UNIVERSIDADE FEDERAL DO ESPÍRITO SANTO

CENTRO DE CIÊNCIAS HUMANAS E NATURAIS

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS

Padrões de diversidade, diferenciação e hibridação no gênero *Stenella*

Drienne Messa Faria

Vitória, ES

Julho, 2018

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Orientador (a): Profª. Drª. Ana Paula Cazerta Farro

Tese submetida ao Programa de Pós-Graduação em Ciências Biológicas (Biologia Animal) da Universidade Federal do Espírito Santo como requisito parcial para a obtenção do grau de Doutor em Biologia Animal.

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Drienne Messa Faria

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RESUMO

Stenella é um gênero da família Delphinidae composto por cinco espécies de distribuição circum-tropical, com exceção de *S. clymene* e *S. frontalis*, endêmicos do Oceano Atlântico. Esse gênero é parafilético, cuja classificação taxonômica e filogénética são consideradas confusas e, possivelmente, refletem a divergência recente (aproximadamente três milhões de anos) entre as suas espécies e eventos de hibridação. Sendo assim, essa tese teve como objetivos testar a hipótese de ocorrência de hibridação entre as espécies do gênero *Stenella* e avaliar a diversidade e diferenciação genética de uma das espécies, *Stenella longirostris longirostris*. Com a utilização de marcadores moleculares mitocondriais foram demonstrados fortes indícios de hibridação entre as espécies *S. clymene* e *S. coeruleoalba* o que reforça que esse processo pode ser um dos responsáveis pela taxonomia complexa do grupo. Não foram encontrados sinais de mistura para as espécies *S. longirostris, S. attenuata* e *S. frontalis*, sendo observados clados monofiléticos para essas espécies. Foi demonstrado que indivíduos de *S. longirostris* do Arquipélago de Fernando de Noronha (associados-a-ilhas) constituem uma população com baixa diversidade genética e alto isolamento genético das demais populações avaliadas. Além dessa população, foi evidenciada a presença de outra população formada por indivíduos amostrados ao longo da costa brasileira (não-associados-a-ilhas). Foi constatada estruturação genética entre populações de *S. longirostris* de diferentes localidades ao longo do mundo. Foi evidenciado também que as duas populações do sudoeste do sul do Oceano Atlântico são altamente diferenciadas de todas as populações de outras localidades do mundo, especialmente das do Oceano Pacífico. Além disso, ficou evidente a baixa diversidade genética e alta diferenciação dos golfinhos-rotadores das ilhas do Avaí. Estes resultados sugerem uma dinâmica de metapopulação com dispersão ou intercâmbio periódico a longa distância entre populações geograficamente distantes de diferentes bacias oceânicas, e, alta fidelidade de sítio a habitats insulares adequados.

Palavras-chave: Delphinidae, introgressão, variabilidade genética, estruturação genética, *Stenella longirostris*.

ABSTRACT

Stenella is a genus of the Delphinidae family composed by five species of cricum-tropical distribution, with the exception of *S. clymene* and *S. frontalis*, endemic to the Atlantic Ocean. This genus is paraphyletic, whose taxonomic classification and phylogenetic relationships are considered confusing and possibly reflect the recent divergence (about three million years) and hybridization events between species. The aim of this thesis was to test the hypothesis of hybridization among species of the genus *Stenella* and to evaluate the genetic diversity and differentiation of one species, *Stenella longirostris*. Using mitochondrial molecular markers, strong evidence of hybridization between S*. clymene* and *S. coeruleoalba* species has been demonstrated, which reinforces that this process may be one of those responsible for the complex taxonomy of the group. No signs of mixing were found for *S. longirostris, S. attenuata and S. frontalis*, and monophyletic clades were observed for those species. It was shown that individuals of *S. longirostris* from the Fernando de Noronha Archipelago (islands-associated) compose a population with low genetic diversity and high genetic isolation from other populations of the world. Besides this population, the presence of another population formed by individuals sampled along the Brazilian coast (non-associated-islands) was displayed. It was also shown that the two populations of southwestern Atlantic Ocean are highly differentiated from all populations in other parts of the world, especially the Pacific Ocean. In addition, it was evident the low genetic diversity and high differentiation of the spinner dolphins of the Hawaiian Islands. These results suggest a metapopulation dynamics with dispersion or periodic interchange among populations geographically distant from different ocean basins, and, high site fidelity to suitable island habitats.

Key-words: Delphinidae, introgression, genetic structuring, genetic variability, *Stenella longirostris*.

Capítulo 1

Phylogenetic pattern of Inter-species hybridization among dolphins of the genus *Stenella* in the Atlantic Ocean

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Phylogenetic pattern of Inter-species hybridization among dolphins of the genus *Stenella* in the Atlantic Ocean

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Abstract

Natural hybridization has been demonstrated as a common process reported for many taxa. Few examples involve dolphins, particularly in the South Atlantic Ocean. Recent studies have demonstrated that this process can cause genetic homogenization and, even, extinction of some species. In contrast, however, hybridization has also been shown to increase the genetic diversity of populations and even be involved in the formation of new species. In this study we employed three genes of the mitochondrial DNA to investigate the occurrence of hybridization and introgression among five species of cetaceans of the genus *Stenella*, all sympatric in the Southwest Atlantic Ocean. We present strong evidences of two hybrids between *S. clymene* and *S. coeruleoalba*, along the Brazilian coast. We also found signs of hybrids between *S. frontalis* and *S. attenuata* in the Northeast Atlantic Ocean, and between *S. frontalis* and *S. longirostris* in the Northwest Atlantic Ocean. All of these putative hybrids were morphologically identified as one species by specialists but exhibited the mitochondrial D-loop identity of another species. Our results demonstrated that ongoing gene flow through hybridization is occurring among species of the genus *Stenella* reinforcing that this process could be one of the reasons for the confusing taxonomy and difficulties in elucidating phylogenetic relationships within this group.

Keywords: Delphinidae, hybrid, introgression*, spinner dolphins, Stenella*.

Introduction

The consequences of hybridization on the evolution of living organisms have been extensively discussed among evolutionists (Arnold 1992; Harrison 1993; Dowling & Secor 1997; Barton 2001; Fitzpatrick 2004). Hybridization can cause genetic homogenization and, even, extinction of some species (Rhymer & Simberloff 1996; Mooney & Cleland 2001; Wolf *et al.* 2001). However, in some cases, hybridization may increase the genetic diversity of populations, providing them with greater adaptability to environmental changes (Buerkle *et al.* 2000). In some cases, hybridization may also lead to the formation of new species, capable of exploring new niches (Baskett & Gomulkiewicz 2011).

Hybridization and incomplete lineage sorting are possible causes for the confusing taxonomy and difficulties in elucidating phylogenetic relationships of a group, such as Cetartiodactyla (Amaral *et al.* 2012). Cetartiodactyla is the order of marine mammals which diverged from their terrestrial ancestors, approximately 53 Ma (Árnason *et al.* 2004), and whose evolution is characterized by events of rapid adaptive radiation. These marine mammals exhibit characteristics that may potentiate the production of viable wild hybrids, such as: prominent karyotype uniformity (Árnason *et al.* 1991); genome permeability; social structure and complex behaviors that contribute to reproductive isolation (Amaral *et al.* 2014). Hybridization has been documented for marine mammals in captivity and in nature with the use of morphological data (Sylvestre & Tasaka 1985; Berubé 2002; Zornetzer & Duffield 2003).

Despite, there are difficulties in the detection of hybridization in mammals, which arise from the occurrence of cryptic hybrids, which may have exactly the same morphotype of one of the parental species, so their

identification is only possible with the use of molecular tools (Mallet 2005). The mitochondrial DNA (mtDNA) consist in an effective molecular marker in the identification of species, reciprocal hybrids and the direction of hybridization events in wild environments (Gunnel *et al.* 2008; Metcalf *et al.* 2008; Broughton *et al.* 2011). Mitochondrial DNA is maternally inherited, has high mutational rates and it is easy to isolate and characterize (Avise 1986; Moritz *et al.* 1987). Molecular evidence of wild hybrids has been presented for several species of Cetartiodactyla (Árnason *et al.* 1991; Spilliaert *et al.* 1991; Bérubé & Aguilar 1998; Baird *et al.* 1998; Silva-Jr *et al.* 2005; Miralles *et al.* 2016) including *Stenella* dolphins (Amaral *et al.* 2014).

Stenella is one of the most abundant and widely distributed genera of the Delphinidae family and is comprised of five species: *Stenella attenuata*, *S. clymene*, *S. coeruleoalba*, *S. frontalis* and *S. longirostris*. *S. attenuata, S. coeruleoalba and S. longirostris* are pantropically distributed, occurring in all oceans of the world, in contrast, *S. clymene* and *S. frontalis* are restricted to the waters of the Atlantic Ocean. Molecular and morphometric data of specimens from North Atlantic, Pacific and Indian oceans demonstrated that the species *S. clymene* may be the result of historic natural hybridization between *S. coeruleoalba* and *S. longirostris* (Amaral *et al*. 2014). All five species are sympatric off the Brazilian coast in the Southwest Atlantic Ocean, allowing the possibility of hybridization between one or more pairs of *Stenella* species in this region. Observation data and underwater photographs of groups of dolphins in the waters around Fernando de Noronha Archipelago, 545 km off the Brazilian coast, indicated the occurrence of two possible hybridization events; one

between *S. longirostris* and *S. attenuata*; and another between *S. longirostris* and *S. clymene* (Silva-Jr *et al.* 2005).

Identification of the *Stenella* species has typically been based on external morphology, however, accurate identification can be difficult due to their close evolutionary relationships (Perrin *et al.* 1981). Molecular identification using mtDNA has been used extensively to identify many cetacean species. Databases, such as the Barcode of Life Data System (Bold) (Ratnasingham & Hebert 2007), GenBank (Benson *et al.* 2013) and DNA Surveillance (Ross *et al.* 2003), contain sequences from all known cetacean species and can be used to help the molecular identification of species.

Several studies have demonstrated uncertainty in the phylogenetic classification of *Stenella* species using both morphologic and molecular methods (mitochondrial and nuclear DNA) leading to confusion over the recognition of these species at the taxonomic level of species, and the paraphyly of this genus (Perrin *et al.* 1987; LeDuc *et al.* 1999; Caballero *et al.* 2008; Kingston *et al.* 2009; McGowen *et al.* 2009; Amaral *et al.* 2012; Steeman *et al.* 2009; Xiong *et al.* 2009). Understanding hybridization within this genus is important to elucidate taxonomic uncertainties of species that have recently diverged and to assist in delineating conservation strategies of populations showing signs of mixing with other taxa. In this study we have used three regions of mtDNA to investigate the hypothesis of some genetic mixture among the species of *Stenella* that occur in sympatry off the Brazilian coast.

Materials and methods

Sampling and DNA extraction

A total of 80 tissue (skin or muscle) samples were collected from the five species of *Stenella: Stenella attenuata* (N = 4), *Stenella coeruleoalba* (N = 9), *Stenella clymene* (N = 13), *Stenella frontalis* (N = 14), *Stenella longirostris* (N = 40) along the Brazilian coast and in the waters around Feranando de Noronha Archipelago. Free-ranging dolphins (N = 45) were sampled using skin swabbing or biopsy methods. Also, samples (N = 35) were collected from stranded animals (Figure 1; Supplementary Appendix 1). All these animals were morphologically identified by specialists.

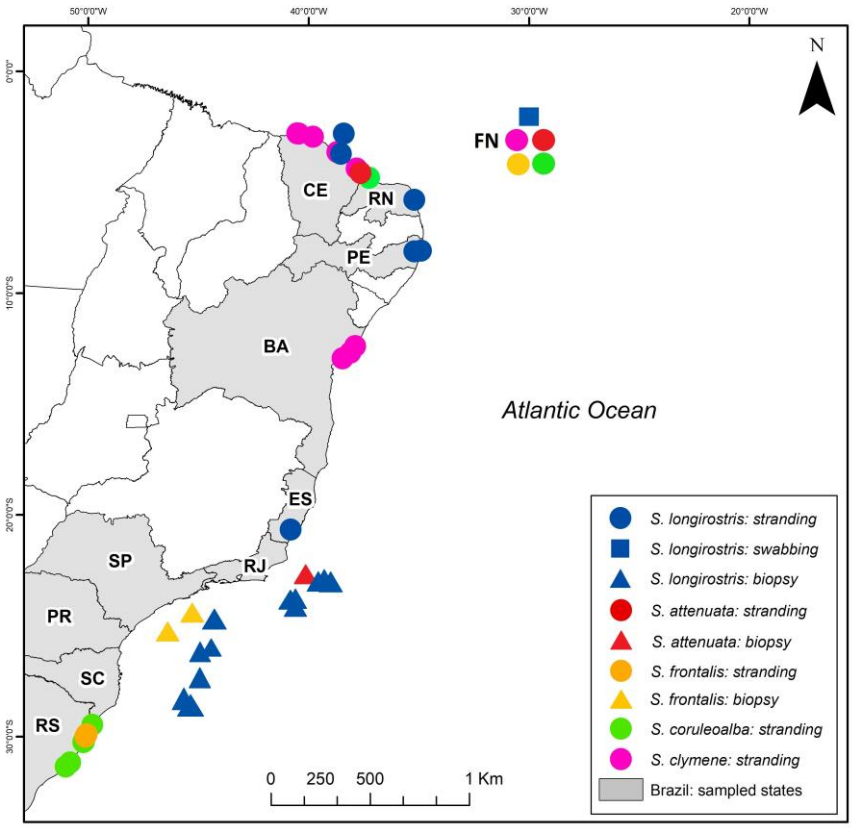

Figure 1 Locations where *Stenella* samples were obtained on the Brazilian coast.

Genomic DNA from muscle samples was extracted following a salt buffer protocol (Bruford *et al.* 1992) and from skin samples using Chelex resin (SIGMA) according to manufacturer's instructions.

Published sequences

For a plylogentic worldwide comparison we included sequences of *Stenella* available in GenBank, that presented the localization information (www.ncbi.nlm.nhi.gov/Genbank): 708 sequences of D-loop (*S. attenuata*, N= 156; *S. clymene*, N= 14; *S. coeruleoalba*, N= 17; *S. frontalis*, N= 268; *S. longirostris*, N= 253); 90 sequences of Cytochrome b (*S. attenuata*, N= 03; *S. clymene*, N= 02*; S. coeruleoalba*, N= 02; *S. frontalis*, N= 03; *S. longirostris*, N= 80); and 31 sequences of Cytochrome oxidase subunit I (*S. attenuata*, N= 05; *S. clymene*, N= 03; *S. coeruleoalba*, N=15; *S. frontalis*, N= 03; *S. longirostris*, N= 05) (Supplementary Appendix 2).

mtDNA sequences

Three mitochondrial DNA (mtDNA) genes were analyzed: the control region (D-loop), and the coding genes of cytochrome b (Cyt b) and cytochrome oxidase subunit I (Cox I). D-loop was amplified with the following primers: KRAdLp 1.5 t-pro (Andrews *et al.* 2006) and dLp5 (Pichler *et al*. 2001). Polymerase Chain Reactions (PCR) were performed in 12.5 µl total volumes containing containing 10-100 ng of the extracted DNA, 10 X Reaction Buffer, 0.2 µM of each dNTP, 2.0 mM $MgCl_2$, 0.5 units of *Taq* DNA polymerase and 0.2 µM of each primer. Thermocycling conditions were: 95ºC for 1 min; followed by

40 cycles of 94ºC for 30 s, 54 ºC for 30 s, and 72 ºC for 30 s; followed by a final 72ºC extension for 15 minutes. Cyt b was amplified with the following primers: L14724 (Palumbi *et al.* 1991) and H15387 (Rosel *et al.* 1994). The amplification reactions were performed with a final volume of 12.5 µl, containing 10-100 ng of the extracted DNA, 10X PCR buffer, 0.15 µM each dNTP, 1.5 mM $MgCl_2$, 1.25 units of *Taq* DNA polymerase and 0.3 µM of each primer. Thermocycler conditions were as follows: 95 ºC for 30 s, followed by 35 cycles of 94 ºC for 30 s, 45 ºC for 30 s and 72 ºC for 7 min; final extension of 72 ºC for 7 minutes. Cox I was amplified with the following primers: COX1F and COX1R (Amaral et al. 2007). Amplification reactions were performed with a final volume of 12.5 µl, containing 10-100 ng of extracted DNA, 10X PCR buffer, 0.16 µM of each dNTP, 1.52 mM $MgCl_2$, one unit of *Taq* DNA polymerase (INVITROGEN) and 0.3 µM of each primer. Thermocycler conditions were as follows: 94 °C for 2 min, followed by 35 cycles of 94 ºC for 45 s, 52 ºC for 45 s and 72 ºC for 1 min; final extension of 72 ºC for 8 minutes. The amplified fragments were visualized on agarose gel (1%) stained with Gelred and photodocumented. The purification of the amplified bands was performed with the enzyme ExoSap-IT (USB Corporation). Purified products were sequenced in both forward and reverse directions using an ABI 310 automated sequencer (Applied Biosystems). Sequencing reactions had a final volume of 10 µL containing 5.06 µL of H_2O, 2.5 µL of 5X Buffer, 0.8 µL of BigDye Terminator CycleSequencing (Applied Biosystems), 0.64 µL of primer and 1.0 µL of PCR product. The conditions of the thermal cycler were as follows: 30 cycles of 96 ºC for 30 s; 55 ºC for 15 s; 60 ºC for 4 minutes.

The software MEGA 6.06 (Tamura *et al*. 2013) was used to edit and align the sequences using the algorithm Muscle, and, to estimate genetic distances among species using Tamura–Nei distance model and 1000 bootstrap replications, including calculation of standard errors. Sequences were compared to BOLD, GenBank and DNA-Surveillance databases to confirm species identity. The diversity indexes for each species were estimated using Arlequin 3.5.2.2 (Excoffier & Lischer 2010). Genealogical relationships among the haplotypes were inferred through Median-Joining analysis as implemented in the Network v 4.6.1.0 program (Bandelt *et al.* 1999).

Phylogenetic analyses

Phylogenetic analyses were conducted using Beast v1.7.4 software package (Drummond *et al.* 2012): 100 million MCMC generations, sampling every 10.000 generations, Yule speciation model. As outgroups, complete mitochondrial genome sequences published on GenBank of three species*:* *Steno bredanensis* (AJF339982), *Globicephala melas* (HM060334) and *Phocoena phocoena* (AJ554063) were used. The best evolutionary model was indicated by the Akaike Information Content (AIC) test implemented in the program jModeltest v2.1.6 (Posada 1998). The program TreeAnnotator v1.7.4 (Drummond *et al.* 2012) was used to summarize the trees obtained into a single tree that best represents the posterior distribution, with a maximum clade credibility and, a burnin value of 1000 and posterior probability limit of 0.5. The program FigTree v1.4.2 (Rambaut 2014) was used to produce and edit the phylogenetic tree figures.

Results

Stenella dolphins from Southwest Atlantic Ocean

All the species exhibited moderate to high levels of variability for all genes analyzed (Table1). Haplotype diversity was highest in *S. coeruleolba* for D-loop, Cyt-b, and concatenated sequence, and highest in *S. clymene* for Cox I and concatenated sequence; lowest in *S. frontalis* for D-loop and Cox I, and in *S. longirostris* for Cyt-b, and in *S. attenuata* for concatenated sequence. Nucleotide diversity was highest in *S. coeruleolba* for D-loop and Cyt-b, and highest in *S. clymene* for Cox I and concatenated file; lowest in *S. frontalis* for D-loop, Cyt-b and concatenated file, and, in *S. longirostris* for Cox I (Table 1).

Table 1 Sample size (N), number of haplotypes (Nh), polymorphic sites (Ps), haplotype diversity (h), and nucleotide diversity (π) of species for sequences of mtDNA. Bp means base pair length**.**

	D-loop (310 bp)					**Cox I (621 bp)**				
	N	Nh	Ps	*h*	*π*	N	Nh	Ps	*h*	*π*
S. attenuata	4	3	11	0.8333	0.020234	4	3	22	0.8333	0.017982
S. clymene	13	12	22	0.9872	0.021062	13	9	43	0.9231	0.019695
S.coeruleoalba	9	9	25	1	0.027244	9	5	16	0.8611	0.008678
S. frontalis	14	4	7	0.7802	0.009968	14	5	13	0.7692	0.006388
S.longirostris	40	14	23	0.7885	0.013410	40	15	14	0.8987	0.005413
	Cyt b (585 bp)					**D-loo + Cyt b + Cox I (1516 bp)**				
	N	Nh	Ps	*h*	*π*	N	Nh	Ps	*h*	*π*
S. attenuata	4	3	11	0.8333	0.009687	4	3	43	0.8333	0.014952
S. clymene	13	9	18	0.9359	0.007015	13	13	82	1.0000	0.015293
S.coeruleoalba	9	8	28	0.9722	0.013295	9	9	69	1.0000	0.014292
S. frontalis	14	6	10	0.8352	0.005354	14	9	30	0.9121	0.006734
S.longirostris	40	15	17	0.7692	0.006623	40	29	54	0.9628	0.007542

Genetic distances revealed values above 2% for almost all comparisons between species. Values below 2% were found between: *S. clymene* X *S. coeruleoalba* for Cyt b, Cox I and concatenated sequence; *S. clymene* X *S. frontalis* for Cyt b; *S. coeruleoalba* X *S. frontalis* for Cyt b and Cox I (Table 2).

Table 2 Genetic distances between species for D-loop, Cyt b, Cox I and concatenated sequence (D-loop + Cyt b+ Cox I) bellow diagonal, and, standard errors (SEs) upper diagonal.

D-loop (310 bp)					
	S. attenuata	*S. clymene*	*S.coruleoalba*	*S. frontalis*	*S.longirostris*
S. attenuata		0,015	0,013	0,014	0,012
S. clymene	83,00%		0,007	0,009	0,010
S.coeruleoalba	6,86%	3,34%		0,008	0,009
S. frontalis	6,69%	3,89%	3,38%		0,010
S.longirostris	5,45%	4,80%	4,11%	4,12%	
Cyt b (585 bp)					
	S. attenuata	*S. clymene*	*S.coruleoalba*	*S. frontalis*	*S.longirostris*
S. attenuata		0,008	0,008	0,008	0,009
S. clymene	3,60%		0,003	0,005	0,009
S.coeruleoalba	3,84%	1,40%		0,004	0,009
S. frontalis	3,47%	1,76%	1,80%		0,008
S.longirostris	4,38%	4,51%	4,63%	3,71%	
Cox I (621 bp)					
	S. attenuata	*S. clymene*	*S.coruleoalba*	*S. frontalis*	*S.longirostris*
S. attenuata		0,007	0,007	0,007	0,007
S. clymene	4,01%		0,003	0,005	0,006
S.coeruleoalba	3,76%	1,96%		0,004	0,006
S. frontalis	3,97%	2,36%	1,79%		0,006
S.longirostris	4,13%	3,41%	3,01%	2,90%	
D-loop + Cyt b + Cox I (1516 bp)					
	S. attenuata	*S. clymene*	*S.coruleoalba*	*S. frontalis*	*S.longirostris*
S. attenuata		0,005	0,005	0,005	0,005
S. clymene	4,69%		0,002	0,003	0,005
S.coeruleoalba	4,44%	1,99%		0,003	0,005
S. frontalis	4,32%	2,44%	2,14%		0,004
S.longirostris	4,49%	4,07%	3,85%	3,44%	

Haplotype networks for all three mtDNA genes showed a clear separation of *S. attenuata*, *S. frontalis* and *S. longirostris* in different haplogroups with at least five mutational steps distinguishing them (Figure 2). In contrast, haplotypes of *S. coeruleoalba* and *S. clymene*, were clustered together. D-loop haplotype network showed one haplotype (D13) represented by two specimens (Scl33, Sco01) shared between *S. clymene* and *S. coeruleoalba,* one *S. clymene* haplotype (D7) present in one specimen (Scl10) nested within the *S. coeruleoalba* group, and, one *S. coeruleoalba* haplotype

(D17) present in one specimen (Sco03) nested with *S. clymene* group. One Cyt b haplotype (Cyt1) and one Cox I haplotype (Cox I12), both present in five specimens (Scl33, Scl34, Scl35, Sco1, Sco03), were shared between *S. coeruleoalba* and *S. clymene*. The Cox I haplotype network also showed that two *S. clymene* haplotypes (Cox I8, present in Scl10 specimen, and Cox I 6 presente in Scl08 specimen) were very distant from the majority of haplotypes of this species (Figure 2).

Bayesian phylogenetic cladograms showed that the topologies were not similar for the three markers analyzed. Clades representing all species were strongly supported for all markers evaluated, with exception of *S. coeruleoalba* for D-loop (Figures 3, 4 and 5). Trees for all markers showed a monophyletic clade for *S. frontalis* and *S. longirostris*. Bayesian phylogenetic cladograms for caoncatenated sequence reflected the same pattern observed for each gene analysed alone (Figure 6). The best evolutionary model indicated by J-Modeltest for D-llop was GTR+G, for Cyt b was GTR+G, for Cox I was HKY+I+G, and, for coancatenate file was GTR+I+G.

The phylogenetic analyses presented four “misplaced” specimens for all markers evaluated, i.e. animals whose morphological identification did not match their respective mtDNA clade. Two specimens of *S. coeruleoalba* (Sco01 and Sco03) always placed in *S. clymene* clade; two *S. clymene* specimens (Scl08 and Scl10) always misplaced in other species clades; one *S. attenuata* specimen (Sat01) placed together with *S. clymene* specimens for Cox I gene (Figures 3, 4, 5).

All these misplaced specimens were stranded dolphins morphologically identified by experts in cetaceans. DNA of all these individuals were compared

to GenBank and DNA-Surveillance databases and showed that mtDNA identity did not match with morphological identity: Sco01 and Sco03 for all three genes; Sat01 for Cox I (Supplementary Appendix 3).

