenBank and in this study. Fragment size: 324 bp.

is strong genetic differentiation from island-associated spinner dolphins from Fernando de Noronha (FN). These findings indicate subtle matrilineally derived structure between SB and wNA and a matrilineally derived isolation between SB and FN.

Environmental variables (e.g., ocean currents), as well as ecological and behavioral differences (e.g., habitat preferences, mating preferences, stability of social groups) can act as barriers to dispersal, contributing to a variety of population structures observed in several cetacean species (Andrews et al. 2010; Hoelzel et al. 2002). The SB spinner dolphins form an offshore group that does not exhibit philopatry (do Amaral et al. 2015). These animals form large and fluid groups, and are polygamous and polyandrous (Perrin 2009). These characteristics favor random mating; thus, reducing the potential for inbreeding and maintaining high genetic diversity, as shown by the low inbreeding coefficient ($F_{IS} = 0.022$). In other words, their open ocean habitat and range promote gene flow, which is consistent with our results showing a single panmictic group within the SB and a moderate level of mitochondrial differentiation among non-island-associated offshore spinner dolphins in a large-scale area.

The mtDNA-based levels of genetic differentiation between SB dolphins and SVG and GM suggested small to moderate genetic

TABLE 2 | Genetic differentiation (F_{ST} and Φ_{ST}) among western Atlantic spinner dolphin geographic groups based on the 324 bp fragment of the mtDNA control region.

(A)

	SB	FN	wNA
SB ($n = 64$)	—	0.467*	0.062*
FN ($n = 162$)	0.438*	—	0.448*
wNA ($n = 84$)	0.053*	0.413*	—

(B)

	SB	FN	GM	SVG
SB ($n = 64$)	—	0.467*	0.129*	0.048*
FN ($n = 162$)	0.439*	—	0.570*	0.467*
GM ($n = 42$)	0.095*	0.508*	—	0.079*
SVG ($n = 39$)	0.071*	0.454*	0.114*	—

Note: F_{ST} (lower diagonal) and Φ_{ST} (upper diagonal). Values with an asterisk (*) have a significant p-value ($p < 0.0001$). (A) Genetic differentiation among Santos Basin (SB), Fernando de Noronha (FN), and western North Atlantic (wNA) spinner dolphins. wNA is formed by spinner dolphins of Saint Vincent and the Grenadines (SVG), east coast of the United States (eUS), and the Gulf of Mexico (GM). (B) Genetic differentiation among Santos Basin (SB), Fernando de Noronha (FN), Saint Vincent and the Grenadines (SVG), and the Gulf of Mexico (GM) spinner dolphins.

TABLE 3 | Nucleotide and haplotypic diversities among western Atlantic spinner dolphin geographic groups based on the 324 bp fragment of the mtDNA control region.

	Haplotype diversity	Nucleotide diversity
BS	0.897 (S.D.: 0.020)	0.015 (S.D.: 0.008)
FN	0.336 (S.D.: 0.043)	0.005 (S.D.: 0.003)
SVG	0.789 (S.D.: 0.036)	0.018 (S.D.: 0.009)
GM	0.930 (S.D.: 0.028)	0.019 (S.D.: 0.010)
eUS	1.000 (S.D.: 0.272)	0.019 (S.D.: 0.015)
wNA[a]	0.915 (S.D.: 0.014)	0.019 (S.D.: 0.010)
wA[b]	0.804 (S.D.: 0.022)	0.015 (S.D.: 0.008)

[a]Diversity calculated by grouping all locations of wNA (SVG, GM, and eUS).
[b]Diversity calculated by grouping all locations of the western Atlantic Ocean (BS, FN, SVG, GM, and eUS).

differentiation (F_{ST} and Φ_{ST} between 0.05 and 0.13) and isolation from FN island-associated dolphins (F_{ST} and $\Phi_{ST} > 0.25$). These results confirm previous findings of philopatry by at least females at FN (Faria et al. 2020) and reveal that SB dolphins are more connected to populations from the Wider Caribbean region. However, the use of nuclear markers, larger sample sizes, and smaller gaps between sampling sites are necessary to test recent genetic connectivity among western Atlantic spinner dolphins. Understanding population connectivity and genetic diversity is essential for population management efforts (Palumbi 2003). The nuclear genetic diversity (microsatellites) of spinner dolphins in the SB was high compared to those from FN (Faria et al. 2020), as well as from individuals from other regions in the Pacific and Indian Oceans (Leslie et al. 2018; Oremus et al. 2007; Viricel et al. 2016). However, these studies did not use the same loci and thus are not directly comparable. When considering the mitochondrial genetic diversity based on the aligned fragment of mtDNA control region (324 bp), SB and wNA spinner dolphins presented high genetic diversity, whereas FN individuals showed the lowest diversity in the western Atlantic Ocean (Faria et al. 2020).

