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Molecular taxonomy and population structure of the rough-toothed dolphin *Steno bredanensis* (Cetartiodactyla: Delphinidae)

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Several delphinid species have shown genetic population structure, both between and within ocean basins. We investigated genetic differentiation in the rough-toothed dolphin, *Steno bredanensis*, using mitochondrial control region sequences from several localities worldwide ($N = 112$). Preliminary analyses indicated high levels of genetic differentiation between the Atlantic and Pacific/Indian Oceans, which were further investigated using complete cytochrome *b* sequences and mitogenomes. Phylogenetic analyses were inconclusive about the existence of cryptic speciation in the genus *Steno*. Notwithstanding this result, analysis of molecular variance and Φ-statistics analyses revealed strong population differentiation not only between the Atlantic and Pacific, but also within the Atlantic, where three populations were detected: Caribbean, southeastern Brazil, and southern Brazil. We propose that these populations be considered management units for conservation purposes. Our results provide the first perspective on the worldwide genetic differentiation of *S. bredanensis*.

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ADDITIONAL KEYWORDS: management units – molecular systematics – southwestern Atlantic – Stenoninae.

INTRODUCTION

The genus *Steno*, described by Gray in 1846, belongs to the order Cetartiodactyla, family Delphinidae, and comprises only one species: the rough-toothed dolphin *Steno bredanensis* (Cuvier in Lesson, 1828). Amongst the delphinids, *S. bredanensis* has greatest genetic affinity with the genus *Sotalia*. Recent molecular data support the existence of the subfamily Stenoninae, which would bring together these two genera (LeDuc, Perrin & Dizon, 1999; Cunha *et al*., 2011).

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The rough-toothed dolphin has a wide distribution range, being found in tropical, subtropical, and warm temperate waters of the Atlantic, Pacific, and Indian Oceans (Miyazaki & Perrin, 1994; West, Mead & White, 2011). Sightings of this species are usually reported in deep waters (Jefferson, 2009). For example, in Hawaii the species shows a preference for waters with depths greater than 1500 m (Gannier & West, 2005; Baird *et al*., 2008). Contrastingly, in the southwestern Atlantic sightings are reported in nearshore, shallow waters, from southern to northeastern Brazil (Ott & Danilewicz, 1996; Flores & Ximenes, 1997; Lodi & Hetzel, 1998; Wedekin *et al*., 2004; Rossi-Santos, Wedekin & Sousa-Lima, 2006; Lodi *et al*., 2012).

Although *S. bredanensis* is classified as 'Least Concern' on the Red List of Threatened Species (IUCN, 2013), in the southwestern Atlantic its coastal habits make the species susceptible to anthropogenic threats such as habitat degradation, chemical and noise pollution, and bycatch (Di Beneditto, Ramos & Lima, 1998; Monteiro-Neto *et al*., 2000; Dorneles *et al*., 2007; Netto & Di Beneditto, 2008; Meirelles *et al*., 2009; Lailson-Brito *et al*., 2012, Lemos *et al*., 2013, Bittencourt *et al*., 2014). These studies suggest that rough-toothed dolphin populations in the southwestern Atlantic may be affected negatively by human impacts upon the coastal zone, but the magnitude of such adverse effects is presently unknown.

The delimitation of genetic populations is a prerequisite to assess demographic parameters and, hence, the threat status of a species (e.g. Allendorf & Luikart, 2007). The population structure of *S. bredanensis* has been studied only in French Polynesia, where genetic differentiation was observed between two islands 170 km apart (Oremus *et al*., 2012). The results of that study suggest site fidelity with little dispersal between populations, which is relevant for their conservation.

This study aimed to describe the population structure of *S. bredanensis*, using mitochondrial control region sequences from individuals collected in four localities in the southwestern Atlantic Ocean and sequences from the Caribbean, Pacific, and Indian Oceans, available from GenBank. As preliminary data analyses indicated large amounts of differentiation between the Atlantic and Pacific, phylogenetic analyses were also performed using cytochrome *b* and mitogenome sequences to test the hypothesis of cryptic or semicryptic speciation in the genus *Steno*. The existence of more than a single species in the genus could have gone undetected, especially considering that the morphological variation of *S. bredanensis* across its range has not been studied yet. Our results provide the first perspective on the worldwide genetic differentiation of rough-toothed dolphins.

MATERIAL AND METHODS

Sampling

Forty-two samples of skin or muscle of *S. bredanensis* were collected from stranded or incidentally caught carcasses as well as through biopsy in four localities in the southwestern Atlantic: Espírito Santo ($N = 4$), Rio de Janeiro ($N = 27$), Santa Catarina ($N = 1$), and Rio Grande do Sul ($N = 10$) (Fig. 1). Samples were stored in 100% ethanol and frozen at –20 °C. For population analyses, 67 control region sequences deposited in GenBank were included, covering most of the species' distribution (southeastern Brazil, $N = 1$, central southern Pacific, $N = 59$; eastern tropical Pacific, $N = 4$; Caribbean, $N = 3$) (Fig. 1, Table 1). For taxonomic analyses, cytochrome *b* sequences from almost all the Delphinidae, published in GenBank were used. The complete mitochondrial genomes of 21 delphinid species (including multiple sequences of some species), available in GenBank, were also used for phylogenetic analyses.

Genetic analysis

Total genomic DNA of all samples was extracted using a DNeasy kit (Qiagen) following the manufacturer's instructions. The sex of biopsied and highly degraded individuals was determined by amplification of the ZFX and ZFY genes through PCR using the protocol of Bérubé & Palsbøll (1996) as adapted by Cunha & Solé-Cava (2007).

A fragment of 550 bp of the mitochondrial control region was amplified by PCR using primers H00034 – TACCAAATGTATGAAACCTCAG (Rosel, Dizon & Heyning, 1994) and Dloop – TCACCCAAAGCTG AARTTCTA (Cunha *et al*., 2005). All PCR reactions were carried out in 25 μL volumes containing 1 unit of GoTaq polymerase (Promega); 0.20 mM deoxynucleotides (dNTP); 2.5 mM $MgCl_2$; 15 μg bovine serum albumin (BSA), and 0.5 μM of each primer. A blank control was included in all PCR experiments.

PCR thermocycling was performed with an initial denaturation step of 3 min at 93 °C, followed by 30 cycles of amplification (1 min at 92 °C, 1 min annealing at 50 °C, and 1 min at 72 °C), and a final extension of 5 min at 72 °C. PCR products were purified using a Illustra GFX PCR DNA and gel band purification kit (GE) and sequenced in both directions in an ABI 3500 automated sequencer (Applied Biosystems). Sequencing reactions were prepared using the specific kit and protocol (BigDye Terminator Sequencing Kit v 3.1 Cycle, Applied Biosystems). Control region haplotype sequences were deposited in GenBank (accession numbers KM260653–7).

