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Seascape Genetics of the Atlantic Spotted Dolphin (*Stenella frontalis*) Based on Mitochondrial DNA

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Abstract

The Atlantic spotted dolphin (*Stenella frontalis*) is endemic to tropical, subtropical, and warm temperate waters of the Atlantic Ocean. Throughout its distribution, both geographic distance and environmental variation may contribute to population structure of the species. In this study, we follow a seascape genetics approach to investigate population differentiation of Atlantic spotted dolphins based on a large worldwide dataset and the relationship with marine environmental variables. The results revealed that the Atlantic spotted dolphin exhibits population genetic structure across its distribution based on mitochondrial DNA control region (mtDNA-CR) data. Analyses based on the contemporary landscape suggested, at both the individual and population level, that the population genetic structure is consistent with the isolation-by-distance model. However, because geography and environmental matrices were correlated, and because in some, but not all analyses, we found a significant effect for the environment, we cannot rule out the addition contribution of environmental factors in structuring genetic variation. Future analyses based on nuclear data are needed to evaluate whether local processes, such as social structure and some level of philopatry within populations, may be contributing to the associations among genetic structure, geographic, and environmental distance.

Subject Area: Tree of Life: Population structure, phylogeography and phylogenomics

***Both authors contributed equally to this work.**

Keywords: Delphininae, isolation-by-distance, isolation-by-environment, isolation-by-resistance, matrilineal marker, population structure

Seascape genetics is a derivation of landscape genetics applied to the marine environment (Selkoe et al. 2008; Riginos and Liggins 2013). In general, landscape genetics aims to understand how spatial factors such as geographic distance and environmental heterogeneity shape genetic differentiation across a species distribution (Manel et al. 2003; Storfer et al. 2007; Holderegger and Wagner 2008; Balkenhol et al. 2009). In addition to these 2 factors, seascape genetic studies also consider the peculiarities of both the marine environment (e.g., fluidity, 3-dimensionality, currents, as well as the temporal and spatial scales of these factors) and marine organisms (e.g., higher dispersal abilities) (Hodel et al. 2018). Isolation-by-distance (IBD) (Wright 1943; Manel et al. 2003), which postulates a positive correlation between genetic differentiation and geographic distances (being either straightline, or Euclidean distances (Wright 1943; Balkenhol et al. 2009), or a re-scaled distance, based on environmental heterogeneity affecting gene flow), is a fundamental model with much relevance in marine systems, especially given the broad distribution of many taxa, often across seemingly homogeneous environments.

Extensive genetic population structure has been reported in many marine species, suggesting more complex recruitment dynamics in marine species than previously assumed (Bierne et al. 2003; Hauser and Carvalho 2008). For example, despite the great dispersal ability of marine top predators such as cetaceans, several studies have shown fine-scale population structure, probably resulting from ecological divergence in species widely distributed such as bottlenose dolphins (*Tursiops* spp.), common dolphins (*Delphinus* spp.), *Stenella* dolphins, and killer whales (*Orcinus orca*) (e.g., Escorza-Treviño et al. 2005; Adams and Rosel 2006; Tezanos-Pinto et al. 2008; Andrews et al. 2010; Amaral et al. 2012a, 2012b; Foote et al. 2016; Barragán-Barrera et al. 2017). This suggests that not only IBD, but other isolation models may also be relevant in marine systems, such as isolation-by-environment (IBE) (Wang and Bradburd 2014) and isolation-by-resistance (IBR) (McRae 2006). The IBE is defined as a pattern in which genetic differentiation increases with environmental differences, independent of geographic distance (Wang and Bradburd 2014). IBR models predict a positive relationship between genetic differentiation and the resistance distance, which provides a more appropriate predictor of genetic differentiation because it accounts for heterogeneity in species' distributions and migration rates, as it incorporates all possible pathways connecting sample pairs (McRae 2006).

Nevertheless, few seascape genetic studies have been conducted for cetacean species (Mendez et al. 2010, 2011; Amaral et al. 2012a) to investigate the impact of IBD, IBE, and IBR models. For example, a correlation between genetic structure and marine productivity and sea surface temperature (SST) was detected in the widely distributed short-beaked common dolphin (*Delphinus delphis*), despite its high mobility (Amaral et al. 2012a). Likewise, in a study of humpback dolphins (*Sousa* spp.), population structure based on mitochondrial DNA control region (mtDNA-CR) data suggested a potential influence of environmental factors in shaping genetic patterns in the western Indian Ocean (Mendez et al. 2011). More specifically,

genetically isolated populations occurred in areas that were environmentally distinct, thereby highlighting the importance of combining molecular markers with high quality environmental data to address questions related to ecological processes in marine species (Mendez et al. 2011).

Here, we focus on the seascape genetics of the Atlantic spotted dolphin (*Stenella frontalis*), a member of the dolphin subfamily Delphininae. This species is endemic to tropical, subtropical, and warm temperate waters of the Atlantic Ocean (Perrin 2009), ranging from 45°N to 35°S in the west Atlantic to the Azores in the eastern Atlantic (Perrin 2009) (Figure 1). The Atlantic spotted dolphin inhabits the continental shelf along most of its distribution, but it also occurs in oceanic waters in the western north Atlantic (WNA) and oceanic islands in the eastern north Atlantic (ENA) (Baumgartner et al. 2001; Moreno et al. 2005; Weir 2010; Barragán-Barrera et al. 2019; Correia et al. 2020). In the western south Atlantic (WSA), a distributional gap in the species distribution is observed between 6°S and 18°S due to a narrowing in the continental shelf and the lack of environmental conditions favorable for the species (Moreno et al. 2005; Caballero et al. 2013; do Amaral et al. 2015).

There is strong evidence that the population in south/southeastern Brazil is geographically, and possibly genetically isolated from the other populations that exhibit a continuous distribution (Moreno et al. 2005; do Amaral et al. 2015). In the WSA, the population, distributed between 21°S and 33°S up to the 1000 m isobath, is separated from individuals that occur in north/northeastern Brazil (out to 6°S) by more than 1300 km (between 6°S and 18°S) and those that occur in the ENA by more than 5000 km (Moreno et al. 2005; do Amaral et al. 2015).

Several lines of evidence suggest great dispersal capabilities for Atlantic spotted dolphins in the North Atlantic (Herzing 1997; Davis et al. 2006; Quérouil et al. 2010); however, distinct regional populations have also been identified based on morphological, genetic, and contaminant analyses, as well as ecological markers (Perrin et al. 1987; Adams and Rosel 2006; Green et al. 2007; Quérouil et al. 2010; Caballero et al. 2013; Viricel and Rosel 2014; Méndez-Fernandez et al. 2018, 2019). However, it is unknown how environmental heterogeneity affects genetic population structure at large scales. This study is the first attempt to investigate the relationship of the marine environment to population structure of this species throughout its distribution in the Atlantic Ocean. Based on a comprehensive review of published mtDNA-CR sequences and their respective geographic coordinates, combined with additional sampling and high-resolution environmental data the aims of this study are: 1) to assess the population structure of Atlantic spotted dolphins across the species' distribution using mtDNA-CR data and 2) to

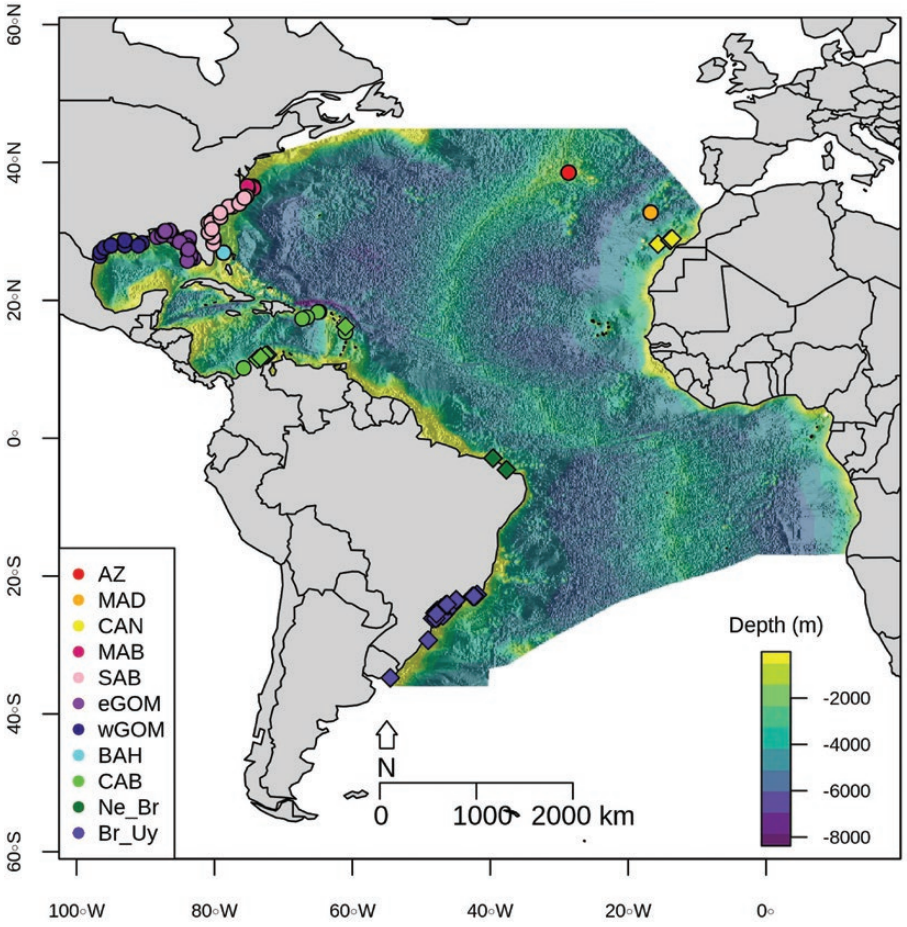

Figure 1. Map of study area and localities of sampled Atlantic spotted dolphins (*Stenella frontalis*). Circles indicate individuals and/or haplotypes sequenced by other studies and diamonds indicate samples collected specifically for this study; symbols are colored according to putative population localities: Azores (AZ), Madeira (MAD), Canary (CAN), Mid-Atlantic Bight (MAB), South Atlantic Bight (SAB), eastern Gulf of Mexico (eGOM), western Gulf of Mexico (wGOM), Bahamas (BAH), Caribbean (CAB), Northeastern Brazil (Ne_Br), Brazil and Uruguay (Br_Uy). Map background represents the bathymetry across the species distribution.