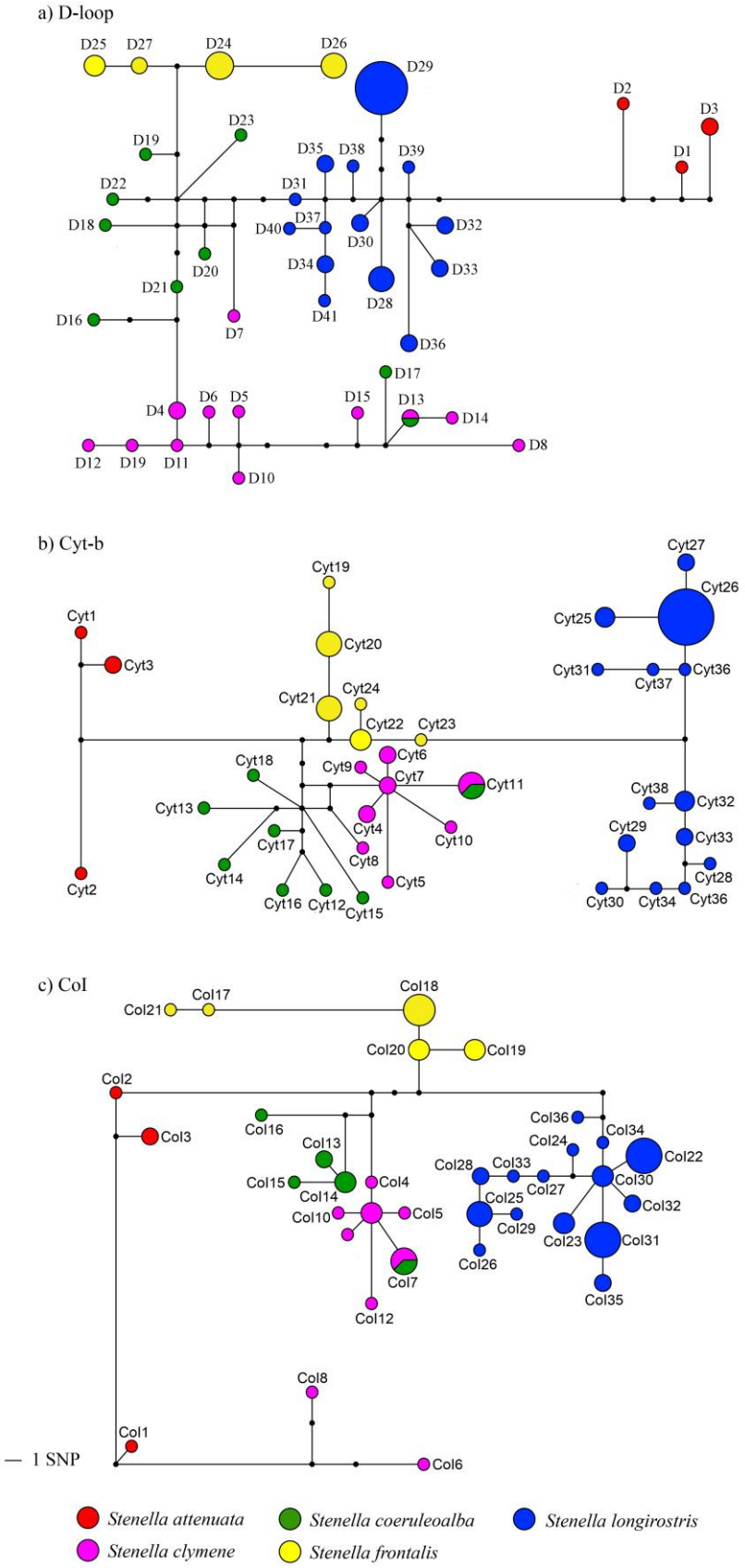

Figure 2 Median-Joining network of mtDNA marker of Southwest Atlantic *Stenella* dolphins: a) D-loop; b) Cyt b; c) Cox I. Each circle corresponds to a haplotype, and its size is proportional to its frequency. Black circles indicate missing or intermediate haplotypes. Lengths of lines connecting haplotypes are proportional to the number of substitutions between haplotypes.

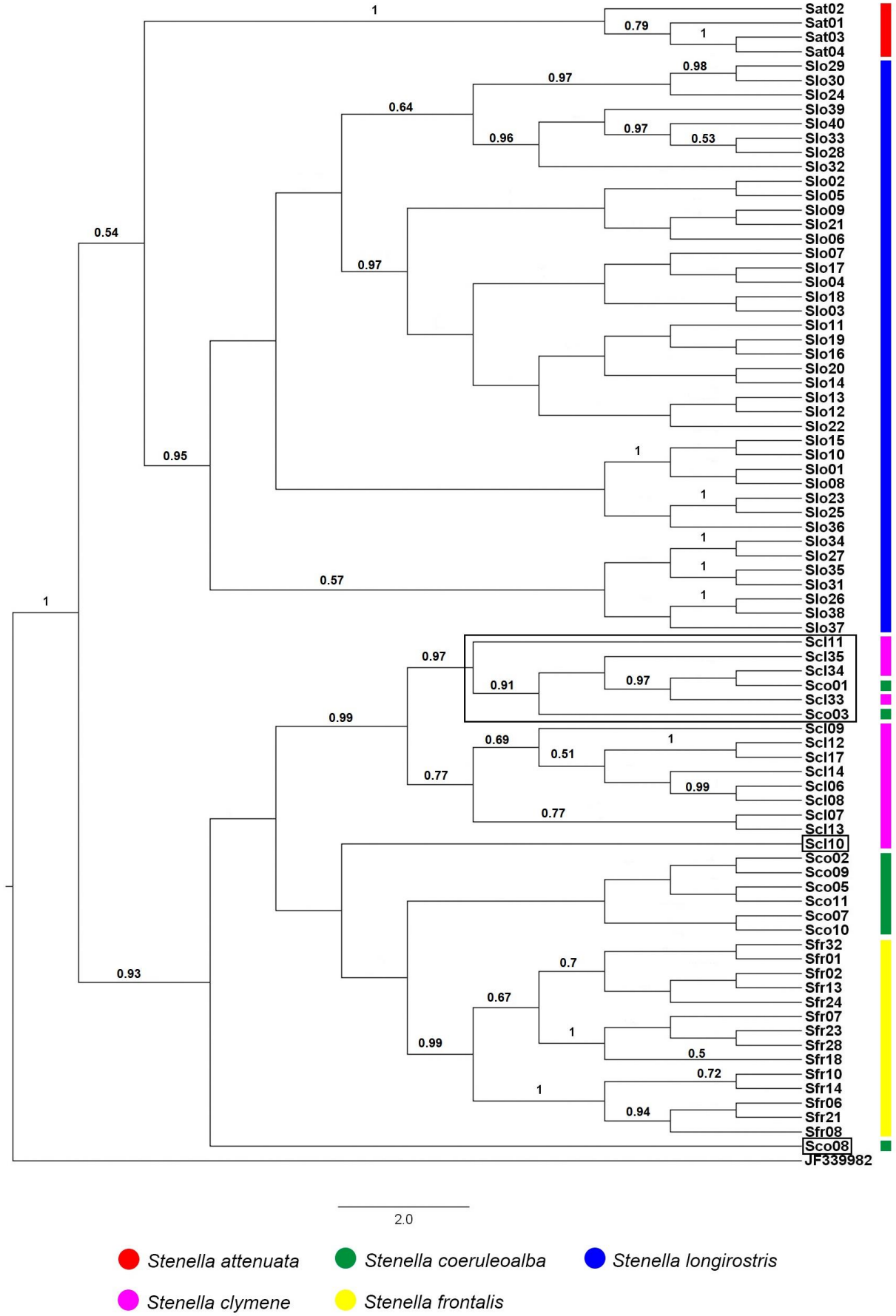

Figure 3 Bayesian cladogram generated by Beast for D-loop marker in Southwest Atlantic *Stenella* specimens. Posterior probability values greater than 0.5 are above nodes. Black boxes indicate misplaced specimens.

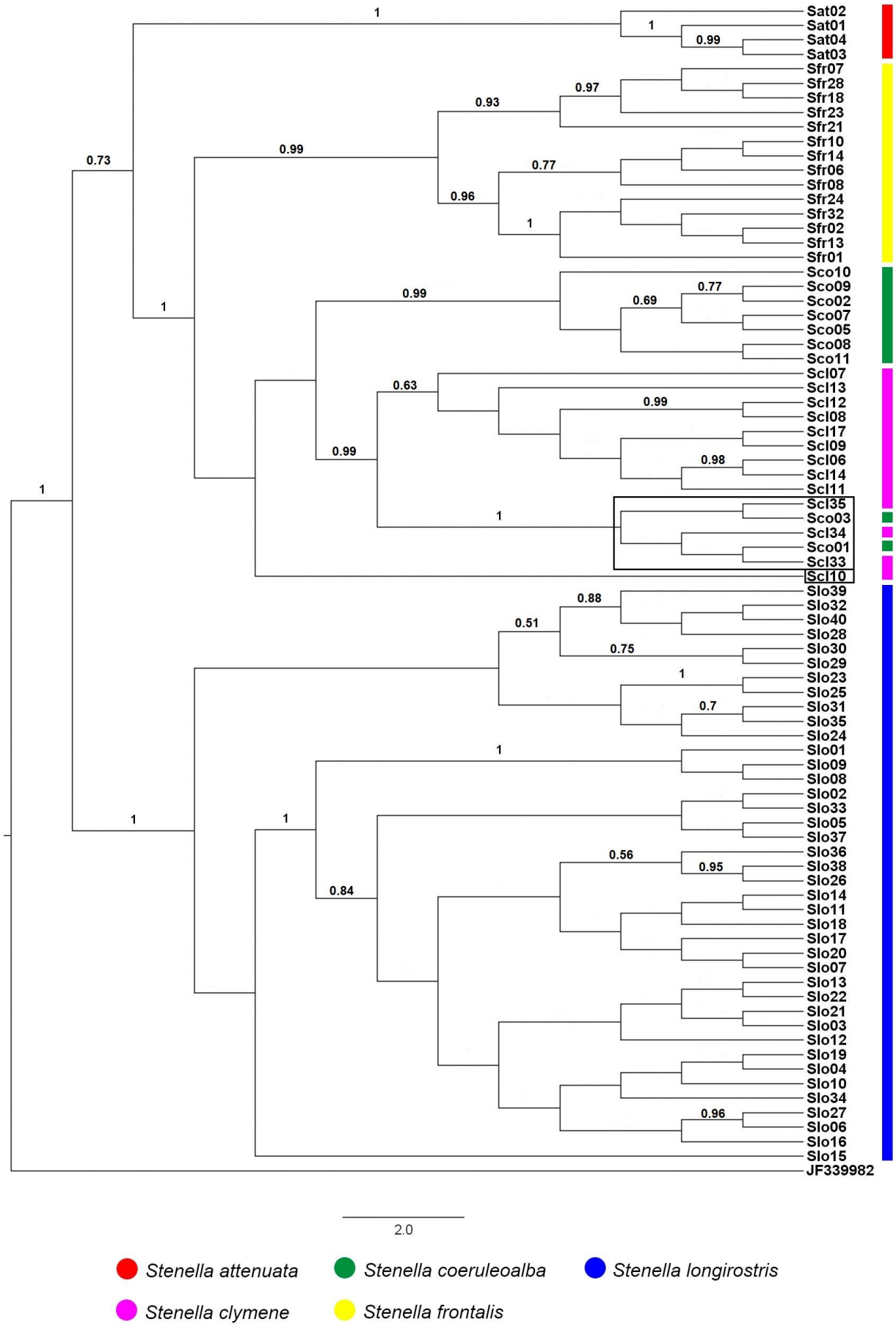

Figure 4 Bayesian cladogram generated by Beast for the Cyt-b gene in Southwest Atlantic *Stenella* specimens. Posterior probability values greater than 0,5 are above nodes. Black boxes indicate misplaced specimens.

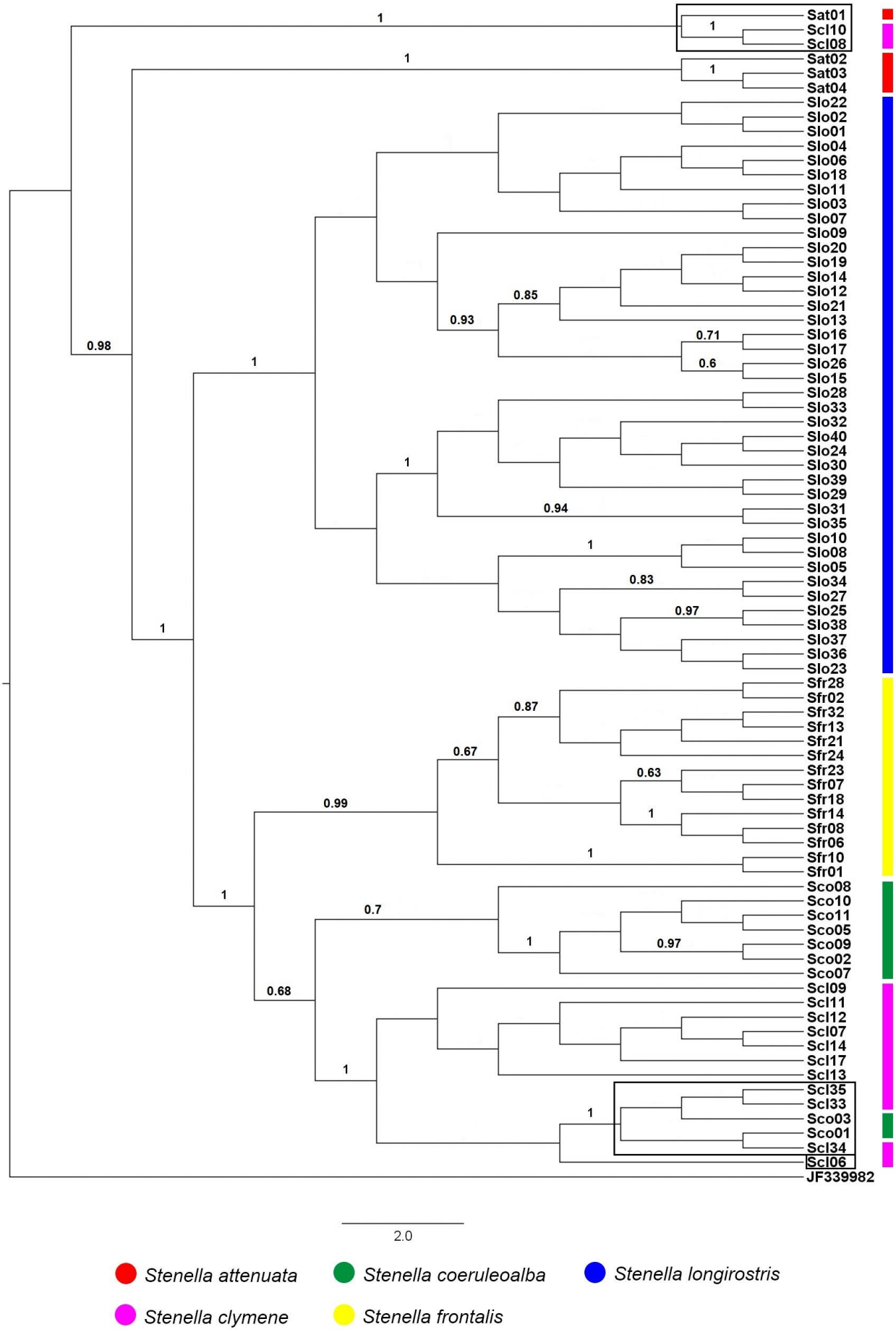

Figure 5 Bayesian cladogram generated by Beast for the Cox I gene in Southwest Atlantic *Stenella* specimens. Posterior probability values greater than 0,5 are above nodes. Black boxes indicate misplaced specimens.

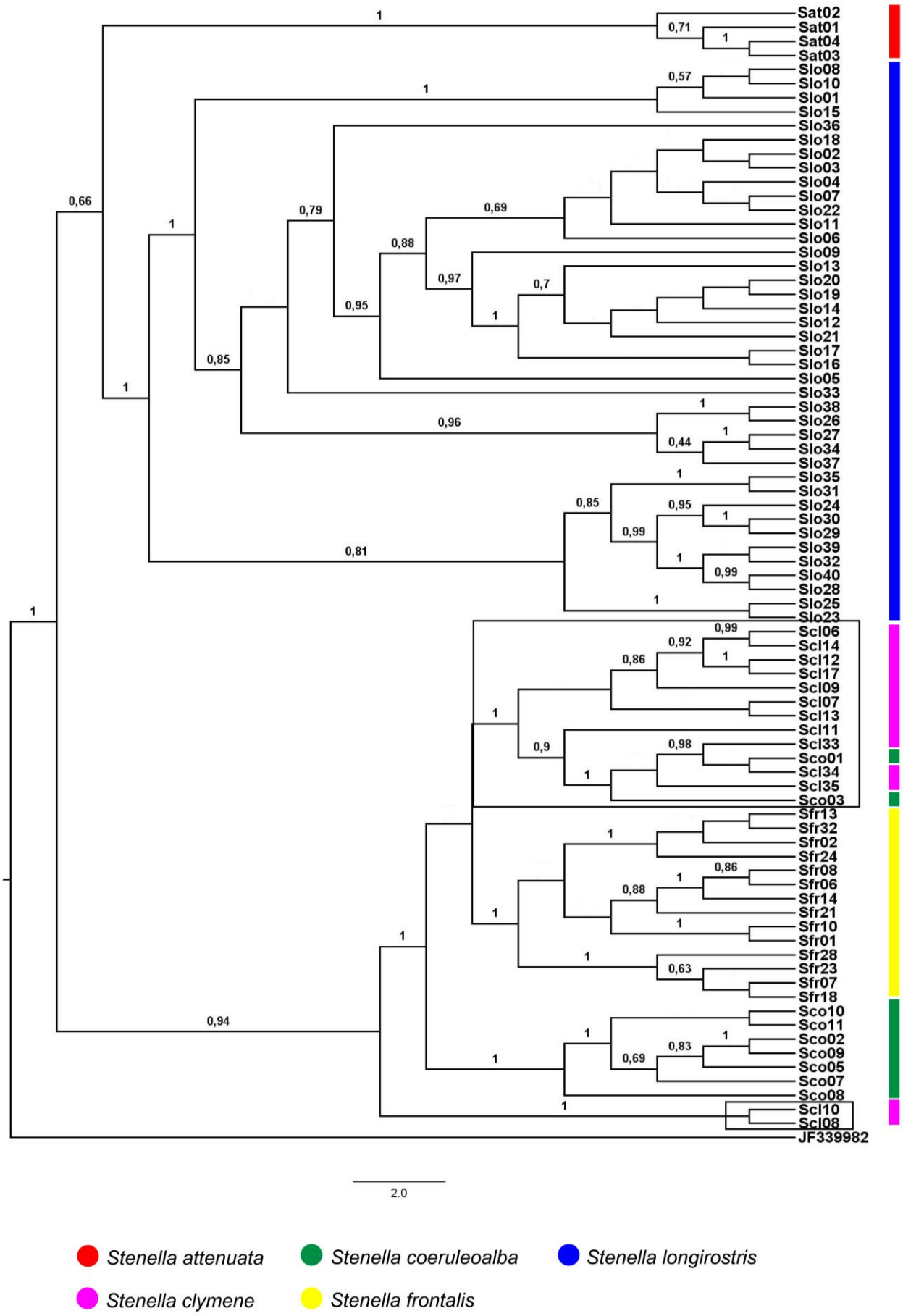

Figure 6 Bayesian cladogram generated by Beast concatenated sequence in Southwest Atlantic *Stenella* specimens. Posterior probability values greater than 0,5 are above nodes. Black boxes indicate misplaced specimens**.**

Ocean basins comparisons

Bayesian phylogenetic comparisons among ocean basins used at total of 1,069 sequences, representing all three oceans (Atlantic, Pacific and Indian). For the sequences downloaded from GenBank we assumed the morphological species identity described in the published paper, and, for the sequences of this study we assumed the morphological species identification made by experts in cetaceans. The names of the haplotypes used here and in the cladograms follow the morphological species identity. The molecular identity (D-loop, Cox I or Cyt-b identity) was determined by comparing the sequences with BOLD, GenBank and *DNA-Surveillance* databases.

For Cyt-b, 170 sequences with a consensus length of 331 bp were used for phylogenetic analyses. 83 haplotypes were identified. The best evolutionary model indicated by J-Modeltest was GTR+G. Well supported clades were identified for *S. attenuata*, *S. frontalis* and *S. longirostris*, but not for *S. clymene* and *S. coeruleoalba* (Figure 7). One haplotype (CYTB_10) in the *S. clymene* clade was represented by sequences from two different morphologically identified species: *S. clymene* (Scl33, Scl34, Scl35) and *S. coeruleoalba* (Sco01, Sco03) (Figure 7). Of those specimens Sco01, Sco03 displayed D-loop identity of *S. clymene* not matching with morphological identity of *S. coeruleoalba*. Four haplotypes (CYTB_07, CYTB_19, CYTB_14 and CYTB_15) did not fall within the main clade for their respective morphologically identified species (Figure 7, 12; Supplementary Appendix 3).

For Cox I, 111 sequences with a consensus length of 613 bp were used for phylogenetic analyses. A total of 57 haplotypes were resolved. The best evolutionary model indicated by J-Modeltest was GTR+I+G. Clades were well

supported for *S. attenuata*, *S. frontalis* and *S. longirostris*. As with Cyt-b, clades of *S. clymene* and *S. coeruleoalba* were not well supported, however, they form a combined clade with some support. One haplotype (COXI_15) nested within the *S. clymene* clade and was represented by sequences of both *S. clymene* (Scl33, Scl34, Scl35) and *S. coeruleoalba* (Sco01, Sco03). Of those, Sco01 and Sco03 displayed d-loop identity of *S. clymene.* The same specimens showed the same pattern when analyzed using Cyt-b. Three *S. coeruleoalba* haplotypes (COXI _31, COXI _26 and COXI _27) formed a separate clade outside of the main *S. coeruleoalba*. The haplotype 27 (COXI _27) has the D-loop identity of *S. clymene*, not matching with morphological identity of *S. coeruleoalba*. Two *S. clymene* haplotypes (COXI _09 and COXI _11) that displayed D-loop identity matching with morphological identity were grouped with one *S. attenuata* haplotype (COXI _01) (whose D-loop identity of *S. clymene* did not match with morphological identity) creating an external clade with no support. Two haplotypes of *S. attenuata*, four of *S. coeruleoalba*, and, four of *S. longirostris* were represented by sequences from different oceans (Figure 8 and 12; Supplementary Appendix 3).

Phylogenetic analyses of D-loop involved 788 sequences with a consensus length of 331 bp. A total of 444 haplotypes were resolved. The best evolutionary model indicated by J-Modeltest was GTR+I+ G. Well supported clades were identified for *S. attenuata*, *S. frontalis* and *S. longirostris*, but, as with the previous two genes (Cox I and Cyt b), not for *S. clymene* and *S. coeruleoalba* (Figure 9).

One haplotype (DLOOP_120) was represented by sequences from specimen identified in this publication as *S. clymene* (Scl33, GQ504147) and in

the field as *S. coeruleoalba* (Sco01). This haplotype was nested within a well-supported clade that contained the majority of *S. clymene* sequences. DLOOP _137(Sco03) from a specimen identified in the field as *S. coeruleoalba* was also nested within this clade. Three haplotypes of *S. clymene* (DLOOP_114, DLOOP_134, DLOOP_129) and two of *S. coeruleoalba* (DLOOP_155, DLOOP_147) were grouped in a clade with moderate support. Of those DLOOP_155 and DLOOP_147 displayed D-loop identity as *S. clymene* on DNA - Surveillance (Figure 10 and 13; Supplementary Appendix 3).

Stenella attenuata sequences resolved into two well supported clades, within which several haplotypes represented by specimens of *S. frontalis* were identified. The majority of these *S. frontalis* haplotypes were from specimen sampled in the Northeast Atlantic Ocean (DLOOP_200, DLOOP_209, DLOOP_177, DLOOP_223, DLOOP_188, DLOOP_214), one was sampled in the Northeast Pacific Ocean (DLOOP_199). All these sequences displayed D-loop identity as *S. attenuata*. Three haplotypes, represented by specimens of two different species, *S. attenuata* and *S. frontalis,* were identified: DLOOP_01, DLOOP_03 and DLOOP_93. The haplotype 01 (DLOOP_01) was represented by seven sequences, of those, five displayed D-loop identity as *S. attenuata* and the other two as *S. frontalis.* The haplotype 03 (DLOOP_03) was represented by ten sequences, of those seven displayed D-loop identities as *S. attenuata* and three as *S. frontalis*. The haplotype 93 (DLOOP_93) was represented by two sequences, one displayed D-loop identity as *S. attenuata* and the other as *S. frontalis* (Figure 11, 12 and 13; Supplementary Appendix 3).

In *S. longirostris* clade it was possible to identify that DLOOP_ 253 was represented by sequences from Northwest Atlantic Ocean of two different

species: KC204736 whose morphological identity of *S. frontalis* did not match with the D-loop identity of *S. longirostris;* and, GQ504169 whose morphological identity of *S. longirostris* did not match with the D-loop identity of *S. frontalis* (Figure 13; Supplementary Appendix 2).

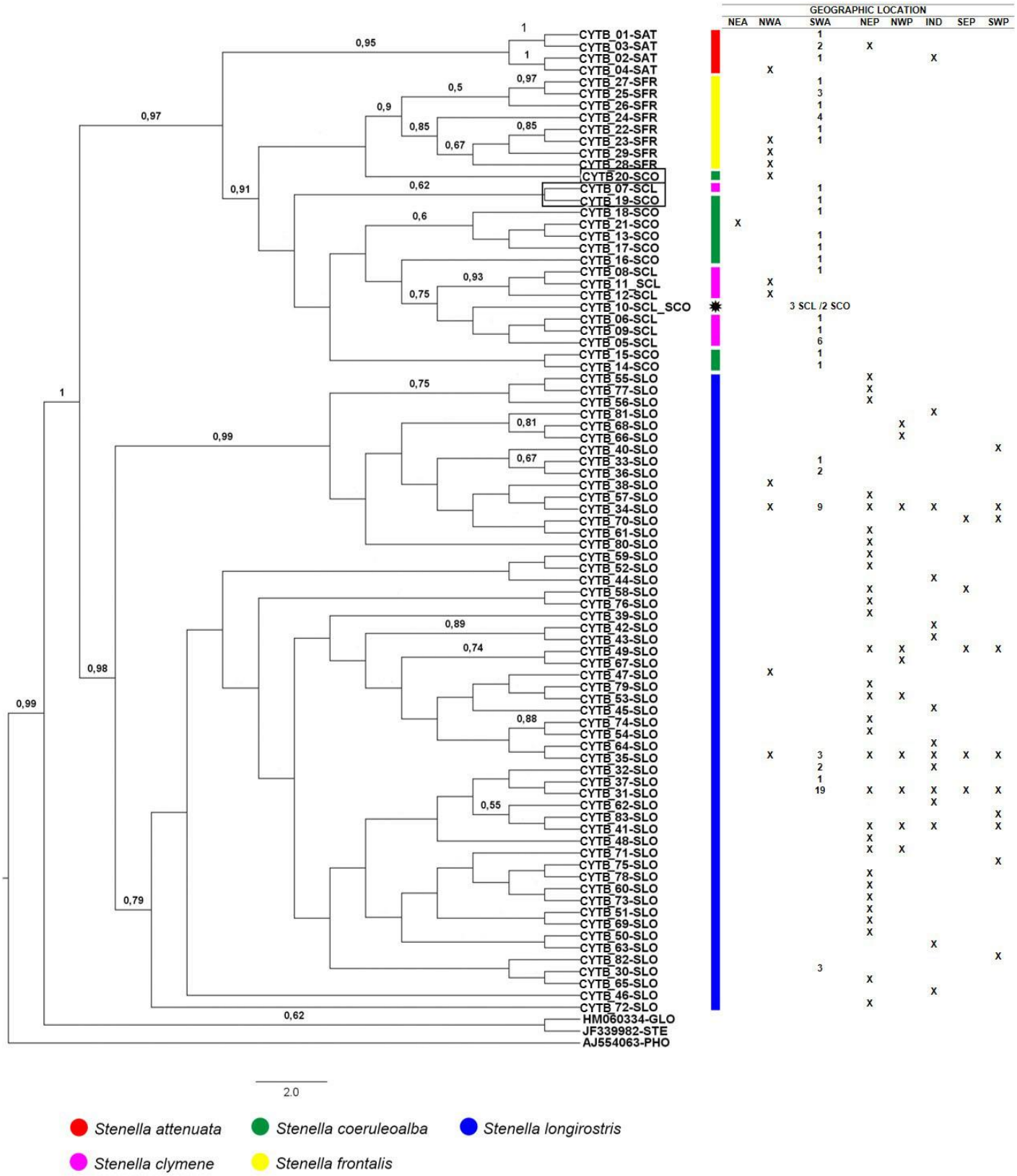

Figure 7 Bayesian cladogram generated by Beast of Cyt-b gene for *Stenella* for ocean basins comparisons. Posterior probability values greater than 0.5 are above nodes. Black boxes indicate misplaced haplotypes. Asterisk indicate haplotypes presents in different species. Table at right display the ocean basin location of each haplotype marked by "X": NEA (Northeast Atlantic Ocean, NWA (Northwest Atlantic Ocean), SWA (Southwest Atlantic Ocean), NEP (Northeast Pacific Ocean), NWP (Northwest Pacific Ocean), IN (Indian Ocean), SEP (Southeast Pacific Ocean), SWP (Southwest Pacific Ocean). The numbers in the SWA column represent the number of specimens for each haplotype.