Data analyses

Sequences were edited using the program SeqMan 7 (DNAStar – Lasergene Inc.) and aligned manually using

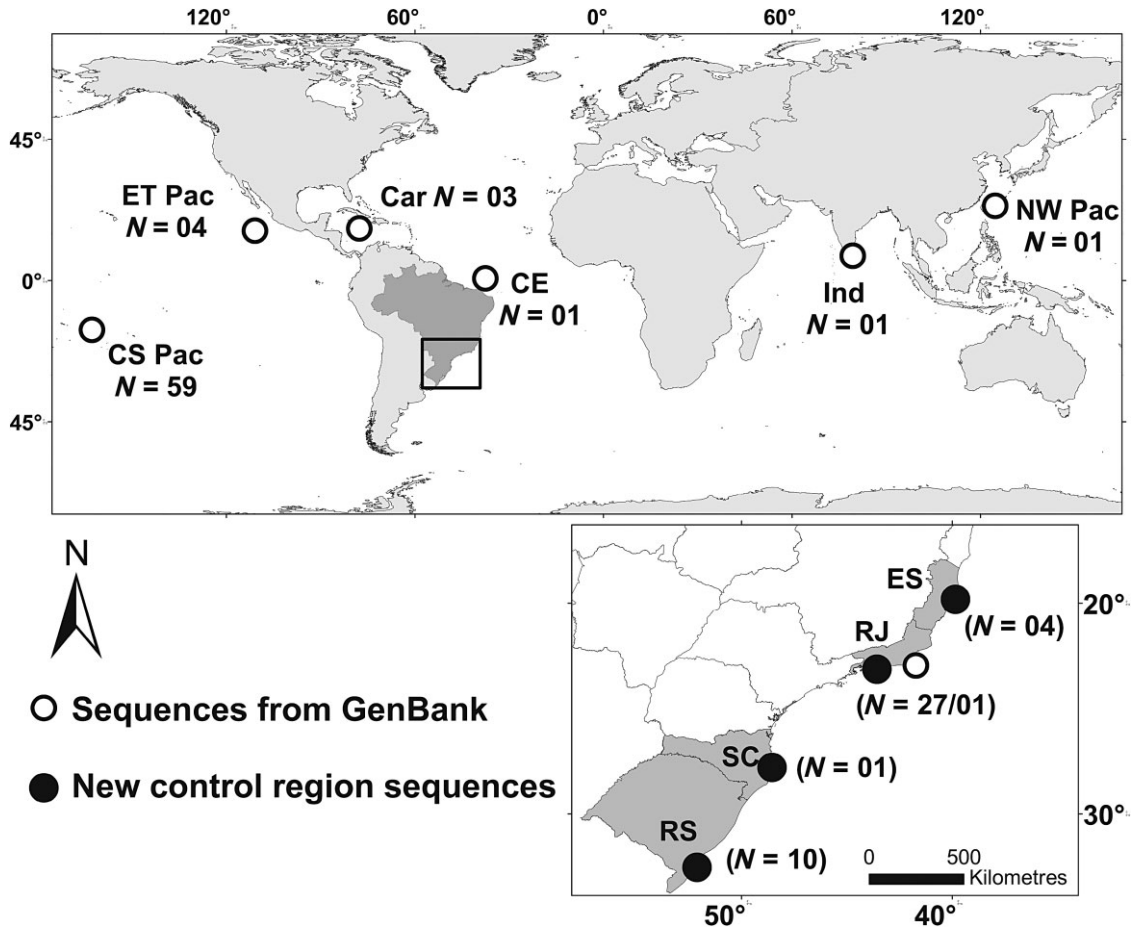

Figure 1. Sampling of *Steno bredanensis* for this study. Black circles, new control region sequences; white circles, sequences available in GenBank. The inset shows sampling localities in the South Western Atlantic (SW Atl). CS Pac, central southern Pacific; ET Pac, eastern tropical Pacific; Car, Caribbean; NW Pac, northwestern Pacific; Ind, Indian Ocean; CE, Ceará State; ES, Espírito Santo State; RJ, Rio de Janeiro State; RS, Rio Grande do Sul State; SC, Santa Catarina State.

Table 1. Control region sequences used in this study, for each analysis

Geographical location	Phylogenetic tree	Haplotype network	Population analyses (AMOVA/Φ_{ST})
SW Atlantic (this study)	42	42	42
SW Atlantic GB	1	1	2
Caribbean GB	3	3	3
ET Pacific GB	4	4	4
CS Pacific GB	59	59	59
NW Pacific GB	1	–	–
Indian Ocean GB	1	–	–

GB, sequences from GenBank.
AMOVA, analysis of molecular variance; Φ_{ST}, Φ-statistics.

MEGA 5 (Tamura *et al.*, 2007). The definition of haplotypes and the estimation of haplotype and nucleotide diversities were carried out in DnaSP 5 (Librado & Rozas, 2009). A median-joining haplotype network of control region sequences was built by the program NETWORK 4.612 (Bandelt, Forster & Röhl, 1999).

For the study of population structure using control region sequences, we used an analysis of molecular variance (AMOVA, Excoffier, Smouse & Quattro, 1992) performed in ARLEQUIN 3.5.1.2 (Excoffier, Laval & Schneider, 2005). This program computes Φ statistics, which are analogous to F statistics (Wright, 1978) but incorporate information about the molecular distance, and separate molecular variance into hierarchical levels, enabling the test of different hypothetical structure scenarios. The significance of the fixation indices was tested by 10 000 permutations of haplotypes,

individuals, or populations between individuals, populations, or groups of populations, respectively. We also used ARLEQUIN to estimate pairwise Φ_{ST} indices and test their significance with 10 000 permutations. A sequential Bonferroni procedure was used to adjust significance for multiple tests (Holm, 1979).

Sequences from GenBank were used for phylogenetic analyses, which followed two approaches: (1) complete cytochrome *b* dataset of almost all delphinid species ($N = 35$); (2) complete mitogenomes of all delphinids available ($N = 21$). MEGA 5 (Tamura *et al*., 2007) was used for building and testing phylogenetic neighbour-joining (NJ) trees, using Kimura two-parameter (K2P) distances and 10 000 bootstrap replicates. Bayesian (B) phylogenetic trees were built by BEAST 1.8.0 (Drummond *et al*., 2012), using a Yule speciation process and the nucleotide substitution model Hasegawa-Kishino-Yano + gamma + invariant sites (HKY + G + I), as selected using jModelTest 2.1.5 (Posada, 2008). Ten million Markov Chain Monte Carlo (MCMC) steps were run, from which 10 000 trees were recorded. The first 1000 trees were regarded as 'burn in' and discarded. After verification that all tree parameters had effective sampling sizes (ESS) > 200, the Maximal Clade Credibility (MCC) search algorithm in TreeAnnotator 1.6.1 (Drummond *et al*., 2012) was used to find the best-supported tree. Trees were visualized using FigTree 1.4 (http://tree.bio.ed.ac.uk/software/figtree/).

