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Initial description of the phylogeography, population structure and genetic diversity of Atlantic spotted dolphins from Brazil and the Caribbean, inferred from analyses of mitochondrial and nuclear DNA

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ABSTRACT

We provide initial information regarding the population structure and genetic diversity of *Stenella frontalis* from the Caribbean and southeastern Brazil from analyses of mitochondrial control region sequences and sequences from the first intron of the α-lactalbumin gene. Comparisons with previously described *S. frontalis* sequences showed a high number of haplotypes shared between populations throughout their distribution range. High diversity was found for southeastern Brazil and Caribbean samples, and population structure analyses indicate significant differentiation among population units at the F_{ST} level, but not at the Φ_{ST} level. Significant differentiation at the F_{ST} level was found between the Caribbean population unit and all other populations units. These results suggest historical or present connectivity between the Azores and Madeira and the southeastern Brazil groups and population differentiation between the Caribbean and southeastern Brazil, supporting the notion of two separate stocks in the waters around the Atlantic coast of South America.

1. Introduction

Atlantic spotted dolphins (*Stenella frontalis*) are endemic to the tropical and warm temperate waters of the Atlantic Ocean, Gulf of Mexico and the Caribbean (Moreno et al., 2005; Perrin, 2002; Perrin et al., 1987). The Atlantic spotted dolphins display geographic variation in terms of body size and degree of spotting (Perrin, 2002). Dolphins from areas along the East Coast of United States and West Africa seem to be heavily spotted, while animals from offshore areas seem to be smaller in size and

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have almost no spotting (Perrin, 2002). Groups seem to be composed of twenty or more individuals, as reported from long-term studies in the Bahamas (Herzing, 1997).

It is unclear how many populations or stocks of Atlantic spotted dolphins exist throughout their distribution range. A recent study by Adams and Rosel (2006), analysing mitochondrial control region sequences and microsatellite genotyping, suggested high structure among the populations off the eastern U.S.A (western North Atlantic) and the Gulf of Mexico. These authors also suggested the presence of particular *S. frontalis* morphotypes in coastal and offshore areas. Another study by Quérouil et al. (2010) found no population differentiation among dolphins from Madeira and Azores. Dolphins in this region appear to be transient between the two archipelagos and also between these archipelagos and open waters. Spotted dolphins are more frequently found in the waters around Azores and Madeira between April and October (Quérouil et al., 2008). In the South Atlantic, very little is known regarding the distribution of Atlantic spotted dolphins (Moreno et al., 2005). They appear to have a discontinuous distribution along the eastern coast of South America, where sightings occur for both near-shore and offshore waters, with depths between 20 and 200 m, with fewer observations in regions with water depths of 1000 m or more (Moreno et al., 2005; Zerbini et al., 2004). It has been suggested that the population in southeastern Brazil (21–33°S) is reproductively isolated from other populations in northeastern Brazil and the Caribbean (north of 6°N) (Moreno et al., 2005). Atlantic spotted dolphin strandings are more common in southeastern Brazil during the warmer months, when the area is under the influence of the warm sea current off Brazil (Moreno et al., 2005). In the Caribbean, Atlantic spotted dolphins have been sighted in waters off the Bahamas (Herzing, 1997; Jefferson and Lynn, 1994), Colombia (Caballero, pers. obs.), Costa Rica (Caballero, pers. obs.), and Puerto Rico and the Virgin Islands (Mignucci-Gianonni et al., 1998). Some behavioural studies have shown inter-specific interactions with common bottlenose dolphins *Tursiops truncatus* in the Bahamas (Herzing, 1996, 1997; Herzing and Johnson, 1997). To date, no studies have investigated the genetic population structure of Atlantic spotted dolphins in the Caribbean or along the coast of Brazil.

The aim of this study is to provide an initial description of the phylogeographic and population structure patterns of Atlantic spotted dolphins in southeastern Brazil and the Caribbean, and to try to define their relationship to other populations found in the Azores, Madeira, Gulf of Mexico and the Western North Atlantic. These analyses consist of examination of a fragment of the non-coding control region (CR) of the mitochondrial DNA and a section of a nuclear gene, the first intron of the α-lactalbumin gene, from a small set of samples from the Caribbean ($n = 6$) and from southeastern Brazil ($n = 9$).