investigate how contemporary, as well as past conditions, of the marine environment affect genetic differentiation of the mtDNA-CR marker based on a seascape genetics framework. Based on previous findings that examined both genetic differentiation and environmental conditions (Viricel and Rosel 2014), we expect to find a correlation between mtDNA-CR variation and environmental heterogeneity and/or geographic distance. In summary, we aim to test how IBD, IBE, and IBR are shaping mtDNA-CR variation of the Atlantic spotted dolphin throughout its distribution.

Materials and Methods

We used DNA sequences from newly collected samples in addition to data available in GenBank, providing a broad geographic coverage across the species' range (Figure 1, Supplementary Table S1). We focused on the mtDNA-CR because it is the most widely used and available genetic marker to evaluate population structure among Atlantic spotted dolphins from different regions of the Atlantic Ocean (Adams and Rosel 2006; Green et al. 2007; Quérouil et al. 2010; Caballero et al. 2013; Viricel and Rosel 2014), as well as a common marker used in cetacean taxonomic studies (Rosel et al. 2017a, b; Schwartz and Boness 2017).

GenBank Sequences and Their Geographic Coordinates

We downloaded all available mtDNA-CR sequences from Atlantic spotted dolphins from GenBank. We only considered in the analyses those with available geographic information in the refereed publications (accession numbers and original references are available in Supplementary Table S1). Geographic coordinates referring to sequences from The Bahamas, Azores, and Madeira Archipelagos were estimated based on the main location of sample collection referenced in the articles (Green et al. 2007; Quérouil et al. 2010). Geographic coordinates from sequences from the WNA and Gulf of Mexico were not available in the published literature but were estimated by matching the published sequence and sampling information with sampling coordinates and dates for published studies available on the Global Biodiversity Information Facility (GBIF) database (GBIF 2018). Sampling information available in GBIF that exactly matched dates and geographic coordinates with the region of sampling presented in Viricel and Rosel (2014) were included in the analyses, and any sequence that did not have coordinates in GBIF or for which the coordinates did not correspond to the sample region reported in Viricel and Rosel (2014) were not considered.

Sampling and DNA Extraction of New Samples

Tissue samples of 108 individuals were obtained from remote dart biopsies, stranded animals, or incidentally captured Atlantic spotted dolphins from different regions of the Atlantic Ocean including: Brazil ($n = 80$), Colombia ($n = 7$), Guadeloupe Island ($n = 1$), Uruguay ($n = 1$), and the Canary Islands ($n = 19$) (Supplementary Table S1). Samples were preserved in different ways, including ethanol 99°GL, sodium chloride-saturated 20% dimethyl sulphoxide, or lyophilized for long-term preservation.

Total genomic DNA was extracted from tissue samples using the DNeasy Blood and Tissue kit (Qiagen), following the manufacturer's protocol, except for the proteinase K digestion step that was extended overnight (Hancock-Hanser et al. 2013). DNA was eluted in lower volumes than recommended to avoid low concentrations of DNA mainly from samples obtained from stranded animals. The quality and concentration was not examined for all samples, but for those that were this was accomplished using the Qubit Fluorometric Quantitation (Thermo Fisher Scientific, Inc.).

Mitochondrial Control Region Sequencing and Alignment

We used 1 µL of DNA (regardless of concentration) to amplify a 650 base pair (bp) portion of the mtDNA-CR using the primers t-Pro-whale M13Dlp1.5 (5′-TGTAAAACGACAGCCAGTTCACC CAAAGCTGRARTTCTA-3′) and Dlp8 (5′-CCATCGWGATGTC TTATTTAAGRGGAA-3′) following the protocol by Tezanos-Pinto et al. (2008) for the amplification reaction and thermal cycler profile. PCR products were cleaned using the Illustra ExoProStar 1-step (GE Healthcare) as recommended by the manufacturer, followed by an incubation period at 37 °C for 30 min and 80 °C for 15 min. Both the forward and reverse strands were sequenced on an ABI-Prism 3500 Genetic Analyzer (Applied Biosystems). We did not find any evidence for heteroplasmy (i.e., sequence variation within the same individual) among the newly sequenced individuals. All sequences obtained from GenBank and those newly obtained in this study were aligned using the software Sequencher, version 5.4.6 (Genes Codes Corporation).

Genetic Diversity and Population Differentiation

Initially, we defined 11 putative populations based on geographic localities across the sampling area (Figure 1), and with reference to the sampling location information available from previous studies (Table 1 and see Supplementary Table S1 for details on relevant literature). Genetic data included individual or haplotype sequences available from GenBank (Supplementary Table S1) as well as the 108 new sequences generated for the first time in this study (see Sampling and DNA extraction section).

Haplotypes were defined using DNAsp 6.0 (Rozas et al. 2017). Molecular diversity indices, including nucleotide and haplotype diversity, were estimated in Arlequin 3.5.2 (Excoffier and Lischer 2015). The relationships among haplotypes were visualized through a median-joining network (Bandelt et al. 1999) constructed using the software PopART v.1.7 (Leigh and Bryant 2015). Neutrality tests (Tajima's D and Fu's FS) were also performed to test for population demographic changes in Arlequin 3.5.2 (Excoffier and Lischer 2015), and significance was assessed through 10 000 permutations.

We used jModelTest 2.1.10 (Darriba et al. 2012) to estimate the best model of nucleotide substitution for the dataset. The most suitable evolution model for our dataset was HKY+I+G. However, because this model was not available in the programs used for estimating genetic divergence, we used the highest rank model (from the jModelTest output) available for subsequent analyses. Therefore, we used the Tamura-Nei model with gamma (TN93+G), setting a value of 0.154 for the shape of the gamma distribution, as suggested by jModelTest. Compared with the original HKY+I+G model, the TN93+G model has an additional transition parameter (A/G ≠ C/T, rather than A/G = C/T), while rate heterogeneity among sites is accounted for using a single parameter (G, rather than I+G).

Population differentiation was tested in Arlequin 3.5.2 (Excoffier and Lischer 2015) by calculating pairwise F_{ST} and Φ_{ST}, and significance was assessed through 10 000 permutations. A Bonferroni correction was applied for multiple comparisons using the function *p.adjust* available in the *stats* package available in the software R 3.4.4 (R Core Team 2018). Nei's estimate of genetic divergence (Nei's dA) was estimated using the *nucleotideDivergence* function of

Table 1. Putative population information, molecular diversity indices, and neutrality tests for Atlantic spotted dolphin (*Stenella frontalis*)

Putative population	Relevant information	*N*	Nh	Exclusive haplotypes	Haplotype diversity	π	Polymorphic sites	Segregating sites	Tajima's *D*	Fu's FS
AZ	Individual sequences from samples collected around the Azores Archipelago (Quérouil et al. 2010)	145	54	31	0.949 (±0.008)	0.021	55	49	−0.7	**−24.065**
MAD	Individual sequences from samples collected around the Madeira Archipelago (Quérouil et al. 2010)	46	31	12	0.981 (±0.008)	0.02	48	42	−1.071	**−15.597**
CAN	Samples collected from animals stranded at different Canary Archipelago islands (Lanzarote, Fuerteventura, and Gran Canaria)	12	11	2	0.985 (±0.04)	0.021	32	27	−0.971	**−3.447**
MAB	Haplotype sequences from individuals collected in coastal and oceanic waters northward of Cape Hatteras (~35°N) (Adams and Rosel 2006; Viricel and Rosel 2014)	17	11	2	0.941 (±0.036)	0.012	17	17	−0.798	**−3.452**
SAB	Haplotype sequences from individuals collected on the continental shelf and southward of Cape Hatteras (~35°N) (Adams and Rosel 2006; Viricel and Rosel 2014)	82	11	5	0.741 (±0.045)	0.01	14	14	0.428	0.297
eGOM	Haplotype sequences from individuals collected on the continental shelf and eastward of Mobile Bay (Adams and Rosel 2006; Viricel and Rosel 2014)	59	17	10	0.914 (±0.017)	0.009	22	20	−0.932	**−5.135**
wGOM	Haplotype sequences from individuals collected on the continental shelf and westward of Mobile Bay (Adams and Rosel 2006; Viricel and Rosel 2014)	15	6	1	0.8 (±0.077)	0.013	13	13	0.227	1.231
BAH	Haplotype sequences from individuals collected in The Bahamas (Green et al. 2007; Green 2008)	93	6	0	0.572 (±0.051)	0.006	8	8	0.968	2.392
CAB	Haplotype sequences collected from the Caribbean Sea (Caballero et al. 2013) and samples collected in La Guajira (Colombia) and Guadeloupe Island	14	11	5	0.934 (±0.061)	0.02	38	31	−1.459	−2.205
Ne_Br	Samples collected from stranded animals in northeastern Brazil	2	2	0	1 (±0.5)	0.053	22	17	0	3.091
Br_Uy	Samples collected between 22°S and 34°S in the western South Atlantic	60	8	3	0.633 (±0.064)	0.007	10	10	0.124	0.255

Significant results for neutrality tests are typed in bold. For population abbreviations, see Figure 1 legend. *N*, number of sequences per population; Nh, the number of haplotypes; π, nucleotide diversity.

the *StrataG* package (Archer et al. 2016) in the software R 3.4.4 (R Core Team 2018).