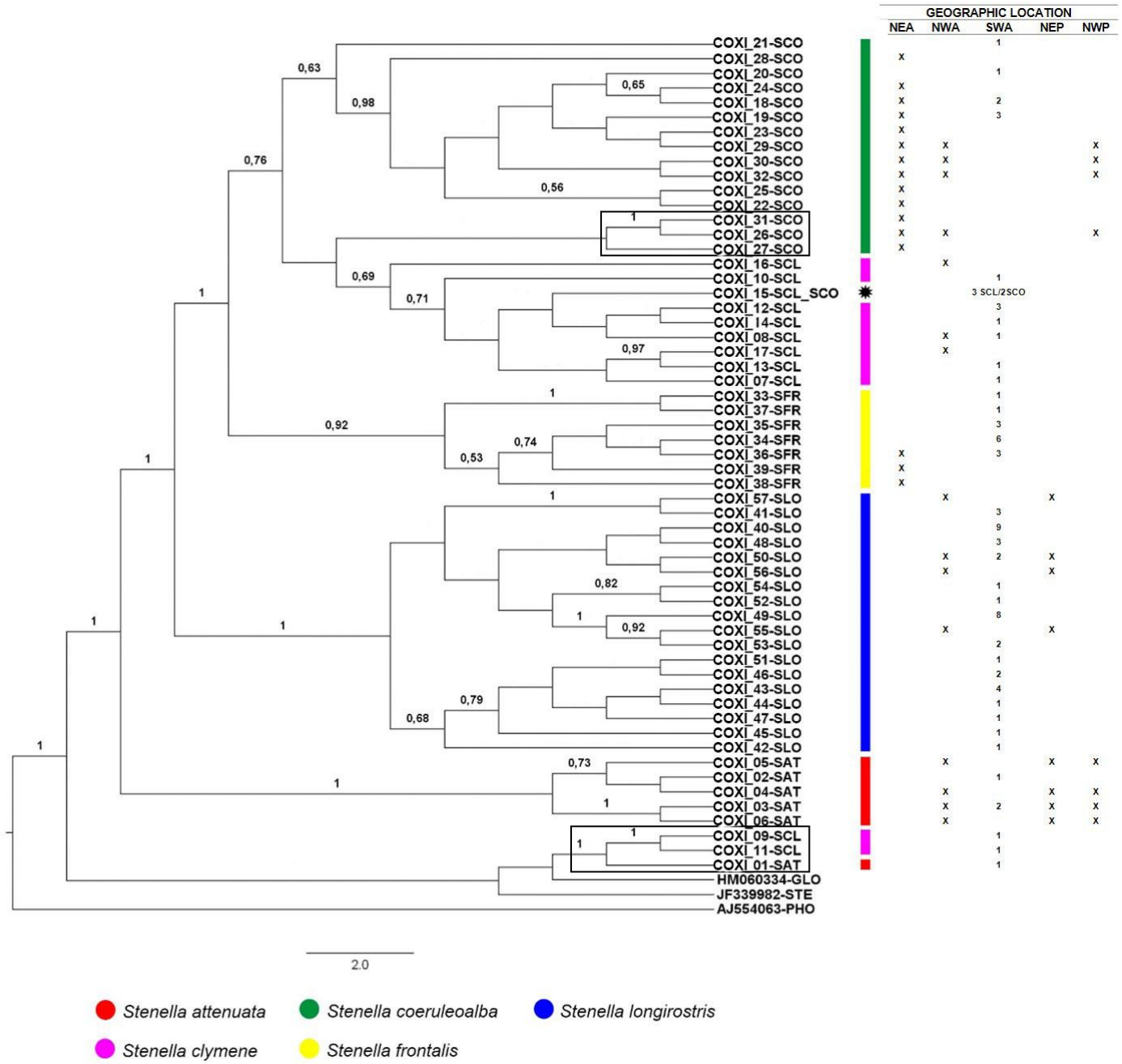

Figure 8 Bayesian cladogram generated by Beast of CoxI gene for *Stenella* for ocean basins comparisons. Posterior probability values greater than 0.5 are above nodes. Black boxes indicate misplaced haplotypes. Asterisk indicate haplotypes presents in different species Table at right display the ocean basin location of each haplotype marked by "X": NEA (Northeast Atlantic Ocean, NWA (Northwest Atlantic Ocean), SWA (Southwest Atlantic Ocean), NEP (Northeast Pacific Ocean), NWP (Northwest Pacific Ocean). The numbers in the SWA column represent the number of specimens for each haplotype.

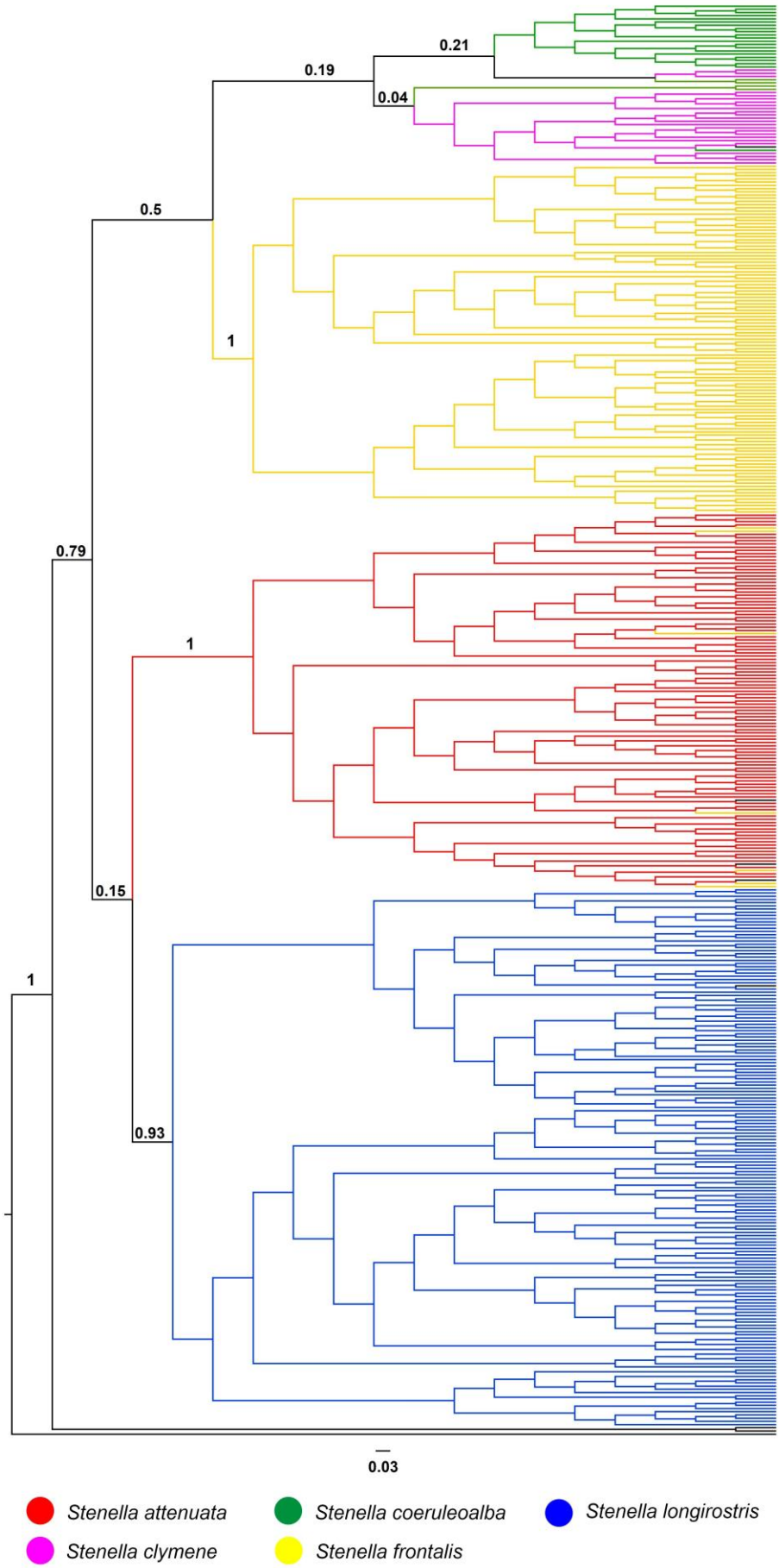

Figure 9 Bayesian cladogram generated by Beast of D-loop sequences for *Stenella* for ocean basins comparisons. Posterior probability values greater than 0.5 are above nodes. Black boxes indicate misplaced haplotypes.

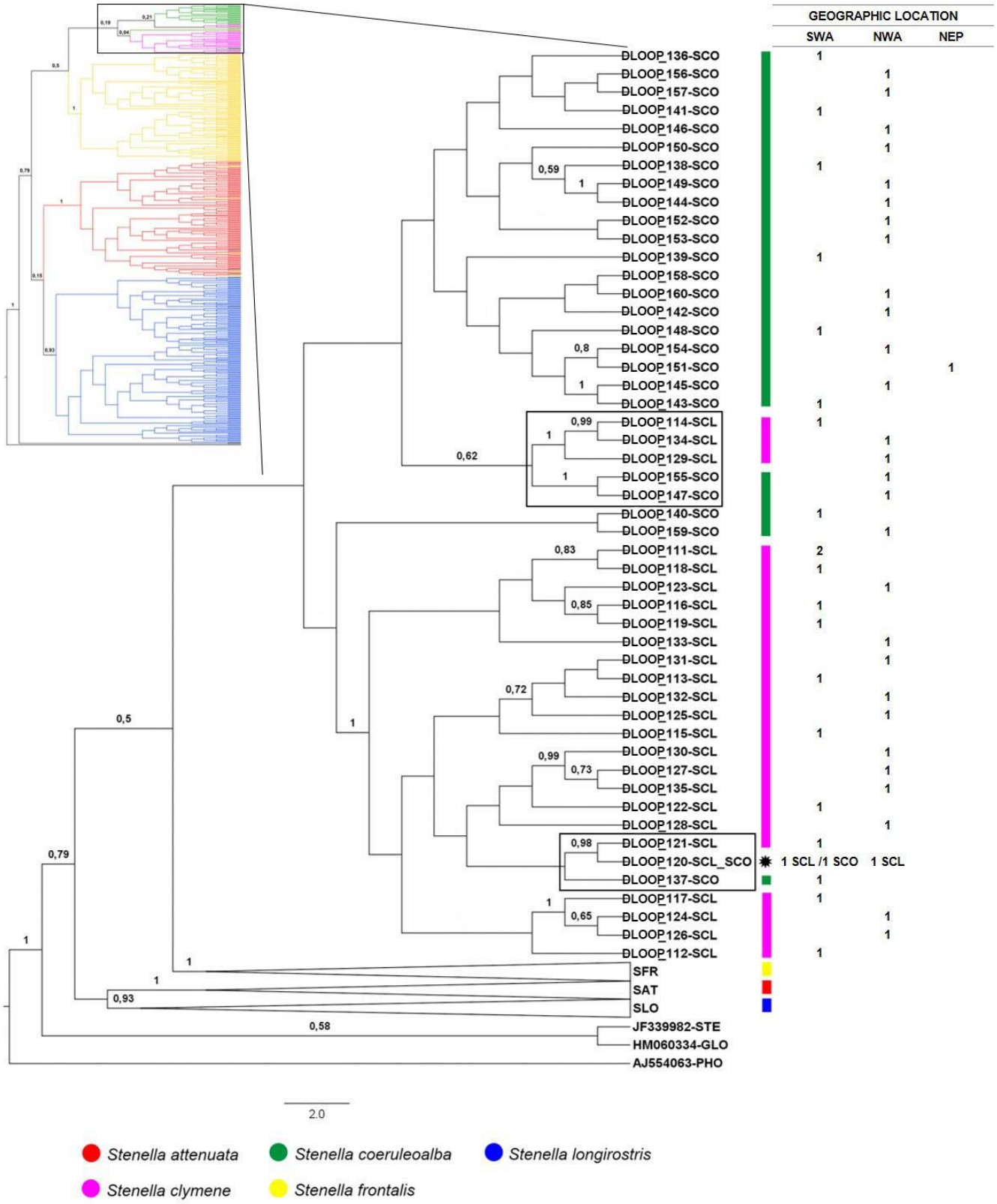

Figure 10 Bayesian cladogram generated by Beast for the clade of *S. clymene* and *S. coeruleoalba* of D-loop marker for ocean basins comparisons. Posterior probability values greater than 0.5 are above nodes. Black boxes indicate misplaced haplotypes. Asterisk indicate haplotypes presents in different species**.** Table at right display the ocean basin location of each haplotype and the number of specimens: SWA (Southwest Atlantic Ocean), NWA (Northwest Atlantic Ocean), NEP (Northeast Pacific Ocean). The numbers in the columns represent the number of specimens for each haplotype.

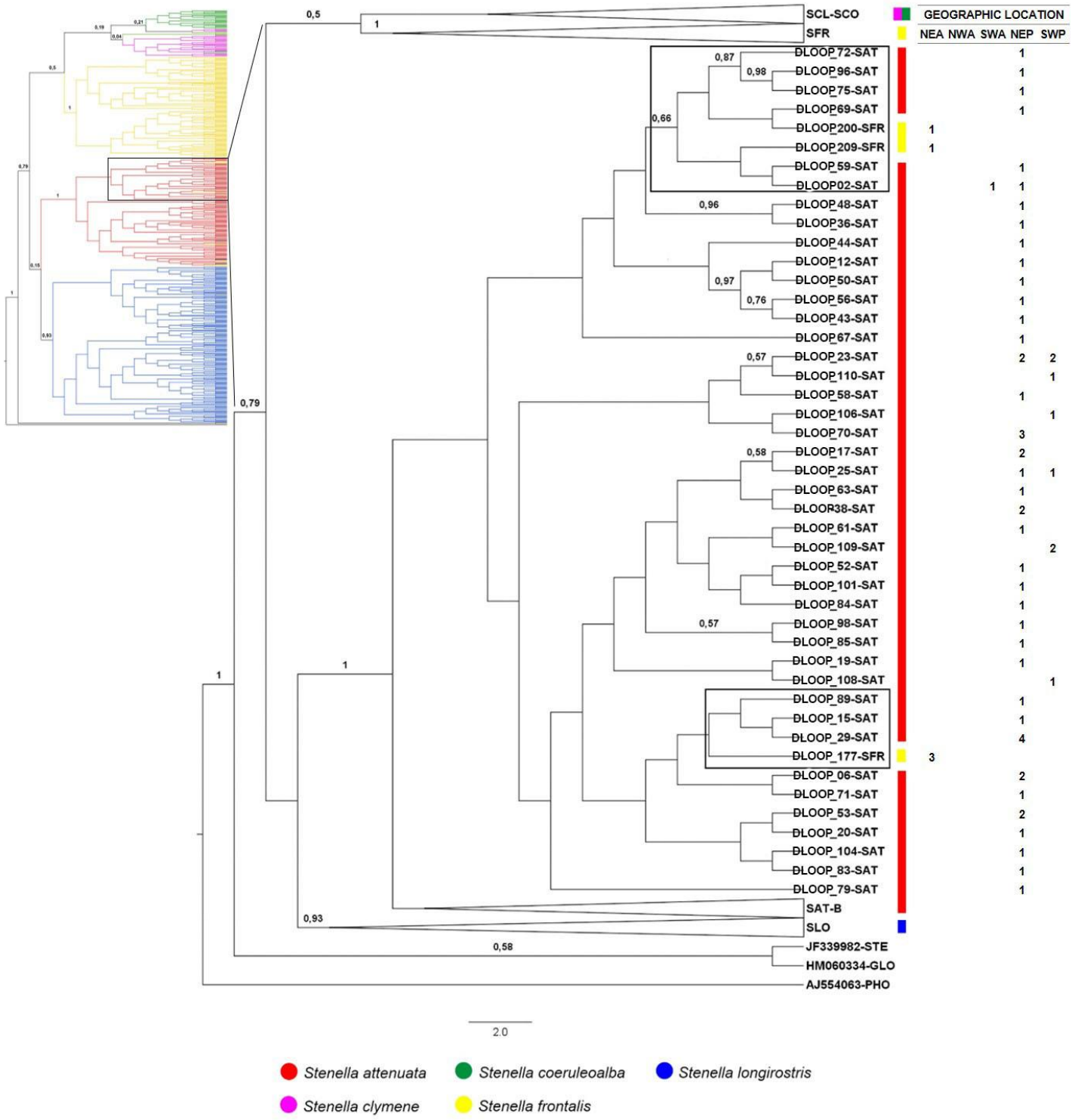

Figure 11 Bayesian cladogram generated by Beast for the first clade of *S. attenuata* of D-loop marker for ocean basins comparisons. Posterior probability values greater than 0.5 are above nodes. Black boxes indicate misplaced haplotypes. Table at right display the ocean basin location of each haplotype and the number of specimens: NEA (Northeast Atlantic Ocean), NWA (Northwest Atlantic Ocean), SWA (Southwest Atlantic Ocean), NEP (Northeast Pacific Ocean), SWP (Southwest Pacific Ocean). The numbers in the columns represent the number of specimens for each haplotype.

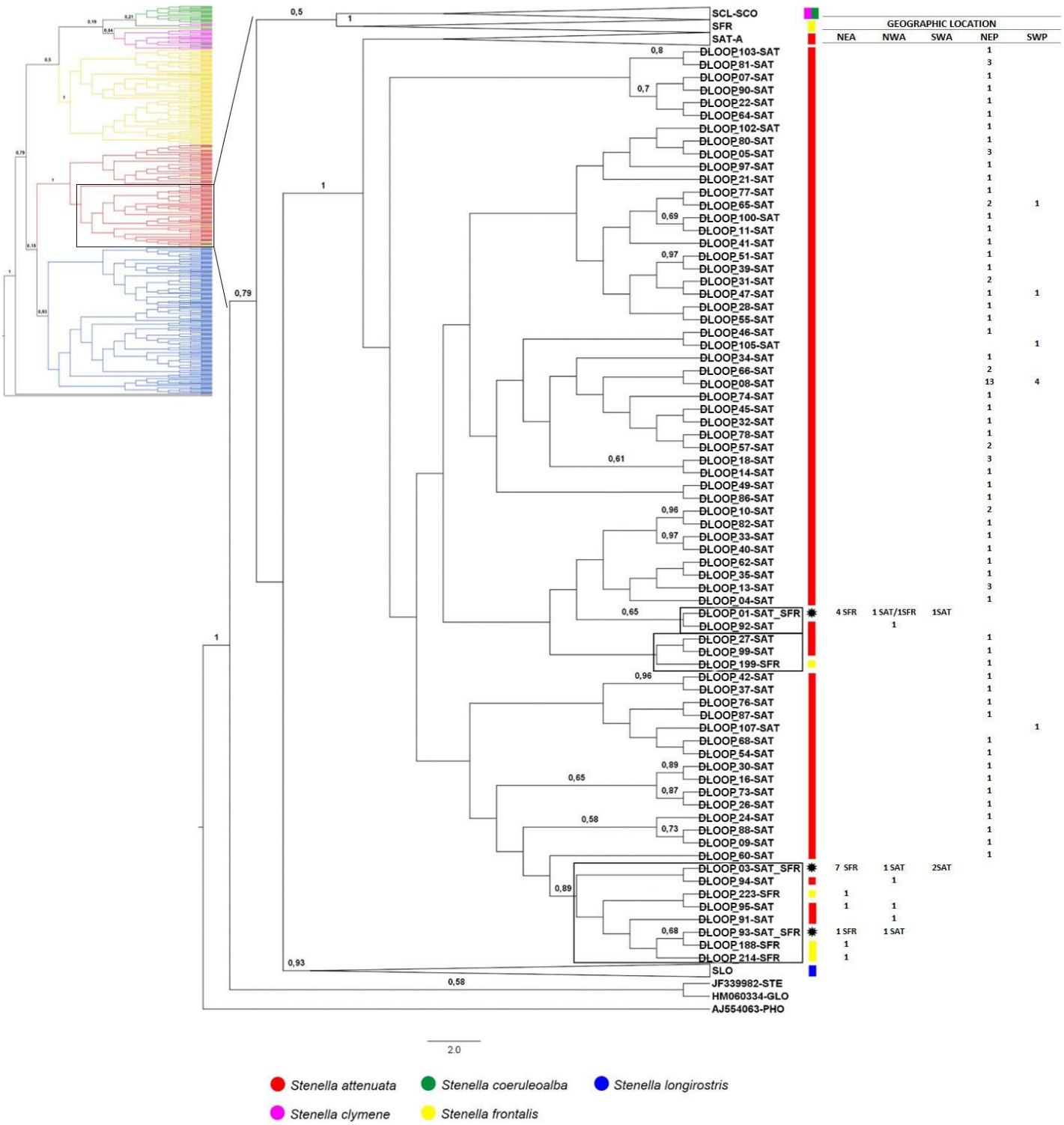

Figure 12 Bayesian cladogram generated in Beast for the second clade of *S. attenuata* of D-loop gene for ocean basins comparisons. Posterior probability values greater than 0.5 are above nodes. Black boxes indicate misplaced haplotypes. Table at right display the ocean basin location of each haplotype and the number of specimens: NEA (Northeast Atlantic Ocean), NWA (Northwest Atlantic Ocean), SWA (Southwest Atlantic Ocean), NEP (Northeast Pacific Ocean), SWP (Southwest Pacific Ocean). The numbers in the columns represent the number of specimens for each haplotype.

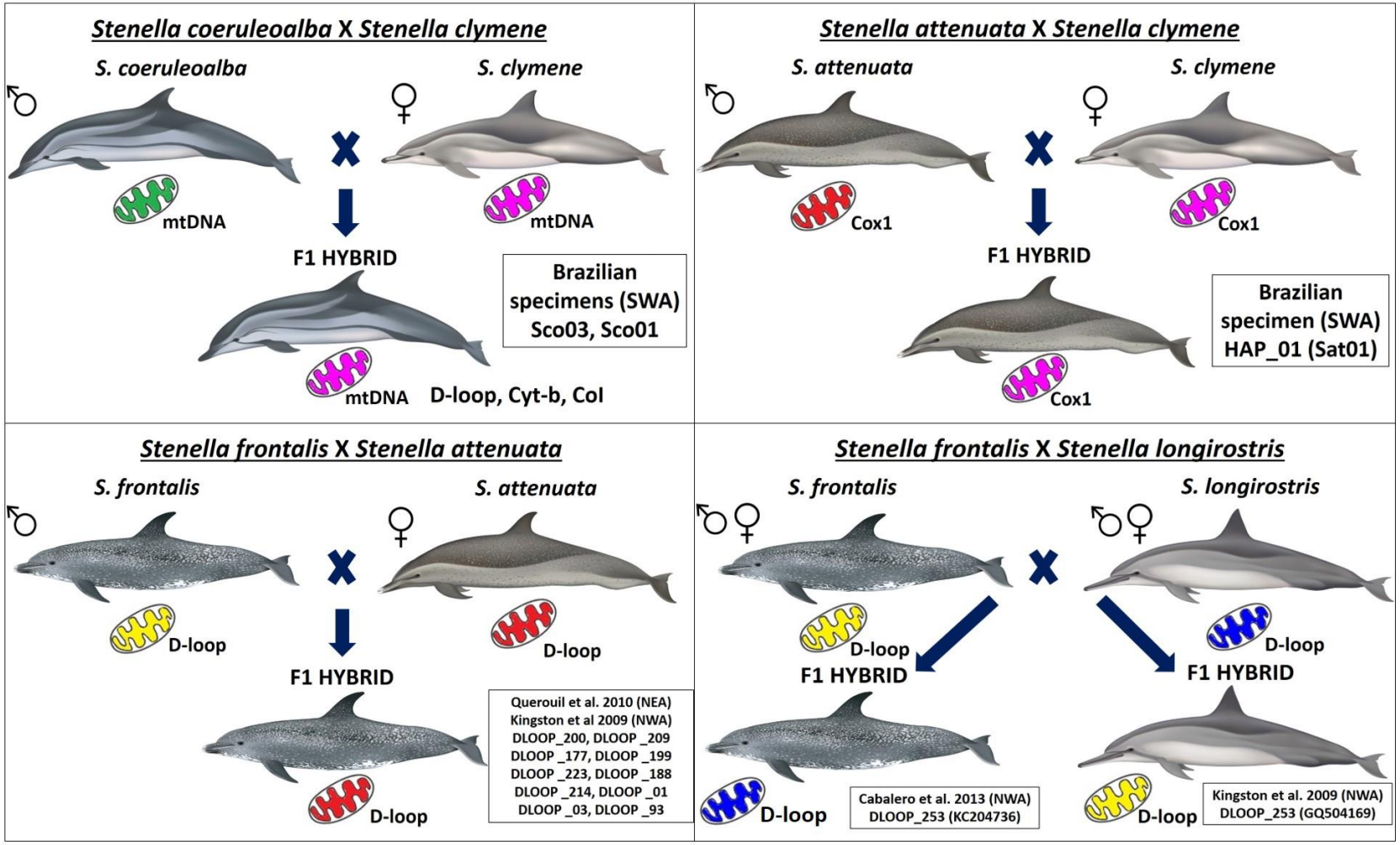

Figure 13 Possible heredograms of hybrids among *Stenella* species: a) hybrid between *S. coeruleoalba* and *S. clymene;* b) hybrid between *S. attenuata* and *S. clymene*; c) hybrid between *S. frontalis* and *S. attenuata*; d) hybrid between *S. frontalis* and *S. longirostris*. The images of the dolphins represent the morphological identity of the specimens and mitochondria, under the dolphins's images, represent the mitochondrial identity. The different colors of mitochondria represent the different species' DNA. In the boxes there are the names of the possible hybrids specimens or the names of the haplotypes representing the possible hybrids specimens. Images have been extracted from the website http://cis.whoi.edu/science/B/whalesounds/index.cf.

Discussion

The results presented here provide evidence of hybridization between *S. clymene* and *S. coeruleoalba*, and, *S. attenuata* and *S. clymene* in the Southwest Atlantic Ocean; between *S. frontalis* and *S. attenuata* in the Northeast Atlantic Ocean; and, between *S. frontalis* and *S. longirostris* in the Northwest Atlantic Ocean.

Generally, hybrids are only recognized in the wild when individuals exhibit morphological characteristics that are intermediate of the two parental species (Hubbs 1955). Although the intermediate morphology is strong evidence of hybridization, it should not be considered in isolation since there is a possibility that hybrids (fertile cases) will backcross with one of the parental species and exhibit dominant morphology of this species and therefore, be "camouflaged" in these populations. In addition, there is an extreme possibility that a hybrid has intermediate morphological characteristics, but phenological characteristics rarely observed in one species (Garofalo *et al.* 2012).

In this study the delimitation of species levels was based on the separation of lineages, according to this concept, species are lineages that evolve separately from another lineage (De Queiroz 2007). The evidence used for this purpose is reciprocal monophyly and distance among haplogroups. Three of the five species of *Stenella*, *S. attenuata*, *S. frontalis* and *S. longirostris* exhibited monophyletic clades in all analyses, for all three markers evaluated and therefore can be considered as species by the above definition. S. *coeruleoalba* and *S. clymene*, on the other hand, showed mixed clades, in all three regions of the mitochondrial DNA evaluated, as previously described in other phylogenetic studies using fewer specimens and different molecular

markers (LeDuc *et al.* 1999; Kingston *et al.* 2009; Amaral *et al.* 2014). Results also revealed that a notable pattern of interspecies admixture can be observed in our mitochondrial phylogenetic cladograms where individuals morphologically identified as one species demonstrated distinct mitochondrial lineages.