2. Materials and methods

2.1. Sample collection

Skin samples were obtained from stranded or entangled dead dolphins (Table 1). Samples were either preserved in 20% dimethyl sulfoxide (DMSO), saturated with sodium chloride, or in 70% ethanol. Samples were obtained from dolphins collected from a total of five Brazilian and Caribbean geographic locations including southeastern Brazil ($n = 9$), Colombia ($n = 1$), Dominica ($n = 1$), Puerto Rico ($n = 3$) and the U.S Virgin Islands ($n = 1$) (Fig. 1). Previously published and available sequences were used for additional phylogeographic comparisons and to find haplotypes shared between the Brazilian and Caribbean samples and other populations in the Eastern Atlantic (Azores and Madeira, Quérouil et al., 2010), northwestern Atlantic (East Coast of the U.S.A), Gulf of México and Bahamas (Adams and Rosel, 2006).

2.2. DNA extraction, mtDNA control region and α-lactalbumin amplification and sequencing

DNA extraction from Caribbean skin samples followed the protocol by Sambrook et al. (1989) modified for small samples by Baker et al. (1994). DNA extractions from Brazilian samples were carried out using a salt buffer protocol and precipitation

Table 1
Sample field codes, sampling date and location, sex, probable age and total length of each dolphin sampled included in this study.

Field code	Date	Location of capture or stranding	Age	Sex	TL (cm)
PA-164[a]	12-Apr-05	Southeastern Brazil 25°59′S/48°16′W	9	F	197
PA165	12-Apr-05	Southeastern Brazil 25°59′S/48°16′W	7	F	184
PA198	18-Dec-05	Southeastern Brazil 24°56′S/47°36′W	0	F	147
PA199	18-Dec-05	Southeastern Brazil 24°56′S/47°36′W	0	M	150
PA205	16-Feb-06	Southeastern Brazil 25°56′S/47°32′W	1	M	158
PA209	03-Apr-06	Southeastern Brazil 26°06′S/47°39′W	2	F	147
PA249	16-Mar-07	Southeastern Brazil 25°16′S/47°46′W	3	F	153
PA254	Mar/Apr 07	Southeastern Brazil NA	–	M	192
PA264	25-Mar-07	Southeastern Brazil 25°08′S/47°47′W	7	M	175
NEPST366	22-Oct-94	Caribbean/US Virgin islands 18°21′N/64°47′W	>18	M	176
NEPST877	8-Jan-04	Caribbean/Puerto Rico 18°23′N/65°43′W	–	F	182
GU01022801	28-Feb-01	Caribbean/Puerto Rico 17°33′N/66°33′W	–	–	–
GU01030102	1-Mar-01	Caribbean/Puerto Rico 17°20′N/67°15′W	–	–	–
Sten 20010612	NA	Caribbean/Dominica 15°25′N/61°20′W	–	–	–
SfronCCIR0103	NA	Caribbean/Colombia 10°10′N/75°46′W	–	–	–

[a] Lactating female.

Fig. 1. Map showing locations where samples sequenced in this study were obtained from, as well as the locations of origin of *Stenella frontalis* mtDNA control region sequences available on Genbank.

with isopropanol (Aljanabi and Martinez, 1997). A portion of about 650 bp of the mitochondrial control region (CR) was amplified using the primers t-Pro-whale M13Dlp1.5 (5′-TGTAAAACGACAGCCAGTTCACCCAAAGCTGRARTTCTA-3′) and Dlp8 (5′- CCATCGWGATGTCTTATTTAAGRGGAA-3′) following the amplification conditions of Baker et al. (1998). A portion of about 600 bp of the first intron of the α-lactalbumin gene was amplified using the primers Lac1R (5′-CTCACTCTCACAGGACATGT-3′) and Lac2F (5′-CCAAAATGATGTCCTTTGTC-3′, Milinkovitch et al., 1998), following the amplification conditions of Caballero et al. (2008). Mitochondrial DNA (MtDNA) control region PCR products were cleaned using polyethyleneglycol. α-Lactalbumin PCR products were separated in a 1.6% agarose gel and bands were cut out and cleaned using the Wizard SV Gel and PCR clean-up system (Promega). All cleaned PCR products were sequenced using the standard protocols of BigDye™ on an ABI 3100 Perkin–Elmer automated capillary sequencer.