Finally, analyses of molecular variance (AMOVA) were computed in Arlequin 3.5.2 (Excoffier and Lischer 2015). The different groups tested were defined based on geographical proximity of putative populations and results from previous studies, to account for all possibilities (Supplementary Table S1). Significance was assessed through 1000 permutations. Due to differences in sampling sizes among populations, a standardized measure of genetic differentiation following Meirmans (2006) was calcuted in the software Genodive 3 (Meirmans 2020).

Environmental and Spatial Data

The study area encompassed almost the complete distribution of Atlantic spotted dolphins across the tropical and subtropical areas of the Atlantic Ocean (Figure 1). A shapefile representing Atlantic spotted dolphin distribution is provided by the IUCN Red List (Hammond et al. 2012). This shapefile was used to delimit the study area after being edited to include a sample collected in Uruguay at the southernmost record for the species in the western Atlantic Ocean (Paro et al. 2014).

Considering what is known about cetacean habitat preference and specific preferences for Atlantic spotted dolphins (Baumgartner et al. 2001; Moreno et al. 2005; Palacios et al. 2013, do Amaral et al. 2015; Barragán-Barrera et al. 2019), 12 static and dynamic environmental layers were selected for seascape analyses (Supplementary Figure S1). These layers encompasses long-term data (~20 years) and were gathered from MARSPEC (Sbrocco and Barber 2013), a resource of ocean climate layers for marine spatial ecology, in ESRI grid format at ~10 km of resolution. Since our analyses focuses on genetic patterns at relatively broad temporal and spatial scales, we expect that the spatial and temporal information are accurate enough for what we seek to analyze in this study.

The correlation among layers was investigated using the function *pairs* from the *raster* package (Hijmans 2019). Layers highly correlated were excluded from the analyses (Supplementary Figure S1). Although the Annual range in SST (Range SST) was strongly negatively correlated (r = −0.81) with Mean annual SST (Mean SST), we retained the Range SST as a layer for analyses because of its importance in representing the interannual variation along the distribution of the species, thus enabling different interpretations about the role of the environment. Therefore, 6 environmental layers were kept in subsequent analyses: Bathymetry (BAT), Slope (SLO), Mean annual sea surface salinity (Mean SSS), Mean SST, and Range SST (Table 2). A principal component analysis (PCA) was performed with these 6 environmental layers in raster format using the function *rasterPCA* from the package *RStoolbox* (Leutner and Horning 2016) to summarize the environmental data within the study area. When the results were plotted it was possible to visualize the environmental heterogeneity across the Atlantic spotted dolphin distribution.

The values for each environmental data were extracted for each sampling location using the function *extract* from the *raster* package (Hijmans 2019). These values were used to calculate if the populations had different medians in relation to each environmental variable. More specifically, we ran a Kruskal–Wallis test with a Bonferroni correction to assess equality of the medians of environmental variables among putative populations, followed by the Dunn test to assess differences between pairwise putative populations. Both tests were performed using the *dunn* package (Dinno 2017).

Table 2. Static and dynamic environmental layers used in this study and their respective abbreviations and units of measure

Environmental layers		Abbreviation	Unit
Static	Bathymetry	BAT	Meters (m)
	Slope	SLO	Degrees (°)
Dynamic	Mean annual sea surface salinity	Mean SSS	psu
	Annual range in sea surface salinity	Range SSS	psu
	Mean annual sea surface temperature	Mean SST	Celsius (°C)
	Annual range in sea surface temperature	Range SST	Celsius (°C)

psu, practical salinity unit.

In the tests including static layers, we excluded populations represented by only one geographical coordinate, and data from stranded animals.

The *maxent* function of the *dismo* package (Hijmans et al. 2017) was used to build Maxent ("Maximum Entropy") species distribution models (Phillips et al. 2006) that were further used as resistance predictors in IBR analyses. Occurrence records used to train the model were derived from sample metadata. We removed duplicate records and records representing the same pixel. An independent data set with Atlantic spotted dolphin occurrences compiled from the literature (do Amaral et al. 2015, 2018; Barragán-Barrera et al. 2019) was built for model evaluation. The same set of environmental layers used to model present conditions (Table 2) was used to transfer the model to Last Glacial Maximum (LGM) conditions. The layers for the paleodata set were gathered from MARSPEC in ESRI grid format at ~10 km of resolution (Sbrocco 2014). Maxent model settings were defined through the *ENMevaluate* function of the package *ENMeval* (Muscarella et al. 2014), which provides species-specific tuning of settings to generate models based on the lowest value of the Akaike information criterion corrected for small samples sizes (AICc; Muscarella et al. 2014). We used the block method to partition occurrences (Muscarella et al. 2014). Across the study area, 10 000 background points were generated using the *randomPoints* function of the *dismo* package (Hijmans et al. 2017). The area under the receiver-operator curve (AUC) was used to evaluate the predictive model's skill, in which an AUC closest to one would be a perfect model and an AUC = 0.5 would indicate that the model performed no better than random (Phillips et al. 2006). The final map outputs were exported in raster format using continuous values from 0% to 100%, representing the estimate of environmental suitability. Analyses were performed in R 3.4.4 (R Core Team 2018).

Seascape Analyses at the Individual Level

Because delimitation of populations a priori based on geography is questionable for cetaceans due to their great dispersal capabilities, we performed analyses at the individual level (i.e., those that do not rely on population designations) using 2 different approaches to test for IBD and IBE: a Procrustes analysis (Wang et al. 2012) and distance-based redundancy analysis (dbRDA; Legendre and Anderson 1999).

A systematic analysis to quantitatively evaluate the similarity of genes and geography was conducted by applying a Procrustes analysis approach to find an optimal transformation that maximizes the similarity between maps of genetic variation and geographic maps

of population locations (Wang et al. 2012). This analysis evaluates evidence for IBD while retaining longitudinal and latitudinal geographic information for each sample (Knowles et al. 2016). A PCA with the mtDNA-CR sequences was conducted to summarize genetic information using the function *dudi.pca* of the *ade4* package (Dray and Dufour 2007), retaining the first 2 axes for the Procrustes analysis. We used the *procrustes* function to rotate the genetic and geographic matrices, and the function *protest* to test the significance of the associations of genetic and geography in the *vegan* package (Oksanen et al. 2017). We also performed an IBE test, applying the *residual* function to estimate the residuals of the Procrustes analysis, which was then used in a second Procustes analysis to test for an association of the residual genetic variation with the principal component 1 (PC1) and principal component 2 (PC2) of the PCA performed with 6 environmental layers. The correspondence of the genetic clusters with the a priori determination of population based on the published literature (Supplementary Table S1) and geography was checked visually.

A dbRDA was used to explicitly test latitude, longitude, geographic Euclidean distance, and environmental predictors against genetic distance. Therefore, we can consider dbRDA as an approach to test for IBD and IBE. To perform these tests, first pairwise genetic distances between individuals were estimated using the *dist.dna* function from the *ape* package (Paradis et al. 2004) using the TN93+G with gamma = 0.154. Three different geographical predictors were tested: latitude, longitude, and pairwise Euclidean distance between individuals. Euclidean distance between individuals was estimated using the function *rdist* from the package *fields* (Nychka et al. 2017). The resulting matrix was reduced to a vector using principal coordinate analysis (Borcard and Legendre 2002) implemented with the *pcnm* function in the *vegan* package (Oksanen et al. 2017). Mean SST and BAT (the environmental layers with the highest percent of contribution in the ecological niche model—see Results), as well as PC1 from the PCA performed with 6 environmental layers (Table 2) were included as environmental predictors. Therefore, the dbRDA included: 1) marginal tests, where the relationship between mtDNA-CR genetic distances and each of the geographical and environmental predictors was analyzed separately, and 2) conditional tests, where the relationship between environmental variables as predictors of mtDNA-CR distances was assessed after controlling for geographical predictors. The dbRDA was performed using the *capscale* function in the *vegan* package (Oksanen et al. 2017); in this function, the dissimilarity data are first ordinated using metric scaling, and the ordination results are analyzed with redundancy analysis. We assessed the significance using the function *anova.cca* from the *vegan* package (Oksanen et al. 2017). Analyses were performed in R 3.4.4 (R Core Team 2018).