RESULTS

Genetic variability

The control region dataset comprised 112 sequences. After alignment, sequences were 423 nucleotides long and contained 28 polymorphic sites. In the total dataset, we observed 19 haplotypes. Overall haplotype diversity (Hd) was 0.839 and nucleotide diversity (π) was 0.019.

The control region haplotype network revealed wide genetic divergence between rough-toothed dolphins of the Atlantic and Pacific/Indian Oceans. Haplotypes from the Atlantic are all closely related, except for H13, observed in a single individual from northeastern Brazil (Ceará State), which grouped with Pacific/Indian haplotypes (Fig. 2). This sequence was available from GenBank and was obtained from a specimen deposited in the Southwest Fisheries Science Center (National Oceanic and Atmospheric Administration/USA; Caballero *et al*., 2008). As confirmation of that sequence was not possible, we decided to exclude it from population analyses.

In the southwestern Atlantic, five of the six control region haplotypes found were new. The exception, H2, had been previously reported in the Caribbean (Albertson *et al*., 2011), and was also observed in a rough-toothed dolphin from Espírito Santo State (ES). The most common haplotype (H16) was more frequent in Rio de Janeiro State (RJ) and was shared by ES and Rio Grande do Sul State (RS). The second most common haplotype, H15, was at its highest frequency in RS and was shared with RJ and Santa Catarina State (SC). Haplotype H17 was found in two individuals of RJ and haplotypes H18 and H19 were each observed in a single individual, from RS and RJ, respectively. Besides haplotype H2, Caribbean samples showed two other haplotypes.

In the Pacific, 11 control region haplotypes were observed: one in the northwestern (Japan), four in the eastern tropical, and five in the central southern Pacific. One of those five haplotypes was shared with the Indian Ocean (H10).

Phylogenetic analyses

A phylogenetic NJ tree built using control region sequences showed a deep divergence between rough-toothed dolphins of the Atlantic and the other regions analysed (Pacific and Indian Oceans) (Fig. 3, p-distance = 0.031, K2P = 0.016).

The phylogenetic NJ and B trees of the complete cytochrome *b* sequences had similar topology and showed modest genetic differentiation between rough-toothed dolphins from the Atlantic and Pacific Oceans (K2P = 0.003, Fig. 4). This divergence is within the range observed for intraspecific comparisons of the Delphinidae using cytochrome *b* (Fig. 5). The NJ and B analyses of mitogenomes revealed a larger genetic divergence between the Atlantic and Pacific/Indian Oceans (K2P = 0.009, Fig. 6). However, considering intra- and interspecific comparisons, this divergence is in the intersection zone where the two distributions overlap (Fig. 7).

Population structure analyses

The single control region sequences from northeastern Brazil (CE) and the Indian and northwestern Pacific Oceans were excluded from population analyses. The sequence from SC, which had the same haplotype as samples from RS, was grouped with those individuals. The population structure hypotheses tested with AMOVA included all possible groupings of the remaining localities.

The AMOVA indicated large differentiation between the Atlantic and Pacific Oceans. The population structure scenario of two populations (Atlantic × Pacific) had a significant Φ_{CT} of 0.769 ($P < 10^{-5}$, Tables 2 and 3). However, two other scenarios had similar significant Φ_{CT} values: three populations, with two in the Atlantic (Pacific/Caribbean/southwestern Atlantic, $\Phi_{CT} = 0.764$, $P < 10^{-5}$); and four populations, with three in the Atlantic (Pacific/Caribbean/ES + RJ/SC + RS, $\Phi_{CT} = 0.747$, $P < 10^{-5}$) (Tables 2 and 3). In addition, the AMOVA rejected the scenarios of panmixia in the Atlantic and also in the Brazilian coast ($\Phi_{ST} = 0.385$ and 0.415,