2.3. Data analyses

2.3.1. MtDNA control region sequence analyses

All sequences were manually edited and aligned using the Geneious v3.6.1 software (Biommatters 2007). Haplotypes were defined using MacClade (Maddison and Maddison, 2000). For phylogeographic comparisons a 368 bp consensus sequence was compiled, analysed and compared with 175 sequences available from Genbank, in order to detect haplotypes shared among populations from around the world. Here, sequences from the Azores, Madeira, northwestern Atlantic, Bahamas, Gulf of Mexico, Caribbean and southeastern Brazil were included. A haplotype network was constructed using the statistical parsimony methodology as implemented in the software TCS Vs. 1.21 (Clement et al., 2000). This method estimates an unrooted tree, providing a 95% plausible set for all sequence type linkages within the tree and considering gaps as a fifth character state.

Population structure analyses well as haplotype and nucleotide diversity calculations were performed in the program Arlequin (Excoffier et al., 1992) and restricted to 368 bp of the control region. Genetic differences among population units established *a priori* (Caribbean, southeastern Brazil, northwestern Atlantic and Gulf of Mexico, Azores and Madeira) were

quantified by an analysis of molecular variance (AMOVA) as implemented in Arlequin (Excoffier et al., 1992) based on conventional F_{ST} and Φ_{ST} statistics, using 10,000 random permutations.

2.3.2. Alpha-lactalbumin sequence analyses

A 600 bp sequence from the first α-lactalbumin nuclear intron was obtained from two independent PCR products for further confirmation of variable sites, as cloning was not possible at this time. Sequence quality was evaluated using the program Phred v.020425 (Ewing and Green, 1998; Ewing et al., 1998). Sequences with Phred score values of 20 (a base call having a probability of more than 1/100 of being incorrectly called) were re-sequenced. Sequences with Phred score values between 20 and 40 (a probability between 1/100 and 1/10,000 of being incorrectly called) were checked by eye. All forward and reverse sequences were edited manually and aligned using Geneious (Biommatters 2007). Electropherograms of nuclear introns were reviewed with Geneious to detect secondary peaks indicative of heterozygotes (Hare and Palumbi, 1999). Heterozygotes were considered authentic if a decline in Phred score values at a specific site was observed, accompanied by a secondary peak with a height 30% of the height of the primary peak (Lento et al., 2003). Alpha-Lactalbumin sequence alleles were submitted to Genbank under accession numbers KC204741–KC204748.

3. Results

3.1. MtDNA control region haplotype determination, population structure and genetic diversity

A total of six control region (CR) sequences were successfully obtained from Caribbean samples, and nine sequences were successfully obtained from Brazilian samples. A total 368 bp of the CR were analysed. On the basis of wider phylogeographic comparisons using 175 sequences previously published for the Azores and Madeira, northwestern Atlantic (East Coast of the U.S.A), Gulf of México and Bahamas, 64 haplotypes were defined by 65 variable sites. Thirty-three haplotypes were shared by more than one individual (Fig. 2). Two samples from the Caribbean (including one sample from the Bahamas published in Adams and Rosel (2006)) shared haplotypes described for the Azores and Madeira (haplotype S and haplotype A, defined as SFA06 and SFM25 respectively by Quérouil et al. (2010)). Six samples from southeastern Brazil shared haplotypes described for the Azores and Madeira (haplotype D, defined as SFA02, haplotype I, defined as SFM12 and haplotype M, defined as SFM04 by Quérouil et al., 2010). Six haplotypes defined for the northwestern Atlantic were shared with the Azores (haplotypes J, Q, R, Y, Z and A1, previously defined as SF35NWA, SF03NWA, SF01NWA, SF02NWA, SF04NWA and SF20NWA respectively). Five new haplotypes were defined for Caribbean samples (one individual each) and three new haplotypes were defined for Brazilian samples (one individual each; Fig. 2). Three haplotypes were defined as ancestral (D, U, Z and CCIR0103). The first four ancestral haplotypes defined were found in the Azores, Madeira, and the western North Atlantic, and the last one was found in one sample from the Colombian Caribbean (Fig. 2). This last haplotype seems to be highly divergent (see Table 1, Supplementary Material for more information). Newly described CR haplotypes from this study were submitted to Genbank as accession numbers KC204733–KC204740 and presented in Table 1 in the Supplementary Material.