Seascape Analyses at the Population Level

A set of linear regression models and Mantel tests (marginal and conditional) were performed at the population level with each population represented by the centroid of the geographic region spanning the different sampled geographic coordinates (Table 1). Several metrics were used to test for IBD, IBE, and IBR.

The Euclidean distance between populations was used as a geographical predictor for genetic structure. It was estimated using the function *rdist* from the package *fields* (Nychka et al. 2017), and the resulting Euclidean distances between populations were transformed into a matrix using the function *dist* of the *stats* package (R Core Team 2018). Four least-cost distance matrices were generated, as well as their respective maps representing least-cost paths: a least-cost distance with no constraint (i.e., the least-cost path ignoring depth constraints), and a least-cost path with constraints computed with a maximum depth constraint of 200, 500, and 1000 m (i.e., paths constrained to waters less than 200, 500, and 1000 m, respectively) (Supplementary Figure S2). These constraints were tested because the species is usually observed over the continental shelf in depths ranging from 20 to 200 m, with a few scattered records up to 1000 m (Moreno et al. 2005). Least-cost distances were computed between pairwise centroids using the *lc.dist* function from the *marmap* package (Pante and Simon-Bouhet 2013).

A matrix representing environmental predictors was generated using the PC1 and PC2 representing the 6 environmental layers (Table 2). In addition, resistance predictors were estimated using the software Circuitscape v. 3.5.8 (Shah and McRae 2008) based on maps of environmental suitability resulting from both contemporary and LGM climatic and geophysical conditions.

Simple linear regression models among 3 different matrices of genetic differentiation data (linearized F_{ST}, Φ_{ST}, and Nei's dA) and predictors were tested using the *lm* function of the *stats* package (R Core Team 2018). The correlation among the 3 genetic distances and geographical, environmental, least-cost, and resistance predictors was estimated using a Mantel test, while partial Mantel was used to test the correlation among genetic matrices and the environmental predictor controlling for geographic and least-cost distances. Mantel and partial Mantel tests were performed using the *mantel* and *mantel.partial* functions, respectively, in the *vegan* package (Oksanen et al. 2017), using Pearson as the correlation method, and significance was assessed with 999 permutations. All R analyses were performed in R 3.4.4 (R Core Team 2018).

Results

mtDNA Sequences and Genetic Diversity

From an initial set of 108 samples, we were able to successfully amplify and sequence 80 samples. Therefore, our final mtDNA-CR data set consisted of 545 sequences, being 80 newly generated in this study (Accession Numbers on GenBank: MN339200–MN339279) and 465 from previous studies (Figure 1, Supplementary Table S1). Some samples obtained from stranded animals had their geographic coordinates slightly modified to capture environmental information from the region, avoiding an inland location.

From an alignment of 344 bp, a total of 103 distinct haplotypes were identified (Figure 2). Maps of the distribution of haplotypes indicated that in the WNA (Figure 2A), Caribbean (Figure 2C), and WSA (Figure 2D), the haplotypes seemed to be more strongly structured among geographically distinct areas, while in the ENA there was less structure but high haplotype diversity (Figure 2B). In the haplotype network, most high-frequency haplotypes were clustered together and mostly sampled in the northern Atlantic, with the exception of one haplotype most common in the Br_Uy (Supplementary Figure S3). Two divergent haplotypes sampled in high frequency were found in BAH and SAB. Most single-frequency haplotypes were sampled in AZ and MAD, showing long branches in regard to the central portion of the network (Supplementary Figure S3).

Genetic diversities based on π and on haplotype diversity varied across regions, being lower in BAH and Br_Uy and higher in the AZ, MAD, CAN, and Ne_Br populations (Table 1). Values of Fu's Fs tended to be negative and were significant in some populations

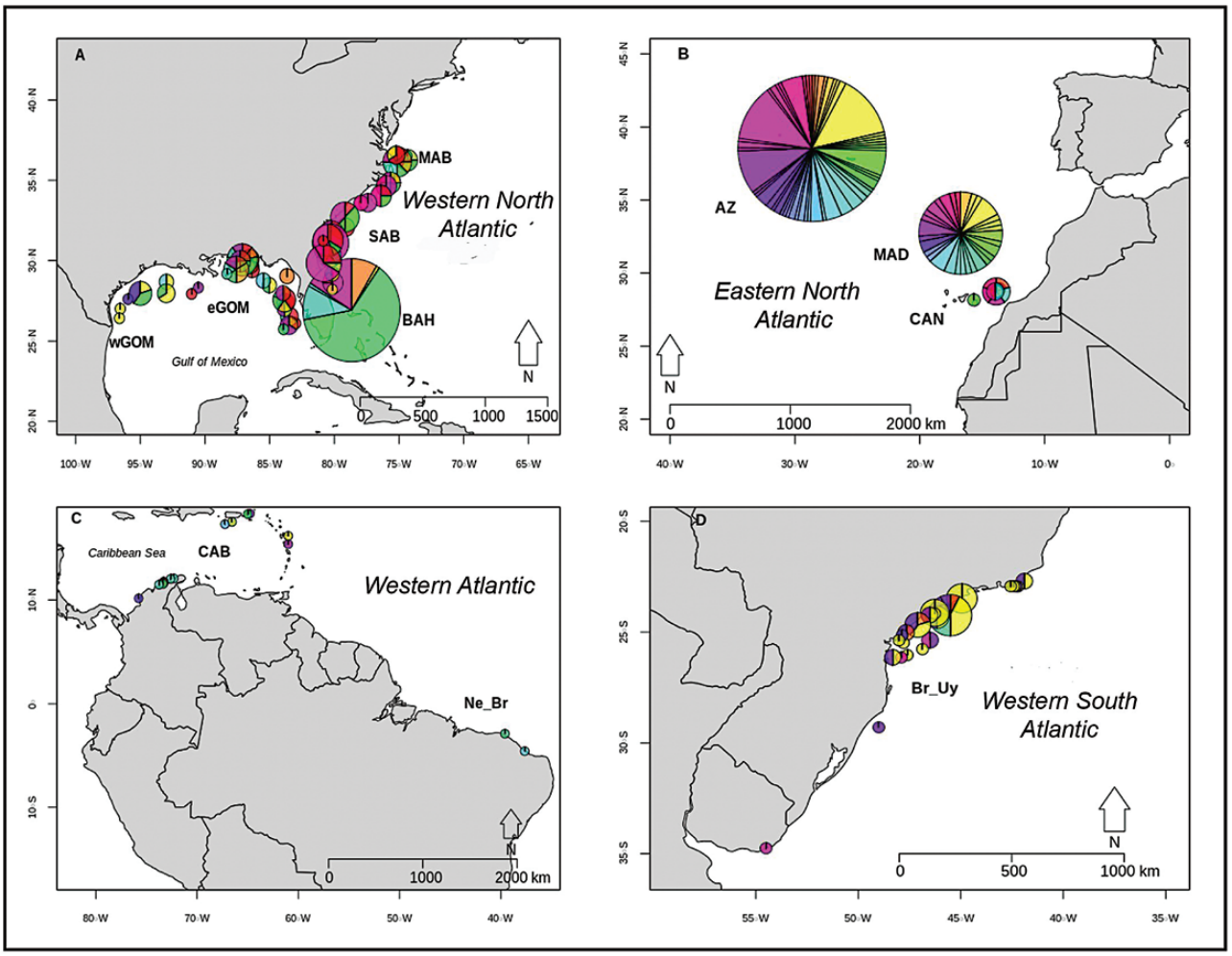

Figure 2. Mitochondrial DNA control region haplotypes recovered among putative populations of Atlantic spotted dolphin (*Stenella frontalis*). The geographic localization of haplotypes is shown by regions: (**A**) western North Atlantic (BAH, $n = 93$; eGOM, $n = 59$; MAB, $n = 17$; SAB, $n = 82$; wGOM, $n = 15$), (**B**) eastern North Atlantic (AZ, $n = 145$; CAN, $n = 12$; MAD, $n = 46$), and (**C**) Caribbean (CAB, $n = 14$) and northeastern Brazil (Ne_Br, $n = 2$), and (**D**) western South Atlantic (Br_Uy, $n = 60$). Each of 103 haplotypes is represented as a different color (see Figure 1 for population labels).

(e.g., AZ, MAD, CAN, MAB, and eGOM), whereas none of the Tajima's D values were statistically significant for any populations (Table 1).

Population Differentiation

For several combinations of populations in different groups, the resulting AMOVA with the highest differentiation among groups were: Group 1: AZ, CAN, MAB, MAD (hereafter named Oceanic), Group 2: SAB, Group 3: BAH, Group 4: eGOM, Group 5: wGOM, Group 6: CAB and Ne_Br (hereafter named CAB+Ne_Br), and Group 7: Br_Uy ($\Phi_{CT} = 0.13007$, $P = 0.004$, $\Phi_{ST} = 0.15372$, $P < 0.00000$, $\Phi_{SC} = 0.02719$, $P = 0.312$, $F'_{CT} = 0.789$, $F'_{SC} = 0.344$) (Supplementary Table S2).