Within the SWA we found two possible hybrids between *S. coeruleoalba* and *S. clymene* (Sco01 and Sco03 specimens) wich displaying a morphological identity consistent with *S. coeruleoalba*, but a genetic identity consistent with *S. clymene* for all three genes evaluated (Dloop, Cyt-B and CoxI). This was shown by both the phylogenetic trees and the haplotype networks, revealing an introgression pattern. These two specimens were identified by specialists in cetaceans that certified the morphological features of *S. coeruleoalba* (Ott & Danilewicz 1996). Niche modelling methods have demonstrated that although the SWA is a sympatric zone for all *Stenella* species each species occupies a different niche (Amaral *et al.* 2015). However, the amount of niches available causes habitat division for each species because of the differences in their environmental necessities and so, just a narrow contact zone among some of these species is demonstrated in the SWA (Amaral *et al.* 2015). Although *S. clymene* was considered to have arisen through natural hybridization between *S. longirostris* and *S. coeruleoalba* (Amaral *et al.* 2014), we did not find any sign of mixture between *S. clymene* and *S. longirostris*, we only found sign of mixture between *S. clymene* and *S. coeruleoalba*. We used a large number of sequences of three genes of the mitochondrial DNA encompassing all ocean basins.

We also found evidence of hybridization between *S. attenuata* and *S. clymene* (Sat01 specimen) in the SWA using CoxI. The COX I gene has been

widely used in the molecular identification of species through the *Barcode* DNA methodology (Herbet *et al.* 2003) and has been efficient, in most cases studied, at correctly identifying organisms (Mallet 2008). For cetaceans, COX I and Cyt-b were efficient in the species identification of almost all species with exception of closely related taxa (*Eubalaena* spp.), but, Cyt-b, routinely used for cetacean species identification, performed better than Cox I (*CoxI)* for some species (Viricel *et al.* 2012). *S. attenuata* and *S. clymene* are known to demonstrate similar environmental constraints and are thought to have a wide degree of niche overlap in the SWA and in the Gulf of Mexico (Davis *et al.* 1998; Baumgartner *et al.* 2001; Amaral *et al.* 2015). Although underwater photographs taken in the Fernando de Noronha Archipelago indicate the occurrence of two possible hybrids, one between *S. longirostris* and *S. attenuata* and another between *S. longirostris* and *S. clymene*, based on the observation of intermediate features between the two species, (Silva-Jr *et al.* 2005) we did not find any signs of hybridization among these species in our genetic analyses.

After incorporating sequences from other ocean basins we found more evidences of possible hybridization, in the Northeast Atlantic Ocean (*S. frontalis* X *S. attenuata)* and in Northwest Atlantic Ocean (*S. frontalis* X *S. longirostris*) using all D-loop sequences available in the GenBank. All sequences used were from specimens morphologically identified and certified by the authors of the papers published in peer-reviewed scientific journals. All these putative hybrids presented D-loop identity inconsistent with the morphological identity displayed in the papers and so were misplaced in different species clades or demonstrated shared haplotypes with another species.

All Atlantic Ocean can be considered a potential hybridization zone for those species since they occur in sympatry (Donnelly *et al.* 2004) and the use of resources and environmental requirements overlap, especially in the South (Amaral *et al.* 2015). All the putative hybrid specimens were identified in areas where the parental species occur in sympatry. In addition, mixed groups have been observed for some *Stenella* species, for example between pantropical spotted (*S. attenuata*) and spinner dolphin (*S. longirostris*) in the eastern tropical Pacific (Perrin *et al.* 1973; Norris *et al.* 1994; Perrin and Gilpatrick 1994; Balance *et al.* 2006) in Hawaiian waters (Psarakos *et al.* 2003), and along the Brazilian continental shelf (Amaral *et al.* 2015).

We suggest the existence of ongoing gene flow among some *Stenella* species as a result of hybridization among different species of this genus. We propose that this phenomenon is a more common evolutionary process in this genus than previously thought. Hybridization has been indicated as one of the possible explanations for the confusing taxonomy and phylogenetic relations in Cetartiodactyla, as well as a reason for the uncertainties about the monophiletysm of *Stenella* and the classification of its species the taxonomic level (Perrin *et al*. 1987; LeDuc *et al.* 1999; Caballero *et al.* 2008; Kingston *et al.* 2009; McGowen *et al.* 2009; Steeman *et al.* 2009; Xiong *et al.* 2009; Amaral *et al.* 2012).

Genealogies often reflect the history of divergence among species when this process is old, nonetheless, there are genealogies that do not reflect the expected divergence (Nunes *et al.* 2010; Zielinski *et al.* 2013). Hybridization can modify bluntly and partially erase the genetic signature of past isolation, common for approximately 25% of plants and 10% of animals (Mallet 2005).

Hybridization and gene flow may occur among species considered to be distinct lineages at the population level and metapopulation (Mayden 1997; de Queiroz 2007) under the condition that they are sufficiently rare to maintain the species as independent evolutionary lineages. Hybridization occurs in most cases in the early stages of speciation while there is still insufficient accumulation of differences to avoid backcrossing (Wu & Ting 2004).

Conservation implications

Our study is the first to evaluate hybridization of Delphinidae in the South Atlantic Ocean, focusing on the five *Stenella* species. A large number of mtDNA sequences of *Stenella* including specimens from all oceans were used to demonstrate that hybridization is occurring among *Stenella* dolphins, and, it is still necessary to evaluate the specimens using nuclear markers to improve our results. Two of the five species, two (*S. attenuata* and *S. coeruleoalba*) are considered to be least concerned by the World Conservation Union (IUCN, Red List of Threatened Species 2017), but three of them (*S. clymene*, *S. frontalis* and *S. longirostris*) are considered data deficient. For all these species information on their biology, ecology, genetic structure and evolutionary history are necessary for the implementation of adequate conservation and management strategies (Nowell & Jackson 1996). These findings have important implications on the understanding of the evolutionary process that contribute to the confusing taxonomy of *Stenella* genus and can help with the conservation implications concerning the delimitation of the species.

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Supporting Information

Appendix 1 Number of specimens of each species analysed in this study, geographic location and sampling method.

	Sample	Species	Localization	Sampling Method
1	Sat 01	*Stenella attenuata*	Fernando de Noronha/PE	Stranding
2	Sat 02	*Stenella attenuata*	Fernando de Noronha/PE	Stranding
3	Sat 03	*Stenella attenuata*	Rio de Janeiro (RJ)	Biopsy
4	Sat 04	*Stenella attenauta*	Ceará (CE)	Stranding
5	Scl 06	*Stenella clymene*	Bahia (BA)	Stranding
6	Scl 07	*Stenella clymene*	Bahia (BA)	Stranding
7	Scl 08	*Stenella clymene*	Bahia (BA)	Stranding
8	Scl 09	*Stenella clymene*	Ceará (CE)	Stranding
9	Scl 10	*Stenella clymene*	Ceará (CE)	Stranding
10	Scl 11	*Stenella clymene*	Ceará (CE)	Stranding
11	Scl 12	*Stenella clymene*	Ceará (CE)	Stranding
12	Scl 13	*Stenella clymene*	Ceará (CE)	Stranding
13	Scl 14	*Stenella clymene*	Ceará (CE)	Stranding
14	Scl 17	*Stenella clymene*	Fernando de Noronha/PE	Stranding
15	Scl 33	*Stenella clymene*	Fernando de Noronha/PE	Stranding
16	Scl 34	*Stenella clymene*	Fernando de Noronha/PE	Stranding
17	Scl 35	*Stenella clymene*	Fernando de Noronha/PE	Stranding
18	Sco 01	*Stenella coeruleoalba*	Fernando de Noronha/PE	Stranding
19	Sco 02	*Stenella coeruleoalba*	Ceará (CE)	Stranding
20	Sco 03 (G0047)	*Stenella coeruleoalba*	Rio Grande do Sul (RS)	Stranding
21	Sco 05	*Stenella coeruleoalba*	Rio Grande do Sul (RS)	Stranding
22	Sco 07	*Stenella coeruleoalba*	Rio Grande do Sul (RS)	Stranding
23	Sco 08	*Stenella coeruleoalba*	Rio Grande do Sul (RS)	Stranding
24	Sco 09	*Stenella coeruleoalba*	Rio Grande do Sul (RS)	Stranding
25	Sco 10	*Stenella coeruleoalba*	Rio Grande do Sul (RS)	Stranding
26	Sco 11	*Stenella coeruleoalba*	Rio Grande do Sul (RS)	Stranding
27	Sfr 01	*Stenella frontalis*	Fernando de Noronha (FN)	Stranding
28	Sfr 02	*Stenella frontalis*	Fernando de Noronha (FN)	Stranding
29	Sfr 06	*Stenella frontalis*	São Paulo (SP)	Biopsy
30	Sfr 07	*Stenella frontalis*	São Paulo (SP)	Biopsy
31	Sfr 08	*Stenella frontalis*	São Paulo (SP)	Biopsy
32	Sfr 10	*Stenella frontalis*	São Paulo (SP)	Biopsy
33	Sfr 13	*Stenella frontalis*	São Paulo (SP)	Biopsy
34	Sfr 14	*Stenella frontalis*	São Paulo (SP)	Biopsy
35	Sfr 18	*Stenella frontalis*	São Paulo (SP)	Biopsy

	Sample	Species	Localization	Sampling Method
36	Sfr 21	*Stenella frontalis*	São Paulo (SP)	Biopsy
37	Sfr 23	*Stenella frontalis*	São Paulo (SP)	Biopsy
38	Sfr 24	*Stenella frontalis*	São Paulo (SP)	Biopsy
39	Sfr 28	*Stenella frontalis*	Rio Grande do Sul (RS)	Stranding
40	Sfr 32	*Stenella frontalis*	Rio Grande do Sul (RS)	Stranding
41	Slo 01	*Stenella longirostris*	Fernando de Noronha (FN)	Skin swabbing
42	Slo 02	*Stenella longirostris*	Fernando de Noronha (FN)	Skin swabbing
43	Slo 03	*Stenella longirostris*	Fernando de Noronha (FN)	Skin swabbing
44	Slo 04	*Stenella longirostris*	Fernando de Noronha (FN)	Skin swabbing
45	Slo 05	*Stenella longirostris*	Fernando de Noronha (FN)	Skin swabbing
46	Slo 06	*Stenella longirostris*	Fernando de Noronha (FN)	Skin swabbing
47	Slo 07	*Stenella longirostris*	Fernando de Noronha (FN)	Skin swabbing
48	Slo 08	*Stenella longirostris*	Fernando de Noronha (FN)	Skin swabbing
49	Slo 09	*Stenella longirostris*	Fernando de Noronha (FN)	Skin swabbing
50	Slo 10	*Stenella longirostris*	Fernando de Noronha (FN)	Skin swabbing
51	Slo 11	*Stenella longirostris*	Fernando de Noronha (FN)	Skin swabbing
52	Slo 12	*Stenella longirostris*	Fernando de Noronha (FN)	Skin swabbing
53	Slo 13	*Stenella longirostris*	Fernando de Noronha (FN)	Skin swabbing
54	Slo 14	*Stenella longirostris*	Fernando de Noronha (FN)	Skin swabbing
55	Slo 15	*Stenella longirostris*	Fernando de Noronha (FN)	Skin swabbing
56	Slo 16	*Stenella longirostris*	Fernando de Noronha (FN)	Skin swabbing
57	Slo 17	*Stenella longirostris*	Fernando de Noronha (FN)	Skin swabbing
58	Slo 18	*Stenella longirostris*	Fernando de Noronha (FN)	Skin swabbing
59	Slo 19	*Stenella longirostris*	Fernando de Noronha (FN)	Skin swabbing
60	Slo 20	*Stenella longirostris*	Fernando de Noronha (FN)	Skin swabbing
61	Slo 21	*Stenella longirostris*	Fernando de Noronha (FN)	Skin swabbing
62	Slo 22	*Stenella longirostris*	Rio Grande do Norte (RN)	Stranding
63	Slo 23	*Stenella longirostris*	Ceará (CE)	Stranding
64	Slo 24	*Stenella longirostris*	Ceará (CE)	Stranding
65	Slo 25	*Stenella longirostris*	Pernambuco (PE)	Stranding
66	Slo 26	*Stenella longirostris*	Pernambuco (PE)	Stranding
67	Slo 27	*Stenella longirostris*	Espírito Santo (ES)	Stranding
68	Slo 28	*Stenella longirostris*	Rio de Janeiro (RJ)	Biopsy
69	Slo 29	*Stenella longirostris*	Rio de Janeiro (RJ)	Biopsy
70	Slo 30	*Stenella longirostris*	Rio de Janeiro (RJ)	Biopsy
71	Slo 31	*Stenella longirostris*	Rio de Janeiro (RJ)	Biopsy
72	Slo 32	*Stenella longirostris*	Rio de Janeiro (RJ)	Biopsy
73	Slo 33	*Stenella longirostris*	Rio de Janeiro (RJ)	Biopsy
74	Slo 34	*Stenella longirostris*	São Paulo (SP)	Biopsy
75	Slo 35	*Stenella longirostris*	Paraná (PR)	Biopsy
76	Slo 36	*Stenella longirostris*	Paraná (PR)	Biopsy
77	Slo 37	*Stenella longirostris*	Paraná (PR)	Biopsy
78	Slo 38	*Stenella longirostris*	Santa Catarina (SC)	Biopsy
79	Slo 39	*Stenella longirostris*	Santa Catarina (SC)	Biopsy
80	Slo 40	*Stenella longirostris*	Santa Catarina (SC)	Biopsy

Appendix 2 Sequences used in this study, name of the species, GenBank accession number, name of the haplotype used in this study, geographic location of the haplotyps and source. NEA (Northeast Atlantic Ocean), NWA (Northwest Atlantic Ocean), SWA (Southwest Atlantic Ocean), NEP (Northeast Pacific Ocean), NWP (Northwest Pacific Ocean), SWP (Southwest Pacific Ocean), SEP (Southeast Pacific Ocean), EP (East Pacific Ocean), EA (East Atlantic Ocean), IN (Indian Ocean), SWI (Southwest Indian Ocean), NEI (Northeast Indian Ocean), IP (Indo Pacific Ocean).

D-loop (331 bp)					
Species		**GenBank**	**Haplotype**	**Localization**	**Source**
S. attenuata	1	XXXXXX	DLOOP1	SWA(Brazil)	This study
S. attenuata	2	XXXXXX	DLOOP2	SWA(Brazil)	This study
S. attenuata	3	XXXXXX	DLOOP3	SWA(Brazil)	This study
S. attenuata	4	DQ150134	DLOOP4	EP	Escorza- Treviño *et al.* 2005
S. attenuata	5	DQ150135	DLOOP5	EP	Escorza- Treviño *et al.* 2005
S. attenuata	6	DQ150136	DLOOP6	EP	Escorza- Treviño *et al.* 2005
S. attenuata	7	DQ150137	DLOOP7	EP	Escorza- Treviño *et al.* 2005
S. attenuata	8	DQ150138	DLOOP7	EP	Escorza- Treviño *et al.* 2005
S. attenuata	9	DQ150139	DLOOP8	EP	Escorza- Treviño *et al.* 2005
S. attenuata	10	DQ150140	DLOOP9	EP	Escorza- Treviño *et al.* 2005
S. attenuata	11	DQ150141	DLOOP10	EP	Escorza- Treviño *et al.* 2005
S. attenuata	12	DQ150142	DLOOP11	EP	Escorza- Treviño *et al.* 2005
S. attenuata	13	DQ150143	DLOOP10	EP	Escorza- Treviño *et al.* 2005
S. attenuata	14	DQ150144	DLOOP12	EP	Escorza- Treviño *et al.* 2005
S. attenuata	15	DQ150145	DLOOP13	EP	Escorza- Treviño *et al.* 2005
S. attenuata	16	DQ150146	DLOOP14	EP	Escorza- Treviño *et al.* 2005
S. attenuata	17	DQ150147	DLOOP15	EP	Escorza- Treviño *et al.* 2005
S. attenuata	18	DQ150148	DLOOP8	EP	Escorza- Treviño *et al.* 2005
S. attenuata	19	DQ150149	DLOOP16	EP	Escorza- Treviño *et al.* 2005
S. attenuata	20	DQ150150	DLOOP2	EP	Escorza- Treviño *et al.* 2005
S. attenuata	21	DQ150151	DLOOP17	EP	Escorza- Treviño *et al.* 2005
S. attenuata	22	DQ150152	DLOOP18	EP	Escorza- Treviño *et al.* 2005
S. attenuata	23	DQ150153	DLOOP19	EP	Escorza- Treviño *et al.* 2005
S. attenuata	24	DQ150154	DLOOP8	EP	Escorza- Treviño *et al.* 2005
S. attenuata	25	DQ150155	DLOOP20	EP	Escorza- Treviño *et al.* 2005
S. attenuata	26	DQ150156	DLOOP21	EP	Escorza- Treviño et al. 2005
S. attenuata	27	DQ150157	DLOOP22	EP	Escorza- Treviño et al. 2005
S. attenuata	28	DQ150158	DLOOP23	EP	Escorza- Treviño et al. 2005
S. attenuata	29	DQ150159	DLOOP24	EP	Escorza- Treviño et al. 2005
S. attenuata	30	DQ150160	DLOOP25	EP	Escorza- Treviño et al. 2005
S. attenuata	31	DQ150161	DLOOP26	EP	Escorza- Treviño et al. 2005
S. attenuata	32	DQ150162	DLOOP27	EP	Escorza- Treviño et al. 2005
S. attenuata	33	DQ150163	DLOOP28	EP	Escorza- Treviño et al. 2005
S. attenuata	34	DQ150164	DLOOP29	EP	Escorza- Treviño et al. 2005
S. attenuata	35	DQ150165	DLOOP30	EP	Escorza- Treviño et al. 2005
S. attenuata	36	DQ150166	DLOOP31	EP	Escorza- Treviño et al. 2005
S. attenuata	37	DQ150167	DLOOP5	EP	Escorza- Treviño et al. 2005
S. attenuata	38	DQ150168	DLOOP32	EP	Escorza- Treviño et al. 2005
S. attenuata	39	DQ150169	DLOOP33	EP	Escorza- Treviño et al. 2005
S. attenuata	40	DQ150170	DLOOP34	EP	Escorza- Treviño et al. 2005
S. attenuata	41	DQ150171	DLOOP35	EP	Escorza- Treviño et al. 2005
S. attenuata	42	DQ150172	DLOOP29	EP	Escorza- Treviño et al. 2005
S. attenuata	43	DQ150173	DLOOP36	EP	Escorza- Treviño et al. 2005
S. attenuata	44	DQ150174	DLOOP8	EP	Escorza- Treviño et al. 2005
S. attenuata	45	DQ150175	DLOOP37	EP	Escorza- Treviño et al. 2005
S. attenuata	46	DQ150176	DLOOP38	EP	Escorza- Treviño et al. 2005
S. attenuata	47	DQ150177	DLOOP39	EP	Escorza- Treviño et al. 2005

D-loop (331 bp)					
Species		**GenBank**	**Haplotype**	**Localization**	**Source**
S. attenuata	48	DQ150178	DLOOP40	EP	Escorza- Treviño et al. 2005
S. attenuata	49	DQ150179	DLOOP41	EP	Escorza- Treviño et al. 2005
S. attenuata	50	DQ150180	DLOOP13	EP	Escorza- Treviño et al. 2005
S. attenuata	51	DQ150181	DLOOP29	EP	Escorza- Treviño et al. 2005
S. attenuata	52	DQ150182	DLOOP42	EP	Escorza- Treviño et al. 2005
S. attenuata	53	DQ150183	DLOOP43	EP	Escorza- Treviño et al. 2005
S. attenuata	54	DQ150184	DLOOP44	EP	Escorza- Treviño et al. 2005
S. attenuata	55	DQ150185	DLOOP45	EP	Escorza- Treviño et al. 2005
S. attenuata	56	DQ150186	DLOOP46	EP	Escorza- Treviño et al. 2005
S. attenuata	57	DQ150187	DLOOP47	EP	Escorza- Treviño et al. 2005
S. attenuata	58	DQ150188	DLOOP48	EP	Escorza- Treviño et al. 2005
S. attenuata	59	DQ150189	DLOOP49	EP	Escorza- Treviño et al. 2005
S. attenuata	60	DQ150190	DLOOP18	EP	Escorza- Treviño et al. 2005
S. attenuata	61	DQ150191	DLOOP8	EP	Escorza- Treviño et al. 2005
S. attenuata	62	DQ150192	DLOOP50	EP	Escorza- Treviño et al. 2005
S. attenuata	63	DQ150193	DLOOP51	EP	Escorza- Treviño et al. 2005
S. attenuata	64	DQ150194	DLOOP52	EP	Escorza- Treviño et al. 2005
S. attenuata	65	DQ150195	DLOOP13	EP	Escorza- Treviño et al. 2005
S. attenuata	66	DQ150196	DLOOP53	EP	Escorza- Treviño et al. 2005
S. attenuata	67	DQ150197	DLOOP23	EP	Escorza- Treviño et al. 2005
S. attenuata	68	DQ150198	DLOOP54	EP	Escorza- Treviño et al. 2005
S. attenuata	69	DQ150199	DLOOP55	EP	Escorza- Treviño et al. 2005
S. attenuata	70	DQ150200	DLOOP56	EP	Escorza- Treviño et al. 2005
S. attenuata	71	DQ150201	DLOOP57	EP	Escorza- Treviño et al. 2005
S. attenuata	72	DQ150202	DLOOP58	EP	Escorza- Treviño et al. 2005
S. attenuata	73	DQ150203	DLOOP59	EP	Escorza- Treviño et al. 2005
S. attenuata	74	DQ150204	DLOOP60	EP	Escorza- Treviño et al. 2005
S. attenuata	75	DQ150205	DLOOP61	EP	Escorza- Treviño et al. 2005
S. attenuata	76	DQ150206	DLOOP62	EP	Escorza- Treviño et al. 2005
S. attenuata	77	DQ150207	DLOOP63	EP	Escorza- Treviño et al. 2005
S. attenuata	78	DQ150208	DLOOP64	EP	Escorza- Treviño et al. 2005
S. attenuata	79	DQ150209	DLOOP65	EP	Escorza- Treviño et al. 2005
S. attenuata	80	DQ150210	DLOOP66	EP	Escorza- Treviño et al. 2005
S. attenuata	81	DQ150211	DLOOP67	EP	Escorza- Treviño et al. 2005
S. attenuata	82	DQ150212	DLOOP68	EP	Escorza- Treviño et al. 2005
S. attenuata	83	DQ150213	DLOOP69	EP	Escorza- Treviño et al. 2005
S. attenuata	84	DQ150214	DLOOP70	EP	Escorza- Treviño et al. 2005
S. attenuata	85	DQ150215	DLOOP7	EP	Escorza- Treviño et al. 2005
S. attenuata	86	DQ150216	DLOOP71	EP	Escorza- Treviño et al. 2005
S. attenuata	87	DQ150217	DLOOP72	EP	Escorza- Treviño et al. 2005
S. attenuata	88	DQ150218	DLOOP73	EP	Escorza- Treviño et al. 2005
S. attenuata	89	DQ150219	DLOOP74	EP	Escorza- Treviño et al. 2005
S. attenuata	90	DQ150220	DLOOP75	EP	Escorza- Treviño et al. 2005
S. attenuata	91	DQ150221	DLOOP18	EP	Escorza- Treviño et al. 2005
S. attenuata	92	DQ150222	DLOOP76	EP	Escorza- Treviño et al. 2005
S. attenuata	93	DQ150223	DLOOP29	EP	Escorza- Treviño et al. 2005
S. attenuata	94	DQ150224	DLOOP8	EP	Escorza- Treviño et al. 2005
S. attenuata	95	DQ150225	DLOOP77	EP	Escorza- Treviño et al. 2005
S. attenuata	96	DQ150226	DLOOP38	EP	Escorza- Treviño et al. 2005
S. attenuata	97	DQ150227	DLOOP8	EP	Escorza- Treviño et al. 2005
S. attenuata	98	DQ150228	DLOOP5	EP	Escorza- Treviño et al. 2005
S. attenuata	99	DQ150229	DLOOP78	EP	Escorza- Treviño et al. 2005
S. attenuata	100	DQ150230	DLOOP79	EP	Escorza- Treviño et al. 2005
S. attenuata	101	DQ150231	DLOOP80	EP	Escorza- Treviño et al. 2005
S. attenuata	102	DQ150232	DLOOP57	EP	Escorza- Treviño et al. 2005
S. attenuata	103	DQ150233	DLOOP81	EP	Escorza- Treviño et al. 2005
S. attenuata	104	DQ150234	DLOOP82	EP	Escorza- Treviño et al. 2005

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Species		**GenBank**	**Haplotype**	**Localization**	**Source**
S. attenuata	105	DQ150235	DLOOP83	EP	Escorza- Treviño et al. 2005
S. attenuata	106	DQ150236	DLOOP84	EP	Escorza- Treviño et al. 2005
S. attenuata	107	DQ150237	DLOOP8	EP	Escorza- Treviño et al. 2005
S. attenuata	108	DQ150238	DLOOP85	EP	Escorza- Treviño et al. 2005
S. attenuata	109	DQ150239	DLOOP8	EP	Escorza- Treviño et al. 2005
S. attenuata	110	DQ150240	DLOOP66	EP	Escorza- Treviño et al. 2005
S. attenuata	111	DQ150241	DLOOP86	EP	Escorza- Treviño et al. 2005
S. attenuata	112	DQ150242	DLOOP87	EP	Escorza- Treviño et al. 2005
S. attenuata	113	DQ150243	DLOOP88	EP	Escorza- Treviño et al. 2005
S. attenuata	114	DQ150244	DLOOP89	EP	Escorza- Treviño et al. 2005
S. attenuata	115	DQ150245	DLOOP90	EP	Escorza- Treviño et al. 2005
S. attenuata	116	DQ845442	DLOOP91	NWA	Kingston et al. 2009
S. attenuata	117	DQ845443	DLOOP92	NWA	Kingston et al. 2009
S. attenuata	118	GQ504120	DLOOP1	NWA	Kingston et al. 2009
S. attenuata	119	GQ504121	DLOOP93	NWA	Kingston et al. 2009
S. attenuata	120	GQ504122	DLOOP3	NWA	Kingston et al. 2009
S. attenuata	121	GQ504123	DLOOP94	NWA	Kingston et al. 2009
S. attenuata	122	GQ504124	DLOOP95	NWA	Kingston et al. 2009
S. attenuata	123	GQ504126	DLOOP53	NEA	Kingston et al. 2009
S. attenuata	124	GQ504127	DLOOP6	NEA	Kingston et al. 2009
S. attenuata	125	GQ504128	DLOOP96	NEA	Kingston et al. 2009
S. attenuata	126	GQ504129	DLOOP8	NEA	Kingston et al. 2009
S. attenuata	127	GQ504130	DLOOP70	NEA	Kingston et al. 2009
S. attenuata	128	GQ504131	DLOOP8	NEA	Kingston et al. 2009
S. attenuata	129	GQ852567	DLOOP97	NEA	Courbis et al. 2014
S. attenuata	130	GQ852568	DLOOP98	NEA	Courbis et al. 2014
S. attenuata	131	GQ852569	DLOOP8	NEA	Courbis et al. 2014
S. attenuata	132	GQ852570	DLOOP99	NEA	Courbis et al. 2014
S. attenuata	133	GQ852571	DLOOP17	NEA	Courbis et al. 2014
S. attenuata	134	GQ852572	DLOOP70	NEA	Courbis et al. 2014
S. attenuata	135	GQ852573	DLOOP100	NEA	Courbis et al. 2014
S. attenuata	136	GQ852574	DLOOP101	NEA	Courbis et al. 2014
S. attenuata	137	GQ852575	DLOOP65	NEA	Courbis et al. 2014
S. attenuata	138	GQ852576	DLOOP102	NEA	Courbis et al. 2014
S. attenuata	139	GQ852577	DLOOP31	NEA	Courbis et al. 2014
S. attenuata	140	GQ852578	DLOOP103	NEA	Courbis et al. 2014
S. attenuata	141	GQ852579	DLOOP8	NEA	Courbis et al. 2014
S. attenuata	142	GU136595	DLOOP104	NEA	Courbis et al. 2014
S. attenuata	143	GU256406	DLOOP95	NEA	Courbis et al. 2014
S. attenuata	144	KP756626	DLOOP105	SWP	Oremus et al. 2015
S. attenuata	145	KP756627	DLOOP65	SWP	Oremus et al. 2015
S. attenuata	146	KP756628	DLOOP106	SWP	Oremus et al. 2015
S. attenuata	147	KP756629	DLOOP8	SWP	Oremus et al. 2015
S. attenuata	148	KP756630	DLOOP107	SWP	Oremus et al. 2015
S. attenuata	149	KP756631	DLOOP8	SWP	Oremus et al. 2015
S. attenuata	150	KP756632	DLOOP8	SWP	Oremus et al. 2015
S. attenuata	151	KP756633	DLOOP8	SWP	Oremus et al. 2015
S. attenuata	152	KP756634	DLOOP47	SWP	Oremus et al. 2015
S. attenuata	153	KP756635	DLOOP25	SWP	Oremus et al. 2015
S. attenuata	154	KP756636	DLOOP108	SWP	Oremus et al. 2015
S. attenuata	155	KP756637	DLOOP23	SWP	Oremus et al. 2015
S. attenuata	156	KP756638	DLOOP23	SWP	Oremus et al. 2015
S. attenuata	157	KP756639	DLOOP109	SWP	Oremus et al. 2015
S. attenuata	158	KP756640	DLOOP109	SWP	Oremus et al. 2015
S. attenuata	159	KP756646	DLOOP110	SWP	Oremus et al. 2015
S. clymene	160	XXXXXX	DLOOP111	SWA(Brazil)	This study
S. clymene	161	XXXXXX	DLOOP112	SWA(Brazil)	This study