A non-hierarchical AMOVA analysis confirmed significant differences among some of the population units defined *a priori* as Azores ($n = 112$), Madeira ($n = 48$), southeastern Brazil ($n = 9$), and Caribbean ($n = 6$). Samples from the northwestern Atlantic, Gulf of Mexico and Bahamas were not included in this analysis, as data on haplotype frequencies was not available from the publication from Adams and Rosel (2006). The Caribbean population unit comprised samples from Puerto Rico, US Virgin Islands, Dominica and Colombia. Significant differentiation among population units was found at the F_{ST} level, but not at the Φ_{ST} level ($F_{ST} = 0.026$, $P < 0.001$, $\Phi_{ST} = 0.015$, $P = 0.077$). In pair-wise comparisons, significant differentiation at the F_{ST} level was found between Azores and Madeira, and between the Caribbean population unit and all other populations units. At the Φ_{ST} level, significant differentiation was only detected between the southeastern Brazil and the Caribbean population units (Table 2). We found high haplotype and nucleotide diversity in all population units considered in this analysis, with the highest haplotype diversity found in the southeastern Brazil population unit and the lowest haplotype and nucleotide diversity found in the Caribbean population unit (Table 2).

3.2. Alpha-lactalbumin allele determination

Eight southeastern Brazil samples and four Caribbean samples were successfully amplified and sequenced for the first intron of the α-lactalbumin gene. One additional *S. frontalis* α-lactalbumin partial gene sequence was available from Genbank as accession number EU121183.1, previously published by Caballero et al. (2008). Seven polymorphic sites defined a minimum of six alleles (Table 3b), but missing information at some polymorphic sites prevented us from being able to define all possible alleles found in all samples included in this analysis. At least four α-lactalbumin alleles were shared between Caribbean and southeastern Brazil samples (Table 3a).

4. Discussion

Here we present an initial description of the genetic diversity and population structure of spotted dolphin from the Caribbean and southeastern Brazil. We analysed partial sequences from the mitochondrial control region and from one nuclear intron from the α-lactalbumin gene. To further understand the phylogeographic patterns for this species,

