Aquatic Mammals 2017, *43*(5), 507-516, DOI 10.1578/AM.43.5.2017.507

An Initial Population Structure and Genetic Diversity Analysis for *Stenella clymene* (Gray, 1850): Evidence of Differentiation Between the North and South Atlantic Ocean

Luana Nara,[1] Ana Carolina Oliveira de Meirelles,[2]
Luciano Raimundo Alardo Souto,[3] Jose Martíns Silva-Jr,[4]
and Ana Paula Cazerta Farro[1]

[1]*Laboratório de Genética e Conservação Animal, Departamento de Ciências Agrárias e Biológicas, CEUNES, Universidade Federal do Espírito Santo, Rodovia BR 101 Norte, Km 60, Bairro Litoranêo, 29932-540 São Mateus, ES, Brazil*
E-mail: luana.nara@gmail.com
[2]*AQUASIS – Associação de Pesquisa e Preservação de Ecossistemas Aquáticos, Praia de Iparana, s/nº, SESC Iparana, 61600-000 Caucaia, CE, Brazil*
[3]*V.I.V.A. – Viver, Informar e Valorizar o Ambiente, Avenida Antônio Carlos Magalhães, nº 1034, Edifício Pituba Park Center, sala 251-A, Itaigara, 41825-000 Salvador, BA, Brasil*
[4]*NGI-FN/ICMBio – Rua Eurico Cavalcanti, s/nº, Vila do Boldró, Caixa Postal nº 50, 53900-000 Fernando de Noronha, PE, Brazil*

Abstract

Information concerning population structure and genetic diversity in *Stenella clymene* is still scarce. Previous studies raised questions regarding the species' position in the genus *Stenella* and suggested that *S. clymene* might be of hybrid origin. The present study analyzed the mitochondrial control region (D-loop), cytochrome oxidase I (CoI), and cytochrome b (Cyt b) of northeastern Brazil individuals and compared them with *S. clymene* sequences from the North Atlantic Ocean and Gulf of Mexico. Brazilian individuals showed high haplotype diversity (D-loop: 1.00/p = 0.02; CoI: 0.99/p = 0.04; Cyt b: 0.96/p = 0.06) and probably constitute one population (South Atlantic Ocean). Significant differentiation and high F_{ST} values (D-loop: F_{ST} = 0.88/p = 0.00; CoI: F_{ST} = 0.70/p = 0.00; Cyt b: F_{ST} = 0.96/p = 0.00) were found between population units from the North and South Atlantic Ocean. For Cyt b, population units from the South Atlantic Ocean and Gulf of Mexico showed significant differentiation, but the F_{ST} value was low (F_{ST} = 0.11/p = 0.0). In addition, the haplotype network suggests connectivity between South Atlantic Ocean and Gulf of Mexico units. More effort focusing on *S. clymene* is needed to better elucidate the patterns of population structure within this species and, thus, provide sufficient data for conservation strategies.

Key Words: D-loop, cytochrome oxidase I, cytochrome b, Clymene dolphin, Delphinidae

Introduction

Stenella clymene, the Clymene dolphin, has a restricted distribution near the equator in the Atlantic Ocean (Jefferson & Curry, 2003). Considered an oceanic species, it is commonly found in deep waters (1,000 to 4,500 m), with cyclonic or confluent circulation (Davis et al., 1998; Weir, 2006), feeding typically on mesopelagic fishes (Jefferson & Curry, 2003). There are reports of significant habitat overlap of *S. clymene* with other *Stenella* sp.—for example, *S. attenuata, S. coeruleoalba*, and *S. longirostris* (Davis et al., 1998; Moreno et al., 2005).

According to the International Union for Conservation of Nature's (IUCN) (2016) *Red List of Endangered Species*, *S. clymene* is under the "Data Deficient" (DD) classification due to the lack of studies focusing on the species. It is uncertain how many populations or stocks of *S. clymene* exist, and there is no information regarding its population structure and diversity overall.

The phylogenetic position of the Clymene dolphin in the *Stenella* genus and in the Delphinidae subfamily remains uncertain. In most studies, the closest species to *S. clymene* is *S. coeruleoalba,* followed by *S. frontalis* (Perrin et al., 2013), but *Delphinus* seems to be closer to *S. clymene* than *S. attenuata* and *S. longirostris* would be (Perrin et al., 2013). Furthermore, Amaral et al. (2014) hypothesized that *S. clymene* originated from a hybridization between *S. longirostris* and *S. coeruleoalba,* as well as the possibility of *S. clymene*

being a recent introgression between *S. clymene* and *S. longirostris*.

Analyses of existent polymorphisms in DNA sequences between individuals of different localities allow for the exploration of evolutionary process and demographic events of a species (Nosil et al., 2009). However, for this species, molecular studies have been difficult as sightings and stranding events are sporadic due to the distribution of *S. clymene* in the deep waters of the Atlantic Ocean. Because most samples available are from stranded individuals, analyses of mitochondrial DNA have been preferred due to its success in low-quality DNA analyses such as that found in samples from stranded individuals (Wan et al., 2004; Frankham et al., 2008).

The aim of this study was to provide initial information about the genetic diversity and population structure of *S. clymene* by comparing the diversity among individuals from the South and North Atlantic Oceans.

Methods

Samples

Samples were collected from 12 individuals (seven male and four female) that stranded in northeastern Brazil (Figure 1). The conditions of the individuals ranged from fresh to an advanced stage of decomposition. Tissues were collected according to the decomposition stage: muscle and skin were obtained from fresh carcasses and stored in ethanol, while internal organs were collected from carcasses with an advanced stage of decomposition and stored in formaldehyde.