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Species		**GenBank**	**Haplotype**	**Localization**	**Source**
S. clymene	162	XXXXXX	DLOOP113	SWA(Brazil)	This study
S. clymene	163	XXXXXX	DLOOP114	SWA(Brazil)	This study
S. clymene	164	XXXXXX	DLOOP115	SWA(Brazil)	This study
S. clymene	165	XXXXXX	DLOOP116	SWA(Brazil)	This study
S. clymene	166	XXXXXX	DLOOP117	SWA(Brazil)	This study
S. clymene	167	XXXXXX	DLOOP118	SWA(Brazil)	This study
S. clymene	168	XXXXXX	DLOOP119	SWA(Brazil)	This study
S. clymene	169	XXXXXX	DLOOP120	SWA(Brazil)	This study
S. clymene	170	XXXXXX	DLOOP121	SWA(Brazil)	This study
S. clymene	171	XXXXXX	DLOOP122	SWA(Brazil)	This study
S. clymene	172	GQ504137	DLOOP123	NWA	Kingston et al. 2009
S. clymene	173	GQ504138	DLOOP124	NWA	Kingston et al. 2009
S. clymene	174	GQ504139	DLOOP125	NWA	Kingston et al. 2009
S. clymene	175	GQ504140	DLOOP126	NWA	Kingston et al. 2009
S. clymene	176	GQ504141	DLOOP127	NWA	Kingston et al. 2009
S. clymene	177	GQ504142	DLOOP128	NWA	Kingston et al. 2009
S. clymene	178	GQ504143	DLOOP129	NWA	Kingston et al. 2009
S. clymene	179	GQ504144	DLOOP130	NWA	Kingston et al. 2009
S. clymene	180	GQ504145	DLOOP131	NWA	Kingston et al. 2009
S. clymene	181	GQ504146	DLOOP132	NWA	Kingston et al. 2009
S. clymene	182	GQ504147	DLOOP120	NWA	Kingston et al. 2009
S. clymene	183	GQ504148	DLOOP133	NWA	Kingston et al. 2009
S. clymene	184	DQ845446	DLOOP134	NWA	Kingston et al. 2009
S. clymene	185	DQ845447	DLOOP135	NWA	Kingston et al. 2009
S. coeruleoalba	186	XXXXXX	DLOOP120	SWA (Brazil)	This study
S. coeruleoalba	187	XXXXXX	DLOOP136	SWA (Brazil)	This study
S. coeruleoalba	188	XXXXXX	DLOOP137	SWA (Brazil)	This study
S. coeruleoalba	189	XXXXXX	DLOOP138	SWA (Brazil)	This study
S. coeruleoalba	190	XXXXXX	DLOOP139	SWA (Brazil)	This study
S. coeruleoalba	191	XXXXXX	DLOOP140	SWA (Brazil)	This study
S. coeruleoalba	192	XXXXXX	DLOOP141	SWA (Brazil)	This study
S. coeruleoalba	193	XXXXXX	DLOOP142	SWA (Brazil)	This study
S. coeruleoalba	194	XXXXXX	DLOOP143	SWA (Brazil)	This study
S. coeruleoalba	195	GQ504149	DLOOP144	NWA	Kingston et al. 2009
S. coeruleoalba	196	GQ504150	DLOOP145	NWA	Kingston et al. 2009
S. coeruleoalba	197	GQ504152	DLOOP146	NWA	Kingston et al. 2009
S. coeruleoalba	198	GQ504153	DLOOP147	NWA	Kingston et al. 2009
S. coeruleoalba	199	GQ504154	DLOOP148	NWA	Kingston et al. 2009
S. coeruleoalba	200	GQ504155	DLOOP149	NWA	Kingston et al. 2009
S. coeruleoalba	201	GQ504156	DLOOP150	NWA	Kingston et al. 2009
S. coeruleoalba	202	GQ504157	DLOOP151	NWA	Kingston et al. 2009
S. coeruleoalba	203	GQ504158	DLOOP152	NWA	Kingston et al. 2009
S. coeruleoalba	204	GQ504159	DLOOP153	NWA	Kingston et al. 2009
S. coeruleoalba	205	GQ504160	DLOOP154	NWA	Kingston et al. 2009
S. coeruleoalba	206	GQ504161	DLOOP155	NWA	Kingston et al. 2009
S. coeruleoalba	207	GQ504162	DLOOP156	NWA	Kingston et al. 2009
S. coeruleoalba	208	GQ504163	DLOOP157	NWA	Kingston et al. 2009
S. coeruleoalba	209	GQ504164	DLOOP158	NWA	Kingston et al. 2009
S. coeruleoalba	210	DQ845440	DLOOP159	NWA	Kingston et al. 2009
S. coeruleoalba	211	DQ845441	DLOOP160	NWA	Kingston et al. 2009
S. frontalis	212	XXXXXX	DLOOP161	SWA (Brazil)	This study
S. frontalis	213	XXXXXX	DLOOP162	SWA (Brazil)	This study
S. frontalis	214	XXXXXX	DLOOP163	SWA (Brazil)	This study
S. frontalis	215	XXXXXX	DLOOP164	SWA (Brazil)	This study
S. frontalis	216	DQ060054	DLOOP165	NWA	Adams et al. 2006
S. frontalis	217	DQ060055	DLOOP166	NWA	Adams et al. 2006
S. frontalis	218	DQ060056	DLOOP161	NWA	Adams et al. 2006

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Species		**GenBank**	**Haplotype**	**Localization**	**Source**
S. frontalis	219	DQ060057	DLOOP167	NWA	Adams et al. 2006
S. frontalis	220	DQ060058	DLOOP168	NWA	Adams et al. 2006
S. frontalis	221	DQ060059	DLOOP169	NWA	Adams et al. 2006
S. frontalis	222	DQ060060	DLOOP170	NWA	Adams et al. 2006
S. frontalis	223	DQ060061	DLOOP171	NWA	Adams et al. 2006
S. frontalis	224	DQ060062	DLOOP172	NWA	Adams et al. 2006
S. frontalis	225	DQ060063	DLOOP173	NWA	Adams et al. 2006
S. frontalis	226	DQ060064	DLOOP174	NWA	Adams et al. 2006
S. frontalis	227	EF546440	DLOOP167	NWA	Green et al. 2007
S. frontalis	228	EF682650	DLOOP165	NEA	Querouil et al. 2010
S. frontalis	229	EF682651	DLOOP175	NEA	Querouil et al. 2010
S. frontalis	230	EF682652	DLOOP176	NEA	Querouil et al. 2010
S. frontalis	231	EF682653	DLOOP175	NEA	Querouil et al. 2010
S. frontalis	232	EF682654	DLOOP175	NEA	Querouil et al. 2010
S. frontalis	233	EF682655	DLOOP174	NEA	Querouil et al. 2010
S. frontalis	234	EF682656	DLOOP177	NEA	Querouil et al. 2010
S. frontalis	235	EF682657	DLOOP178	NEA	Querouil et al. 2010
S. frontalis	236	EF682658	DLOOP1	NEA	Querouil et al. 2010
S. frontalis	237	EF682659	DLOOP3	NEA	Querouil et al. 2010
S. frontalis	238	EF682660	DLOOP179	NEA	Querouil et al. 2010
S. frontalis	239	EF682661	DLOOP161	NEA	Querouil et al. 2010
S. frontalis	240	EF682662	DLOOP180	NEA	Querouil et al. 2010
S. frontalis	241	EF682663	DLOOP181	NEA	Querouil et al. 2010
S. frontalis	242	EF682664	DLOOP182	NEA	Querouil et al. 2010
S. frontalis	243	EF682665	DLOOP175	NEA	Querouil et al. 2010
S. frontalis	244	EF682666	DLOOP183	NEA	Querouil et al. 2010
S. frontalis	245	EF682667	DLOOP93	NEA	Querouil et al. 2010
S. frontalis	246	EF682668	DLOOP161	NEA	Querouil et al. 2010
S. frontalis	247	EF682669	DLOOP165	NEA	Querouil et al. 2010
S. frontalis	248	EF682670	DLOOP184	NEA	Querouil et al. 2010
S. frontalis	249	EF682671	DLOOP163	NEA	Querouil et al. 2010
S. frontalis	250	EF682672	DLOOP165	NEA	Querouil et al. 2010
S. frontalis	251	EF682673	DLOOP185	NEA	Querouil et al. 2010
S. frontalis	252	EF682674	DLOOP175	NEA	Querouil et al. 2010
S. frontalis	253	EF682675	DLOOP186	NEA	Querouil et al. 2010
S. frontalis	254	EF682676	DLOOP187	NEA	Querouil et al. 2010
S. frontalis	255	EF682677	DLOOP165	NEA	Querouil et al. 2010
S. frontalis	256	EF682678	DLOOP165	NEA	Querouil et al. 2010
S. frontalis	257	EF682679	DLOOP174	NEA	Querouil et al. 2010
S. frontalis	258	EF682680	DLOOP180	NEA	Querouil et al. 2010
S. frontalis	259	EF682681	DLOOP188	NEA	Querouil et al. 2010
S. frontalis	260	EF682682	DLOOP189	NEA	Querouil et al. 2010
S. frontalis	261	EF682683	DLOOP161	NEA	Querouil et al. 2010
S. frontalis	262	EF682684	DLOOP165	NEA	Querouil et al. 2010
S. frontalis	263	EF682685	DLOOP167	NEA	Querouil et al. 2010
S. frontalis	264	EF682686	DLOOP190	NEA	Querouil et al. 2010
S. frontalis	265	EF682687	DLOOP191	NEA	Querouil et al. 2010
S. frontalis	266	EF682688	DLOOP192	NEA	Querouil et al. 2010
S. frontalis	267	EF682689	DLOOP175	NEA	Querouil et al. 2010
S. frontalis	268	EF682690	DLOOP181	NEA	Querouil et al. 2010
S. frontalis	269	EF682691	DLOOP161	NEA	Querouil et al. 2010
S. frontalis	270	EF682692	DLOOP181	NEA	Querouil et al. 2010
S. frontalis	271	EF682693	DLOOP193	NEA	Querouil et al. 2010
S. frontalis	272	EF682694	DLOOP194	NEA	Querouil et al. 2010
S. frontalis	273	EF682695	DLOOP175	NEA	Querouil et al. 2010
S. frontalis	274	EF682696	DLOOP167	NEA	Querouil et al. 2010
S. frontalis	275	EF682697	DLOOP195	NEA	Querouil et al. 2010

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Species		**GenBank**	**Haplotype**	**Localization**	**Source**
S. frontalis	276	EF682698	DLOOP3	NEA	Querouil et al. 2010
S. frontalis	277	EF682699	DLOOP161	NEA	Querouil et al. 2010
S. frontalis	278	EF682700	DLOOP161	NEA	Querouil et al. 2010
S. frontalis	279	EF682701	DLOOP196	NEA	Querouil et al. 2010
S. frontalis	280	EF682702	DLOOP197	NEA	Querouil et al. 2010
S. frontalis	281	EF682703	DLOOP1	NEA	Querouil et al. 2010
S. frontalis	282	EF682704	DLOOP161	NEA	Querouil et al. 2010
S. frontalis	283	EF682705	DLOOP3	NEA	Querouil et al. 2010
S. frontalis	284	EF682706	DLOOP175	NEA	Querouil et al. 2010
S. frontalis	285	EF682707	DLOOP175	NEA	Querouil et al. 2010
S. frontalis	286	EF682708	DLOOP193	NEA	Querouil et al. 2010
S. frontalis	287	EF682709	DLOOP181	NEA	Querouil et al. 2010
S. frontalis	288	EF682710	DLOOP181	NEA	Querouil et al. 2010
S. frontalis	289	EF682711	DLOOP175	NEA	Querouil et al. 2010
S. frontalis	290	EF682712	DLOOP189	NEA	Querouil et al. 2010
S. frontalis	291	EF682713	DLOOP198	NEA	Querouil et al. 2010
S. frontalis	292	EF682714	DLOOP189	NEA	Querouil et al. 2010
S. frontalis	293	EF682715	DLOOP199	NEA	Querouil et al. 2010
S. frontalis	294	EF682716	DLOOP200	NEA	Querouil et al. 2010
S. frontalis	295	EF682717	DLOOP195	NEA	Querouil et al. 2010
S. frontalis	296	EF682718	DLOOP189	NEA	Querouil et al. 2010
S. frontalis	297	EF682719	DLOOP175	NEA	Querouil et al. 2010
S. frontalis	298	EF682720	DLOOP171	NEA	Querouil et al. 2010
S. frontalis	299	EF682721	DLOOP3	NEA	Querouil et al. 2010
S. frontalis	300	EF682722	DLOOP165	NEA	Querouil et al. 2010
S. frontalis	301	EF682723	DLOOP171	NEA	Querouil et al. 2010
S. frontalis	302	EF682724	DLOOP161	NEA	Querouil et al. 2010
S. frontalis	303	EF682725	DLOOP201	NEA	Querouil et al. 2010
S. frontalis	304	EF682726	DLOOP166	NEA	Querouil et al. 2010
S. frontalis	305	EF682727	DLOOP161	NEA	Querouil et al. 2010
S. frontalis	306	EF682728	DLOOP184	NEA	Querouil et al. 2010
S. frontalis	307	EF682729	DLOOP202	NEA	Querouil et al. 2010
S. frontalis	308	EF682730	DLOOP203	NEA	Querouil et al. 2010
S. frontalis	309	EF682731	DLOOP161	NEA	Querouil et al. 2010
S. frontalis	310	EF682732	DLOOP165	NEA	Querouil et al. 2010
S. frontalis	311	EF682733	DLOOP166	NEA	Querouil et al. 2010
S. frontalis	312	EF682734	DLOOP177	NEA	Querouil et al. 2010
S. frontalis	313	EF682735	DLOOP161	NEA	Querouil et al. 2010
S. frontalis	314	EF682736	DLOOP204	NEA	Querouil et al. 2010
S. frontalis	315	EF682737	DLOOP161	NEA	Querouil et al. 2010
S. frontalis	316	EF682738	DLOOP205	NEA	Querouil et al. 2010
S. frontalis	317	EF682739	DLOOP175	NEA	Querouil et al. 2010
S. frontalis	318	EF682740	DLOOP175	NEA	Querouil et al. 2010
S. frontalis	319	EF682741	DLOOP161	NEA	Querouil et al. 2010
S. frontalis	320	EF682742	DLOOP161	NEA	Querouil et al. 2010
S. frontalis	321	EF682743	DLOOP206	NEA	Querouil et al. 2010
S. frontalis	322	EF682744	DLOOP165	NEA	Querouil et al. 2010
S. frontalis	323	EF682745	DLOOP207	NEA	Querouil et al. 2010
S. frontalis	324	EF682746	DLOOP167	NEA	Querouil et al. 2010
S. frontalis	325	EF682747	DLOOP208	NEA	Querouil et al. 2010
S. frontalis	326	EF682748	DLOOP1	NEA	Querouil et al. 2010
S. frontalis	327	EF682749	DLOOP161	NEA	Querouil et al. 2010
S. frontalis	328	EF682750	DLOOP165	NEA	Querouil et al. 2010
S. frontalis	329	EF682751	DLOOP161	NEA	Querouil et al. 2010
S. frontalis	330	EF682752	DLOOP165	NEA	Querouil et al. 2010
S. frontalis	331	EF682753	DLOOP209	NEA	Querouil et al. 2010
S. frontalis	332	EF682754	DLOOP210	NEA	Querouil et al. 2010

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Species		**GenBank**	**Haplotype**	**Localization**	**Source**
S. frontalis	333	EF682755	DLOOP165	NEA	Querouil et al. 2010
S. frontalis	334	EF682756	DLOOP193	NEA	Querouil et al. 2010
S. frontalis	335	EF682757	DLOOP179	NEA	Querouil et al. 2010
S. frontalis	336	EF682758	DLOOP211	NEA	Querouil et al. 2010
S. frontalis	337	EF682759	DLOOP212	NEA	Querouil et al. 2010
S. frontalis	338	EF682760	DLOOP181	NEA	Querouil et al. 2010
S. frontalis	339	EF682761	DLOOP189	NEA	Querouil et al. 2010
S. frontalis	340	EF682762	DLOOP213	NEA	Querouil et al. 2010
S. frontalis	341	EF682763	DLOOP181	NEA	Querouil et al. 2010
S. frontalis	342	EF682764	DLOOP214	NEA	Querouil et al. 2010
S. frontalis	343	EF682765	DLOOP202	NEA	Querouil et al. 2010
S. frontalis	344	EF682766	DLOOP180	NEA	Querouil et al. 2010
S. frontalis	345	EF682767	DLOOP215	NEA	Querouil et al. 2010
S. frontalis	346	EF682768	DLOOP207	NEA	Querouil et al. 2010
S. frontalis	347	EF682769	DLOOP165	NEA	Querouil et al. 2010
S. frontalis	348	EF682770	DLOOP165	NEA	Querouil et al. 2010
S. frontalis	349	EF682771	DLOOP180	NEA	Querouil et al. 2010
S. frontalis	350	EF682772	DLOOP216	NEA	Querouil et al. 2010
S. frontalis	351	EF682773	DLOOP210	NEA	Querouil et al. 2010
S. frontalis	352	EF682774	DLOOP190	NEA	Querouil et al. 2010
S. frontalis	353	EF682775	DLOOP217	NEA	Querouil et al. 2010
S. frontalis	354	EF682776	DLOOP181	NEA	Querouil et al. 2010
S. frontalis	355	EF682777	DLOOP1	NEA	Querouil et al. 2010
S. frontalis	356	EF682778	DLOOP167	NEA	Querouil et al. 2010
S. frontalis	357	EF682779	DLOOP165	NEA	Querouil et al. 2010
S. frontalis	358	EF682780	DLOOP219	NEA	Querouil et al. 2010
S. frontalis	359	EF682781	DLOOP174	NEA	Querouil et al. 2010
S. frontalis	360	EF682782	DLOOP165	NEA	Querouil et al. 2010
S. frontalis	361	EF682783	DLOOP179	NEA	Querouil et al. 2010
S. frontalis	362	EF682784	DLOOP175	NEA	Querouil et al. 2010
S. frontalis	363	EF682785	DLOOP219	NEA	Querouil et al. 2010
S. frontalis	364	EF682786	DLOOP165	NEA	Querouil et al. 2010
S. frontalis	365	EF682787	DLOOP161	NEA	Querouil et al. 2010
S. frontalis	366	EF682788	DLOOP189	NEA	Querouil et al. 2010
S. frontalis	367	EF682789	DLOOP3	NEA	Querouil et al. 2010
S. frontalis	368	EF682790	DLOOP189	NEA	Querouil et al. 2010
S. frontalis	369	EF682791	DLOOP165	NEA	Querouil et al. 2010
S. frontalis	370	EF682792	DLOOP161	NEA	Querouil et al. 2010
S. frontalis	371	EF682793	DLOOP161	NEA	Querouil et al. 2010
S. frontalis	372	EF682794	DLOOP161	NEA	Querouil et al. 2010
S. frontalis	373	EF682795	DLOOP180	NEA	Querouil et al. 2010
S. frontalis	374	EF682796	DLOOP187	NEA	Querouil et al. 2010
S. frontalis	375	EF682797	DLOOP220	NEA	Querouil et al. 2010
S. frontalis	376	EF682798	DLOOP163	NEA	Querouil et al. 2010
S. frontalis	377	EF682799	DLOOP221	NEA	Querouil et al. 2010
S. frontalis	378	EF682800	DLOOP189	NEA	Querouil et al. 2010
S. frontalis	379	EF682801	DLOOP174	NEA	Querouil et al. 2010
S. frontalis	380	EF682802	DLOOP222	NEA	Querouil et al. 2010
S. frontalis	381	EF682803	DLOOP161	NEA	Querouil et al. 2010
S. frontalis	382	EF682804	DLOOP223	NEA	Querouil et al. 2010
S. frontalis	383	EF682805	DLOOP165	NEA	Querouil et al. 2010
S. frontalis	384	EF682806	DLOOP161	NEA	Querouil et al. 2010
S. frontalis	385	EF682807	DLOOP224	NEA	Querouil et al. 2010
S. frontalis	386	EF682808	DLOOP225	NEA	Querouil et al. 2010
S. frontalis	387	EF682809	DLOOP202	NEA	Querouil et al. 2010
S. frontalis	388	EF682810	DLOOP226	NEA	Querouil et al. 2010
S. frontalis	389	EF682811	DLOOP226	NEA	Querouil et al. 2010

D-loop (331 bp)					
Species		**GenBank**	**Haplotype**	**Localization**	**Source**
S. frontalis	390	EF682812	DLOOP3	NEA	Querouil et al. 2010
S. frontalis	391	EF682813	DLOOP161	NEA	Querouil et al. 2010
S. frontalis	392	EF682814	DLOOP184	NEA	Querouil et al. 2010
S. frontalis	393	EF682815	DLOOP165	NEA	Querouil et al. 2010
S. frontalis	394	EF682816	DLOOP190	NEA	Querouil et al. 2010
S. frontalis	395	EF682817	DLOOP181	NEA	Querouil et al. 2010
S. frontalis	396	EF682818	DLOOP211	NEA	Querouil et al. 2010
S. frontalis	397	EF682819	DLOOP161	NEA	Querouil et al. 2010
S. frontalis	398	EF682820	DLOOP227	NEA	Querouil et al. 2010
S. frontalis	399	EF682821	DLOOP175	NEA	Querouil et al. 2010
S. frontalis	400	EF682822	DLOOP180	NEA	Querouil et al. 2010
S. frontalis	401	EF682823	DLOOP175	NEA	Querouil et al. 2010
S. frontalis	402	EF682824	DLOOP181	NEA	Querouil et al. 2010
S. frontalis	403	EF682825	DLOOP3	NEA	Querouil et al. 2010
S. frontalis	404	EF682826	DLOOP202	NEA	Querouil et al. 2010
S. frontalis	405	EF682827	DLOOP228	NEA	Querouil et al. 2010
S. frontalis	406	EF682828	DLOOP167	NEA	Querouil et al. 2010
S. frontalis	407	EF682829	DLOOP177	NEA	Querouil et al. 2010
S. frontalis	408	EF682830	DLOOP229	NEA	Querouil et al. 2010
S. frontalis	409	EF682831	DLOOP230	NEA	Querouil et al. 2010
S. frontalis	410	EF682832	DLOOP231	NEA	Querouil et al. 2010
S. frontalis	411	EF682833	DLOOP232	NEA	Querouil et al. 2010
S. frontalis	412	EF682834	DLOOP229	NEA	Querouil et al. 2010
S. frontalis	413	EF682835	DLOOP233	NEA	Querouil et al. 2010
S. frontalis	414	EF682836	DLOOP195	NEA	Querouil et al. 2010
S. frontalis	415	EF682837	DLOOP234	NEA	Querouil et al. 2010
S. frontalis	416	EF682838	DLOOP221	NEA	Querouil et al. 2010
S. frontalis	417	EF682839	DLOOP175	NEA	Querouil et al. 2010
S. frontalis	418	EF682840	DLOOP191	NEA	Querouil et al. 2010
S. frontalis	419	GQ504170	DLOOP235	NWA	Kingston et al. 2009
S. frontalis	420	GQ504171	DLOOP236	NWA	Kingston et al. 2009
S. frontalis	421	GQ504172	DLOOP237	NWA	Kingston et al. 2009
S. frontalis	422	GQ504173	DLOOP166	NWA	Kingston et al. 2009
S. frontalis	423	GQ504174	DLOOP191	NWA	Kingston et al. 2009
S. frontalis	424	GQ504175	DLOOP238	NWA	Kingston et al. 2009
S. frontalis	425	GQ504176	DLOOP239	NWA	Kingston et al. 2009
S. frontalis	426	GQ504177	DLOOP184	NWA	Kingston et al. 2009
S. frontalis	427	GQ504178	DLOOP189	NWA	Kingston et al. 2009
S. frontalis	428	GQ504179	DLOOP175	NWA	Kingston et al. 2009
S. frontalis	429	GQ504180	DLOOP180	NWA	Kingston et al. 2009
S. frontalis	430	GQ504181	DLOOP240	NWA	Kingston et al. 2009
S. frontalis	431	GQ504182	DLOOP241	NWA	Kingston et al. 2009
S. frontalis	432	GQ504183	DLOOP221	NWA	Kingston et al. 2009
S. frontalis	433	GQ504184	DLOOP242	NWA	Kingston et al. 2009
S. frontalis	434	GQ504185	DLOOP243	NWA	Kingston et al. 2009
S. frontalis	435	GQ504186	DLOOP244	NWA	Kingston et al. 2009
S. frontalis	436	GQ504187	DLOOP245	NWA	Kingston et al. 2009
S. frontalis	437	GQ504188	DLOOP246	NWA	Kingston et al. 2009
S. frontalis	438	GQ504189	DLOOP247	NWA	Kingston et al. 2009
S. frontalis	439	GQ504190	DLOOP248	NWA	Kingston et al. 2009
S. frontalis	440	GQ504191	DLOOP249	NWA	Kingston et al. 2009
S. frontalis	441	GQ504192	DLOOP250	NWA	Kingston et al. 2009
S. frontalis	442	GQ504193	DLOOP181	NWA	Kingston et al. 2009
S. frontalis	443	GQ504194	DLOOP251	NWA	Kingston et al. 2009
S. frontalis	444	GQ504195	DLOOP252	NWA	Kingston et al. 2009
S. frontalis	445	KC204733	DLOOP163	SWA	Caballero et al. 2013
S. frontalis	446	KC204734	DLOOP166	SWA	Caballero et al. 2013