Fixation indices were significantly different from zero among almost all pairwise comparisons (see Table 3 for pairwise Φ_{ST}; pairwise F_{ST} showed qualitatively similar results, see Supplementary Table S3). The lowest significant Φ_{ST} value after Bonferroni correction ($\Phi_{ST} = 0.041$, $P < 0.0001$) was obtained between Br_Uy and Oceanic populations, while the highest was between CAB+Ne_Br and SAB ($\Phi_{ST} = 0.409$, $P < 0.0001$) (Table 3). The lowest F_{ST} value after Bonferroni correction was obtained between eGOM and Oceanic populations ($F_{ST} = 0.042$, $P < 0.0001$), while the highest was between BAH and Br_Uy ($F_{ST} = 0.388$, $P < 0.0001$) (Supplementary Table S3). Nei's dA values ranged from 0.0007 to 0.019. The lowest value was obtained between eGOM and wGOM (Nei's dA = 0.00071); almost all comparisons among Br_Uy and the remaining populations resulted in Nei's dA values higher than 0.01 (except with SAB) (Supplementary Table S4).

Environmental Analyses

Environmental heterogeneity was summarized by PCA performed with 6 environmental layers (Table 2, Supplementary Figure S4). The WNA, ENA, and WSA seemed to have similar environmental conditions (represented by shades of pink in the Supplementary Figure 4), as did the equatorial zone in the central portion of Atlantic Ocean (represented by shades of green in the Supplementary Figure S4). The Gulf of Mexico exhibited distinct conditions similar to those found in the South Atlantic Bight (represented by shades of orange in the Supplementary Figure S4). Coastal waters from north South America and the Caribbean seemed to have similar features from those found in western Africa (represented by shades of yellow in the Supplementary Figure S4).

Table 3. Pairwise φ_{ST} values obtained between the Atlantic spotted dolphin (*Stenella frontalis*) populations for the mtDNA control region marker

Φ_{ST}	OCE	SAB	eGOM	wGOM	BAH	CAB+Ne_Br	Br_Uy
OCE		0.000	0.000	0.756	0.000	0.945	0.000
SAB	**0.152**		0.000	0.000	0.000	0.000	0.000
eGOM	**0.046**	**0.326**		0.378	0.000	0.000	0.000
wGOM	0.058	**0.391**	0.095		0.000	1.000	0.000
BAH	**0.148**	**0.400**	**0.170**	**0.309**		0.000	0.000
CAB+Ne_Br	0.037	**0.409**	**0.132**	0.053	**0.310**		0.000
Br_Uy	**0.041**	**0.234**	**0.081**	**0.227**	**0.232**	**0.224**	

P values are in the upper diagonal and significant values (significant values are those with $P \leq 0.05$) are typed in bold below the diagonal. BAH, Bahamas; Br_Uy, Brazil and Uruguay; CAB, Caribbean; eGOM, eastern Gulf of Mexico; Ne_Br, Northeastern Brazil; OCE, oceanic; SAB, South Atlantic Bight; wGOM, western Gulf of Mexico.

The median values for the 6 environmental variables showed significant differences for each putative population (Supplementary Table S5), suggesting that each population is adapted to different conditions. However, pairwise comparisons revealed that some populations were not differentiated based on some environmental layers (Supplementary Tables S6 and S7). In relation to static layers, CAB had the highest median BAT (654 m depth) (Supplementary Figure S5A); however, individuals from MAB were recorded in waters up to 2000 m depth (Supplementary Figure S5A). Medians from the remaining populations ranged from 39 to 92 m depth (Supplementary Figure S5A). Almost all populations seem to occupy flat areas with low SLO values, with the exception of CAB and MAB that exhibited higher values in this layer (Supplementary Figure S5B).

In relation to dynamic layers, CAN and Ne_Br occupied waters with the highest Mean SSS (more than 36 psu), as well as the lowest Range SSS (0.19 and 0.68 psu, respectively) (Supplementary Figure S5C,D). MAB occupied waters with the lowest Mean SSS (33 psu) but an intermediate Range SSS (2.24 psu). The remaining populations occupied waters with Mean SSS around 35 psu, and Range SSS ranging from 0.90 to 3.7 psu (Supplementary Figure S5C,D). CAB and Ne_Br occupied waters with the highest Mean SST (more than 27 °C), as well as the lowest Range SSS (3.2 °C and 2 °C, respectively) (Supplementary Figure S5E,F). CAN and MAB occupied waters with the lowest Mean SST (20.5 °C and 18 °C, respectively), and MAB had the highest Range SST, around 13 °C (Supplementary Figure S5E,F). The remaining populations occupied waters with Mean SST between 23 °C and 25 °C, where Range SST ranging from 5 °C to 10 °C (Supplementary Figure S5E,F).

In relation to the ecological niche models, we used 98 records of presence for model training, and 302 records of presence for model testing. The best configuration model (showing the lowest AICc value) included a hinge feature class and a regularization multiplier equal to 3.5. Environmental layers with the highest percent of contribution to the model were BAT and Mean SST. Both AUC training and AUC testing values were higher than 0.9, indicating a good model performance.

Prediction based on contemporary conditions suggested high environmental suitability along the continental shelf of Africa and around oceanic islands in the eastern Atlantic (Supplementary Figure S6). In the western Atlantic, high suitability was recovered mainly in the continental shelves of the WNA, Gulf of Mexico, as well as the continental shelf from southeastern Brazil to Uruguay (Supplementary Figure S6). In the Caribbean Sea, spots of high suitability were recovered in the continental shelf areas of Colombia and Venezuela. The resistance distance matrix based on the environmental suitability for contemporary conditions indicated multiple pathways among all populations (results not showed).

When the model was transferred to LGM conditions, high environmental suitability was recovered in specific areas in the western Atlantic, such as southeastern Brazil, the Antilles, Gulf of Mexico, and South Atlantic Bight; high environmental suitability was also recovered in the and western Africa mainly close to Guinea and Guinea-Bissau (Supplementary Figure S7). Because sea level reduced 120 m during the LGM, many areas of high suitability in the contemporary period (Supplementary Figure S6) were not available for the species in the LGM (Supplementary Figure S7). Therefore, the resistance distance matrix based on the environmental suitability for LGM conditions resulted in a limited set of potential pathways connecting populations because the centroids of some populations (i.e., AZ, BAH, Br_Uy, Ne_Br, and SAB) were not represented in the LGM suitability map due to the exposure of the continental shelf (results not showed).

Seascape Analyses at the Individual Level

Procrustes analysis revealed that genetic differentiation was significantly associated with geographic distance ($t = 0.1719$, $P < 0.001$), although there is a large proportion of genetic variation that is not explained by geography. Specifically, individuals are genetically more similar to each other than expected based on geographic location alone (shown by the long lines of deviation connecting the sampling localities to the position of individuals in genetic PC space) (Figure 3A). The subsequent Procrustes analysis showed that the residual from the association between genetic and geographic distance was significantly associated with differences in the environmental conditions of sampled localities ($t = 0.1015$, $P = 0.02$). In summary, the environment contributes to the patterns of genetic difference even after controlling for genetic differentiation predicted by geographic distance (Figure 3B). This association was detected even though the environmental space occupied by the sampled individuals and populations was a small subset (see the cloud of points represented in Figure 3B) of the environmental space considering the broad distribution of the species (Figure 1). Note that individuals tend to form clusters in environmental space matching our population designations and previous studies (Figure 3B). Within the Br_Uy population, the most displaced sample (i.e., the one with the greatest deviation under an isolation by distance model) is from the southernmost record in the WSA. In general, Procrustes results suggested both an IBD and IBE patterns.

Marginal dbRDA tests detected an IBD pattern due to significant associations of genetic variation with different measures of