Additionally, to perform a broader analysis of *S. clymene*'s relationship within the genus, we included sequences from other *Stenella* sp. from GenBank (Figure 1; Table S1—This table is available in the supplementary material for this article on the Supplementary Material page of the *Aquatic Mammals* website: www.aquaticmammalsjournal.org/index.php?option=com_content&view=article&id=10&Itemid=147).

DNA Extraction, Amplification, and Sequencing

Different DNA extraction protocols were used according to the tissue type and storage solution: salt buffer protocol (Bruford et al., 1992) was used for muscle; Chelex resin protocol was used for skin; a phenol-chloroform protocol (Sheppard et al., 1992) was used for degraded tissue; and Mesquita et al.'s (2001) protocol was applied with adaptations for tissues preserved in formaldehyde (see "DNA Extraction Protocol . . ." in the supplementary material for this article on the Supplementary Material page of the *Aquatic Mammals* website: www.aquaticmammalsjournal.org/index.php?option=com_content&view=article&id=10&Itemid=147).

The D-loop control region was amplified following Pichler et al. (2001), with primers dLp1.5 (5' TCACCCAAAGCTGRARTTTA 3') and dLp5 (5' CCATCGWGATGTCTTATTTAA-GR GGAA 3'); and reactions were performed in 12.5 µl volumes containing 10 to 100 ng of extracted DNA, 10x PCR buffer (Invitrogen), 2 mM $MgCl_2$, 0.12 µM of each primer, 0.05 mM dNTP, and 1U/µl Taq polymerase (Invitrogen). The thermocycle profiles consisted of an initial denaturation step at 95° C for 1 min, 40 cycles of 94° C for 30 s, 54° C for 30 s, and a final extension step at 72° C for 5 min. The cytochrome oxidase I (CoI) gene was amplified following Amaral et al. (2007a), with primers COX1F (5' TGCCTACTCGGCCATTTTAC 3') and COX1R (5' TGAAACCCAGGAAGCCAATA 3'). Amplification reactions were performed in 12.5 µl volumes containing 10 to 100 ng of extracted DNA, 10x PCR buffer (Invitrogen), 1.5 mm $MgCl_2$, 0.15 mm dNTPs, 0.3 µm of each primer, and 1 U/µL Taq polymerase. The thermocycle profiles consisted of an initial denaturation step at 94° C for 2 min, followed by 35 cycles of 45 s at 94° C, 45 s at 52° C, 1 min at 72° C, and a final extension step for 8 min at 72° C. The cytochrome b (Cyt b) gene was amplified using primers described in Palumbi et al. (1991): 5' TGACTTGAARAAC CAYCG TTG 3' and 5' CCTTTTCCGGTTTACAAGAC 3'. Amplification reactions were performed in 12.5 µl volumes containing 10 to 100 ng of extracted DNA, 10x PCR buffer (Invitrogen), 1.52 mM $MgCl_2$, 0.3 µM of each primer, 0.04 mM dNTP, and 1 U/µl Taq polymerase (Invitrogen). The thermocycle profiles for the Cyt b gene consisted of an initial denaturation step at 94° C for 3 min, followed by 35 cycles of 45 s at 94° C, 45 s at 48° C, 1 min at 72° C, and a final extension step for 5 min at 72° C.

The mitochondrial genes were purified with Exonuclease I (10 u/µl), Shrimp alkaline phosphatase (1 u/µl), and Exo/Sap (1/1). They were incubated for 15 min at 37° C and 15 min at 80° C. Sequenced reactions were performed with 1 µl of PCR product, 5.06 µl of ultrapure water, 2.5 µl of buffer, 0.8 µl of BigDye Terminator CycleSequencing (Applied Biosystems), and 0.64 µl of primer (5 µM) for both strands, forward and reverse. The thermocycle profiles consisted of an initial denaturation step at 96° C, followed by 25 cycles of 10 s at 96° C, 5 s at 48° C, and 4 min at 60° C. To precipitate the DNA, we added 80 µl of isopropyl alcohol (75%), centrifuged at 13,000 rpm for 25 min, and discarded the supernatant. The pellet was washed with 250 µl of ethanol (70%) being centrifuged at 13,000 rpm for 5 min. The supernatant was discarded, and the pellets were dried on the thermocycler for 3 min at 95° C. The

Figure 1. Locations where *Stenella clymene* samples were obtained on the Brazilian coast, as well as the sequences available from GenBank

samples were sequenced on an ABI310 automated sequencer (Applied Biosystems).

Data Analyses

All DNA sequences were aligned using the algorithm *MUSCLE* (Robert, 2004) and manually edited in the software *MEGA*, Version 6 (Kumar et al., 1994; Tamura et al., 2013). To confirm the species, sequences were compared with GenBank and DNA Surveillance (Ross et al., 2003) databases.

Population structure analyses were conducted, and estimates of haplotype (H) and nucleotide (π) diversity were obtained using the software *Arlequin*, Version 3.5 (Excoffier et al., 2005). Genetic differences among population units (North Atlantic Ocean, South Atlantic Ocean, and Gulf of Mexico) were quantified by Analysis of Molecular Variance (AMOVA) based on conventional F_{ST} statistics with 10,000 random permutations using the *Arlequin* software. Haplotype networks were constructed using Median-Joining calculations as implemented in *Network* (Bandelt et al., 1999).

For an insight into *S. clymene*'s relationship to its genus, we included GenBank sequences of other *Stenella* sp., for both CoI and Cyt b, to estimate a species tree of the genus. As outgroups, we used sequences from *Megaptera novaeangliae* (Borowski, 1781) (GenBank AP006467 for CoI and Cyt b) and *Pontoporia blainvillei* (Gervais & d'Orbigny, 1844) (GenBank EU496358 for CoI and GenBank AF334488 for Cyt b). We only included sequences that had a reference article submitted (see Table S1).