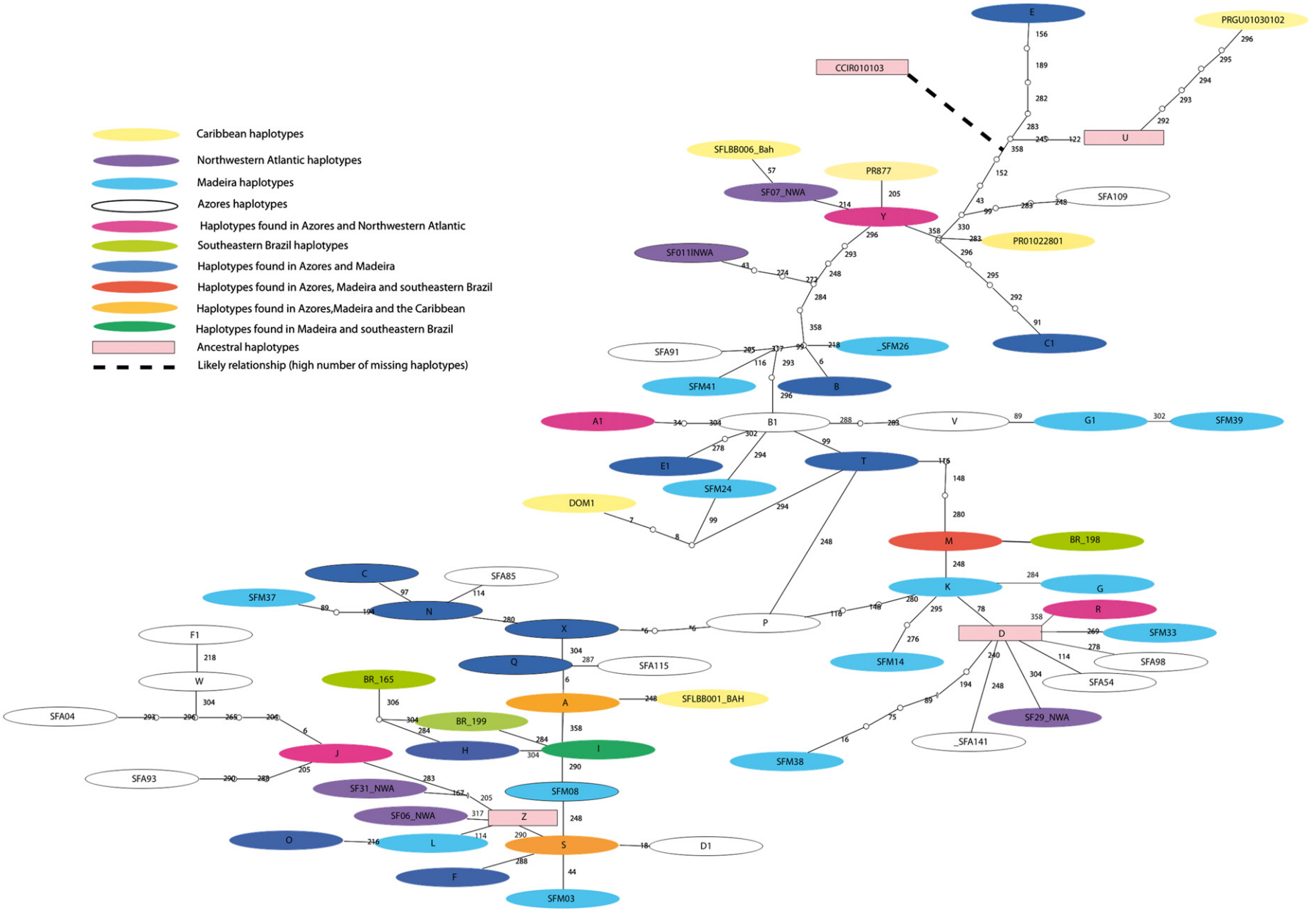

Fig. 2. Parsimony network of *Stenella frontalis* mtDNA control region haplotypes. Small white circles represent missing or extinct haplotypes. Numbers on branches represent site changes.

Table 2
Pairwise F_{ST} (below diagonal) and Φ_{ST} (above diagonal) values for control region among *Stenella frontalis* population units. Probability values based on 10,000 permutations shown in italics. Significantly different values ($P < 0.05$) in bold. Haplotype (h) and nucleotide (π) % ± standard deviation (SD) are shown on the diagonal for each population unit. Sample sizes for each population unit are shown (n).

F_{ST}	Φ_{ST}			
	Azores $n = 112$	Madeira $n = 48$	Southeastern Brazil $n = 9$	Caribbean $n = 6$
Azores	$h = 0.96 \pm 0.007$ $\pi = 0.022 \pm 0.011$	*0.045 (0.780)*	*0.177 (0.224)*	0.462 *(0.950)*
Madeira	*0.013* ***(0.0026)***	$h = 0.97 \pm 0.015$ $\pi = 0.019 \pm 0.010$	*0.510 (0.0951)*	0.159 *(0.977)*
South/southeastern Brazil	*0.063 (0.280)*	*0.0381 (0.373)*	$h = 1 \pm 0.052$ $\pi = 0.027 \pm 0.015$	*0.930* ***(0.010)***
Caribbean	0.089 ***(0.0004)***	0.082 ***(0.0006)***	0.097 ***(0.038)***	$h = 0.80 \pm 0.110$ $\pi = 0.009 \pm 0.006$

comparisons were made with previously published information on populations from the Azores and Madeira and from the northwestern Atlantic, Gulf of Mexico and the Bahamas.

4.1. Genetic diversity estimates

We found high levels of genetic diversity (nucleotide and haplotype diversity) among the Caribbean and southeastern Brazil samples analysed, similar to the values obtained from the Azores and Madeira population units. The haplotype and nucleotide diversity for the Caribbean samples were lower than for the other population units analysed, but this may be due to the low number of samples analysed from this area compared to other population units, particularly from the Azores. It was interesting to detect a higher degree of genetic diversity in the southeastern Brazil population unit, similar to that found for the Azores and Madeira population units, given the small number of samples analysed (9). High genetic diversity was also found at the nuclear level, when analysing 600 bp of the first intron of the nuclear α-lactalbumin gene for Caribbean and southeastern Brazil samples.

4.2. Population structure and connectivity among population units

A relatively high number of haplotypes was shared between the different population units considered in this study. There was a high number of shared haplotypes between the Azores, Madeira and southeastern Brazil population units and less haplotype sharing between the Caribbean, western North Atlantic and Gulf of Mexico with the Azores and Madeira groups.