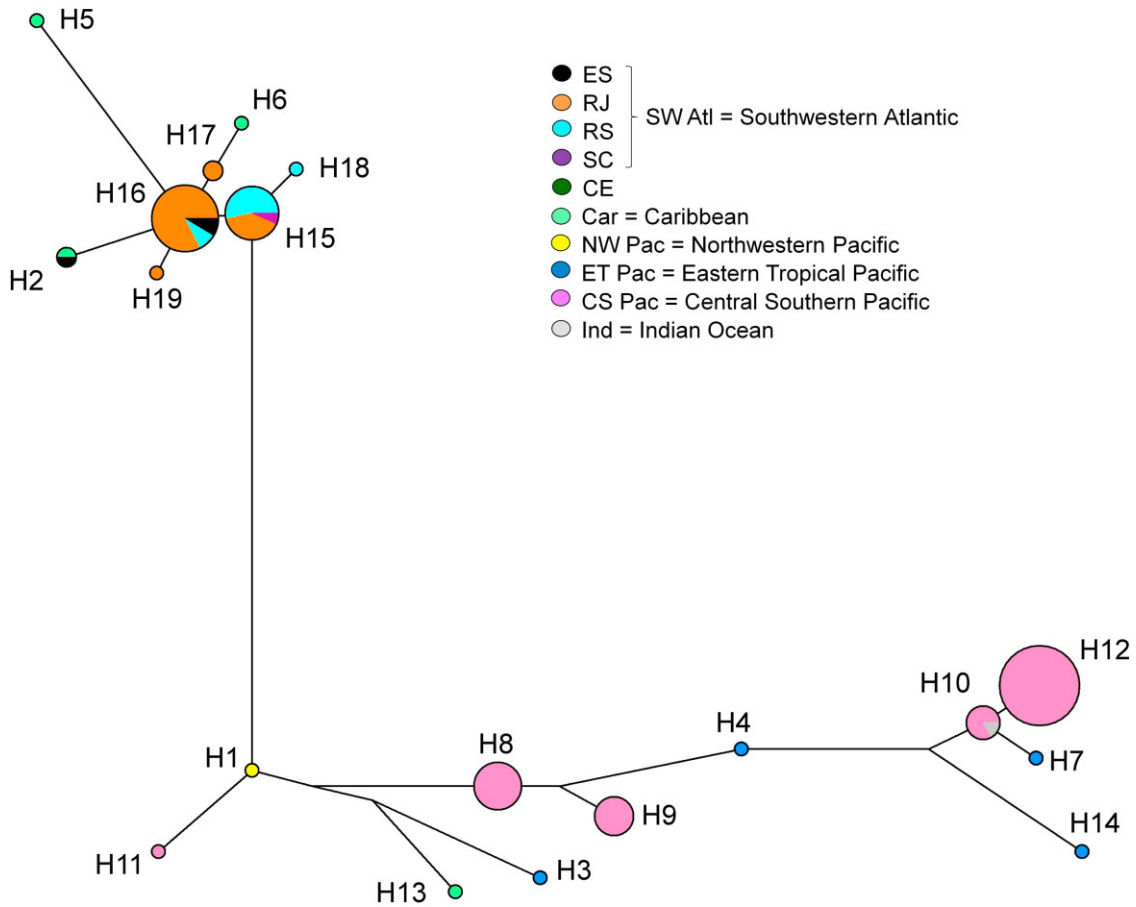

Figure 2. Median-joining network of *Steno bredanensis* mtDNA control region haplotypes ($N = 112$). Circle size is proportional to frequency. Branch length reflects molecular distance. CE, Ceará State; ES, Espírito Santo State; RJ, Rio de Janeiro State; RS, Rio Grande do Sul State; SC, Santa Catarina State.

respectively, both with $P < 10^{-5}$, Table 2). When only samples from the Atlantic and Brazilian coast were considered, the scenario of three populations was significant: Caribbean/RJ + ES (southeastern Brazil)/SC + RS (southern Brazil) ($\Phi_{CT} = 0.386$; $P = 0.02$, Table 2). These two latter results give support to the existence of at least four populations worldwide. Although panmixia in Brazil was rejected, it was not possible to test any scenario for the Brazilian populations owing to insufficient power of the analysis (see Fitzpatrick, 2009).

Most pairwise Φ_{ST} comparisons were significantly different from zero (Table 4). Thus, Φ_{ST} analysis favoured four populations worldwide: three in the Atlantic (Caribbean/RJ + ES/SC + RS) and one in the Pacific (central south Pacific + eastern tropical Pacific).

DISCUSSION

This is the first study on the genetic population structure of rough-toothed dolphins in the Atlantic Ocean. Our preliminary results indicated large amounts of genetic differentiation between the Atlantic and Pacific/Indian Oceans, prompting the need for molecular taxonomic analyses. However, phylogenetic analyses using cytochrome *b* and complete mitogenome data were not conclusive about the existence of more than one *Steno* species. Population analyses, by contrast, showed that rough-toothed dolphins worldwide and in the Atlantic are not panmictic.

PHYLOGENETIC ANALYSES

The mitogenomic phylogenetic trees revealed considerable genetic distance between rough-toothed dolphins from the Atlantic and Pacific, but the observed divergence was within the intersection zone where intra- and interspecific distances overlap. However, only one of the six delphinid species for which more than one mitogenome is available had K2P distances higher than *S. bredanensis* (0.009): *Tursiops aduncus* (0.013). A recent study suggested that *T. aduncus* may comprise

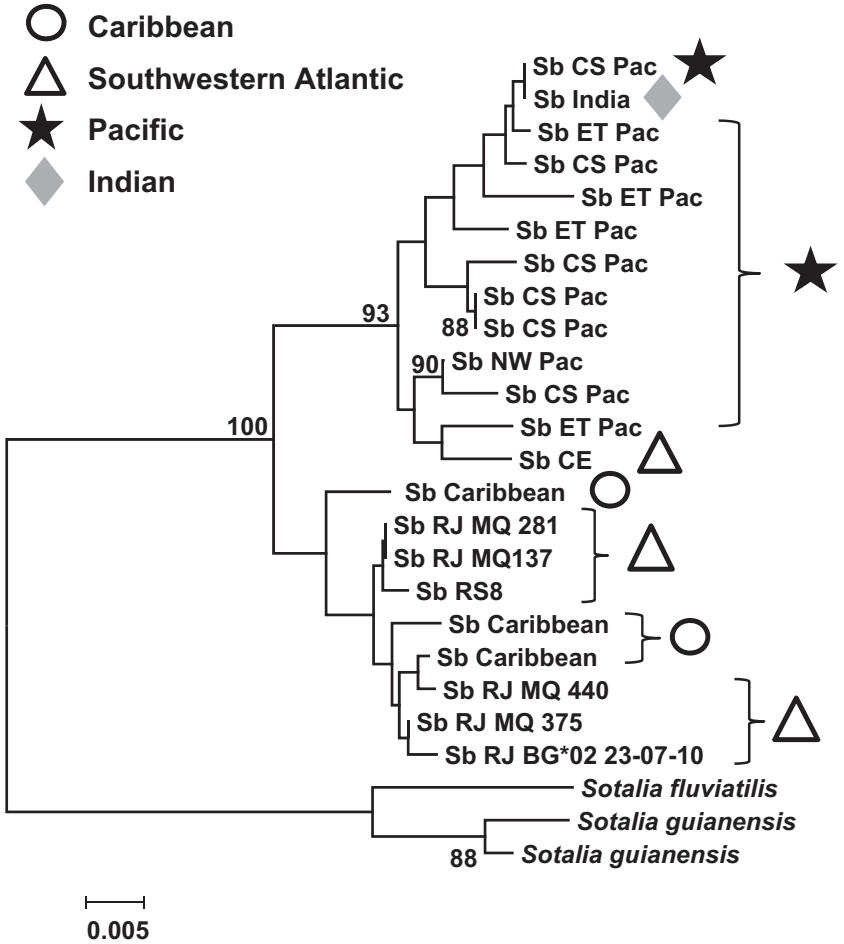

Figure 3. Phylogenetic tree (neighbour-joining, Kimura two-parameter) showing the genetic divergence between sequences of the control region of the Atlantic (ES, Espírito Santo State; RJ, Rio de Janeiro State; SC, Santa Catarina State; RS, Rio Grande do Sul State) and other regions analysed (Pacific and Indian Oceans). Numbers at nodes correspond to bootstrap values >75% (10 000 replicates). CS Pac, central southern Pacific; ET Pac, eastern tropical Pacific; NW Pac, northwestern Pacific; CE, Ceará State; Sb, *Steno bredanensis*. MQ and BG are field codes for samples from RJ. The scale bar shows the length of branch that corresponds to a Kimura two-parameter distance of 0.005.

different species (Moura *et al*., 2013); therefore, the right tail of the intraspecific distribution may be significantly overestimated. At the same time, the left tail of the distribution of interspecific values is probably skewed as a result of values of zero for comparisons between the two *Globicephala* species, which have been shown to be paraphyletic, possibly because of hybridization or recent divergence (Oremus *et al*., 2009). Intraspecific distances for the other three species (*Peponocephala electra*, *Feresa attenuata*, and *Pseudorca crassidens*) were in the range 0.000–0.002.