The best evolutionary model for each mitochondrial gene region was obtained through the *JModeltest*, Version 2.1.6 program (Guindon & Gascuel, 2003; Darriba et al., 2012), using the Akaike information criterion (AIC) test. To estimate a species tree of the genus *Stenella*, 10 million Monte Carlo Markov Chain (MCMC) generations sampling every 1,000 generations were run in the program *BEAST*, Version 1.8.1 (Drummond et al., 2012); the evolutionary model applied for both CoI and Cyt b was HKY + G, with the Yule process as the species tree prior and a strict

molecular clock with an uncorrelated lognormal distribution. *TreeAnnotator*, Version 1.8.1 (Drummond et al., 2012) was subsequently used to summarize the obtained trees in a single tree, with a maximum clade credibility, a burning value of 1,000, and a posterior probability limit of 0.5. *FigTree*,Version 1.4.3 (Rambaut, 2009) was used to edit and produce the tree figures.

Results

For all mitochondrial regions, AMOVA and genetic distance results showed no significant genetic differentiation between individuals from different locations in Brazil (D-loop: F_{ST} = -0.29/p = 0.9; CoI: F_{ST} = -0.25/p = 0.7; Cyt b: F_{ST} = 0.25/p = 0.12); therefore, these individuals were considered as a single South Atlantic Ocean (SAO) population. GenBank sequences included for D-loop and CoI were all described as a North Atlantic Ocean (NAO) population. For the D-loop, both SAO and NAO showed high haplotype and nucleotide diversity. As for CoI, both SAO and NAO presented high haplotype diversity, but the NAO had lower nucleotide diversity when compared to SAO (Table 1).

As we included the GenBank sequences for Cyt b, AMOVA and genetic distance results considering all GenBank sequences as one population unit showed higher intrapopulation distance (F_{ST} = 0.92/p = 0.04) than interpopulation distance (F_{ST} = 0.08/p = 0.04). Thus, for Cyt b analyses, we considered NAO and the Gulf of Mexico (GOM) as separate population units. For Cyt b, SAO and NAO exhibited higher nucleotide diversity when compared to GOM, while NAO and GOM had similar haplotype diversity that was higher than SAO (Table 1).

AMOVA results were significant for all mitochondrial genes: D-loop, F_{ST} = 0.88 (p = 0.00); CoI, F_{ST} = 0.70 (p = 0.00); and Cyt b, F_{ST} = 0.86 (p = 0.00). For Cyt b, the F_{ST} values between NAO and SAO (F_{ST} = 0.96/p = 0.00) and between NAO and GOM (F_{ST} = 0.93/p = 0.00) were higher than between SAO and GOM (F_{ST} = 0.11/p = 0.00).

Haplotype networks support the results of differentiation between individuals from the northern and southern localities of the Atlantic Ocean (Figure 2). Note that for Cyt b, despite SAO and GOM being separated, there were individuals maintaining a connection between these populations.

As for the phylogenetic tree, in Figures 3 and 4, for both CoI and Cyt b, the closest species to *S. clymene* is *S. coeruleoalba* followed by *S. frontalis.* For CoI, we have a strongly supported clade for *S. clymene*, but individuals SAO03 (KX346587) and SAO05 (KX346585) are clustered isolated outside any clade, including *S. clymene* (Figure 3). We also have a supported clade for *S. clymene* for Cyt b. Again, the individual SAO05 (KX346595) is outside the clade, but now it is clustered with the *S. clymene* individual KF691995 from the GOM (Figure 4). In addition, the *S. clymene* individual KF691958 from the GOM is clustered in the *S. longirostris* clade (Figure 4).

Discussion

Even with a low sample size, the haplotype and nucleotide diversity of *S. clymene* were relatively high for all molecular markers and geographic localities evaluated as found in previous Delphinidae studies (Natoli et al., 2005; Adams & Rosel, 2006; Amaral et al., 2007b; Quérouil et al., 2007; Caballero et al., 2013; Stockin et al., 2014).The high haplotype diversity together with the low nucleotide diversity found in CoI for NAO individuals and in Cyt b for SAO individuals may be an indication of genetic bottleneck events followed by population expansion. Generally, the haplotype network has a star pattern, which supports more clearly the hypothesis that new haplotypes

Table 1. Sample size (*N*), base par (Bp), polymorphic sites (Ps), haplotype diversity (H), and nucleotide diversity (π) of *Stenella clymene* in the Atlantic Ocean; SA = South Atlantic, NA = North Atlantic, and GOM = Gulf of Mexico.

		N	Ps	H	π
D-loop (400 bp)	SA	8	14	1.0 ± 0.02	0.02 ± 0.01
	NA	14	24	1.0 ± 0.02	0.02 ± 0.01
CoI (636 bp)	SA	10	44	0.99 ± 0.04	0.0234 ± 0.1
	NA	3	4	1.0 ± 0.27	0.0042 ± 0.01
Cyt b (783 bp)	SA	9	19	0.97 ± 0.06	0.006 ± 0.03
	NA	3	11	1.0 ± 0.27	0.0112 ± 0.001
	GOM	12	51	0.83 ± 0.08	0.0128 ± 0.007