It is important to understand how populations can adapt to changes in the marine environment due to heavy human exploitation (Barrett and Schluter 2008). Genetic diversity is an important part of species adaptation to these changes. Although this study estimated genetic variability only through neutral markers, panmixia can favor high genetic variability of adaptive markers in SB spinner dolphins, consequently allowing these animals to be more adaptive to anthropogenic impacts and environmental changes. Future studies using genomic data are necessary to better understand how this species can adapt to a variety of anthropogenic impacts (Kardos et al. 2021; Manlik et al. 2019; Sommer 2005).

From a genetic standpoint, our data suggests that SB spinner dolphins are less vulnerable than island-associated populations, particularly when compared to FN. However, the protection and promotion of high genetic variability throughout the genome of spinner dolphins are crucial for their ability to survive and reproduce in the face of the range of predictable and non-predictable environmental threats they could face (Kardos et al. 2021). These spinner dolphins reside in the oceanographic basin in Brazil, which has a high production capacity of oil and natural gas but has not yet reached its full potential for exploration (Petrobras 2022). Consequently, the region will likely continue to suffer strong anthropogenic impacts. We reiterate the importance of systematic monitoring of spinner dolphins and other cetacean species in the SB, as has been done by the PMC-BS. Such monitoring will allow a better understanding of these animals and the threats they face, promoting the establishment of appropriate management strategies. Encouraging effective public conservation policies is also extremely important to prevent human actions from leading to the loss of genetic diversity of marine mammals in the SB.

5 | Conclusions

The spinner dolphins from the SB form a panmictic cluster with high genetic diversity for both nuclear (microsatellite) and mitochondrial (control region) markers. The SB spinner dolphins have connectivity with other not island-associated spinner dolphins of the western North Atlantic (Saint Vincent and the Grenadines, U.S. Atlantic, and Gulf of Mexico), but possible isolation from island-associated spinner dolphins of Fernando de Noronha (based on mtDNA). These results reinforce previous findings of geographic fidelity of FN spinner dolphins, at least by females. However, it is still necessary to evaluate the connectivity between the different areas based on nuclear DNA. The results of this study indicate that the SB spinner dolphins have high genetic variability, which can potentially help with their resilience to environmental impacts. However, maintaining this variability is important for the viability of these dolphins. The systematic monitoring of this offshore group conducted by

PMC-BS should continue as it provides valuable information that can assist in developing necessary public management policies and evaluating the effectiveness of conservation measures.

Author Contributions

Thaís Leal: conceptualization, data curation, formal analysis, investigation, methodology, validation, visualization, writing – original draft, writing – review and editing. **Ana Lúcia Cypriano-Souza:** data curation, funding acquisition, investigation, methodology, project administration, resources, validation, writing – review and editing. **Paulo C. Simões-Lopes:** conceptualization, project administration, supervision, writing – review and editing. **Sandro L. Bonatto:** data curation, funding acquisition, methodology, project administration, resources, writing – review and editing. **Jeremy J. Kiszka:** data curation, resources, writing – review and editing. **Ruth Y. Ewing:** data curation, resources, writing – review and editing. **Adam Lache:** investigation, writing – review and editing. **Maria C. Cartolano:** funding acquisition, writing – review and editing. **Ana P. B. Costa:** conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, supervision, validation, writing – review and editing. [Correction added on May 8, 2025, after first online publication: Author contribution details for Ana P. B. Costa have been updated.]

Acknowledgments

We thank the Projeto de Monitoramento de Cetáceos na Bacia de Santos (PMC-BS, SB Cetacean Monitoring Project), executed by PETROBRAS and defined in the Federal Environmental Licensing conducted by IBAMA for the production and flow of oil and natural gas in the SB, for having provided the samples used in this study. PMC-BS is a monitoring program required by Brazil's federal environmental agency, IBAMA, for the environmental licensing process of the oil production and transport by Petrobras at the Santos Basin pre-salt province (process no. 02001.114279/2017-80, ACCTMB no. 657/2015). We thank all the marine mammal observers who were responsible for biopsy collection and the crucial support of the crew of R/V Sea Route. We also acknowledge Lynsey Wilcox (NOAA/SEFSC) for help with some of the statistical analyses and information about GM and eUS haplotypes. We are thankful to Lynne Fieber and Michael Schmale (University of Miami) for all the support and lab space provided, which allowed us to conduct the genetic analyses of the SVG samples. This is contribution #1827 from the Institute of Environment at Florida International University. St. Vincent and Grenadines samples (SVG) permitted through NMFS Marine Mammal Protection Act Permit (No. 24359) and St. Vincent and the Grenadines export CITES permit (VC3729). The findings and conclusions presented herein represent the opinions of the authors and do not reflect the views of the U.S. Department of Commerce, NOAA, NMFS.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The haplotypes found in this study were deposited in GenBank under the accession numbers MZ463903–MZ463932 and PV453762–PV453784.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.