Table 3
a. Seven polymorphic sites defining a minimum of six α-lactalbumin (first intron, 590 bp) alleles among 13 *Stenella frontalis* samples from southeastern Brazil and the Caribbean. (?) indicates missing data. (BR) refers to southeastern Brazil samples, (PR) refers to samples from Puerto Rico and (CC) refers to samples from the Colombian Caribbean. b. Inferred α-lactalbumin (first intron, 590 bp) alleles detected among 13 *S. frontalis* samples.

a

Sample ID	Polymorphic sites (from a total of 590 bp)						
	4	6	3	3	4	5	5
	1	3	4	4	3	6	8
			5	8	5	0	8
NEPST366 (PR)	C/T	C	C/T	G	C	G	A
PA249, PA254, PA165 (BR)	?	?	C/T	G	C	?	?
PA205, PA264 (BR)	C	C	C/T	G	C	G	A
GU01022801 (PR)	?	?	T	G	C	G	A
CCIR0103 (CC)	?	T	C	G/A	C	G	A
PA199 (BR)	?	?	T	G	C	G	T
PA164 (BR), NEPST877 (PR)	C	C	T	A	T	G	A
PA198 (BR)	?	?	T	A	T	G	A
GU0130102 (PR)	?	?	T	A	T	C	A

b

Allele ID	Polymorphic sites (from a total of 590 bp)
α-lactalbumin (first intron) allele 1	CCTGCGA
α-lactalbumin (first intron) allele 2	CCCGCGA
α-lactalbumin (first intron) allele 3	CCTATGA
α-lactalbumin (first intron) allele 4	TCTATGA
α-lactalbumin (first intron) allele 5	CCCATGA
α-lactalbumin (first intron) allele 6	TCCATGA

In the AMOVA analyses, no significant population structuring was found between Azores and Madeira population units and the southeastern Brazil population unit, while significant population structuring was found between the Caribbean and all other population units considered in this analysis. Due to the small sample size of the Caribbean population unit, caution is advised when interpreting the significant differentiation detected between this population unit and all others included. Small sample sizes may bias F_{ST} estimates particularly if, by chance, highly differentiated haplotypes were included in the analyses (Kalinowski, 2005). In general, it has been recommended to use at least 20 samples to have more confident F_{ST} estimates (Kalinowski, 2005), particularly for species that may have high levels of gene flow, as may be the case for widely distributed marine species (Waples, 1998). We also found significant differentiation between the Madeira and Azores population units at the F_{ST} in this analysis, which is different from the results of Quérouil et al. (2010). This result may be an artefact due to differences in the number of CR sequences from Azores and Madeira included in their study (144 from Azores and 48 from Madeira) and the present study (112 from Azores and 48 from Madeira).

At the nuclear level, a high number of α-lactalbumin first intron alleles were shared between Caribbean and southeastern Brazil samples. Assuming that these population units are, indeed, genetically isolated, this finding might be interpreted as the result of not enough time for nuclear DNA to diverge between *S. frontalis* population units due to lower mutation rates in nuclear DNA when compared to mitochondrial DNA (Caballero et al., 2008).