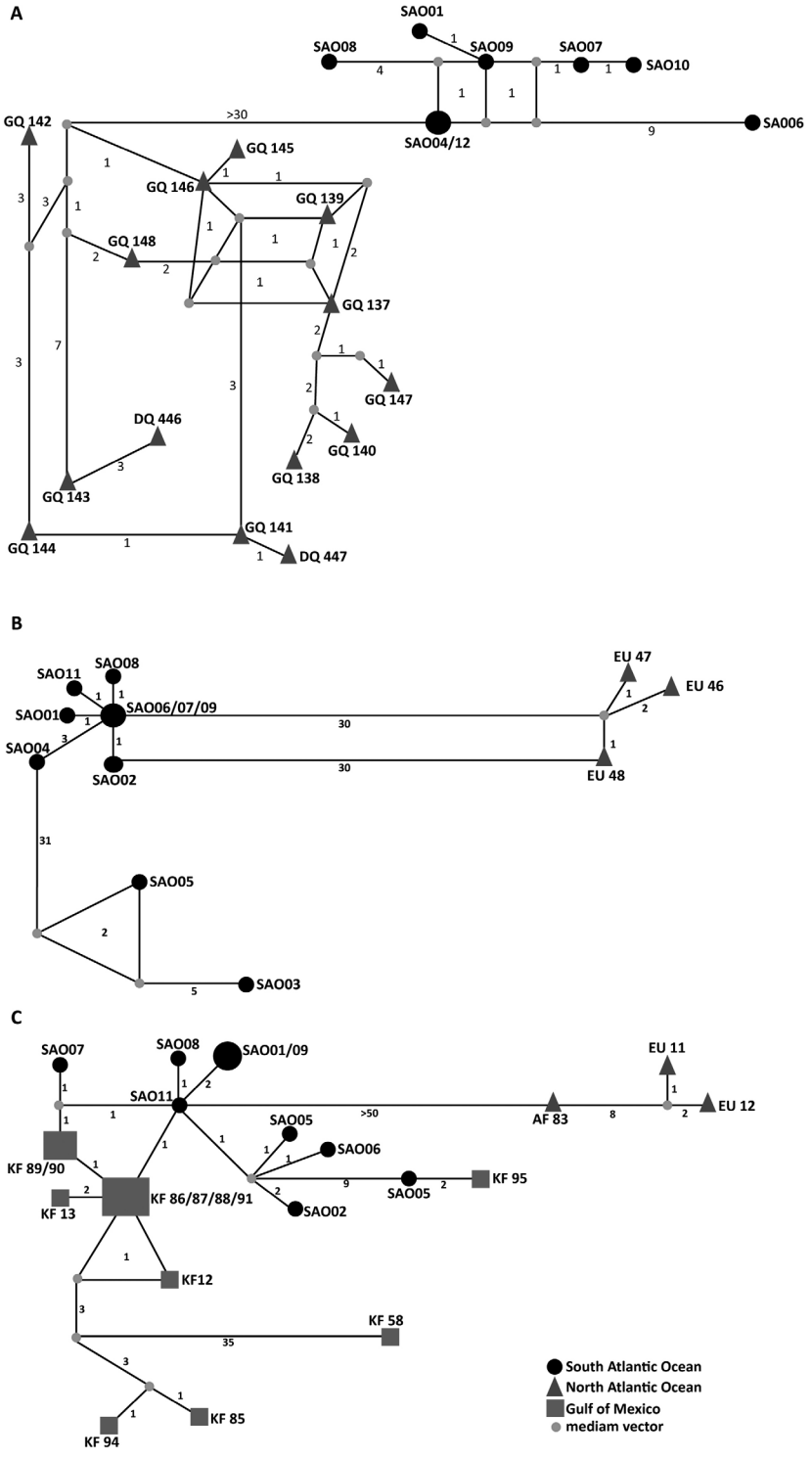

Figure 2. *S. clymene* haplotype network for mtDNA regions D-loop (A), CoI (B), and Cyt b (C). Circle diameter is proportional to relative frequency of haplotypes; numbers within the lines represent mutational step numbers.