D-loop (331 bp)					
Species		**GenBank**	**Haplotype**	**Localization**	**Source**
S. frontalis	447	KC204735	DLOOP162	SWA	Caballero et al. 2013
S. frontalis	448	KC204736	DLOOP253	NWA	Caballero et al. 2013
S. frontalis	449	KC204737	DLOOP248	NWA	Caballero et al. 2013
S. frontalis	450	KC204738	DLOOP254	NWA	Caballero et al. 2013
S. frontalis	451	KC204739	DLOOP1	NWA	Caballero et al. 2013
S. frontalis	452	KC204740	DLOOP255	NWA	Caballero et al. 2013
S. frontalis	453	EU121116	DLOOP254	NWA	Caballero et al. 2008
S. frontalis	454	JX414567	DLOOP256	NWA	Viricel et al. 2014
S. frontalis	455	JX414568	DLOOP190	NWA	Viricel et al. 2014
S. frontalis	456	JX414569	DLOOP257	NWA	Viricel et al. 2014
S. frontalis	457	JX414570	DLOOP258	NWA	Viricel et al. 2014
S. frontalis	458	JX414571	DLOOP161	NWA	Viricel et al. 2014
S. frontalis	459	JX414572	DLOOP259	NWA	Viricel et al. 2014
S. frontalis	460	JX414573	DLOOP260	NWA	Viricel et al. 2014
S. frontalis	461	JX414574	DLOOP210	NWA	Viricel et al. 2014
S. frontalis	462	JX414575	DLOOP261	NWA	Viricel et al. 2014
S. frontalis	463	JX414576	DLOOP262	NWA	Viricel et al. 2014
S. frontalis	464	JX414577	DLOOP179	NWA	Viricel et al. 2014
S. frontalis	465	JX414578	DLOOP195	NWA	Viricel et al. 2014
S. frontalis	466	JX414579	DLOOP165	NWA	Viricel et al. 2014
S. frontalis	467	JX414580	DLOOP178	NWA	Viricel et al. 2014
S. frontalis	468	JX414581	DLOOP263	NWA	Viricel et al. 2014
S. frontalis	469	JX414582	DLOOP264	NWA	Viricel et al. 2014
S. frontalis	470	JX414583	DLOOP265	NWA	Viricel et al. 2014
S. frontalis	471	JX414584	DLOOP266	NWA	Viricel et al. 2014
S. frontalis	472	JX414585	DLOOP194	NWA	Viricel et al. 2014
S. frontalis	473	JX414586	DLOOP267	NWA	Viricel et al. 2014
S. frontalis	474	JX414587	DLOOP268	NWA	Viricel et al. 2014
S. frontalis	475	JX414588	DLOOP269	NWA	Viricel et al. 2014
S. frontalis	476	JX414589	DLOOP270	NWA	Viricel et al. 2014
S. frontalis	477	JX414590	DLOOP271	NWA	Viricel et al. 2014
S. frontalis	478	JX414591	DLOOP272	NWA	Viricel et al. 2014
S. frontalis	479	JX414592	DLOOP273	NWA	Viricel et al. 2014
S. frontalis	480	JX414593	DLOOP274	NWA	Viricel et al. 2014
S. frontalis	481	JX414594	DLOOP275	NWA	Viricel et al. 2014
S. frontalis	482	JX414595	DLOOP276	NWA	Viricel et al. 2014
S. frontalis	483	JX414596	DLOOP277	NWA	Viricel et al. 2014
S. longirostris	484	XXXXXX	DLOOP278	SWA (Brazil)	This study
S. longirostris	485	XXXXXX	DLOOP279	SWA (Brazil)	This study
S. longirostris	486	XXXXXX	DLOOP280	SWA (Brazil)	This study
S. longirostris	487	XXXXXX	DLOOP281	SWA (Brazil)	This study
S. longirostris	488	XXXXXX	DLOOP282	SWA (Brazil)	This study
S. longirostris	489	XXXXXX	DLOOP283	SWA (Brazil)	This study
S. longirostris	490	XXXXXX	DLOOP284	SWA (Brazil)	This study
S. longirostris	491	XXXXXX	DLOOP285	SWA (Brazil)	This study
S. longirostris	492	XXXXXX	DLOOP286	SWA (Brazil)	This study
S. longirostris	493	XXXXXX	DLOOP287	SWA (Brazil)	This study
S. longirostris	494	XXXXXX	DLOOP288	SWA (Brazil)	This study
S. longirostris	495	XXXXXX	DLOOP289	SWA (Brazil)	This study
S. longirostris	496	XXXXXX	DLOOP290	SWA (Brazil)	This study
S. longirostris	497	XXXXXX	DLOOP291	SWA (Brazil)	This study
S. longirostris	498	DQ845444	DLOOP292	NWA	Kingston et al. 2009
S. longirostris	499	DQ845445	DLOOP282	NWA	Kingston et al. 2009
S. longirostris	500	EU121117	DLOOP293	SEPO	Caballero et al. 2008
S. longirostris	501	GQ504125	DLOOP294	EA	Kingston et al. 2009
S. longirostris	502	GQ504165	DLOOP295	NWA	Kingston et al. 2009
S. longirostris	503	GQ504166	DLOOP296	NWA	Kingston et al. 2009

D-loop (331 bp)					
Species		**GenBank**	**Haplotype**	**Localization**	**Source**
S. longirostris	504	GQ504167	DLOOP297	NWA	Kingston et al. 2009
S. longirostris	505	GQ504168	DLOOP298	NWA	Kingston et al. 2009
S. longirostris	506	GQ504169	DLOOP253	NWA	Kingston et al. 2009
S. longirostris	507	GU253256	DLOOP299	NEP	Andrews et al. 2010
S. longirostris	508	GU253257	DLOOP300	NEP	Andrews et al. 2010
S. longirostris	509	GU253258	DLOOP301	NEP	Andrews et al. 2010
S. longirostris	510	GU253259	DLOOP302	NEP	Andrews et al. 2010
S. longirostris	511	GU253260	DLOOP303	NEP, SWP	Andrews et al. 2010
S. longirostris	512	GU253261	DLOOP304	NEP	Andrews et al. 2010
S. longirostris	513	GU253262	DLOOP305	NEP, SWP	Andrews et al. 2010
S. longirostris	514	GU253263	DLOOP306	NEP, SWP	Andrews et al. 2010
S. longirostris	515	GU253264	DLOOP307	NEP	Andrews et al. 2010
S. longirostris	516	GU253265	DLOOP308	NEP	Andrews et al. 2010
S. longirostris	517	GU253266	DLOOP309	NEP	Andrews et al. 2010
S. longirostris	518	GU253267	DLOOP310	NEP	Andrews et al. 2010
S. longirostris	519	GU253268	DLOOP289	NEP	Andrews et al. 2010
S. longirostris	520	GU253269	DLOOP311	NEP	Andrews et al. 2010
S. longirostris	521	GU253270	DLOOP312	NEP	Andrews et al. 2010
S. longirostris	522	GU253271	DLOOP313	NEP	Andrews et al. 2010
S. longirostris	523	GU253272	DLOOP314	NEP	Andrews et al. 2010
S. longirostris	524	GU253273	DLOOP315	NEP	Andrews et al. 2010
S. longirostris	525	GU253274	DLOOP316	NEP	Andrews et al. 2010
S. longirostris	526	GU253275	DLOOP317	SWP	Andrews et al. 2010
S. longirostris	527	GU253276	DLOOP318	SWP	Andrews et al. 2010
S. longirostris	528	GU253277	DLOOP319	SWP	Andrews et al. 2010
S. longirostris	529	GU253278	DLOOP320	SWP	Andrews et al. 2010
S. longirostris	530	GU253279	DLOOP321	SWP	Andrews et al. 2010
S. longirostris	531	GU253280	DLOOP322	SWP	Andrews et al. 2010
S. longirostris	532	GU253281	DLOOP323	SWP	Andrews et al. 2010
S. longirostris	533	GU253282	DLOOP324	SWP	Andrews et al. 2010
S. longirostris	534	GU253283	DLOOP325	SWP	Andrews et al. 2010
S. longirostris	535	GU253284	DLOOP326	SWP	Andrews et al. 2010
S. longirostris	536	KC160997	DLOOP312	NEP	Andrews et al. 2013
S. longirostris	537	KC160998	DLOOP299	NEP	Andrews et al. 2013
S. longirostris	538	KC160999	DLOOP306	NEP, NWP, SEP	Andrews et al. 2013
S. longirostris	539	KC161000	DLOOP327	EP	Andrews et al. 2013
S. longirostris	540	KC161001	DLOOP328	EP	Andrews et al. 2013
S. longirostris	541	KC161002	DLOOP329	EP	Andrews et al. 2013
S. longirostris	542	KC161003	DLOOP330	EP	Andrews et al. 2013
S. longirostris	543	KC161004	DLOOP331	EP	Andrews et al. 2013
S. longirostris	544	KC161005	DLOOP332	EP	Andrews et al. 2013
S. longirostris	545	KC161006	DLOOP333	EP	Andrews et al. 2013
S. longirostris	546	KC161007	DLOOP334	EP	Andrews et al. 2013
S. longirostris	547	KC161008	DLOOP335	EP	Andrews et al. 2013
S. longirostris	548	KC161009	DLOOP329	EP	Andrews et al. 2013
S. longirostris	549	KC161010	DLOOP336	EP	Andrews et al. 2013
S. longirostris	550	KC161011	DLOOP337	EP	Andrews et al. 2013
S. longirostris	551	KC161012	DLOOP338	EP	Andrews et al. 2013
S. longirostris	552	KC161013	DLOOP339	EP	Andrews et al. 2013
S. longirostris	553	KC161014	DLOOP340	EP	Andrews et al. 2013
S. longirostris	554	KC161015	DLOOP341	EP	Andrews et al. 2013
S. longirostris	555	KC161016	DLOOP342	IP	Andrews et al. 2013
S. longirostris	556	KC161017	DLOOP343	EP	Andrews et al. 2013
S. longirostris	557	KC161018	DLOOP314	NEP	Andrews et al. 2013
S. longirostris	558	KC161019	DLOOP344	EP	Andrews et al. 2013
S. longirostris	559	KC161020	DLOOP345	EP	Andrews et al. 2013
S. longirostris	560	KC161021	DLOOP346	SWI	Andrews et al. 2013

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Species		**GenBank**	**Haplotype**	**Localization**	**Source**
S. longirostris	561	KC161022	DLOOP347	EP	Andrews et al. 2013
S. longirostris	562	KC161023	DLOOP348	EPO	Andrews et al. 2013
S. longirostris	563	KC161024	DLOOP349	EP	Andrews et al. 2013
S. longirostris	564	KC161025	DLOOP350	EP	Andrews et al. 2013
S. longirostris	565	KC161026	DLOOP351	EP	Andrews et al. 2013
S. longirostris	566	KC161027	DLOOP352	EP	Andrews et al. 2013
S. longirostris	567	KC161028	DLOOP353	EP	Andrews et al. 2013
S. longirostris	568	KC161029	DLOOP354	EP	Andrews et al. 2013
S. longirostris	569	KC161030	DLOOP355	EP	Andrews et al. 2013
S. longirostris	570	KC161031	DLOOP356	EP	Andrews et al. 2013
S. longirostris	571	KC161032	DLOOP321	EP	Andrews et al. 2013
S. longirostris	572	KC161033	DLOOP357	EP	Andrews et al. 2013
S. longirostris	573	KC161034	DLOOP358	EP	Andrews et al. 2013
S. longirostris	574	KC161035	DLOOP340	EP	Andrews et al. 2013
S. longirostris	575	KC161036	DLOOP341	EP	Andrews et al. 2013
S. longirostris	576	KC161037	DLOOP283	NEI, SWI, IP	Andrews et al. 2013
S. longirostris	577	KC161038	DLOOP303	NEP, SEP, SWP	Andrews et al. 2013
S. longirostris	578	KC161039	DLOOP301	NEP	Andrews et al. 2013
S. longirostris	579	KC161040	DLOOP300	NEP	Andrews et al. 2013
S. longirostris	580	KC161041	DLOOP296	NWA	Andrews et al. 2013
S. longirostris	581	KC161042	DLOOP282	NWA	Andrews et al. 2013
S. longirostris	582	KC161043	DLOOP359	NWP	Andrews et al. 2013
S. longirostris	583	KC161044	DLOOP360	NWP	Andrews et al. 2013
S. longirostris	584	KC161045	DLOOP361	NWP	Andrews et al. 2013
S. longirostris	585	KC161046	DLOOP362	NWP	Andrews et al. 2013
S. longirostris	586	KC161047	DLOOP363	IP	Andrews et al. 2013
S. longirostris	587	KC161048	DLOOP364	IP	Andrews et al. 2013
S. longirostris	588	KC161049	DLOOP365	IP	Andrews et al. 2013
S. longirostris	589	KC161050	DLOOP366	IP	Andrews et al. 2013
S. longirostris	590	KC161051	DLOOP367	NEI	Andrews et al. 2013
S. longirostris	591	KC161052	DLOOP368	NEI	Andrews et al. 2013
S. longirostris	592	KC161053	DLOOP369	NEI	Andrews et al. 2013
S. longirostris	593	KC161054	DLOOP304	NEP	Andrews et al. 2013
S. longirostris	594	KC161055	DLOOP284	NWA	Andrews et al. 2013
S. longirostris	595	KC161056	DLOOP370	NWA	Andrews et al. 2013
S. longirostris	596	KC161057	DLOOP307	NWP, NEP	Andrews et al. 2013
S. longirostris	597	KC161058	DLOOP308	NEP, NWP	Andrews et al. 2013
S. longirostris	598	KC161059	DLOOP371	NWP	Andrews et al. 2013
S. longirostris	599	KC161060	DLOOP372	NWP	Andrews et al. 2013
S. longirostris	600	KC161061	DLOOP373	NWP	Andrews et al. 2013
S. longirostris	601	KC161062	DLOOP374	NWP	Andrews et al. 2013
S. longirostris	602	KC161063	DLOOP375	NWP	Andrews et al. 2013
S. longirostris	603	KC161064	DLOOP311	NEP	Andrews et al. 2013
S. longirostris	604	KC161065	DLOOP376	NEP	Andrews et al. 2013
S. longirostris	605	KC161066	DLOOP324	NEP, SEP, SWP	Andrews et al. 2013
S. longirostris	606	KC161067	DLOOP323	NEP, SEP, SWP	Andrews et al. 2013
S. longirostris	607	KC161068	DLOOP317	SWP	Andrews et al. 2013
S. longirostris	608	KC161069	DLOOP318	SWP	Andrews et al. 2013
S. longirostris	609	KC161070	DLOOP319	SWP	Andrews et al. 2013
S. longirostris	610	KC161071	DLOOP305	SWP	Andrews et al. 2013
S. longirostris	611	KC161072	DLOOP320	SWP, SEP	Andrews et al. 2013
S. longirostris	612	KC161073	DLOOP321	SWP, SEP	Andrews et al. 2013
S. longirostris	613	KC161074	DLOOP322	SWP	Andrews et al. 2013
S. longirostris	614	KC161075	DLOOP325	SWP	Andrews et al. 2013
S. longirostris	615	KC161076	DLOOP326	SWP	Andrews et al. 2013
S. longirostris	616	KC161077	DLOOP313	NWP	Andrews et al. 2013
S. longirostris	617	KC161078	DLOOP377	EP	Andrews et al. 2013

D-loop (331 bp)					
Species		**GenBank**	**Haplotype**	**Localization**	**Source**
S. longirostris	618	KC161079	DLOOP378	EP	Andrews et al. 2013
S. longirostris	619	KC161080	DLOOP379	EP	Andrews et al. 2013
S. longirostris	620	KC161081	DLOOP380	EP	Andrews et al. 2013
S. longirostris	621	KC161082	DLOOP381	EP	Andrews et al. 2013
S. longirostris	622	KC161083	DLOOP382	EP	Andrews et al. 2013
S. longirostris	623	KC161084	DLOOP383	EP	Andrews et al. 2013
S. longirostris	624	KC161085	DLOOP346	EP	Andrews et al. 2013
S. longirostris	625	KC161086	DLOOP384	EP	Andrews et al. 2013
S. longirostris	626	KC161087	DLOOP346	EP	Andrews et al. 2013
S. longirostris	627	KC161088	DLOOP385	EP	Andrews et al. 2013
S. longirostris	628	KC161089	DLOOP386	EP	Andrews et al. 2013
S. longirostris	629	KC161090	DLOOP387	EP	Andrews et al. 2013
S. longirostris	630	KC161091	DLOOP388	EP	Andrews et al. 2013
S. longirostris	631	KC161092	DLOOP389	EP	Andrews et al. 2013
S. longirostris	632	KC161093	DLOOP390	IP	Andrews et al. 2013
S. longirostris	633	KC161094	DLOOP391	IP	Andrews et al. 2013
S. longirostris	634	KC161095	DLOOP392	IP	Andrews et al. 2013
S. longirostris	635	KC161096	DLOOP393	NWP	Andrews et al. 2013
S. longirostris	636	KC161097	DLOOP394	NWP	Andrews et al. 2013
S. longirostris	637	KC161098	DLOOP355	NWP	Andrews et al. 2013
S. longirostris	638	KC161099	DLOOP395	EP	Andrews et al. 2013
S. longirostris	639	KC161100	DLOOP396	EP	Andrews et al. 2013
S. longirostris	640	KC161101	DLOOP397	EP	Andrews et al. 2013
S. longirostris	641	KC161102	DLOOP398	EP	Andrews et al. 2013
S. longirostris	642	KC161103	DLOOP399	EP	Andrews et al. 2013
S. longirostris	643	KC161104	DLOOP400	EP	Andrews et al. 2013
S. longirostris	644	KC161105	DLOOP401	EP	Andrews et al. 2013
S. longirostris	645	KC161106	DLOOP357	EP	Andrews et al. 2013
S. longirostris	646	KC161107	DLOOP313	EP	Andrews et al. 2013
S. longirostris	647	KC161108	DLOOP402	EP	Andrews et al. 2013
S. longirostris	648	KC161109	DLOOP314	EP	Andrews et al. 2013
S. longirostris	649	KC161110	DLOOP403	EP	Andrews et al. 2013
S. longirostris	650	KC161111	DLOOP404	EP	Andrews et al. 2013
S. longirostris	651	KC161112	DLOOP405	EP	Andrews et al. 2013
S. longirostris	652	KC161113	DLOOP406	SWI	Andrews et al. 2013
S. longirostris	653	KC161114	DLOOP280	SWI	Andrews et al. 2013
S. longirostris	654	KC161115	DLOOP407	SWI	Andrews et al. 2013
S. longirostris	655	KC161116	DLOOP408	SEP	Andrews et al. 2013
S. longirostris	656	KC161117	DLOOP310	SEP	Andrews et al. 2013
S. longirostris	657	KC161118	DLOOP293	SEP	Andrews et al. 2013
S. longirostris	658	KC161119	DLOOP409	SEP	Andrews et al. 2013
S. longirostris	659	KC161120	DLOOP410	SEP	Andrews et al. 2013
S. longirostris	660	KC161121	DLOOP323	SEP	Andrews et al. 2013
S. longirostris	661	KC161122	DLOOP411	SEP	Andrews et al. 2013
S. longirostris	662	KC161123	DLOOP412	SEP	Andrews et al. 2013
S. longirostris	663	KC161124	DLOOP393	SEP	Andrews et al. 2013
S. longirostris	664	KC161125	DLOOP413	SEP	Andrews et al. 2013
S. longirostris	665	KX905105	DLOOP406	SWI	Viricel et al. 2016
S. longirostris	666	KX905106	DLOOP414	SWI	Viricel et al. 2016
S. longirostris	667	KX905107	DLOOP415	SWI	Viricel et al. 2016
S. longirostris	668	KX905108	DLOOP314	SWI	Viricel et al. 2016
S. longirostris	669	KX905109	DLOOP283	SWI	Viricel et al. 2016
S. longirostris	670	KX905110	DLOOP416	SWI	Viricel et al. 2016
S. longirostris	671	KX905111	DLOOP417	SWI	Viricel et al. 2016
S. longirostris	672	KX905112	DLOOP418	SWI	Viricel et al. 2016
S. longirostris	673	KX905113	DLOOP395	SWI	Viricel et al. 2016
S. longirostris	674	KX905114	DLOOP419	SWI	Viricel et al. 2016

D-loop (331 bp)					
Species		**GenBank**	**Haplotype**	**Localization**	**Source**
S. longirostris	675	KX905115	DLOOP420	SWI	Viricel et al. 2016
S. longirostris	676	KX905116	DLOOP421	SWI	Viricel et al. 2016
S. longirostris	677	KX905117	DLOOP346	SWI	Viricel et al. 2016
S. longirostris	678	KX905118	DLOOP422	SWI	Viricel et al. 2016
S. longirostris	679	KX905119	DLOOP423	SWI	Viricel et al. 2016
S. longirostris	680	KX905120	DLOOP406	SWI	Viricel et al. 2016
S. longirostris	681	KX905121	DLOOP280	SWI	Viricel et al. 2016
S. longirostris	682	KX905122	DLOOP424	SWI	Viricel et al. 2016
S. longirostris	683	KX905123	DLOOP395	SWI	Viricel et al. 2016
S. longirostris	684	KX905124	DLOOP314	SWI	Viricel et al. 2016
S. longirostris	685	KX905125	DLOOP345	SWI	Viricel et al. 2016
S. longirostris	686	KX905126	DLOOP425	SWI	Viricel et al. 2016
S. longirostris	687	KX905127	DLOOP426	SWI	Viricel et al. 2016
S. longirostris	688	KX905128	DLOOP427	SWI	Viricel et al. 2016
S. longirostris	689	KX905129	DLOOP428	SWI	Viricel et al. 2016
S. longirostris	690	KX905130	DLOOP429	SWI	Viricel et al. 2016
S. longirostris	691	KX905131	DLOOP314	SWI	Viricel et al. 2016
S. longirostris	692	KX905132	DLOOP279	SWI	Viricel et al. 2016
S. longirostris	693	KY457781	DLOOP359	NWP	Martien et al. 2014
S. longirostris	694	KY457782	DLOOP430	NWP	Martien et al. 2014
S. longirostris	695	KY457783	DLOOP360	NWP	Martien et al. 2014
S. longirostris	696	KY457784	DLOOP306	NWP	Martien et al. 2014
S. longirostris	697	KY457785	DLOOP362	NWP	Martien et al. 2014
S. longirostris	698	KY457786	DLOOP307	NWP	Martien et al. 2014
S. longirostris	699	KY457787	DLOOP431	NWP	Martien et al. 2014
S. longirostris	700	KY457788	DLOOP304	NWP	Martien et al. 2014
S. longirostris	701	KY457789	DLOOP432	NWP	Martien et al. 2014
S. longirostris	702	KY457790	DLOOP371	NWP	Martien et al. 2014
S. longirostris	703	KY457791	DLOOP301	NWP	Martien et al. 2014
S. longirostris	704	KY457792	DLOOP310	NWP	Martien et al. 2014
S. longirostris	705	KY457793	DLOOP313	NWP	Martien et al. 2014
S. longirostris	706	KY457794	DLOOP433	NWP	Martien et al. 2014
S. longirostris	707	KY457795	DLOOP361	NWP	Martien et al. 2014
S. longirostris	708	KY457796	DLOOP434	NWP	Martien et al. 2014
S. longirostris	709	KY457797	DLOOP323	NWP	Martien et al. 2014
S. longirostris	710	KY457798	DLOOP414	NWP	Martien et al. 2014
S. longirostris	711	KY457799	DLOOP435	NWP	Martien et al. 2014
S. longirostris	712	KY457800	DLOOP436	NWP	Martien et al. 2014
S. longirostris	713	KY457801	DLOOP437	NWP	Martien et al. 2014
S. longirostris	714	KY457802	DLOOP311	NWP	Martien et al. 2014
S. longirostris	715	KY457803	DLOOP314	NWP	Martien et al. 2014
S. longirostris	716	KY457804	DLOOP309	NWP	Martien et al. 2014
S. longirostris	717	EF558737	DLOOP410	SEP	Oremus et al. 2007
S. longirostris	718	EF558738	DLOOP408	SEP	Oremus et al. 2007
S. longirostris	719	EF558739	DLOOP310	SEP	Oremus et al. 2007
S. longirostris	720	EF558740	DLOOP293	SEP	Oremus et al. 2007
S. longirostris	721	EF558741	DLOOP409	SEP	Oremus et al. 2007
S. longirostris	722	EF558742	DLOOP323	SEP	Oremus et al. 2007
S. longirostris	723	EF558743	DLOOP321	SEP	Oremus et al. 2007
S. longirostris	724	EF558744	DLOOP364	SEP	Oremus et al. 2007
S. longirostris	725	EF558745	DLOOP320	SEP	Oremus et al. 2007
S. longirostris	726	EF558746	DLOOP306	SEP	Oremus et al. 2007
S. longirostris	727	EF558747	DLOOP324	SEP	Oremus et al. 2007
S. longirostris	728	EF558748	DLOOP323	SEP	Oremus et al. 2007
S. longirostris	729	EF558749	DLOOP412	SEP	Oremus et al. 2007
S. longirostris	730	EF558750	DLOOP411	SEP	Oremus et al. 2007
S. longirostris	731	EF558751	DLOOP308	SEP	Oremus et al. 2007