Cytochrome *b* analysis had a denser taxon sampling, including all recognized delphinid species (except for *Sousa teuszii*). The divergence between the Atlantic and Pacific rough-toothed dolphins was modest, lower than other intraspecific comparisons (e.g. *Orcaella brevirostris*, *Delphinus capensis*, *Lagenorhynchus acutus*, *Delphinus delphis*, *Globicephala macrorhynchus*, *Lagenodelphis hosei*, *Lagenorhynchus obliquidens*, *Lagenorhynchus obscurus*, *Orcinus orca*, *Sousa chinensis*, *Stenella attenuata*, *Stenella longirostris*, *T. aduncus*, and *Tursiops truncatus*). Despite this, it is also worth noting that most of the right tail of the intraspecific distribution refers to comparisons between *T. truncatus* from several localities across the globe, which are believed to represent more than a single species (Moura *et al*., 2013). In any case, as there are many more cytochrome *b* sequences than mitogenomes from each of the delphinid species, the cytochrome *b* histogram depicts better the variability within and between species.

Although mitochondrial markers are reputed to be good markers for mammalian taxonomy, a mitochondrial tree may not always correctly depict the species tree. Mitochondrial markers behave as a single locus and, therefore, may result in trees that are more affected by stochastic lineage sorting. Introgression is another

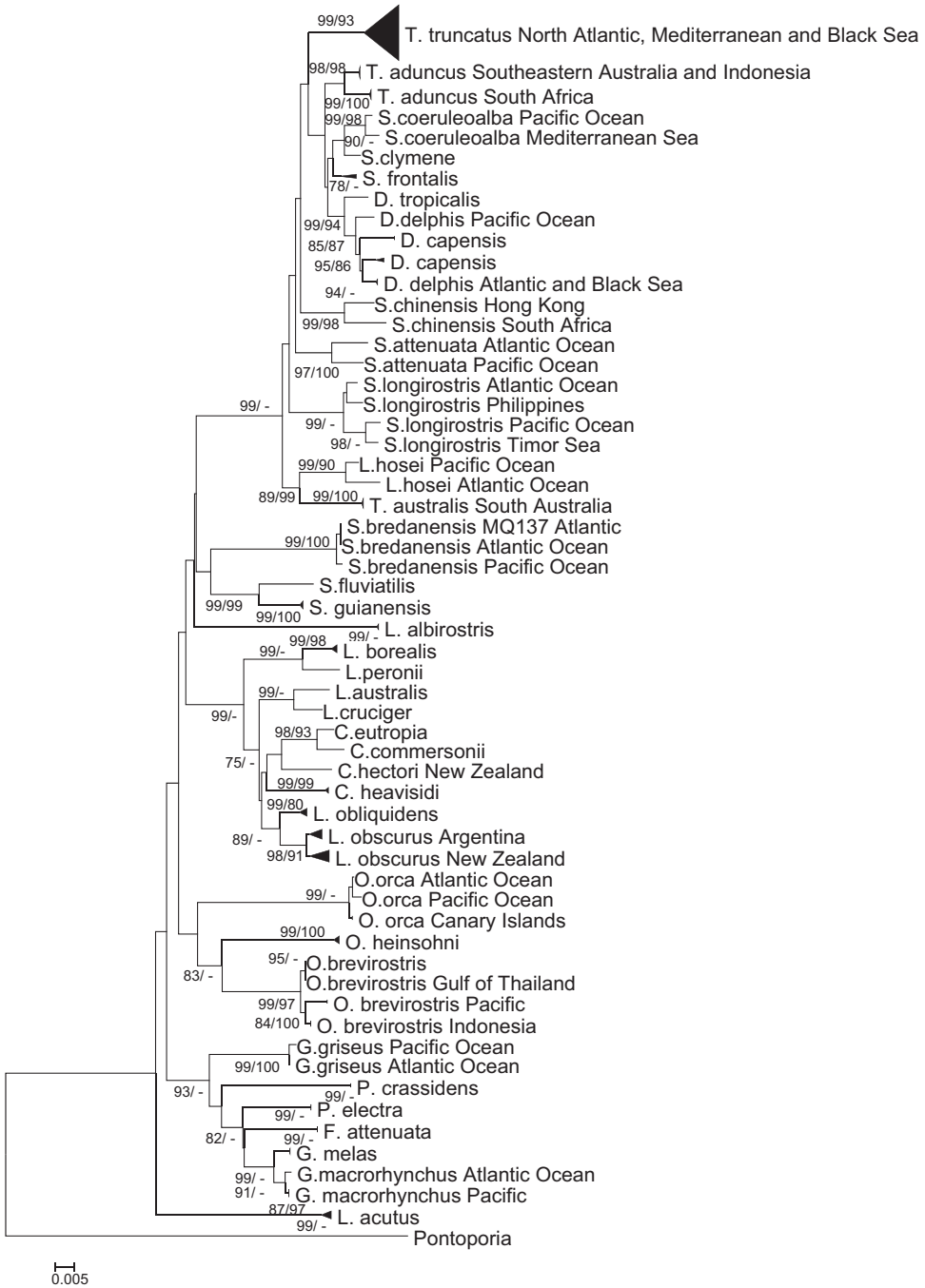

Figure 4. Phylogenetic neighbour-joining (NJ) tree of delphinid cytochrome *b* sequences. Numbers above branches indicate bootstrap/posterior probability values >75% (NJ, Kimura two-parameter/Bayesian, Hasegawa-Kishino-Yano + gamma + invariant sites).

possible source of noise in mitochondrial trees. Consequently, basing taxonomic decisions on a single locus is risky, and usually at least two independent lines of evidence (such as genetics and morphology) are required to sustain a claim in favour of species recognition. Morphological data could help to settle the taxonomic issue raised by the mitochondrial divergence found between Atlantic and Pacific/Indian rough-toothed dolphins. Unfortunately, however, morphological variation across the species range has not been studied yet. Therefore, combining our phylogenetic analyses and the fact that morphological data are still lacking, we concluded that the available data are not sufficient to support the existence of cryptic speciation in *Steno*,