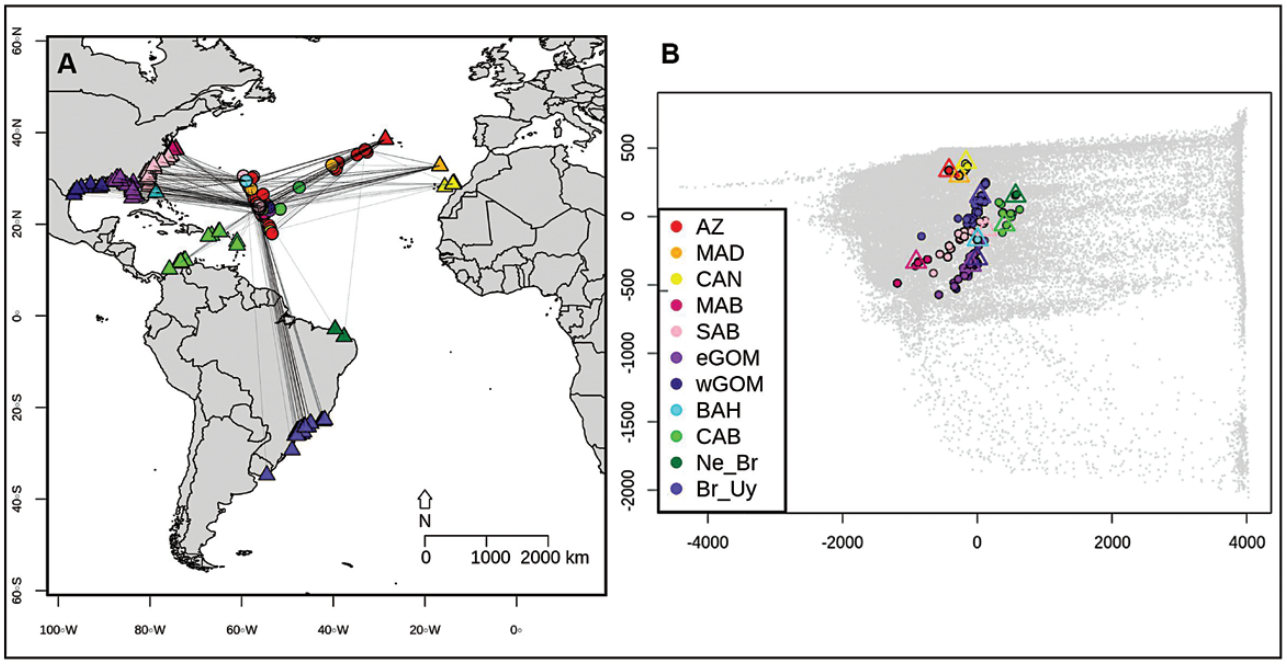

Figure 3. Representation of Procrustes analyses. (**A**) Graphic of the deviations of individuals genetically from expected values based on the geographic locations individuals were collected from, shown as lines connecting the geographic origin of each sample (triangles) and the position of individuals in the genetic PC (dots) and color coded based on the sampled population (see Figure 1 for population labels). The length of lines represents the magnitude of the deviation in the genetic PC-space from the expected pattern of genetic variation based on geography. (**B**) The environmental space occupied by the sampled individuals and populations was a small subset of the environmental space associated with broad distribution of the species (Figure 1). Nevertheless, environmental variation was significantly associated with genetic distance after controlling for geography (see text for details). Colored circles represent the position of genetic samples environmentally relative to the small gray circles that represent the environmental space of study area (Figure 1); the centroid of each putative population is represented as a hollow triangle.

geographic distances, although each variable explained a small percentage of the genetic variance among sampled individuals (Table 4). Conditional dbRDA showed a similar pattern of significance but lower fit to the genetic variance for the environmental measures (Supplementary Table S8).

Seascape Analyses at the Population Level

Simple linear regression models among 3 different matrices of genetic differentiation (linearized F_{ST}, Φ_{ST}, and Nei's dA) among the putative populations and geographical, environmental, least-cost, and resistance matrices revealed some significant results (Table 5). However, most variables showed low correlations (though statistically significant); the highest correlation was that between Φ_{ST} and the least-cost distances with 500 m constraint (R^2 = 0.192) (Table 5). These results are qualitatively similar to the Mantel tests results in the sense that none of the variables showed high associations (Supplementary Tables S9 and S10). Mantel tests between the Nei's dA genetic matrix with either the least-cost distance with no restriction (r = 0.37, P = 0.02) or the resistance matrix based on the suitability map of contemporary conditions (r = 0.41, P = 0.04) were significant, indicating a correlation between geographical and resistance predictors and genetic divergence (Supplementary Table S9). None of the conditional tests (i.e., partial Mantel tests that controlled for an association between genetic and geographic distance) were statistically significant (Supplementary Table S10).

Discussion

In this study, we evaluated the genetic differentiation of the Atlantic spotted dolphin along its distribution using the most comprehensive mtDNA-CR dataset for the species. Furthermore, we investigated the relationship between matrilineal genetic structure, geography, and contemporary and past environmental heterogeneity using a seascape genetics approach. Our results showed that both geography and contemporary environment conditions have a relationship with genetic structure. The results also suggest that geographic distances could be more influential on a large-scale, while environmental heterogeneity could be more influential on a smaller geographic scale, in agreement with Viricel and Rosel (2014).

Genetic Structure

Although we included individuals from new geographic regions that had not been sampled before, molecular diversity and fixation indices were relatively similar to those from previous studies (Adams and Rosel 2006; Green et al. 2007; Quérouil et al. 2010; Caballero et al. 2013; Viricel and Rosel 2014). However, unlike previous studies, we found different genetic structure among putative populations, possibly due to increased sample sizes. Applying the thresholds for Nei's dA values to identify populations, subspecies and species based on the mtDNA-CR suggested by Rosel et al. (2017a), we found 7 populations that could represent different units. It is noteworthy that levels of differentiation between most populations were greater than the threshold considered to designate subspecies (i.e., Nei's dA > 0.004; Supplementary Table S4) (Rosel et al. 2017a). Although we do not consider the results from a single genetic marker to be sufficient to propose that these populations represent subspecies, our results do suggest an important degree of genetic structure among 7 populations of the Atlantic spotted dolphin: an Oceanic population (including individuals from AZ, MAD, CAN, and MAB), BAH, Br_Uy, SAB, eGOM, and wGOM possibly with CAB+Ne_Br.

Further analyses using nuclear markers will be important to indicate the degree of evolutionary isolation between these populations and to corroborate, or not, the idea that they might represent distinct evolutionary trajectories.

Genetic Structure: Western South Atlantic

It is important to consider topographic features of the continental shelf (the preferred habitat of *S. frontalis* in South America), as well the dynamics of the western boundary currents flowing along it, to better understand the genetic structure of the Atlantic spotted dolphin in the WSA and the Caribbean. Branches of the eastward flowing South Equatorial Current approach the coast of South America, and at approximately 10°30′S the North Brazilian Current is formed, where some branches of the South Equatorial Current converge. The North Brazilian Current flows northward toward the Amazon River mouth with relatively high speed (Castro and Miranda 1998). Also, at approximately 10°S, another branch of the South Equatorial Current feeds the Brazilian Current, which flows southwestward with lower speed (Castro and Miranda 1998). In northeastern Brazil, the continental shelf is very narrow, especially between 8°S and 15°S, where it has a width of approximately 15 km, reaching only 10 km at certain parts (Castro and Miranda 1998). In this sense, due to oceanographic features, Costa et al. (2017) proposed that northern Brazil cetacean fauna seems to be more similar to that of the southern Caribbean than northeastern Brazil. Indeed, the absence of Atlantic spotted dolphins for approximately 1500 km between 6°S and 18°S in northeastern Brazil represents an important barrier to gene flow between individuals from south/southeastern Brazil and those in north/northeastern Brazil. The absence of Atlantic spotted dolphin is well recognized in northeastern Brazil, and this pattern was also recovered from our analyses (Figure 1; Moreno et al. 2005; do Amaral et al. 2015).

Atlantic spotted dolphins in north/northeastern Brazil are more likely to be related to those in the Caribbean basin than to those in south/southeastern Brazil, in agreement with our genetic findings. However, in our study, only 2 samples from northeastern Brazil were analyzed. One of these samples had Haplotype 45, which was only recovered in 4 Brazilian samples collected off the 50 m isobath; the second had Haplotype 53, which was also found in the Oceanic population (Supplementary Table S1). Thus, the relationship between north/northeastern Brazil individuals with other populations is still an open question and additional sampling, together nuclear molecular markers are needed.

On the other hand, Atlantic spotted dolphins from southeastern Brazil, following the southward displacement of the Brazil Current, seldom reach extreme south Brazil and Uruguayan waters during

Table 4. Results of marginal distance-based redundancy analyses (i.e., after controlling for geography) between individual pairwise genetic distances and predictors

	Marginal dbRDA tests			
	Variable	*F*-statistics	*P*	%Variance
Individual pairwise genetic distances	Euclidean distance	**4.44**	**0.001**	**2.8**
	Latitude	1.0955	0.308	0.1
	Longitude	**12.99**	**0.001**	**0.8**
	Mean SST	**8.478**	**0.001**	**0.6**
	BAT	**5.176**	**0.032**	**0.3**
	PC1	**5.272**	**0.025**	**0.3**

Significant values (significant values are those with $P \leq 0.05$) are in bold. BAT, bathymetry; SST, sea surface temperature; PC1, principal component 1.

Table 5. Simple linear regression between linearized F_{ST}, φ_{ST}, and Nei's estimate of net divergence (dA) matrices and geography, least-cost and resistance matrices

	Simple linear regression			
	Variable	Slope	Adjusted R^2	*P*
Nei's dA	Euclidean distance	1428	0.002	0.291
	Resistance based on cENM	**3639.1**	**0.149**	**0.002**
	Resistance based on LGM ENM	5696.7	0.006	0.316
	LC no constraint	**173970.8**	**0.128**	**0.004**
	LC 200 m constraint	−9.837e+11	−0.016	0.746
	LC 500 m constraint	−1.212e+12	−0.015	0.673
	LC 1000 m constraint	−1.198e+12	−0.015	0.643
Φ_{ST}	Euclidean distance	−29.5	−0.009	0.4729
	Resistance based on cENM	−55.1	0.023	0.136
	Resistance based on LGM ENM	−298.7	−0.017	0.396
	LC no constraint	−817.5	−0.015	0.669
	LC 200 m constraint	**−2.996e+11**	**0.188**	**0.0006**
	LC 500 m constraint	**−2.874e+11**	**0.192**	**0.0004**
	LC 1000 m constraint	**−2.570e+11**	**0.190**	**0.0005**
$F_{ST}/(1 - F_{ST})$	Euclidean distance	9.6	−0.018	0.843
	Resistance based on cENM	20.8	−0.014	0.635
	Resistance based on LGM ENM	−136.4	−0.052	0.590
	LC no constraint	2680.6	0.009	0.230
	LC 200 m constraint	**−3.137e+11**	**0.145**	**0.002**
	LC 500 m constraint	**−3.043e+11**	**0.152**	**0.002**
	LC 1000 m constraint	**−2.730e+11**	**0.151**	**0.002**

Significant values (significant values are those with $P \leq 0.05$) are in bold. cENM, contemporary ecological niche model for contemporary condition; LGM ENM, ecological niche model for Last Glacial Maximum condition; LC, least-cost.

summer (Moreno et al. 2005, Paro et al. 2014). This distribution information was used to consider samples from south/southeastern Brazil and Uruguay as a single population (Br_Uy), inhabiting coastal waters from approximately 22°S to 34°S in the WSA. The genetic analyses of individuals sampled in this area revealed lower diversity indices and genetic differentiation in relation to both fixation indices (Table 3; Supplementary Table S3), supporting findings from a previous study (Caballero et al. 2013). It is worth noting the low number of haplotypes and nucleotide diversity recovered in this isolated population despite the large sample size in this region (n = 60). Conversely, other populations with more limited sampling size, such as CAB (n = 14), were more diverse and possibly more abundant than the isolated Br_Uy population (Table 1). Here again, Nei's dA values were higher than those considered to designate subspecies (Rosel et al. 2017a) in almost all pairwise comparisons (Supplementary Figure S4), suggesting a relatively strong reproductive isolation of this population, as already suggested (Moreno et al. 2005). Moreno (2002) found that 12 of 32 skull measurements differed significantly between specimens from the north Atlantic and WSA. Moreover, animals from the WSA have an average of three more teeth in each tooth row than the animals from the north Atlantic.