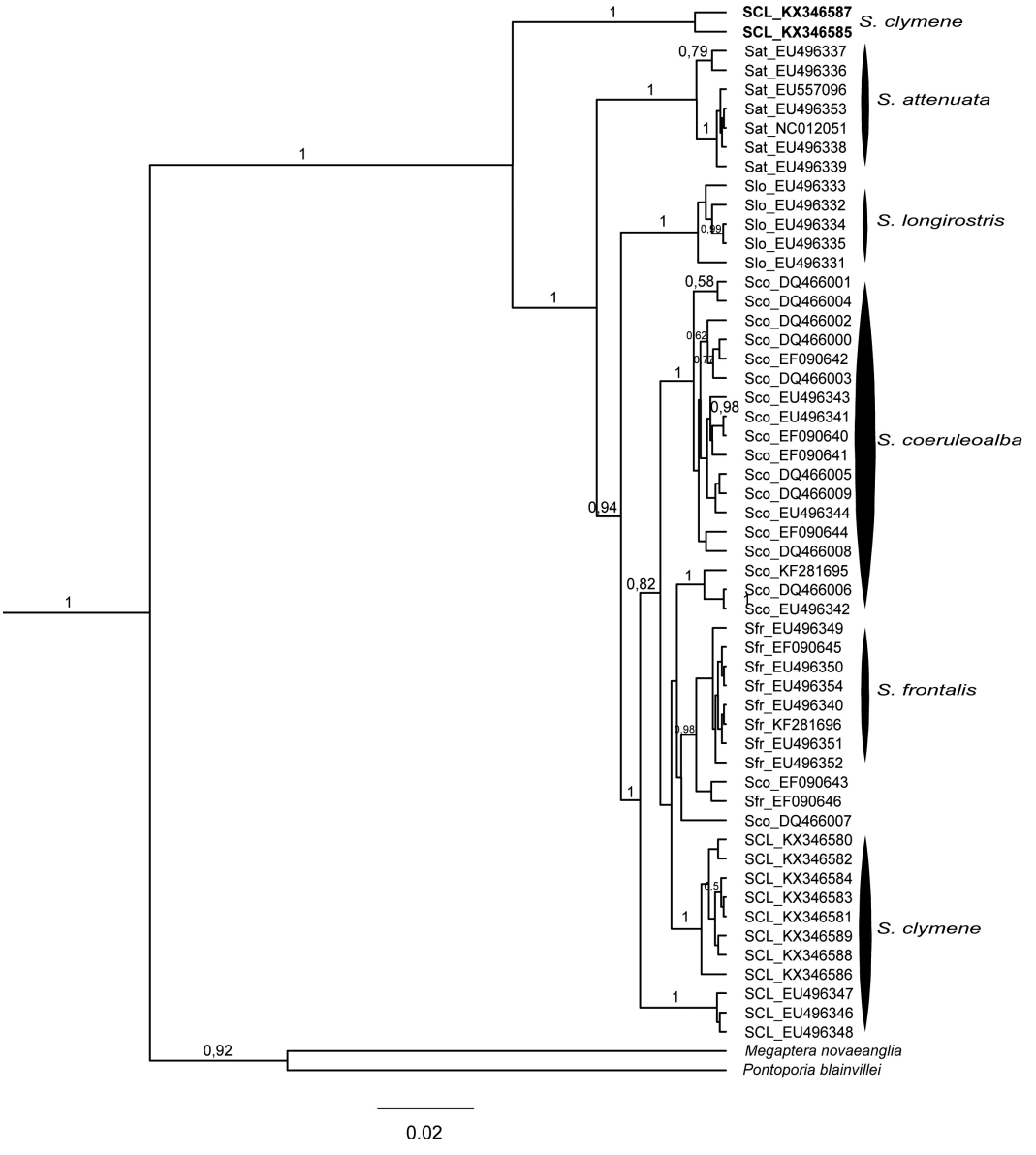

Figure 3. Species tree estimated for CoI with the *BEAST* method. Posterior probability values are above nodes.

apparently recently diverged from a single ancestral haplotype. A similar hypothesis has been proposed for other cetacean species (Hoelzel et al., 2002, 2007; Luca et al., 2009).

Analyses of all mitochondrial markers showed large genetic differentiation between the NAO and SAO populations, and between NAO and GOM. However, the F_{ST} value between SAO and GOM was substantially lower, which is also reflected in the haplotype network where there are distances of only one mutational step between some SAO and GOM haplotypes. This structure, separating the SAO and GOM populations, may be sustained or not if more samples are included in a future analysis.

In a study of population structure in *S. frontalis*, Adams & Rosel (2006) found similar differentiation between NAO and GOM populations and suggested that the most likely hypothesis is the influence of the distribution of their prey. The physical barrier that could prevent dispersal