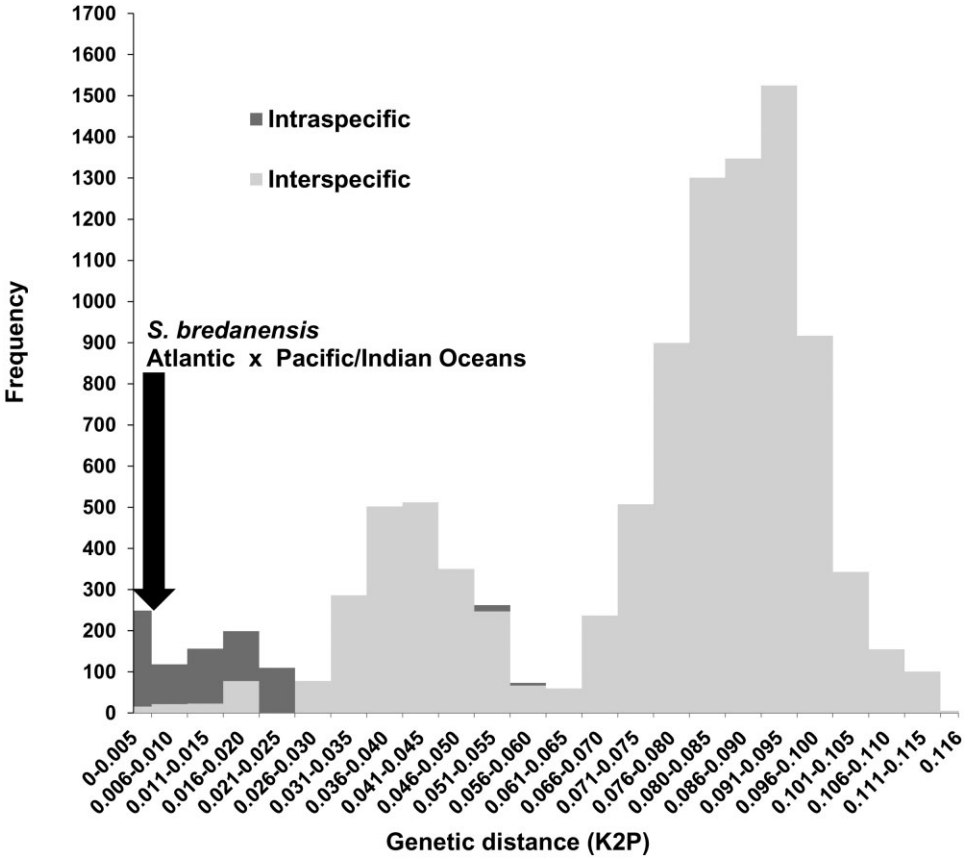

Figure 5. Intra- and interspecific genetic distances (Kimura two-parameter, K2P) in the cytochrome *b* sequences of delphinids, and the divergence between *Steno bredanensis* in the Atlantic and Pacific/Indian Oceans.

and decided to treat the Atlantic and Pacific/Indian populations as conspecific. However, the large amount of differentiation between mitochondrial lineages from these ocean basins deserves further examination, using nuclear markers as well as morphological data.

Population structure analyses

The AMOVAs revealed large amounts of genetic differentiation between rough-toothed dolphins from the Atlantic and Pacific Oceans, indicating that gene flow between these ocean basins is insignificant.

The existence of genetic structure in *S. bredanensis* worldwide could be anticipated because the only previous genetic study on the species detected microscale population differentiation in French Polynesia (Oremus *et al.*, 2012). Restrictions to gene flow between two islands 170 km apart were observed using control region sequences (450 bp, $F_{ST} = 0.63$, $P < 0.001$; $\Phi_{ST} = 0.58$, $P < 0.001$) and microsatellites (14 loci, $F_{ST} = 0.009$, $P < 0.001$; $R_{ST} = 0.15$, $P < 0.05$). The authors suggested that the significant genetic divergence between rough-toothed dolphins from the two islands is explained by site fidelity and low dispersal (Oremus *et al.*, 2012).

Amongst the delphinids with circumglobal distribution, only a few have had their genetic population structure studied. A general pattern in these studies is the existence of genetic differentiation between distinct ocean basins. For example, the long-finned pilot whale (*Globicephala melas*) showed strong population differentiation amongst sampling sites in the southwestern Pacific (New Zealand, Tasmania) and the North Atlantic (USA, England, Scotland, and the Faeroe Islands, $F_{ST} = 0.495$, $P < 0.001$; $\Phi_{ST} = 0.429$, $P < 0.001$) (Oremus *et al.*, 2009). The same study verified that the short-finned pilot whale (*G. macrorhynchus*) is also structured between the Atlantic and Pacific basins, with AMOVA supporting four populations (North Japan, South Japan, Atlantic, and South Pacific) ($F_{ST} = 0.392$, $P < 0.001$; $\Phi_{ST} = 0.598$, $P < 0.001$).

The common dolphin (genus *Delphinus*) is also found in the Atlantic, Pacific and Indian Oceans. Analyses of mitochondrial and nuclear sequences and microsatellites showed significant genetic differentiation between the Atlantic and Pacific Oceans and within