The results from the AMOVA analysis of CR sequences provided some interesting initial information regarding connectivity between the population units analysed in this study. Based on our results and bearing in mind the small number of samples available to date, we propose that the Caribbean and the southeastern Brazil *S. frontalis* belong to separate stocks based on significant differentiation found at the F_{ST} and Φ_{ST} levels. This conclusion is based on the fact that haplotypes found to date in the Caribbean and southeastern Brazil population units differ not only in their frequency but also on their nucleotide composition. This finding provides further support to the notion of a discontinuous distribution of this species and the presence of more than one stock or population along the Atlantic Coast of South America, as suggested by previous morphometric analyses from skulls belonging to dolphins from the Caribbean and from southeastern Brazil (Moreno, 2002; Moreno et al., 2005). We recommend to investigate this possible population differentiation further using increased sample sizes as well as additional molecular markers. If this stock differentiation along the Atlantic Coast of South America is confirmed, it would imply that management and conservation programs would need to manage these stocks as separate units. The lack of differentiation found between the population units from Azores and Madeira and the southeastern Brazil population unit is puzzling but may reflect historic or present migratory connectivity between these regions in the Atlantic Ocean, and, in practical terms, means that with the data available to date, Atlantic spotted dolphins in the Central Atlantic (i.e. Azores–Madeira–southeastern Brazil) should be managed as one stock. Previous studies have shown that *S. frontalis* in the Azores and Madeira are transient and are found around these islands from April to October (Quérouil et al., 2008, 2010). Moreno et al. (2005) reported that most strandings of this species in the Coast of southeastern Brazil occur from October to January and that the warm current off Brazil may influence this distribution. Also, it has been reported that surface sightings for this species in southeastern Brazil may occur in offshore waters with depths of up to 1000 m (Moreno et al., 2005). Taking this information into consideration and the apparently anti-phasic seasonal distribution of Atlantic spotted dolphins in the Azores and Madeira vs. southeastern Brazil, one could hypothesize that there is migration of offshore groups between these areas that could be contributing to increased gene flow between this population units and/or coastal spotted dolphin populations. Another possibility is that the different population units from the Azores and Madeira and southeastern Brazil represent presently isolated communities, but that share a common evolutionary history mediated by male and female gene flow, as has been shown previously by Oremus et al. (2007) for spinner dolphins (*Stenella longirostris*) around islands in French Polynesia. Testing these two hypotheses warrants further investigation with increased sample sizes and additional molecular markers (e.g. microsatellites), in order to clarify the degree of present or historical connectivity between what is believed to be different Atlantic spotted dolphins stocks.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bse.2012.12.016.

References

Adams, L.D., Rosel, P.E., 2006. Population differentiation of the Atlantic spotted dolphin (*Stenella frontalis*) in the western North Atlantic, including the Gulf of Mexico. Marine Biology 148, 671–681.

Aljanabi, S.M., Martinez, I., 1997. Universal and rapid SALT-extraction of high quality genomic DNA for PCR-based techniques. Nucleic Acids Research 25, 4694–5693.

Baker, C.S., Flórez-González, L., Abernethy, R.B., Rosenbaum, H.C., Slade, R.W., Capella, J., Bannister, J.L., 1998. Mitochondrial DNA variation and maternal gene flow among humpback whales of the Southern Hemisphere. Marine Mammal Science 14, 721–737.

Baker, C.S., Slade, R.W., Bannister, J.L., Abernethy, R.B., Weinrich, W.T., Lien, J., Urban, J., Corkeron, P., Calambokidis, J., Vasquez, O., Palumbi, S.R., 1994. Hierarchical structure of mitochondrial DNA gene flow among humpback whales *Megaptera novaeangliae*, world-wide. Molecular Ecology 3, 313–327.

Caballero, S., Jackson, J., Mignucci-Giannoni, A.A., Barrios-Garrido, H., Beltrán-Pedreros, S., Motniel-Villalobos, M.G., Robertson, K.M., Baker, C.S., 2008. Molecular systematics of South American dolphins *Sotalia*: sister taxa determination and phylogenetic relationships, with insights into a multi-locus phylogeny of the Delphinidae. Molecular Phylogenetics and Evolution 46, 252–268.

Clement, M., Posada, D., Crandall, K.A., 2000. TCS: a computer program to estimate gene genealogies. Molecular Ecology 9, 1657–1659.

Ewing, B., Green, P., 1998. Base-calling of automated sequencer traces using *Phred*. II. Error probabilities. Genome Research 8, 186–194.

Ewing, B., Hillier, L., Wendl, M.C., Green, P., 1998. Base-calling of automated sequencer traces using *Phred*. I. Accuracy assessment. Genome Research 8, 175–185.

Excoffier, L., Smouse, P.E., Quattro, J.M., 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131, 479–491.

Hare, M.P., Palumbi, S.R., 1999. The accuracy of heterozygous base calling from diploid sequence and resolution of haplotypes using allele-specific primers. Molecular Ecology 8, 1750–1752.

Herzing, D.L., 1996. Vocalizations and associated underwater behavior of free-ranging Atlantic spotted dolphins, *Stenella frontalis* and bottlenose dolphins, *Tursiops truncatus*. Aquatic Mammals 22, 61–79.

Herzing, D.L., 1997. The life history of free-ranging Atlantic spotted dolphins (*Stenella frontalis*): age classes, color phases, and female reproduction. Marine Mammal Science 13, 576–595.