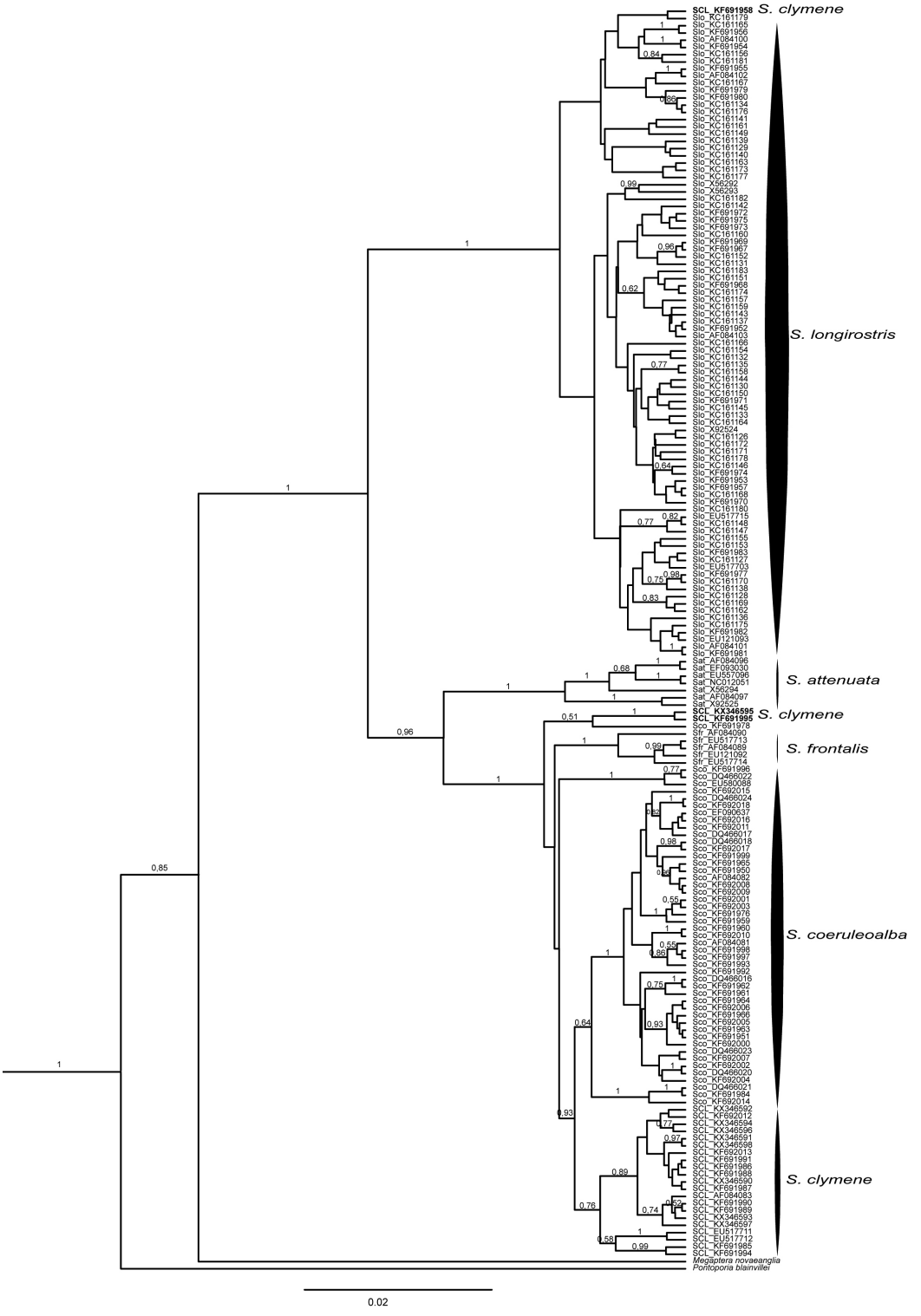

Figure 4. Species tree estimated for Cyt b with the *BEAST* method. Posterior probability values are above nodes.

between GOM and NAO would be the ocean streams, but dolphins are highly agile organisms, and water currents are not likely to be a barrier for them; however, their prey would have limited dispersal because of water currents (Dowling & Brown, 1993; Hoelzel, 1994). The same situation would apply to *S. clymene* considering most of its prey are mesopelagic species (Jefferson & Curry, 2003) whose distribution is influenced by vertical stratification of water bodies (Davis et al., 1998).

An interesting fact that should be highlighted in the analysis of Cyt b haplotype networks is that the individual KF691995 from GOM is closer to SAO05 from SAO than to any other individual from GOM (Figure 2). An explanation for such a result could be the existence of gene flow between the SAO and GOM stocks. The occurrence of a seasonal migration of *S. clymene* from the SAO to the GOM region is possible if considering the study of Stramma et al. (1995). They reported that during the austral spring in the northern Brazil current, there is an undercurrent of intense speed in the upper 1,000 m of the water column (depth within the range preferred by *S. clymene*) that can influence phyto- and zooplankton migration and, consequently, species of other trophic levels, especially mesopelagic fish with vertical migration that have been recognized as *S. clymene*'s prey (Jefferson & Curry, 2003). Therefore, the observed genetic difference might be related to spatial separation of *S. clymene* individuals by oceanographic processes.

Silva-Jr et al. (2005) reported an individual with physical characteristics of both *S. longirostris* and *S. clymene* and suggested hybridization between these species. Later, Amaral et al.'s (2014) molecular analysis suggested that *S. clymene* might be a hybrid of *S. coeruleoalba* and *S. longirostris*, and they also proposed the possibility of a recent introgression between *S. clymene* and *S. longirostris*. In our Cyt b analyses, there was only one individual of *S. clymene* close to *S. longirostris* (KF691958 from GOM; Figure 4), which was different from results presented by Amaral et al. (2014) where two individuals were clustered with *S. longirostris*. Considering that in our analyses we used all of the sequences from the Amaral et al. (2014) study, our results might be dissimilar to theirs because of the inclusion of more *S. clymene* individuals from other localities. The second individual from GOM that was with the *S. longirostris* clade in the Amaral et al. (2014) study now is probably clustered with the *S. clymene* individual from SAO included from our samples. Therefore, our result weakens the possibility of a recent introgression between *S. clymene* and *S. longirostris*. In addition, in our phylogenetic analyses (Figures 3 & 4), we found *S. clymene* closely related to *S. coeruleoalba* (as reported by LeDuc et al., 1999; May-Collado & Agnarsson, 2006; Agnarsson & May-Collado, 2008; Möller et al., 2008; McGowen et al., 2009; Bilgmann et al., 2011; McGowan, 2011). Thus, we must improve the *S. clymene* DNA sample collection to better understand its genetics and relationships to other species in the *Stenella* genus.

Conclusions

Our results indicate that *S. clymene* individuals from northeastern Brazil have high haplotype diversity and probably constitute one population. Additionally, our results suggest that there might be three well-defined populations in the Atlantic Ocean: (1) North Atlantic Ocean, (2) South Atlantic Ocean, and (3) Gulf of Mexico. In addition, there are probably individuals that move between the SAO and GOM populations, while the NAO population seems to be isolated.

Acknowledgments

We want to thank all of the volunteers who helped collect the samples included in this study, Leonora Pires Costa for allowing us to use her laboratory for some of the analysis, and especially Drienne Messa Faria for all of the assistance through the whole process. We wish to thank Petrobras for providing funds through the Programa Petrobras Socioambiental (Process: 0080.0094052.14.2).

Literature Cited

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. https://doi.org/10.1007/s00227-005-0094-2

Agnarsson, I., & May-Collado, L. J. (2008). The phylogeny of Cetartiodactyla: The importance of dense taxon sampling, missing data, and the remarkable promise of cytochrome b to provide reliable species-level phylogenies. *Molecular Phylogenetics and Evolution*, *48*, 964-985. https://doi.org/10.1016/j.ympev.2008.05.046

Amaral, A. R., Sequeira, M., & Coelho, M. M. (2007a). A first approach to the usefulness of cytochrome c oxidase I barcodes in the identification of closely related delphinid cetacean species. *Marine and Freshwater Research*, *58*, 505-510. https://doi.org/10.1071/MF07050

Amaral, A. R., Sequeira, M., Martínez-Cedeira, J., & Coelho, M. M. (2007b). New insights on population genetic structure of *Delphinus delphis* from the northeast Atlantic and phylogenetic relationships within the genus inferred from two mitochondrial markers. *Marine Biology*, *151*, 1967-1976. https://doi.org/10.1007/s00227-007-0635-y