D-loop (331 bp)					
Species		**GenBank**	**Haplotype**	**Localization**	**Source**
S. longirostris	732	EF558752	DLOOP303	SEP	Oremus et al. 2007
S. longirostris	733	EF558753	DLOOP393	SEP	Oremus et al. 2007
S. longirostris	734	EF558754	DLOOP323	SEP	Oremus et al. 2007
S. longirostris	735	EF558755	DLOOP322	SEP	Oremus et al. 2007
S. longirostris	736	EF558756	DLOOP438	SEP	Oremus et al. 2007
S. longirostris	737	EF558757	DLOOP304	SEP	Oremus et al. 2007
S. longirostris	738	EF558758	DLOOP393	SEP	Oremus et al. 2007
S. longirostris	739	EF558759	DLOOP439	SEP	Oremus et al. 2007
S. longirostris	740	EF558760	DLOOP350	SEP	Oremus et al. 2007
S. longirostris	741	EF558761	DLOOP440	SEP	Oremus et al. 2007
S. longirostris	742	EF558762	DLOOP441	SEP	Oremus et al. 2007
S. longirostris	743	EF558763	DLOOP434	SEP	Oremus et al. 2007
S. longirostris	744	EF558764	DLOOP442	SEP	Oremus et al. 2007
S. longirostris	745	EF558765	DLOOP314	SEP	Oremus et al. 2007
S. longirostris	746	EF558766	DLOOP443	SEP	Oremus et al. 2007
S. longirostris	747	EF558767	DLOOP323	SEP	Oremus et al. 2007
S. longirostris	748	KP756642	DLOOP444	SWP	Oremus et al. 2015
S. longirostris	749	KP756643	DLOOP308	SWP	Oremus et al. 2015
S. longirostris	750	KP756644	DLOOP408	SWP	Oremus et al. 2015
Cyt-b (331 bp)					
Species		**GenBank**	**Haplotype**	**Localization**	**Source**
S. attenuata	1	XXXXX	CYTB1	SWA (Brazil)	This study
S. attenuata	2	XXXXX	CYTB2	SWA (Brazil)	This study
S. attenuata	3	XXXXX	CYTB3	SWA (Brazil)	This study
S. attenuata	4	EF438304	CYTB1	IN	Jayasankar et al. 2009
S. attenuata	5	AF084096	CYTB3	EP	Leduc et al. 1999
S. attenuata	6	AF084097	CYTB4	NWA	Leduc et al. 1999
S. clymene	7	XXXXXX	CYTB5	SWA (Brazil)	This study
S. clymene	8	XXXXXX	CYTB6	SWA (Brazil)	This study
S. clymene	9	XXXXXX	CYTB7	SWA (Brazil)	This study
S. clymene	10	XXXXXX	CYTB8	SWA (Brazil)	This study
S. clymene	11	XXXXXX	CYTB9	SWA (Brazil)	This study
S. clymene	12	XXXXXX	CYTB10	SWA (Brazil)	This study
S. clymene	13	EU517711	CYTB11	NWA	Viricel et al. 2012
S. clymene	14	EU517712	CYTB12	NWA	Viricel et al. 2012
S. coeruleoalba	15	XXXXX	CYTB10	SWA (Brazil)	This study
S. coeruleoalba	16	XXXXX	CYTB10	SWA (Brazil)	This study
S. coeruleoalba	17	XXXXX	CYTB13	SWA (Brazil)	This study
S. coeruleoalba	18	XXXXX	CYTB14	SWA (Brazil)	This study
S. coeruleoalba	19	XXXXX	CYTB15	SWA (Brazil)	This study
S. coeruleoalba	20	XXXXX	CYTB16	SWA (Brazil)	This study
S. coeruleoalba	21	XXXXX	CYTB17	SWA (Brazil)	This study
S. coeruleoalba	22	XXXXX	CYTB18	SWA (Brazil)	This study
S. coeruleoalba	23	XXXXX	CYTB19	SWA (Brazil)	This study
S. coeruleoalba	24	EU580088	CYTB20	NWA	Viricel et al. 2012
S. coeruleoalba	25	AF084082	CYTB21	NEA	Leduc et al. 1999
S. frontalis	26	XXXXX	CYTB22	SWA (Brazil)	This study
S. frontalis	27	XXXXX	CYTB23	SWA (Brazil)	This study
S. frontalis	28	XXXXX	CYTB24	SWA (Brazil)	This study
S. frontalis	29	XXXXX	CYTB25	SWA (Brazil)	This study
S. frontalis	30	XXXXX	CYTB26	SWA (Brazil)	This study
S. frontalis	31	XXXXX	CYTB27	SWA (Brazil)	This study
S. frontalis	32	EU121092	CYTB28	NWA	Leduc et al. 1999
S. frontalis	33	EU517713	CYTB23	NWA	Leduc et al. 1999
S. frontalis	34	EU517714	CYTB29	NWA	Caballero et al. 2008
S. longirostris	35	XXXXX	CYTB30	SWA (Brazil)	This study
S. longirostris	36	XXXXX	CYTB31	SWA (Brazil)	This study

Cyt-b (331 bp)					
Species		**GenBank**	**Haplotype**	**Localization**	**Source**
S. longirostris	37	XXXXX	CYTB32	SWA (Brazil)	This study
S. longirostris	38	XXXXX	CYTB33	SWA (Brazil)	This study
S. longirostris	39	XXXXX	CYTB34	SWA (Brazil)	This study
S. longirostris	40	XXXXX	CYTB35	SWA (Brazil)	This study
S. longirostris	41	XXXXX	CYTB36	SWA (Brazil)	This study
S. longirostris	42	XXXXX	CYTB37	SWA (Brazil)	This study
S. longirostris	43	AF084100	CYTB38	NWA	Leduc et al. 1999
S. longirostris	44	AF084101	CYTB39	EP	Leduc et al. 1999
S. longirostris	45	AF084102	CYTB40	NWP	Leduc et al. 1999
S. longirostris	46	AF084103	CYTB41	IN	Leduc et al. 1999
S. longirostris	47	DQ232770	CYTB42	IN	Jayasankar et al. 2008
S. longirostris	48	DQ270182	CYTB35	IN	Jayasankar et al. 2008
S. longirostris	49	EF057433	CYTB35	IN	Jayasankar et al. 2008
S. longirostris	50	EF057434	CYTB43	IN	Jayasankar et al. 2008
S. longirostris	51	EF057436	CYTB44	IN	Jayasankar et al. 2008
S. longirostris	52	EF057437	CYTB45	IN	Jayasankar et al. 2008
S. longirostris	53	EF057438	CYTB45	IN	Jayasankar et al. 2008
S. longirostris	54	EF203445	CYTB41	IN	Jayasankar et al. 2008
S. longirostris	55	EF203446	CYTB31	IN	Jayasankar et al. 2008
S. longirostris	56	EF203447	CYTB46	IN	Jayasankar et al. 2008
S. longirostris	57	EF203448	CYTB34	IN	Jayasankar et al. 2008
S. longirostris	58	EF203449	CYTB31	IN	Jayasankar et al. 2008
S. longirostris	59	EF203450	CYTB34	IN	Jayasankar et al. 2008
S. longirostris	60	EF446613	CYTB44	IN	Jayasankar et al. 2008
S. longirostris	61	EF446614	CYTB31	IN	Jayasankar et al. 2008
S. longirostris	62	EU204619	CYTB34	IN	Jayasankar et al. 2008
S. longirostris	63	EU517703	CYTB35	NWA	Viricel et al. 2012
S. longirostris	64	EU517715	CYTB47	NWA	Viricel et al. 2012
S. longirostris	65	KC161126	CYTB48	NEP	Andrews et al. 2013
S. longirostris	66	KC161127	CYTB35	EP, NEP, NWP, SEP, SWP	Andrews et al. 2013
S. longirostris	67	KC161128	CYTB49	EP, NEP, NWP, SEP, SWP,	Andrews et al. 2013
S. longirostris	68	KC161129	CYTB34	EP, NEP, NWA, NWP, NEI, NWA, SEP, SWP, IP	Andrews et al. 2013
S. longirostris	69	KC161130	CYTB31	EP, NWP, NEP, SEP, SWP, NWP, IP	Andrews et al. 2013
S. longirostris	70	KC161131	CYTB50	EP	Andrews et al. 2013
S. longirostris	71	KC161132	CYTB31	EP	Andrews et al. 2013
S. longirostris	72	KC161133	CYTB51	EP	Andrews et al. 2013
S. longirostris	73	KC161134	CYTB34	EP	Andrews et al. 2013
S. longirostris	74	KC161135	CYTB52	EP	Andrews et al. 2013
S. longirostris	75	KC161136	CYTB53	EP	Andrews et al. 2013
S. longirostris	76	KC161137	CYTB31	EP, NEP, SEP, SWP, IP	Andrews et al. 2013
S. longirostris	77	KC161138	CYTB54	EP	Andrews et al. 2013
S. longirostris	78	KC161139	CYTB55	EP	Andrews et al. 2013
S. longirostris	79	KC161140	CYTB56	EP	Andrews et al. 2013
S. longirostris	80	KC161141	CYTB57	EP	Andrews et al. 2013
S. longirostris	81	KC161142	CYTB31	EP	Andrews et al. 2013
S. longirostris	82	KC161143	CYTB41	EP, NWP, SWP, IP	Andrews et al. 2013
S. longirostris	83	KC161144	CYTB58	EP, SEP	Andrews et al. 2013
S. longirostris	84	KC161145	CYTB59	EP	Andrews et al. 2013
S. longirostris	85	KC161146	CYTB60	EP, NEP	Andrews et al. 2013
S. longirostris	86	KC161147	CYTB35	NWA	Andrews et al. 2013
S. longirostris	87	KC161148	CYTB47	NWA	Andrews et al. 2013
S. longirostris	88	KC161149	CYTB61	NWA	Andrews et al. 2013
S. longirostris	89	KC161150	CYTB31	NWPO	Andrews et al. 2013
S. longirostris	90	KC161151	CYTB62	IPO	Andrews et al. 2013
S. longirostris	91	KC161152	CYTB63	EP, IP, NWP	Andrews et al. 2013
S. longirostris	92	KC161153	CYTB64	IP	Andrews et al. 2013
S. longirostris	93	KC161154	CYTB32	NEI	Andrews et al. 2013

Cyt-b (331 bp)					
Species		**GenBank**	**Haplotype**	**Localization**	**Source**
S. longirostris	94	KC161155	CYTB35	NEI	Andrews et al. 2013
S. longirostris	95	KC161156	CYTB34	NEI	Andrews et al. 2013
S. longirostris	96	KC161157	CYTB65	NEI	Andrews et al. 2013
S. longirostris	97	KC161158	CYTB52	NEP, NWP	Andrews et al. 2013
S. longirostris	98	KC161159	CYTB65	NEP	Andrews et al. 2013
S. longirostris	99	KC161160	CYTB31	NWP	Andrews et al. 2013
S. longirostris	100	KC161161	CYTB66	NWP	Andrews et al. 2013
S. longirostris	101	KC161162	CYTB67	NWP	Andrews et al. 2013
S. longirostris	102	KC161163	CYTB68	NWP	Andrews et al. 2013
S. longirostris	103	KC161164	CYTB69	NEP	Andrews et al. 2013
S. longirostris	104	KC161165	CYTB70	SEP, SWP	Andrews et al. 2013
S. longirostris	105	KC161166	CYTB71	EP, NWP	Andrews et al. 2013
S. longirostris	106	KC161167	CYTB72	EP	Andrews et al. 2013
S. longirostris	107	KC161168	CYTB73	EP	Andrews et al. 2013
S. longirostris	108	KC161169	CYTB49	EP	Andrews et al. 2013
S. longirostris	109	KC161170	CYTB74	EP	Andrews et al. 2013
S. longirostris	110	KC161171	CYTB75	EP	Andrews et al. 2013
S. longirostris	111	KC161172	CYTB76	EP	Andrews et al. 2013
S. longirostris	112	KC161173	CYTB77	EP	Andrews et al. 2013
S. longirostris	113	KC161174	CYTB78	EP	Andrews et al. 2013
S. longirostris	114	KC161175	CYTB79	EP	Andrews et al. 2013
S. longirostris	115	KC161176	CYTB80	EP	Andrews et al. 2013
S. longirostris	116	KC161177	CYTB81	SWI	Andrews et al. 2013
S. longirostris	117	KC161178	CYTB31	SWI	Andrews et al. 2013
S. longirostris	118	KC161179	CYTB34	SWI	Andrews et al. 2013
S. longirostris	119	KC161180	CYTB45	SWI	Andrews et al. 2013
S. longirostris	120	KC161181	CYTB34	SWI	Andrews et al. 2013
S. longirostris	121	KC161182	CYTB82	SEP	Andrews et al. 2013
S. longirostris	122	KC161183	CYTB83	SEP	Andrews et al. 2013
Cox I (613 bp)					
Species	**1**	**GenBank**	**Haplotype**	**Localization**	**Source**
S. attenuata	2	XXXXX	COXI1	SWA (Brazil)	This study
S. attenuata	3	XXXXX	COXI2	SWA (Brazil)	This study
S. attenuata	4	XXXXX	COXI3	SWA (Brazil)	This study
S. attenuata	5	EU496336	COXI4	NWA, EP, NWP	Viricel et al.2012
S. attenuata	6	EU496337	COXI5	NWA, EP, NWP	Viricel et al.2012
S. attenuata	7	EU496338	COXI3	NWA, EP, NWP	Viricel et al.2012
S. attenuata	8	EU496339	COXI6	NWA, EP, NWP	Viricel et al.2012
S. attenuata	9	EU496353	COXI3	NWA, EP, NWP	Viricel et al.2012
S. clymene	10	XXXXXX	COXI7	SWA (Brazil)	This study
S. clymene	11	XXXXXX	COXI8	SWA (Brazil)	This study
S. clymene	12	XXXXXX	COXI9	SWA (Brazil)	This study
S. clymene	13	XXXXXX	COXI10	SWA (Brazil)	This study
S. clymene	14	XXXXXX	COXI11	SWA (Brazil)	This study
S. clymene	15	XXXXXX	COXI12	SWA (Brazil)	This study
S. clymene	16	XXXXXX	COXI13	SWA (Brazil)	This study
S. clymene	17	XXXXXX	COXI14	SWA (Brazil)	This study
S. clymene	18	XXXXXX	COXI15	SWA (Brazil)	This study
S. clymene	19	EU496346	COXI16	NWA	Viricel et al.2012
S. clymene	20	EU496347	COXI17	NWA	Viricel et al.2012
S. clymene	21	EU496348	COXI8	NWA	Viricel et al.2012
S. coeruleoalba	22	XXXXX	COXI15	SWA (Brazil)	This study
S. coeruleoalba	23	XXXXX	COXI18	SWA (Brazil)	This study
S. coeruleoalba	24	XXXXX	COXI19	SWA (Brazil)	This study
S. coeruleoalba	25	XXXXX	COXI20	SWA (Brazil)	This study
S. coeruleoalba	26	XXXXX	COXI21	SWA (Brazil)	This study
S. coeruleoalba	27	DQ466000	COXI18	NEA	Amaral et al. 2007

Cox I (613 bp)					
Species	**1**	**GenBank**	**Haplotype**	**Localization**	**Source**
S. coeruleoalba	28	DQ466001	COXI22	NEA	Amaral et al. 2007
S. coeruleoalba	29	DQ466002	COXI23	NEA	Amaral et al. 2007
S. coeruleoalba	30	DQ466003	COXI24	NEA	Amaral et al. 2007
S. coeruleoalba	31	DQ466004	COXI25	NEA	Amaral et al. 2007
S. coeruleoalba	32	DQ466005	COXI19	NEA	Amaral et al. 2007
S. coeruleoalba	33	DQ466006	COXI26	NEA	Amaral et al. 2007
S. coeruleoalba	34	DQ466007	COXI27	NEA	Amaral et al. 2007
S. coeruleoalba	35	DQ466008	COXI28	NEA	Amaral et al. 2007
S. coeruleoalba	36	DQ466009	COXI19	NEA	Amaral et al. 2007
S. coeruleoalba	37	EU496341	COXI29	NWA, NEA, NWP	Viricel et al. 2012
S. coeruleoalba	38	EU496342	COXI26	NWA, NEA, NWP	Viricel et al. 2012
S. coeruleoalba	39	EU496343	COXI30	NWA, NEA, NWP	Viricel et al. 2012
S. coeruleoalba	40	EU496344	COXI32	NWA, NEA, NWP	Viricel et al. 2012
S. coeruleoalba	41	KF281695	COXI31	NEA	Alfonsi et al. 2013
S. frontalis	42	XXXXX	COXI33	SWA (Brazil)	This study
S. frontalis	43	XXXXX	COXI34	SWA (Brazil)	This study
S. frontalis	44	XXXXX	COXI35	SWA (Brazil)	This study
S. frontalis	45	XXXXX	COXI36	SWA (Brazil)	This study
S. frontalis	46	XXXXX	COXI37	SWA (Brazil)	This study
S. frontalis	47	EF090645	COXI36	NEA	Amaral et al. 2007
S. frontalis	48	EF090646	COXI38	NEA	Amaral et al. 2007
S. frontalis	49	KF281696	COXI39	NEA	Alfonsi et al. 2013
S. longirostris	50	XXXXX	COXI40	SWA (Brazil)	This study
S. longirostris	51	XXXXX	COXI41	SWA (Brazil)	This study
S. longirostris	52	XXXXX	COXI42	SWA (Brazil)	This study
S. longirostris	53	XXXXX	COXI43	SWA (Brazil)	This study
S. longirostris	54	XXXXX	COXI44	SWA (Brazil)	This study
S. longirostris	55	XXXXX	COXI45	SWA (Brazil)	This study
S. longirostris	56	XXXXX	COXI46	SWA (Brazil)	This study
S. longirostris	57	XXXXX	COXI47	SWA (Brazil)	This study
S. longirostris	58	XXXXX	COXI48	SWA (Brazil)	This study
S. longirostris	59	XXXXX	COXI49	SWA (Brazil)	This study
S. longirostris	60	XXXXX	COXI50	SWA (Brazil)	This study
S. longirostris	61	XXXXX	COXI51	SWA (Brazil)	This study
S. longirostris	62	XXXXX	COXI52	SWA (Brazil)	This study
S. longirostris	63	XXXXX	COXI53	SWA (Brazil)	This study
S. longirostris	64	XXXXX	COXI54	SWA (Brazil)	This study
S. longirostris	65	EU496331	COXI55	NWA, ETP	Viricel et al.2012
S. longirostris	66	EU496332	COXI56	NWA, ETP	Viricel et al.2012
S. longirostris	67	EU496333	COXI57	NWA, ETP	Viricel et al.2012
S. longirostris	68	EU496334	COXI50	NWA, ETP	Viricel et al.2012
S. longirostris	69	EU496335	COXI50	NWA, ETP	Viricel et al.2012

Appendix 3 Blasts of GenBank and DNA - Surveillance for misplaced haplotypes and haplotypes represented by sequences of different species.

D-loop (331 bp)						
Haplotype	**Sequence ID**	**Morphological identity**	**DNA-Surveillance**	**GenBank**	**Localization**	**Author**
DLOOP_120	Scl_33	Scl	SclyZ4185 0.0112	100% Scl	SWA	This study
	GQ504147	Scl	SclyZ4185 0.0117	99% Scl	NWA	Kingston et al. 2009
	Sco_01	Sco	SclyZ4185 0.0112	100% Scl	SWA	This study
DLOOP_121	Scl_34	Scl	SclyZ4185 0.0015	99% Scl	SWA	This study
DLOOP_137	Sco_03(G0047)	Sco	SclyZ4185 0.0112	99% Scl	SWA	This study
DLOOP_114	Scl_10	Scl	Scly8 0,0037	99% Scl	SWA	This study
DLOOP_134	DQ845446	Scl	Scly8 0.0	99% Scl	NWA	Kingston et al 2009
DLOOP_129	GQ504143	Scl	Scly8 0.0078	99% Scl	NWA	Kingston et al 2009
DLOOP_155	GQ504161	Sco	Scly8 0.0394	99%Sco	NWA	Kingston et al 2009
DLOOP_147	GQ504153	Sco	Scly8 0.0353	99%Sco	NWA	Kingston et al 2009
DLOOP_200	EF682716	Sfr	SattNP0.PR 0.0336	99% Sat	NEA	Querouil et al. 2010
DLOOP_209	EF682753	Sfr	SattNP0.PR 0.0222	99% Sat	NEA	Querouil et al. 2010
DLOOP_177	EF682656	Sfr	Satt5 0.0336	98% Sat	NEA	Querouil et al. 2010
	EF682734	Sfr	Satt5 0.0336	98% Sat	NEA	Querouil et al. 2010
	EF682829	Sfr	Satt5 0.0336	98% Sat	NEA	Querouil et al. 2010
DLOOP_199	EF682715	Sfr	Satt5 0.0185	98% Sat	NEA	Querouil et al. 2010
DLOOP_223	EF682804	Sfr	Satt1 0.0038	97% Sat	NEA	Querouil et al. 2010
DLOOP_188	EF682681	Sfr	Satt1 0.0148	99% Sat	NEA	Querouil et al. 2010
DLOOP_214	EF682764	Sfr	Satt5 0.011	99% Sat	NEA	Querouil et al. 2010
DLOOP_01	Sat_01	Sat	Satt 5 0,0074	100% Sat	SWA	This study
	GQ504120	Sat	Satt5 0.0078	99% Sat	NWA	Kingston et al 2009
	EF682658	Sfr	Satt5 0.0078	99% Sat	NEA	Querouil et al. 2010
	EF682703	Sfr	Satt5 0.0078	99% Sat	NEA	Querouil et al. 2010
	EF682748	Sfr	Satt5 0.0078	99% Sat	NEA	Querouil et al. 2010
	EF682777	Sfr	Satt5 0.0078	99% Sat	NEA	Querouil et al. 2010
	KC204739	Sfr	Satt5 0.0078	99% Sat	NWA	Caballero et al. 2013
DLOOP_3	Sat_03	Sat	Satt1 0,0037	99% Sat	SWA	This study
	Sat_04	Sat	Satt1 0,0037	99% Sat	SWA	This study
	GQ504122	Sat	Satt1 0.0039	99% Sat	NWA	Kingston et al 2009
	EF682659	Sfr	Satt1 0.0039	99% Sat	NEA	Querouil et al. 2010
	EF682698	Sfr	Satt1 0,0037	99% Sat	NEA	Querouil et al. 2010
	EF682705	Sfr	Satt1 0.0039	99% Sat	NEA	Querouil et al. 2010
	EF682721	Sfr	Satt1 0.0039	99% Sat	NEA	Querouil et al. 2010
	EF682789	Sfr	Satt1 0.0039	99% Sat	NEA	Querouil et al. 2010
	EF682812	Sfr	Satt1 0.0039	99% Sat	NEA	Querouil et al. 2010
	EF682825	Sfr	Satt1 0.0039	99% Sat	NEA	Querouil et al. 2010
DLOOP_93	GQ504121	Sat	Satt1 0.0039	99% Sat	NWA	Kingston et al 2009
	EF682667	Sfr	Satt1 0.0039	99% Sat	NEA	Querouil et al. 2010
DLOOP_253	KC204736	Sfr	Slon03FP01 0.0155	99 % Slo	NWA	Caballero et al. 2013
	GQ504169	Slo	SfrontCCIR0103 0,0	100% Sfr	NWA	Kingston et al 2009
CYTB_10	Scl_33	Scl	Scly3.LD 0.0029	99% Scl	SWA	This study
	Scl_34	Scl	Scly3.LD 0.0029	99% Scl	SWA	This study
	Scl_35	Scl	Scly3.LD 0.0029	99% Scl	SWA	This study
	Sco_01	Sco	Scly3.LD 0,0024	99% Scl	SWA	This study
	Sco_03	Sco	Scly3.LD 0,0024	99% Scl	SWA	This study
CYTB_20	EU580088	Sco	SfroA0.LD 0.0125	99% Sco	NWA	Viricel et al. 2012
CYTB_07	Scl_10	Scl	Scly3.LD 0,0087	99% Scl	SWA	This study
CYTB_19	Sco_11	Sco	ScoeCO01 0,0098	99% Sco	SWA	This study
CYTB_14	Sco_05	Sco	ScoeCO01 0,0122	99% Sco	SWA	This study

D-loop (331 bp)						
Haplotype	**Sequence ID**	**Morphological identity**	**DNA-Surveillance**	**GenBank**	**Localization**	**Author**
CYTB_15	Sco_07	Sco	ScoeCO01 0,049	99% Sco	SWA	This study
COXI_15	Scl_33	Scl	-	99% Scl	SWA	This study
	Scl_34	Scl	-	99% Scl	SWA	This study
	Scl_35	Scl	-	99% Scl	SWA	This study
	Sco_01	Sco	-	99% Scl	SWA	This study
	Sco_03	Sco	-	99% Scl	SWA	This study
COXI_31	KF281695	Sco	-	99% Sco	NEA	Alfonsi et al. 2013
COXI_26	DQ466006	Sco	-	99% Sco	NEA	Amaral et al. 2007
	EU496342	Sco	-	99% Sco	NWA, GOM, NEA, NWP	Viricel et al. 2012
COXI_27	DQ466007	Sco	-	99% Scl	NEA	Amaral et al. 2007
COXI_09	Scl_08	Scl	-	99% Scl	SWA	This study
COXI_11	Scl_10	Scl	-	99% Scl	SWA	This study
COXI_01	Sat_01	Sat	-	98% Scl	SWA	This study

Capítulo 2

Low mtDNA diversity in a highly differentiated population of spinner dolphin (*Stenella longirostris*, Gray 1828) from the Fernando de Noronha Archipelago, Brazil

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Low mtDNA diversity in a highly differentiated population of spinner dolphin (*Stenella longirostris*, Gray 1828) from the Fernando de Noronha Archipelago, Brazil

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Abstract

Spinner dolphins (*Stenella longirostris*, Gray 1828) are widely distributed in tropical waters around the word. Although found in larger pelagic groups in the Eastern Tropical Pacific, elsewhere in the Pacific they are found in small and genetically isolated populations associated with islands. To assess genetic diversity and population structure of an island-associated population in the South Atlantic Ocean we surveyed 162 spinner dolphins throughout the Fernando de Noronha Archipelago of the northeast coast of Brazil using ten microsatellite loci and sequencing 413bp of the mtDNA control region. Eleven mtDNA haplotypes were identified and haplotype diversity (*h*) and nucleotide diversity (π) were 0.3747 and 0.0060, respectively. Median-Joining Network revealed the presence of two very divergent haplotypes and F-statistics indicated some heterogeneity between two sampling years. Microsatellite loci analyzed were polymorphic (H_o: 0.767; H_e: 0,764) but, revealed no detectable substructure by sampling period or overall. We also compared the mtDNA haplotypes from Noronha to 159 haplotypes representing 893 individuals from 14 locations worldwide. We found that the two common haplotypes from Noronha were absent in all other populations. These comparisons showed that Noronha spinner dolphins are more differentiated than other island populations suggesting that they compose society with strong site fidelity.

Keywords: spinner dolphin, cetaceans, conservation, D-loop, genetic variability

Introduction

The spinner dolphin *(Stenella longirostris,* Gray 1828*)* is widely distributed in tropical waters around the world (Galver 2002; Oremus *et al*. 2007; Andrews *et al*. 2010; Viricel *et al.* 2016). This wide distribution is reflected in considerable intraspecific variation in several morphological characters and ecological parameters. Four subspecies are recognized: Gray's spinner subspecies *(Stenella longirostris longirostris)* generally associated to tropical islands of Indian, Atlantic and Pacific Oceans (Perrin 1990; Perrin *et al.* 1999). The dwarf subspecies *(Stenella longirostris roseiventris)*, considerably smaller (140 cm), is found in waters of the Gulf of Thailand, Indonesia and southern China. The Central American subspecies (*Stenella longirostris centroamericana*) whose distribution covers coastal waters of the Pacific Ocean in Central America; the Eastern subspecies (*Stenella longirostris orientalis*) a morphologically distinct form in the Eastern Pacific Ocean found in tropical and coastal ocean waters of Mexico.

Gray's spinner dolphins (here after referred to as spinner dolphins) are found in oceanic waters of Atlantic, Pacific and Indian oceans and around volcanic islands and atolls, such as those of Hawaii, French Polynesia and Fernando de Noronha. These dolphins can travel great distances (from 300 to 1000 miles) and are able to disperse between islands and atolls (Martin *et al*. 1990; Würsig *et al*. 1994 a; Würsig *et al*. 1994 b; Wells & Gannon 2005). This ability could have contributed to variations in the levels of dispersal and genetic divergence showed by the species around the world.

Gray's subspecies are the only spinner dolphin ecotype known to inhabit the South Atlantic Ocean. Along the Brazilian coast the distribution of the

spinner dolphin covers almost the entire length of the country, reaching 30° S, in waters up to 1000 m deep with low productivity and along the continental shelf break where temperatures range from 24ºC to 28ºC, seeming to be displaced when the warm Brazil current meets the cold Malvinas Current (Secchi & Siciliano 1995; Moreno *et al*. 2005; Amaral *et al.* 2015). The Fernando de Noronha Archipelago, north-eastern Brazil, is considered a natural refuge of the species, where large groups of 600 individuals on average are observed daily (Silva & Silva-Jr 2009).

Gray's ecotype from the Fernando de Noronha Archipelago (hereafter referred to as Noronha spinner dolphins) congregate in these islands to use their calm waters during the day to rest, play, care for their young and take refuge from sharks; at night, they move out into deep sea waters to feed (Silva-Jr 1996; Silva & Silva-Jr 2009). While in the Pacific Ocean there are several rest areas along the coast of the Islands and even within atolls (Norris & Dohl, 1980), in the Atlantic Ocean the Fernando the Noronha Archipelago encompasses the only area in the South West Atlantic Ocean available with resources and resting areas to support the large number of spinner dolphins (Silva & Silva-Jr 2009).