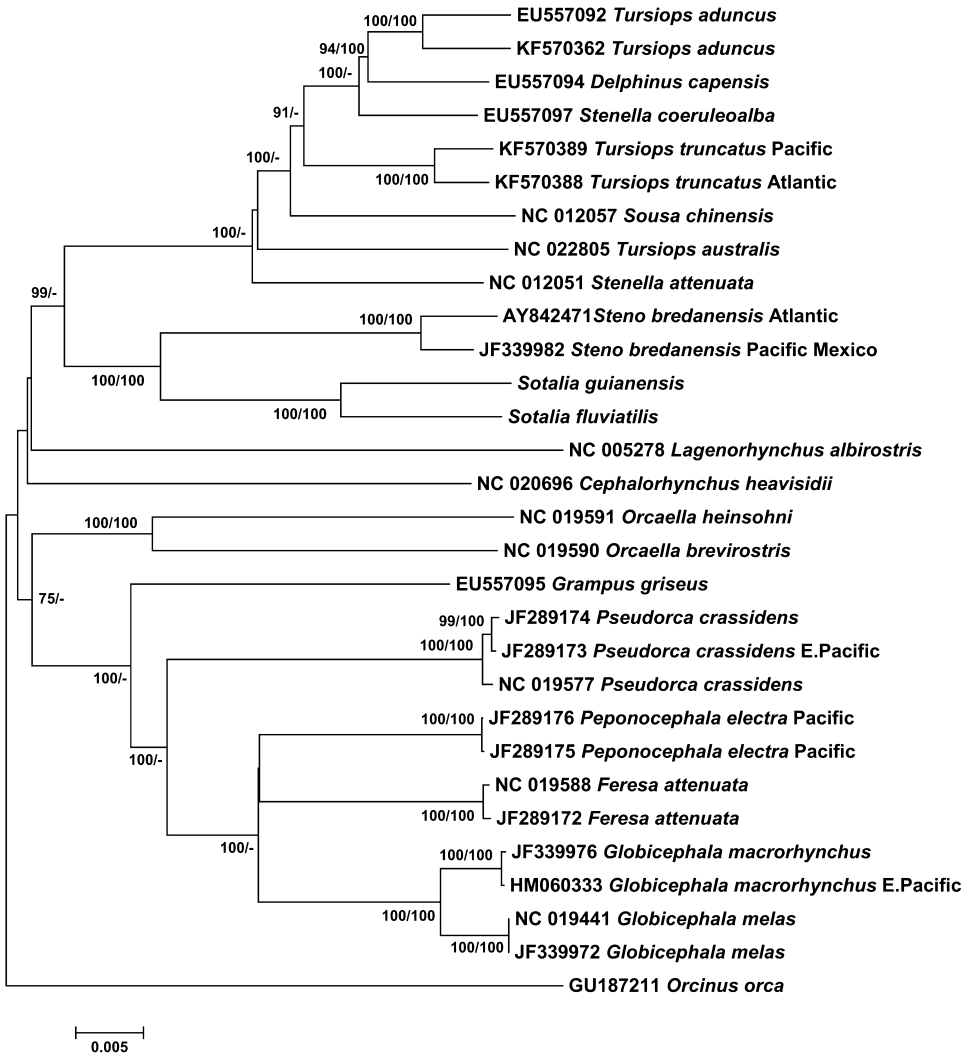

Figure 6. Phylogenetic neighbour-joining (NJ) tree of delphinid mitogenomes. Numbers above branches indicate bootstrap/posterior probability values >75% (NJ, Kimura two-parameter/Bayesian, Hasegawa-Kishino-Yano + gamma + invariant sites).

localities in the same ocean basin (Amaral *et al*., 2012a, b), a result in agreement with previous studies using mitochondrial and microsatellite data (Natoli *et al*., 2006; Mirimin *et al*., 2009).

In the present study, AMOVA and Φ_{ST} analyses also showed that there are restrictions to gene flow amongst rough-toothed dolphins from the Atlantic Ocean. Combining the two analyses, three Atlantic populations were detected (Caribbean, ES + RJ, and SC + RS), and should be provisionally accepted until the issue is further investigated using higher resolution genetic markers.

Delphinids can exhibit significant genetic differentiation across small geographical distances, such as that found here between ES + RJ and SC + RS (*c*. 700 km). Along the coast of Brazil, for example, Cunha *et al*. (2005) reported three populations of *Sotalia guianensis*, using preliminary control region sequence data: north, northeastern, and south–southeastern ($\Phi_{CT} = 0.628$, $P < 10^{-5}$). Also in the southwestern Atlantic, Fruet *et al*., (2014) detected significant genetic differentiation in *T. truncatus* from southern Brazil, Uruguay, and Argentina, using microsatellites ($F_{ST} = 0.46$, $P < 0.001$) and mitochondrial DNA ($\Phi_{ST} = 0.43$, $P < 0.0001$). Microscale genetic differentiation has been detected in other regions in *T. truncatus* (Tezanos-Pinto *et al*., 2009; Richards *et al*., 2013), *T. aduncus* (Wiszniewski *et al*., 2010), and *D. delphis* (Bilgmann *et al*., 2008; Möller *et al*., 2011). Finally, like *S. bredanensis*, the spinner dolphin *Stenella longirostris* is also structured amongst the six islands of French Polynesia (mtDNA: $F_{ST} = 0.143$; $\Phi_{ST} = 0.129$, $P < 0.001$; microsatellite: $F_{ST} = 0.029$, $P < 0.001$, Oremus *et al*., 2007).

The significant genetic structuring observed here in rough-toothed dolphins from the Atlantic mirrors a study

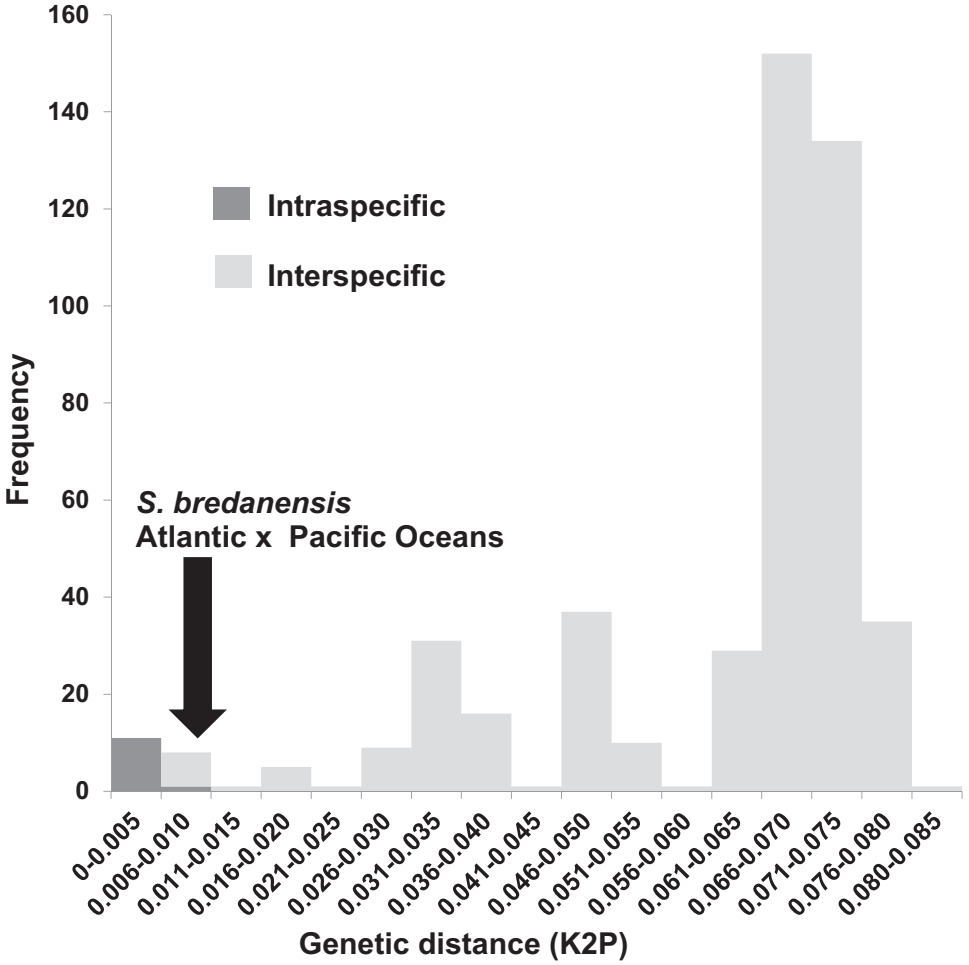

Figure 7. Intra- and interspecific genetic distances (Kimura two-parameter, K2P) in the mitogenomes of delphinids, and the divergence between *Steno bredanensis* in the Atlantic and Pacific Oceans.