Amaral, A. R., Lovewell, G., Coelho, M. M., Amato, G., & Rosenbaum, H. C. (2014). Hybrid speciation in a marine mammal: The Clymene dolphin (*Stenella clymene*). *PLOS ONE*, *9*(1), 1-8. https://doi.org/10.1371/journal.pone.0083645

Bandelt, H. J., Forster, P., & Rohl, A. (1999). Median-joining networks for inferring intraspecific phylogenies. *Molecular Biotechnology*, *16*, 37-48. https://doi.org/10.1093/oxfordjournals.molbev.a026036

Bilgmann, K., Möller, L. M., Harcourt, R. G., Kemper, C. M., & Beheregaray, L. B. (2011). The use of carcasses for the analysis of cetacean population genetic structure: A comparative study in two dolphin species. *PLOS ONE*, *6*(5), 22-24. https://doi.org/10.1371/journal.pone.0020103

Bruford, M. W., Hanotte, O., Brookfield, J. F. Y., & Burke, T. (1992). Single-locus and DNA fingerprinting. In A. R. Hoelzel (Ed.), *Molecular genetic analyses of populations: A practical approach* (pp. 225-269). Oxford, UK: IRL Press.

Caballero, S., Santos, M. C. O., Sanches, A., & Mignucci-Giannoni, A. A. (2013). 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. *Biochemical Systematics and Ecology*, *48*, 263-270. https://doi.org/10.1016/j.bse.2012.12.016

Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). *jModelTest 2*: More models, new heuristics and parallel computing. *Nature Methods*, *9*, 772. https://doi.org/10.1038/nmeth.2109.

Davis, R. W., Fargion, G. S., May, N., Leming, T. D., Baumgartner, M., Evans, W. E., . . . Mullin, K. (1998). Physical habitat of cetaceans along the continental slope in the north-central and western Gulf of Mexico. *Marine Mammal Science*, *14*, 490-507. https://doi.org/10.1111/j.1748-7692.1998.tb00738.x

Dowling, T., & Brown, W. (1993). Population structure of the bottlenose dolphin (*Tursiops truncatus*) as determined by restriction endonuclease analysis of mitochondrial DNA. *Marine Mammal Science*, *9*, 138-155. https://doi.org/10.1111/j.1748-7692.1993.tb00439.x

Drummond, A. J., Suchard, M. A., Xie, D., & Rambaut, A. (2012). Bayesian phylogenetics with *BEAUti* and the *BEAST* 1.7. *Molecular Biology and Evolution*, *29*, 1969-1970. https://doi.org/10.1093/molbev/mss075

Excoffier, L., Laval, G., & Schneider, S. (2005). *Arlequin* (Version 3.0): An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, *1*, 47-50.

Frankham, R., Ballou, J. D., & Briscoe, D. A. (2008). *Fundamentos de genética da conservação* [A primer of conservation genetics]. Riberão Preto, São Paulo: Sociedade Brasileira de Genética.

Guindon, S., & Gascuel, O. (2003). A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology*, *52*(5), 696-704. https://doi.org/10.1080/10635150390235520

Hoelzel, A. R. (1994). Genetics and ecology of whales and dolphins. *Annual Review of Ecology, Evolution, and Systematics*, 377-399. https://doi.org/10.1146/annurev.es.25.110194.002113

Hoelzel, A. R., Hey, J., Dahlheim, M. E., Nicholson, C., Burkanov, V., & Black, N. (2007). Evolution of population structure in a highly social top predator, the killer whale. *Molecular Biology and Evolution*, *24*, 1407-1415. https://doi.org/10.1093/molbev/msm063

Hoelzel, A. R., Natoli, A., Dahlheim, M. E., Olavarria, C., Baird, R. W., & Black, N. A. (2002). Low worldwide genetic diversity in the killer whale (*Orcinus orca*): Implications for demographic history. *Proceedings of the Royal Society B: Biological Sciences*, *269*, 1467-1473. https://doi.org/10.1098/rspb.2002.2033

International Union for Conservation of Nature (IUCN). (2016). *IUCN red list of threatened species*. Retrieved from www.iucnredlist.org

Jefferson, T. A., & Curry, B. E. (2003). *Stenella clymene*. *Mammalian Species*, *726*, 1-5. https://doi.org/10.1644/726

Kumar, S., Tamura, K., & Nei, M. (1994). *MEGA*: *Molecular Evolutionary Genetics Analysis* software for microcomputers. *Computer Applications in the Biosciences*, *10*, 189-191. https://doi.org/10.1093/bioinformatics/10.2.189

LeDuc, R. G., Perrin, W. F., & Dizon, A. E. (1999). Phylogenetic relationships among the Delphinid cetaceans based on full cytochrome B sequences. *Marine Mammal Science*, *15*, 619-648. https://doi.org/10.1111/j.1748-7692.1999.tb00833.x

Luca, M., Westgate, A., Rogan, E., Rosel, P., Read, A., Coughlan, J., & Cross, T. (2009). Population structure of short-beaked common dolphins (*Delphinus delphis*) in the North Atlantic Ocean as revealed by mitochondrial and nuclear genetic markers. *Marine Biology*, *156*, 821-834. https://doi.org/10.1007/s00227-008-1120-y

May-Collado, L., & Agnarsson, I. (2006). Cytochrome b and Bayesian inference of whale phylogeny. *Molecular Phylogenetics and Evolution*, *38*, 344-354. https://doi.org/10.1016/j.ympev.2005.09.019

McGowen, M. R. (2011). Toward the resolution of an explosive radiation: A multilocus phylogeny of oceanic dolphins (Delphinidae). *Molecular Phylogenetics and Evolution*, *60*(3), 345-357. https://doi.org/10.1016/j.ympev.2011.05.003