Other delphinids have also shown a similar pattern of isolation in the WSA, such as the clymene dolphin (*Stenella clymene*) (Nara et al. 2017), *Delphinus* sp. (Amaral et al. 2012b), the Guiana dolphin (*Sotalia guianensis*) (Caballero et al. 2018) and the rough-toothed dolphin (*Steno bredanensis*) (da Silva et al. 2015). In some cases, as with the Lahille bottlenose dolphin (*Tursiops gephyreus*), the differences were sufficient to lead some authors to propose a different species (Wickert et al. 2016; Hohl et al. 2020).

In a previous study, the population from southern Brazil was considered part of a single population in Atlantic (including Azores, Madeira, and southeastern Brazil) due to the large number of haplotypes shared (Caballero et al. 2013). Here, we consider a much larger dataset, which showed low genetic diversity and differences between Br_Uy and all putative populations along the species range, including the Caribbean. Therefore, the different conclusions reached by different studies reflect both differences in the criteria used to evaluate population status, as well as differences in the statistical power to detect genetic differentiation. It is worth mentioning that haplotype sharing could be due to other factors like incomplete lineage sorting from sharing a common ancestry rather than contemporary genetic exchange (Jefferson and Wang 2011). As such, and also based on ecological evidence (do Amaral et al. 2015, Méndez-Fernandez et al. 2019), we suggest that from a conservationist point of view the south/southeastern Brazil population deserves special attention, at least until more data (e.g., nuclear and morphological data) becomes available, which will allow for more formal model-based tests of population/taxonomic designations (see discussions in Huang and Knowles 2016; Sukumaran and Knowles 2017). Furthermore, these populations could be vulnerable to general threats, such as those triggered by pollution, climate change, and fisheries overexploitation.

The Southern Brazilian Bight from Cabo Frio (23°S) to Cabo de Santa Marta (28°S) seems to be the core habitat of this isolated population of the Atlantic Spotted dolphin in Brazil. This region has a high human population density, and the urbanization and industrialization in the states of São Paulo and Rio de Janeiro exerts great pressure on the environment of this area. Indeed, there are studies showing high levels of contaminants in Atlantic spotted dolphin of southeastern Brazil (Leonel et al. 2012; Kehrig et al. 2017; Mendéz-Fernandez et al. 2018; Lavandier et al. 2019). Furthermore, the Port of Santos supports intense ship traffic, and southeastern Brazil is the main area of oil and gas exploitation in Brazil (Santos et al. 2018). Moreover, the Southern Brazilian Bight is one of the most productive and exploited coastal regions of Brazil (Vansconcelos and Gasalla 2001; Paes and Moraes 2007) and congregates one of the biggest and most diversified industrial and artisanal fishing fleets in Brazil (Barreto et al. 2017), with several fish stocks already overexploited.

Genetic Structure: Caribbean and North Atlantic

Caballero et al. (2013) found no differentiation in pairwise comparisons of CAB with Azores and Madeira based on Φ_{ST}. Our genetic findings based on both fixation indices (F_{ST} and Φ_{ST}) grouped CAB and Ne_Br. Our results with Φ_{ST} showed no differentiation between CAB+Ne_Br and the Oceanic population, nor compared to the wGOM. F_{ST} also suggested no differentiation between CAB+Ne_Br and the Oceanic population, nor in the comparison between the eGOM and CAB+Ne_Br. We are aware of the limitations in using such a limited sample size to represent these groups, and we recognize that we may be missing haplotype diversity that is truly present in this area. However, after an exhaustive sampling effort, only 2 individuals from Ne_Br and 14 from CAB were obtained. Therefore, additional sampling of individuals and nuclear data will be needed to effectively assess genetic connectivity or potential structure of the Caribbean and populations mainly from the North Atlantic and north/northeastern Brazil.

Our results confirm the differentiation of the Oceanic population from the SAB, which is consistent with previous studies (e.g., Adams and Rosel 2006; Viricel and Rosel 2014). We did not find differentiation between the Oceanic population and wGOM based on Φ_{ST} (Table 3) and unlike Viricel and Rosel (2014), we also did not find differentiation between eGOM and wGOM based on Φ_{ST}.

The BAH population showed differentiation from all populations in both fixation indices (Figure 3, Supplementary Table S3), and Nei's dA values were all above the subspecies threshold (Supplementary Table S4). Previous genetic analyses showed that individuals from BAH are not genetically differentiated from those collected across the Gulf of Mexico and South Atlantic Bight, probably due to the Gulf Stream flow (Green 2008). However, we recovered higher differentiation between BAH and wGOM, as well as between BAH and SAB; while lower differentiation was recovered between BAH and eGOM for all estimated genetic metrics. The different sample size and different definition of populations could be explanations for these different findings among our study and that performed by Green (2008). Green (2008) compared BAH samples (n = 93) with only those from the study of Adams and Rosel (2006) that included individuals from the WNA and Gulf of Mexico (n = 199). However, additional sequences from the WNA have become available in the study conducted by Viricel and Rosel (2014) (n = 422), which recognized 2 populations in the Gulf of Mexico. In our study, we included sequences published considering these studies, therefore we had a high number of samples in relation to the study of Green (2008) and we considered a different population definition (as explained in the Materials and Methods section).

An alternative explanation for the differences found in relation to previous studies could involve the philopatry observed in dolphins occurring in The Bahamas (Herzing 1997, Elliser and Herzing 2012, 2014). In the Gulf of Mexico, indirect evidence of site fidelity was reported by Viricel and Rosel (2014), who collected samples from the same individual twice over 5 years. In Brazil, a relatively high number of individuals from southeastern Brazil were re-identified in the same location over a 2-year sampling period (Santos et al. 2018),

reinforcing an important role for social structure on population genetic structure.

Seascape Genetics Analyses

The Atlantic basin presented heterogeneous conditions across the Atlantic spotted dolphin distribution (Supplementary Figure S4), and our univariate analysis showed that Atlantic spotted dolphin populations indeed occupy different environments across their distribution in relation to the 6 environmental layers analyzed (Supplementary Figure S5). A significant association between genetic, geographic, and environmental distances was detected using the Procrustes analyses conducted at the individual level (i.e., without a priori designated populations), although the strength of the associations varied when considering the effects of geography and environment. The factors better fit the observed genetic differences varied across populations. For example, the genetic differentiation for Br_Uy seems to be best explained by geography compared to other populations because a higher deviation is observed in the genetic PC-space in regard to the expected pattern of genetic variation based on geography; whereas, populations from the North Atlantic seem to have a lower deviation from the expected pattern (Figure 3A). When environmental variables are considered, population clusters were determined by BAT (PC1-axis) and Mean SST (PC2-axis) (Figure 3B). These clusters also correspond with our a priori division of putative population designations based on the literature (except for a few individuals that seem to be dislocated from their counterparts and/or centroids, see Figure 3B). These findings suggest that Atlantic spotted dolphins may have environmental preferences and therefore environmental heterogeneity at small spatial scales contributes to genetic isolation. However, this analysis also revealed that some populations that occupy similar environmental spaces are not genetically close, probably due to geographical distance (e.g., the proximity of Br_Uy with SAB and MAB populations). Taken together, this indicates that the environment has a smaller effect than (i.e., does not override) the influence of geography on genetic differentiation at large geographic scales.

The dbRDA, also conducted at the individual level, showed that geographic Euclidean distance explained 2.8% of the genetic variation, while environmental predictors, such as BAT, Mean SST, or even all environmental variables analyzed here represented by PC1 explained less than 0.6% (Table 4). Conditional dbRDA also showed the same pattern (Supplementary Table S8). In other words, when controlling for geography, only a small percentage of genetic variation is explained by the environment (e.g., the highest variation of 0.5% observed in conditional tests was between genetic and Mean SST controlled for latitude). Although these results agree with those from the Procrustes analyses, in which geography explains mtDNA-CR variance better than the environment, favoring an IBD pattern, in both cases there is a large genetic variance that is not explained. This could reflect the limited power of analyzing a single locus like the mtDNA-CR, for which a high stochastic variance may obscure weaker associations.