Table 2. Analysis of molecular variance results of population structure hypotheses tested and the rejected scenarios of panmixia

Scenarios tested	Φ statistics	*P*
Scenarios of panmixia		
Atlantic	0.385 (Φ_{ST})	10^{-5}
Brazil	0.415 (Φ_{ST})	10^{-5}
Worldwide		
2 populations: Atlantic × Pacific	**0.769** (Φ_{CT})	**10^{-5}**
3 populations: Caribbean/SW Atlantic/Pacific	**0.764** (Φ_{CT})	**10^{-5}**
4 populations: RJ + ES/SC + RS/Caribbean/ET Pacific + CS Pacific	**0.747 (Φ_{CT})**	**10^{-5}**
5 populations: RJ/ES/SC + RS/Caribbean/ET Pacific + CS Pacific	0.696 (Φ_{CT})	10^{-4}
5 populations: RJ + ES × SC + RS × Caribbean × ET Pacific × CS Pacific	0.781* (Φ_{CT})	10^{-5}
Atlantic only		
3 populations: SC + RS/RJ + ES/Caribbean	0.386 (Φ_{CT})	0.01723

Significant results with the largest Φ_{CT} values are shown in bold.
*Overestimated, because $\Phi_{SC} = -1.836$.
ES, Espírito Santo State; RJ, Rio de Janeiro State; SC, Santa Catarina State; RS, Rio Grande do Sul State; ET, east tropical; CS, central south.

Table 3. Detailed analysis of molecular variance results for the most likely population structure scenarios

Source of variation	Sum of squares	Variance component	Percentage variation	Φ statistics	*P*
(A) 4 populations: Caribbean/RJ + ES/SC + RS/CS Pacific + ET Pacific					
Between groups = populations	286.165	4.498	74.70	**0.747**	10^{-5}
Amongst localities within groups	5.122	0.166	2.75		
Within localities	138.398	1.356	22.53		
(B) 3 populations: Caribbean/SW Atlantic/Pacific					
Between groups = populations	282.169	4.968	76.37	**0.764**	10^{-5}
Amongst localities within groups	9.118	0.179	2.76		
Within localities	138.398	1.356	20.85		
(C) 2 populations: Atlantic/Pacific					
Between groups = populations	279.488	5.166	76.87	**0.769**	10^{-5}
Amongst localities within groups	11.799	0.197	2.93		
Within localities	138.398	1.356	20.18		

Significant results with the largest Φ_{CT} values are shown in bold. ES, Espírito Santo State; RJ, Rio de Janeiro State; SC, Santa Catarina State; RS, Rio Grande do Sul State; ET, east tropical; CS, central south.

Table 4. Pairwise fixation index (Φ_{ST}) values amongst sampling localities. Significant values ($P < 0.008$) are marked with an *

	Car	ES	RJ	RS	ET Pac	CS Pac
Car	0.000					
ES	−0.044	0.000				
RJ	0.451*	0.131	0.000			
RS	0.524*	0.607*	0.465*	0.000		
ET Pac	0.528	0.706	0.896*	0.822*	0.000	
CS Pac	0.694*	0.746*	0.798*	0.753*	0.142	0.000

Car, Caribbean; ES, Espírito Santo State; RJ, Rio de Janeiro State; RS, Rio Grande do Sul State; ET Pac, eastern tropical Pacific; CS Pac, central south Pacific.

on the Atlantic spotted dolphin (*Stenella frontalis*). Analyses of control region sequences showed genetic differentiation between the Caribbean and southwestern Atlantic ($F_{ST} = 0.097$ and $\Phi_{ST} = 0.930$, $P < 0.05$; Caballero *et al*., 2013). However, spotted dolphins from the southwestern Atlantic were not different from those sampled in the Madeira and Azores Islands, which could indicate recent population fragmentation or ongoing long-distance connectivity as supported by sightings of this species in deep waters (Caballero *et al*., 2013).

In most of the sampled area in Brazil, *S. bredanensis* occurs sympatrically with *Sotalia guianensis* (from ES to SC). A study using control region sequences of *So. guianensis* reported a single haplotype from RJ to SC ($N = 20$, Cunha *et al*., 2005). This homogeneity in the control region has been confirmed with increased sampling (H. A. Cunha, unpubl. data), and has been attributed to a founder effect in the southern portion of the species distribution (Cunha *et al*., 2005). The fact that *S. bredanensis* presents variability across the same region implies that the two species experienced different evolutionary pathways, or that *S. bredanensis* could have been in the area for longer than *So. guianensis*. In the present study, the southeastern Brazil population (ES + RJ) had haplotype and nucleotide diversities roughly twice the values found for the southern population (SC + RS) ($Hd = 0.514$ and $\pi = 0.00152$; $Hd = 0.345$ and $\pi = 0.00086$, respectively), which could indicate that the southern population is either smaller or younger. However, the lower diversity may also be simply a result of the difference in sample sizes ($N = 31$ and $N = 11$).

It is important to note that population structure results may change when more sequences are available. More specifically, the clustering of Central Southern Pacific and Eastern Tropical Pacific observed here may be a result of the small number of sequences from the latter (only four). With the inclusion of more samples, it may be possible to detect population differentiation across the Pacific, as found in the Atlantic. In addition, samples from the Indian Ocean will enable comparisons with the Pacific and Atlantic basins.

The use of hypervariable, biparentally inherited microsatellites may also improve analyses of the population structure of rough-toothed dolphins.

Implications for conservation

The genetic differentiation between rough-toothed dolphins from the Atlantic and Pacific deserves further attention, as it may indicate cryptic speciation in the genus.

Our data revealed genetic population structure of rough-toothed dolphins both worldwide (Atlantic × Pacific Oceans) and also within the Atlantic Ocean basin, where three populations were detected (ES + RJ, SC + RS, and Caribbean). The restricted gene flow amongst these areas shows that they are demographically independent and should be considered distinct management units (MUs, *sensu* Moritz, 1994). In the southwestern Atlantic, rough-toothed dolphins have a coastal distribution and are exposed to several anthropogenic threats (Di Beneditto *et al*., 1998; Monteiro-Neto *et al*., 2000; Dorneles *et al*., 2007; Meirelles *et al*., 2009; Lailson-Brito *et al*., 2012). With respect to the delimitation of southwestern Atlantic MUs, basic demographic data should be gathered to allow for a proper assessment of the population status and to define conservation strategies aimed at the maintenance of genetic diversity.

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