Herzing, D.L., Johnson, C.M., 1997. Interspecific interactions between Atlantic spotted dolphins (*Stenella frontalis*) and bottlenose dolphins (*Tursiops truncatus*) in the Bahamas, 1985–1995. Aquatic Mammals 23, 85–99.

Jefferson, T.A., Lynn, S.K., 1994. Marine mammal sightings in the Caribbean Sea and the Gulf of Mexico, summer 1991. Caribbean Journal of Science 30, 83–89.

Kalinowski, S.T., 2005. Do polymorphic loci require large sample sizes to estimate genetic distances? Heredity 94, 33–36.

Lento, G.M., Baker, C.S., David, V., Yuhki, N., Gales, N.J., O'Brien, S.J., 2003. Automated single-strand conformation polymorphism reveals low diversity of a Major Histocompatibility Complex Class II gene in the threatened New Zealand sea lion. Molecular Ecology Notes 3, 346–349.

Maddison, D.R., Maddison, W.P., 2000. MacClade: Analysis of Phylogeny and Character Evolution. Sinauer, Sunderland, MA, USA.

Mignucci-Gianonni, A.A., Hoberg, E.P., Siegel-Causey, D., Williams, E.H., 1998. Metazoan parasites and other symbionts of cetaceans in the Caribbean. Journal of Parasitology 84, 939–946.

Milinkovitch, M.C., Bérubé, M., Palsboll, P.J., 1998. Cetaceans are highly specialized artiodactyls. In: Thewissen, J.G.M. (Ed.), The Emergence of Whales: Evolutionary Patterns in the Origin of Cetacea. Plenum, New York, NY, pp. 113–131.

Moreno, I.B., 2002. Padrão de distribuição dos golfinhos do gênero Stenella (Delphinidae: Cetacea) no oceano Atlântico sul-ocidental e morfometria craniana dos Golfinhos-Pintados (Stenella frontalis e S. attenuata). MSc thesis. Biologia. Pontifícia Universidade Católica do Rio Grande do Sul, Rio Grande do Sul, Brazil.

Moreno, I.B., Zerbini, A.N., Danilewicz, D., Santos, M.C.O., Simoes-Lopes, P.C., Lailson-Brito, J., Azevedo, A.F., 2005. Distribution and habitat characteristics of dolphins of the genus *Stenella* (Cetacea: Delphinidae) in the southwest Atlantic Ocean. Marine Ecology Progress Series 300, 229–240.

Oremus, M., Poole, M.M., Steel, D., Baker, C.S., 2007. Isolation and interchange among insular spinner dolphin communities in the South Pacific revealed by individual identification and genetic diversity. Marine Ecology Progress Series 336, 275–289.

Perrin, W.F., 2002. Stenella frontalis. Mammalian Species, 1–6.

Perrin, W.F., Mitchell, E.D., Mead, J.G., Caldwell, D.K., Caldwell, M.C., vanBree, P.J.H., Dawbin, W.H., 1987. Revision of the spotted dolphins, *Stenella* spp. Marine Mammal Science 3, 99–170.

Quérouil, S., Freitas, L., Cascao, I., Alves, F., Dinis, A., Almeida, J.R., Prieto, R., Borras, S., Matos, J.A., Mendonca, D., Santos, R.S., 2010. Molecular insight into the population structure of common and spotted dolphins inhabiting the pelagic waters of the Northeast Atlantic. Marine Biology 157, 2567–2580.

Quérouil, S., Silva, M., Cascao, I., Magalhaes, S., Seabra, M.I., Machete, M.A., Santos, R.S., 2008. Why do dolphins form mixed-species associations in the Azores. Ethology 114, 1183–1194.

Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular Cloning: a Laboratory Manual, second ed. Cold Spring Harbor Laboratory Press, New York, U.S.A.

Waples, R.S., 1998. Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. Journal of Heredity 89, 438–450.

Zerbini, A.N., Secchi, E.R., Bassoi, M., Dalla-Rosa, L., Higa, A., deSouza, L., Moreno, I.B., Möller, L.M., Caon, G., 2004. Distribuição e abundância relativa de cetáceos na Zona Econômica Exclusiva da região sudeste-sul do Brasil. Série Documentos Revizee Score Sul, Instituto Oceanográfico – USP, São Paulo, Brazil.