McGowen, M. R., Spaulding, M., & Gatesy, J. (2009). Molecular phylogenetics and evolution divergence date estimation and a comprehensive molecular tree of extant cetaceans. *Molecular Phylogenetics and Evolution*, *53*(3), 891-906. https://doi.org/10.1016/j.ympev.2009.08.018

Mesquita, R. A., Anzai, E. K., Oliveira, R. N., & Nunes, F. D. (2001). Avaliação de três métodos de extração de DNA de material parafinado para amplificação de DNA genômico pela técnica da PCR [Evaluation of three methods of DNA extraction from paraffin-embedded material for the amplification of genomic DNA by means of the PCR technique]. *Pesquisa Odontológica Brasileira*, *15*, 314-319. https://doi.org/10.1590/S1517-74912001000400008

Möller, L. M., Bilgmann, K., Charlton-Robb, K., & Beheregaray, L. (2008). Multi-gene evidence for a

new bottlenose dolphin species in southern Australia. *Molecular Phylogenetics and Evolution*, *49*, 674-681. https://doi.org/10.1016/j.ympev.2008.08.011

Moreno, I. B., Zerbini, A. N., Danilewicz, D., Santos, M. C. O., Simões-Lopes, P. C., Lailson-Brito, J., Jr., & 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. https://doi.org/10.3354/meps300229

Natoli, A., Birkun, A., Aguilar, A., Lopez, A., & Hoelzel, A. H. (2005). Habitat structure and the dispersal of male and female bottlenose dolphins (*Tursiops truncatus*). *Proceedings of the Royal Society B: Biological Sciences*, *272*, 1217-1226. https://doi.org/10.1098/rspb.2005.3076

Nosil, P., Funk, D. J., & Ortiz-Barrientos, D. (2009). Divergent selection and heterogeneous genomic divergence. *Molecular Ecology*, *18*, 375-402. https://doi.org/10.1111/j.1365-294X.2008.03946.x

Palumbi, S., Martin, A., Romano, S., McMillan, W. O., Stice, L., & Grabowski, D. (1991). *The simple fool's guide to PCR: Version 2.0*. Honolulu: University of Hawaii.

Perrin, W. F., Rosel, P. E., & Cipriano, F. (2013). How to contend with paraphyly in the taxonomy of the delphinine cetaceans? *Marine Mammal Science*, *29*, 567-588. https://doi.org/10.1111/mms.12051

Pichler, F. B., Robineau, D., Goodall, R. N., Meÿer, M. A., Olivarría, C., & Baker, C. S. (2001). Origin and radiation of Southern Hemisphere coastal dolphins (genus *Cephalorhynchus*). *Molecular Ecology*, *10*, 2215-2223. https://doi.org/10.1046/j.0962-1083.2001.01360.x

Quérouil, S., Silva, M. A., Freitas, L., Prieto, R., Magalhães, S., Dinis, A., . . . Santos, R. S. (2007). High gene flow in oceanic bottlenose dolphins (*Tursiops truncatus*) of the North Atlantic. *Conservation Genetics*, *8*, 1405-1419. https://doi.org/10.1007/s10592-007-9291-5

Rambaut, A. (2009). FigTree *v1.3.1*. Retrieved from http://tree.bio.ed.ac.uk

Robert, E. C. (2004). *MUSCLE*: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, *32*, 1792-1797. https://doi.org/10.1093/nar/gkh340

Ross, H. A., Lento, G. M., Dalebout, M. L., Goode, M., McLaren, G. E. P., Rodrigo, A. G., . . . Baker, C. S. (2003). DNA surveillance: Web-based molecular identification of whales, dolphins, and porpoises. *Journal of Heredity*, *94*, 111-114. https://doi.org/10.1093/jhered/esg027

Sheppard, W. S., Steck, G. J., & McPheron, B. A. (1992). Geographic populations of the medfly may be differentiated by mitochondrial DNA variation. *Experientia*, *48*, 1010-1013. https://doi.org/10.1007/BF01919155

Silva-Jr, J. M., Silva, F. J. L., & Sazima, I. (2005). Two presumed interspecific hybrids in the genus *Stenella* (Delphinidae) in the Tropical West Atlantic. *Aquatic Mammals*, *31*(4), 468-472. https://doi.org/10.1578/AM.31.4.2005.468

Stockin, K. A., Amaral, A. R., Latimer, J., Lambert, D. M., & Natoli, A. (2014). Population genetic structure and taxonomy of the common dolphin (*Delphinus* sp.) at its southernmost range limit: New Zealand waters. *Marine Mammal Science*, *30*, 44-63. https://doi.org/10.1111/mms.12027

Stramma, L., Fischer, J., & Reppin, J. (1995). The North Brazil undercurrent. *Deep-Sea Research Part I: Oceanographic Research Papers*, *42*(5), 773-795. https://doi.org/10.1016/0967-0637(95)00014-W

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). *MEGA6*: *Molecular Evolutionary Genetics Analysis* Version 6.0. *Molecular Biology and Evolution*, *30*, 2725-2729. https://doi.org/10.1093/molbev/mst197

Wan, Q. H., Wu, H., Fujihara, T., & Fang, S. (2004). Which genetic marker for which conservation genetics issue? *Electrophoresis*, *25*, 2165-2176. https://doi.org/10.1002/elps.200305922

Weir, C. R. (2006). First confirmed records of Clymene dolphin, *Stenella clymene* (Gray, 1850), from Angola and Congo, south-east Atlantic Ocean. *Ketos Ecology*, *41*(2), 297-300. https://doi.org/10.1080/15627020.2006.11407366