In this study, the main aim of the ecological niche modeling was to provide a map of suitability to estimate the resistance matrix for further IBR analyses and these models were generated only considering the geographic coordinates for samples analyzed in this study. Therefore, these models most likely reflect the environmental suitability of these specific sets of records, and extrapolations should be considered with caution. Nevertheless, the resulting map of environmental suitability for contemporary conditions corroborates that Atlantic spotted dolphins primarily inhabit coastal environments throughout most of their range (Baumgartner et al. 2001; Moreno et al. 2005; Weir 2010; do Amaral et al. 2015; Barragán-Barrera et al. 2019; Correia et al. 2020). Our results are in agreement with the idea proposed by Barragán-Barrera et al. (2019) that this species is not very cosmopolitan in the Atlantic Ocean, showing restricted movements across the northern and southern portions.

The regression analyses detected a significant relationship between genetic and environmental resistance derived from the ecological niche models performed with a contemporary condition. Although our analyses have not found a relationship between genetics and past environmental conditions, is interesting observe that much of the continental shelf was exposed when the sea level dropped 120 m in the Atlantic Ocean, and many areas that are currently occupied by Atlantic spotted dolphins were unavailable during the LGM (Supplementary Figure S7). Haplotypes from the Oceanic population are spread out across all populations (Figure 2), and a high genetic diversity and signal of population expansion was recovered in Azores, Madeira, and Canary Archipelagos, as well as Mid-Atlantic Bight individuals (Table 1). Therefore, we hypothesize that dolphins from this Oceanic population were able to recolonize different areas of the continental shelf that have similar environmental features in the WNA and WSA after events of sea transgression in the last 21 000 years, as has been suggested by others considering more local studies (e.g., genetic differences in the Gulf of Mexico; see Viricel and Rosel 2014). The influence of sea level changes in shaping genetic structure is an ad hoc explanation in other delphinid species as well (Amaral et al. 2012b; Barragán-Barrera et al. 2017; do Amaral et al. 2018).

The different methods used here both at the individual level (e.g., Procrustes and dbRDA analyses) and at the population level (simple linear regression and Mantel tests) detected a multivariate species–environment relationship. All tests suggested a significant pattern not only of IBD, but also of IBE and IBR. The influence of geography seems to be stronger at larger spatial scales, explaining the highest differentiation of the Br_Uy population, whereas, environment (primarily BAT and Mean SST) seems to be important to segregate individuals that are geographically closer to each other in the WNA, which is consistent with suggestions that different genetic clusters are associated with distinct habitats in terms of depth and SST (Viricel and Rosel 2014).

These scale-dependent factors could potentially explain some differences observed across studies considering other species. For example, a pattern of IBD was observed at large spatial scales for widespread species of *Delphinus*, indicating that the stronger genetic differentiation observed in short-beaked common dolphins from different oceans may actually represent an effect of geographic distance. Conversely, when a smaller geographic scale was considered (i.e., within each ocean basin), genetic differentiation was explained by oceanographic variables (Amaral et al. 2012a). Furthermore, SST was the strongest predictor associated with population divergence of short-beaked common dolphins in the Atlantic Ocean basin (Amaral et al. 2012a). The analyses conducted by Mendez et al. (2011) for humpback dolphins along the western Indian Ocean did not show patterns of IBD or IBE. However, Mendez et al. (2011) observed an overlap between genetic and environmental breaks, suggesting that these environmental breaks could have some influence on the genetic structure of humpback dolphin populations in a non-linear or proportional manner that was not recovered by the analyses performed that rely more on linear relationships. For the franciscana (*Pontoporia blainvillei*), seascape analyses at their southern range

in the WSA indicated genetic patterns consistent with IBD only for mtDNA data; whereas, the nuclear data showed no evidence of IBD (Mendez et al. 2010). The authors suggested that female philopatry could drive IBD and explain the lack of significance of IBE for mtDNA data. Mendez et al. (2010) suggested that environmental distance, but not geographical distance, could be influencing dispersal patterns in mobile marine organisms with no strong behavioral ties to their natal sites (i.e., males in some cetacean species). Environmental variables such as depth, SST, SSS, productivity, and turbidity are widely recognized factors affecting cetacean species distributions by influencing their prey distributions (Baumgartner et al. 2001; Palacios et al. 2013). These variables had some explanatory power in predicting genetic variation, with depth and SST being the most important environmental predictors for the Atlantic spotted dolphin (Baumgartner et al. 2001; Viricel and Rosel 2014; do Amaral et al. 2015; Barragán-Barrera et al. 2019).

Because cetaceans are highly social species, it is possible that the observed population structure also depends on sociality, group interactions, and/or philopatry (Hoelzel 1998; Mendez et al. 2010). Habitat association, foraging specializations, and kin interactions can also lead to discontinuous relationships between genetic and geographic distance (Möller et al. 2011). With our data, it was not possible to evaluate how social and genetic structure interact (neither was it our aim). However, there is circumstantial evidence to support the hypothesis that social and behavioral features could also play a role in explaining genetic structure among populations. For example, genetic structure documented here corresponds to evidence of strong social structure for this species in different regions, such as in southeastern Brazil (Santos et al. 2018), the Gulf of Mexico (Viricel and Rosel 2014), and The Bahamas (Elliser and Herzing 2012, 2014).

Conclusions

This study supports that the Atlantic spotted dolphin exhibits population structure (based on mtDNA-CR data), and that such structure is more related to geography at a large spatial scale and to the environment at a smaller spatial scale. Genetic diversity varied from low in The Bahamas and south/southeastern Brazil and Uruguay to high in oceanic dolphins from Azores, Madeira, Canary Islands, and Mid-Atlantic Bight. Genetic structure was recovered among 7 groups: 1) Oceanic dolphins (Azores, Canary, Madeira, and Mid-Atlantic Bight), 2) South Atlantic Bight, 3) eastern Gulf of Mexico, 4) western Gulf of Mexico, 5) The Bahamas, 6) Caribbean and northeastern Brazil, and 7) south/southeastern Brazil and Uruguay.

We reinforce the need for future studies to determine the taxonomic status of the populations proposed here using several lines of evidence, particularly nuclear data, to assess potential units relevant for conservation purposes (Huang and Knowles 2016). Furthermore, ecological markers could be used as a complementary tool to determine population structure of spotted dolphins in an ecological time frame from months to years, therefore shorter than genetic markers (Mendéz-Fernandéz et al. 2019). We also strongly recommend an additional directed sampling effort in northern Brazil and the Caribbean waters. The inclusion of individuals from the eastern South Atlantic (Africa), which were never previously analyzed, is also crucial to properly consider the whole range of genetic structure in this species.

The Southern Brazilian Bight (23°S to 28°S) is probably the core habitat of the isolated population of the Atlantic Spotted dolphin in Brazil, and we reinforce that it may be appropriate to consider this population separately for management purposes due to anthropogenic threats faced by the dolphins in this region.

Supplementary Material

Supplementary data are available at *Journal of Heredity* online.

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Author Contributions

D.C.B.B., R.M.G., N.F.C., S.C., P.M.F., M.C.O.S., C.R., R.R., S.S., V.M., M.C., A.C.O.M., V.F.T., and I.B.M. provided samples. K.B.A, N.J.R.F., I.B.M., L.L.K., and A.R.A conceptualized the article and designed the experiments. K.B.A. performed the experiment and analysis. K.B.A, N.J.R.F., I.B.M., L.L.K., and A.R.A wrote the article. D.C.B.B., R.M.G., P.M.F., S.C., P.M.F., M.C.O.S, S.S., A.C.O.M., and V.F.T. reviewed the early versions of the article. I.B.M. provided the main funding. I.B.M., N.J.R.F., L.L.K., and A.R.A supervised the article. All authors reviewed the final article.

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical Approval

All applicable international, national, and/or institutional guidelines for the care and use of animals and sampling were followed in the current study. All samples were collected under permission of the corresponding local authorities of each coauthor. In Brazil, all methods and research conducted in this study were carried out under the guidelines stipulated by Instituto Chico Mendez de Conservação da Biodiversidade (ICMBio) and Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA) under the following permits: SISBIO n° 23521-1 (holder I.B.M.), SISBIO n° 70710–1 (holder R. A. H. D.), SISBIO n° 37206-3 (holder M.C.O.S.), SISBIO n° 14104 (holder AQUASIS). In Colombia, samples were collected under Resolution 1177 of Marco Permit for Specimen Collection of Wildlife Biodiversity Non Commercial Purposes of Scientific Research. This permit was provided by the National Authority for Environmental Licenses (ANLA) to Universidad de los Andes. In Canarias Archipelago, samples were collected under permit n°1/55803 (holder V.M.). In Guadeloupe Island, samples were collected under permit n° 2010–235 (holder Association Evasion Tropicale). In Uruguay, samples were collected under Resolución Dinara 2211/05 to V.F.T. The activity of access to the Genetic Heritage is registered under the Sistema Nacional de Gestão do Patrimônio Genético d do Conhecimento Tradicional Associado (SisGen) number AB0E956.

Data Availability

All relevant data are within the paper and its Supporting Information file. New sequences were deposited in GenBank (Accession Numbers: MN339198-MN339279). Data available from the Dryad Digital Repository: (doi:10.5061/dryad.cvdncjt50).

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