The social structure of the spinner dolphin is considered to be transient, with groups merging and splitting up continuously, forming the so-called "fission-fusion" groups (Lammers 2004) in which the roles alternate between individuals and coalitions or groups (Norris 1994). Noronha spinner dolphins display two particularities recorded through photographs and videotapes. First, they probably use two distinct feeding zones at night, one closer to the archipelago (east side route) and other more distant (west side route). Second,

they form distinct groups for copulation, a smaller group consisting of two to six males and one female, like the Hawaiian spinner dolphins, and a larger group formed by 50 to 100 individuals (many males and females) who display different behaviors like loud whistles, clicks, burst pulsed signals and sometimes aggressive behavior: opening their beaks, biting and bumping each other and leaving distinct scratches on the skin (Silva Jr 2005).

Despite the wide occurrence of spinner dolphins around the world, there are relatively few studies of genetic structure of island-associated populations worldwide and none in the South Atlantic Ocean (Andrews *et al*. 2006; Oremus *et al*. 2007; Farro *et al.* 2008b; Andrews *et al*. 2010; Andrews *et al*. 2013; Viricel *et al*. 2016). Here, we examine sequences of mitochondrial DNA (mtDNA) and 10 microsatellite loci to evaluate the genetic diversity of this species in the Fernando de Noronha Archipelago, and test the hypothesis of the existence of genetic structuring in the South Atlantic Ocean and haplotypes sharing among individuals from different oceans.

Materials and methods

Study area and sample collection

The Fernando de Noronha Archipelago is composed of 17 islands, located in the South Atlantic Ocean, coast of Brazil with a total area of 26 km^2 (Funatura 1990). The main island has 17 km^2 distributed along the northeast-southwest direction (Figure 1). The archipelago is located at the fork of the South Equatorial current, which runs westbound with waters characterized by high salinity, low concentrations of sediments, organic matter, nutrients and

plankton (Funatura 1990), high transparency, with depth of 87 m light extinction and averaged temperature of 27 ºC (Linsker 2003).

A total of 162 skin samples were collected from Noronha spinner dolphins by the skin swabbing method (Harlin *et al.* 1999; Farro *et al.* 2008a) in in four different years: 2004 (N=1); 2006 (N=35); 2009 (N=67); 2012 (N=59). Skin samples were stored in 70% alcohol (Supplementary Appendix 1).

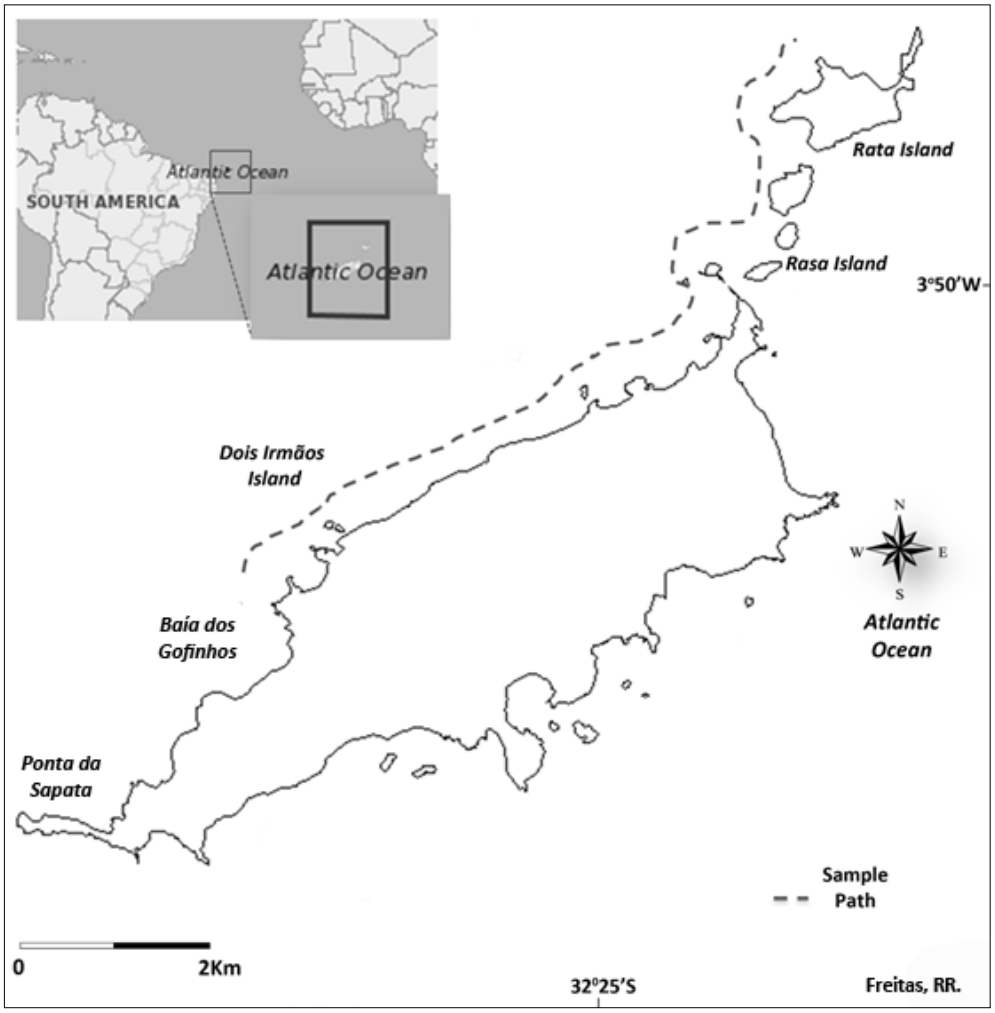

Figure 1 Fernando de Noronha Archipelago, coast of Brazil. Dashed lines indicate the path of spinner dolphins during the collection of the skin samples utilized in the study.

Dataset of different oceans

To compare the mtDNA haplotypes of the Noronha spinner dolphins worldwide, excluding the samples collected in Fernando de Noronha Archipelago, we reconstructed haplotypes from a total 159 sequences available in GenBank from five published papers (www.ncbi.nlm.nhi.gov/Genbank). These haplotypes represent 893 specimens of Gray's spinner subspecies of 19 different locations of the world (Hawaiian Islands, Society Islands, 3-Islands, Guam, Saipan, Rota, American Samoa, Samoa, Zanzibar, Mayotte, La Reunión, Palmyra, Philippines, Maldives, Indonesia, Gulf of Mexico, Taiwan and North Atlantic) (Figure 2) (Supplementary Appendix 2).

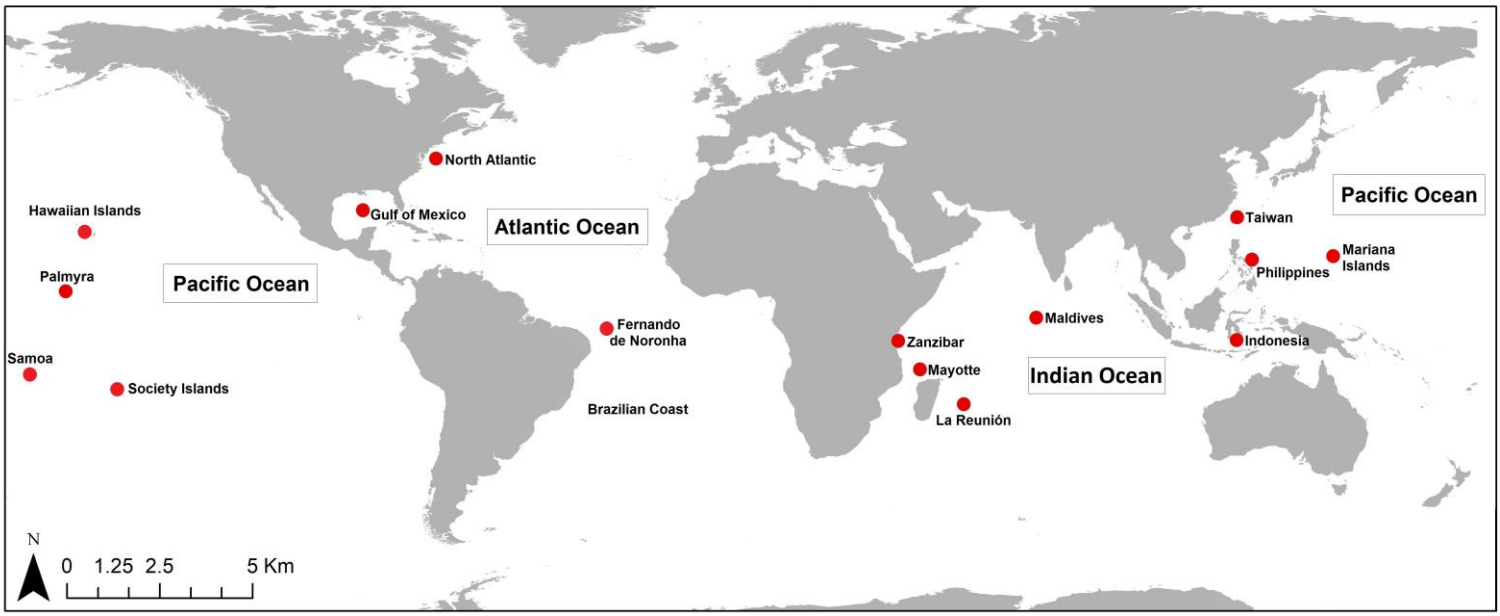

Figure 2 Map showing sampling locations for spinner dolphins analyzed in this study.

To reduce the complexity of the comparisons some locations were pooled, reducing the 19 island samples to 14 locations in total: Fernando de Noronha, Hawaiian Islands, Society Islands, Mariana Islands (3-Islands + Guam + Saipan + Rota), Samoa (American Samoa + Independent Samoa), Zanzibar,

Mayotte, La Reunión, Palmyra, Philippines, Maldives, Indonesia, Taiwan, and, North Atlantic (Gulf of Mexico + North Atlantic).

DNA extraction and sex determination

Genomic DNA was extracted from skin samples using Chelex resin (SIGMA) according to manufacturer's instructions. The sex of each Noronha spinner dolphins was determined by the amplification of ZFX and SRY genes: primers ZFX0582 and ZFX0923 (Bérube & Palsboll 1996); PMSRYF (Richard *et al.*1994) and TtSRYR (Rosel *et al.* 2003). The PCR reactions were carried out according to Rosel *et al.* 2003, with the following modifications: 1 x Reaction Buffer, 150 µM of each dNTP, 1.5 mM $MgCl_2$, 1.5 u of *Taq* polymerase (INVITROGEN) and 0.3 µM each primer, with the exception of the reverse for the SRY (0.06 µM). The final volume of the reaction was 25 µL. The amplification was carried out under the following conditions: 92° C for 30 sec, followed by 35 cycles of 94° C for 30 sec, 51° C for 45 sec and 72° C for 45 seconds. The fragments were separated on agarose gel 2.5 %, stained with GelRed (Biotium).

Microsatellite loci and analyses

Ten microsatellite loci were evaluated, seven specific of *S. longirostris*, (Table. 1) and amplification reactions were carried out with the multiplex PCR Kit from Qiagen, 1.5 µL of DNA and 0.1 µM of each primer in a final volume of 5 µL. Cycle conditions were: 95°C for 15 min, followed by 35 cycles of 94°C for 4

min, annealing temperature for 1 min and 30 s, and 72°C for 1 min and 30s, followed by a final 72°C extension for 15 min.

Capillary electrophoresis of PCR products was performed on ABI 3730 sequencer (Applied Biosystems). Fragment sizes were identified using GeneMapper software 5.0 (Applied Biosystems). The average non-exclusion probability for the identity of individuals was assessed using Cervus 3.0.3 (Kalinowski *et al*. 2007) and potential duplicate samples were searched by comparing their genotypes.

Linkage disequilibrium and Hardy-Weinberg equilibrium were evaluated using GENEPOP on the Web (Raymond & Rousset 1995; Rousset 2008). Microsatellite loci were also tested for the presence of null alleles and/or genotyping errors, using the Microchecker 2.2.0.3 (Van Oosterhout *et al.* 2004), with Bonferroni correction.

Table 1 Microsatellite loci used to access genetic diversity of spinner dolphin (*Stenella longirostris*). Locus name (Locus), primers sequences (Sequence 5'-3'), range of amplified fragments size (base pairs, bp), fluorescent primer labeling (dye), annealing temperature (T) and original loci development (reference).

Locus	Sequence 5'-3'	pb	dye	T (ºC)	Reference
SD8	TGGCCGTTATAAATAGAGC GACAACAGTTTGGCAGTG	185-218	Fam	52ºC	Galver 2002
SL1-25	TTGATTTTCTGACTTCTTGGG CTCCGATATTGCCTTTACC	109-125	Fam	54ºC	Galver 2002
SL8-49	CATCTGTTCTTTGAATAGAGG ACCCATTCTGGTTCACC	138-168	Hex	52ºC	Galver 2002
SL9-69	TTCCAAACATACCCCTGCC ACTAGATGCCACTTGCACC	109-125	Hex	54ºC	Galver 2002
SL10-26	GCTATGTTATATCTATCTTCC TTAGGGCATTAATTTGAGTGC	128-172	Hex	52ºC	Galver 2002
SLO15	CGTCAAACTCCATCAAGACATC ATCTCCACCACAAGACACCAC	218-252	Fam	52ºC	Farro et al. 2008b
SLO1	CAAACCAAAAGCAAACACACAC CATCTCTATCAGCCATGTCCAA	140-166	Fam	54ºC	Farro et al. 2008b
415-416	GTTCCTTTCCTTACA ATCAATGTTTGTCAA	222-234	Fam	50ºC	Amos et al. (1993)
EV1	CCCTGCTCCCCATCTC ATAAACTCTAATACACTTCCTCCAAC	115-197	Fam	52ºC	Valescchi & Amos (1996)
EV94	ATCCTATTGGTCCTTTTCTGC AATAGATAGTGATGATGATTCACAC	198-261	Hex	52ºC	Valescchi & Amos (1996)

Only the polymorphic loci were used in analyzes of genetic diversity. Total number of alleles (K), mean number of alleles (Nam), number of private alleles (Nap), observed (Ho) and expected (He) heterozygosity, genetic diversity (Dg) and inbreeding coefficient (FIS) were calculated using Fstat v.2.9.3.2 (Goudet 2001) and Arlequin 3.5.2.2 (Excoffier & Lischer 2010). Contemporary effective population size (Ne) was estimated using a method based on linkage disequilibrium (Waples 1989; Waples and Do 2010) as implemented in NeEstimator 2.0 (Do *et al.* 2014). A P_{crit} value of 0.02 and 95% confidence intervals was chosen to reduce the potential bias for low frequency alleles (Waples & Do 2010). A test for past or recent events of population bottleneck based on allele frequencies were performed using the software Bottleneck 1.2.02 (Cornuet & Luikart 1996). The one-tailed Wilcoxon sign-rank test for heterozygote excess was used under the stepwise mutational model (SMM) and the mutational biphasic model (TPM; 30 variations, mutational model 70 gradual, 10000 interactions). A probability level of 0.05 was considered significant (Piry *et al. 1999*).

Temporal population structuring among the Noronha spinner dolphins sampled during the years 2006, 2009 and 2012 was investigated using Arlequin 3.5.2.2 (Excoffier & Lischer 2010). The year 2004 was not analyzed due to just one individual sampled. Pairwise $F_{ST,}$ based on number of differences, was estimated with 10000 random generations.

Population structure was also investigated in a Bayesian clustering analysis to estimate the most probable number of populations using Structure 2.3.2 (Pritchard *et al.* 2000) using an admixture model without prior population information and a correlated allele frequencies model. The burn-in length was

set at 10^4 steps, followed by 500.000 repetitions of Markov Chain Simulation and Monte Carlo (MCMC). Ten independent executions were performed for each value of K varying between one and ten to test the consistency of estimates of P (XK). The number of clusters that best fit in the data was assessed and visualized using Structure Harvester (Earl & VonHoldt, 2012) (available at http://taylor0.biology.ucla.edu/structureHarvester/), a web-based program for collating results generated by the program Structure 2.3.2 (Pritchard *et al.* 2000).

mtDNA sequences and analyses

An approximately 550bp fragment of the mtDNA control region (D-loop) was amplified with the following primers: KRAdLp 1.5 t-pro (Andrews *et al.* 2006) and dLp5 (Pichler *et al*. 2001). Polymerase Chain Reactions (PCR) were performed in 12,5 µl total volumes containing 1X Reaction Buffer, 200 µM of each dNTP, 2.0 mM $MgCl_2$, 0.5 units of *Taq* DNA polymerase (INVITROGEN) and 0.2 µM of each primer. Cycle conditions were as follows: 95ºC for 1 min; followed by 40 cycles of 94ºC for 30 s, 54 ºC for 30 s, and 72 ºC for 30 s; followed by a final 72ºC extension for 15 min. Amplification products were sequenced in both forward and reverse directions, with an ABI 310 automated sequencer (Applied Biosystems), and the sequences were aligned and edited manually using the algorithm Muscle of MEGA 6.06 (Tamura *et al*. 2013).

Arlequin 3.5.2.2 (Excoffier & Lischer 2010) was used to calculate nucleotide (π) and haplotypic (h) diversities and mismatch distribution, to test selective neutrality in mtDNA control region and population structuring.

Selective neutrality in mtDNA control region was tested by the Tajima test (1989) and Fu (1997) using a coalescent simulation algorithm (10 000 steps).

Temporal population structuring was investigated among Noronha spinner dolphins sampled during the years 2006, 2009 and 2012, the year 2004 was removed from this analysis due just one individual sampled. Pairwise F_{ST} and Φ_{ST} were estimated with 10 000 random generations.

Genetic differentiation was also assessed among Noronha spinner dolphins and other locations of the world using AMOVA, Pairwise F_{ST} and Φ_{ST} using Arlequin 3.5.2.2 (Excoffier & Lischer 2010), using haplotype frequencies only and pairwise difference respectively. A Median-Joining analysis among haplotypes was estimated through Network 4.5 (Bandelt *et al*. 1999; http: www.fluxus-enginering.com), the MP option was used to reduce the complexity (Bandelt *et al*. 1999).

Results

Noronha spinner dolphins Genetic Diversity and Population Structure

Sex identification

From the analysis of 162 individuals, we identified the sex of 137 individuals (84 males and 53 females). The remainder of samples failed to amplify (Supplementary Appendix 1).

Microsatellite loci

Of the 162 skin samples, 92 provided genotypes for 7-10 loci. Three loci were not used in the analysis: SL8-49 was monomorphic; SL10-26 revealed the

presence of null alleles; and SL01 was discarded because of evidence of linkage disequilibrium. The 7 other microsatellite loci were moderately variable, e.g. k= 4-15 (Table 2). The probability of identity was 1, 6 X 10^{-9}. None of the samples had matching genotypes. Three loci showed significant departure from Hardy Weinberg equilibrium, with a deficiency in homozygotes (Table 2).

Table 2 Genetic diversity of Noronha spinner dolphins for seven microsatellite loci. (*N*: sample size for each locus; *k*: total number of alleles; H_O: observed heterozygosity; H_e: expected heterozygosity; *PIC*: polymorphic information content; *Ra*: allelic richness; *Dg*: genetic diversity; F_{IS}: inbreeding coefficient; *F (Null):* estimation of frequency of null alleles); Hardy-Weinberg Equilibrium P (HWE). (*) Statistically significant values for HWE P < 0.05**.**

Loci	*N*	*k*	H_o	H_e	PIC	*Ra*	*Dg*	F_{IS}	F(Null)	P(HWE)
415-416	76	9	0.763	0.801	0.767	8.835	0.801	0.047	+0.0194	0.20377
EV1	66	12	0.758	0.825	0.795	12.000	0.825	0.082	+0.0440	0.07913
EV94	81	13	0.840	0.841	0.818	12.774	0.841	0.002	+0.0004	0.29335
SD8	91	15	0.846	0.829	0.805	13.878	0.829	-0.021	-0.0139	0.02913*
SLI-25	91	10	0.813	0.758	0.732	9.904	0.758	-0.073	-0.0450	0.01974*
SL9	90	7	0.756	0.701	0.646	6.663	0.701	-0.078	-0.0449	0.01108*
SLO15	83	4	0.590	0.594	0.529	3.959	0.594	0.006	+0.0083	0.73308

Using NeEstimator, contemporary effective population size (Ne) for Noronha spinner dolphins was estimated to be 491.3 (95% CI 153.1 - ∞; harmonic mean sample size: 70.8). The Wilcoxon one-tailed test revealed that the Noronha spinner dolphins went through a possible bottleneck event based on the TPM mutational model (P= 0.00391), but not under SMM model (P=0.3475).

The temporal genetic structuring test applied between individuals sampled in different years showed no statically significant F_{ST} values. The Bayesian clustering analysis revealed no detectable substructure by sampling

period or overall. The greatest likelihood was K = 1, and, K = 2 showed all individuals to have similarly mixed ancestries (Figure 3).

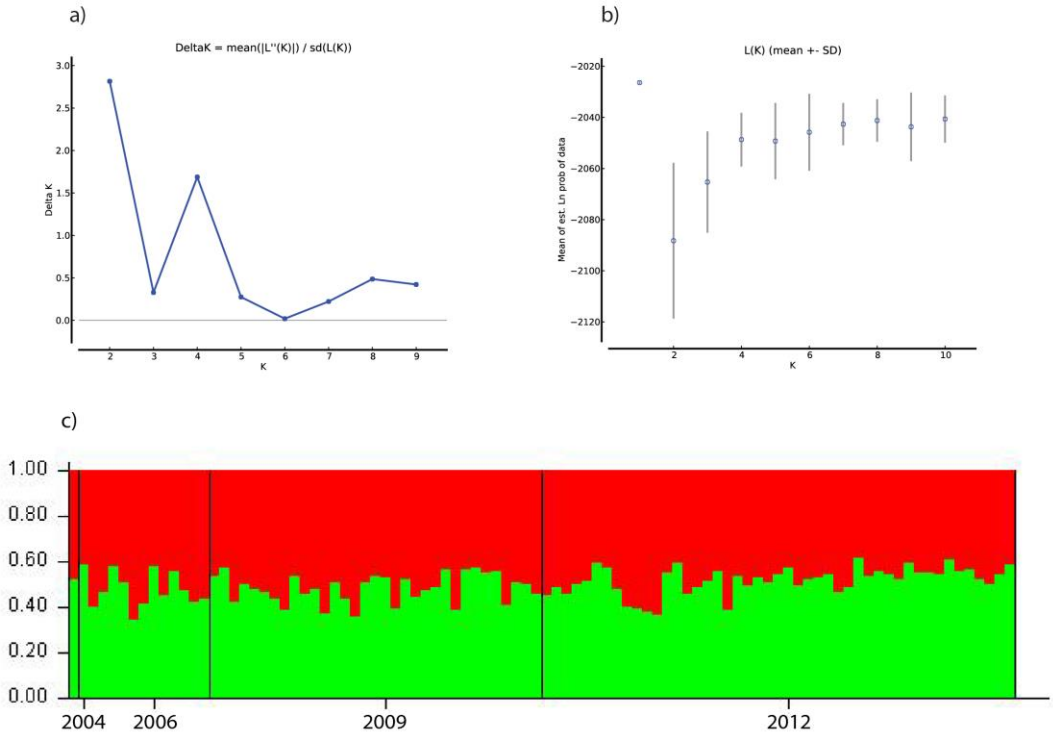

Figure 3 Bayesian clustering analysis inferred with the program Structure 2.3.2 for seven microsatellite loci: (a) ΔK from Evanno's method is shown between successive K values and (b) mean log probability (Lk) is given for each K tested and the (c) assignment probabilities of individuals to putative population clusters at K = 2 according to sampling year (2004, 2006, 2009, 2012).

mtDNA

Sequences of a 413 bp fragment of the mtDNA control region were obtained for all 162 samples of Noronha Spinner dolphins and revealed 27 variable sites (20 transitions and eight transversions) defining 11 haplotypes (NOR); GenBank accession numbers: XXXXXX – XXXXXX (Table 3).

Haplotype diversity (*h*) and nucleotide diversity (*π*) were 0.3747 (+/- 0.04450) and 0.006005 (+/- 0.003609), respectively.

Table 3 Twenty-seven variable sites over 413 bp of the mitochondrial control region determining eleven Noronha spinner dolphins (FN) haplotypes. Absolute frequency: total and by sampling year (2004, 2006, 2009, 2012). *Sequence used as reference.

	Variable sites	**Absolute frequency**				
D-loop 413 bp	111112222222233333344444 191223635666789245789001 11 815469892367875619756978023	**2004**	**2006**	**2009**	**2012**	**Total**
***NOR1**	AAGCACATACTCCTCGCACTTAAAAAT	1	22	52	51	126
NOR2	.....GTCTT.T...TC.....	-	8	12	4	24
NOR3	CC..T.GC.....	-	1	-	-	1
NOR4	.....TC.....C....G.....	-	1	-	-	1
NOR5	.....G...GGGGGA	-	1	-	-	1
NOR6	.....TTCT...T.....	-	1	-	-	1
NOR7	.....G.G.	-	-	1	-	1
NOR8	..A.....	-	-	1	2	3
NOR9	.....GG.G.	-	-	2	-	2
NOR10	...T.....TTCT...T.....	-	-	-	1	1
NOR11	.....G.....	-	-	-	1	1
Total		1	34	68	59	162

Median-Joining network showed two very divergent haplotypes: NOR1 (N=126) and NOR2 (N=24); NOR1 was shared among individuals from 2004, 2006, 2009 and 2012; NOR2 was shared among the individuals of 2006, 2009 and 2012; NOR2 is distant from the majority of haplotypes by eight mutational steps (Figure 4).

The mismatch distribution chart of the Noronha spinner dolphins mtDNA presented a bimodal curve (Figure 5). The neutrality tests were not significant: Fu's *Fs* (Fs= 0.17703, $P < 0.58940$) and Tajima's *D (*D= -1.36902, $P < 0.05580$). The temporal genetic structuring test applied for individuals sampled in different years showed significant F_{ST} values for mtDNA control region between 2006 and 2012 (F_{ST}: 0.087; $P \leq 0.006$).

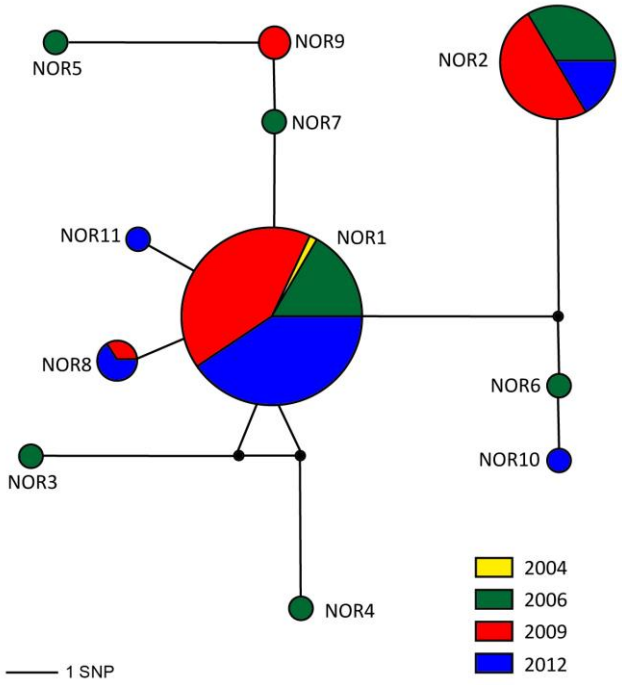

Figure 4 Temporal Median-Joining network including the individuals from 2004, 2006, 2009 and 2012 from the Noronha spinner dolphins (FN). Each circle corresponds to a haplotype, and its size is proportional to its frequency in the population. Black circles indicate potential intermediate haplotypes not sampled. Different colors represent the sampling years. Lengths of lines connecting haplotypes are proportional to the number nucleotide differences between haplotypes.

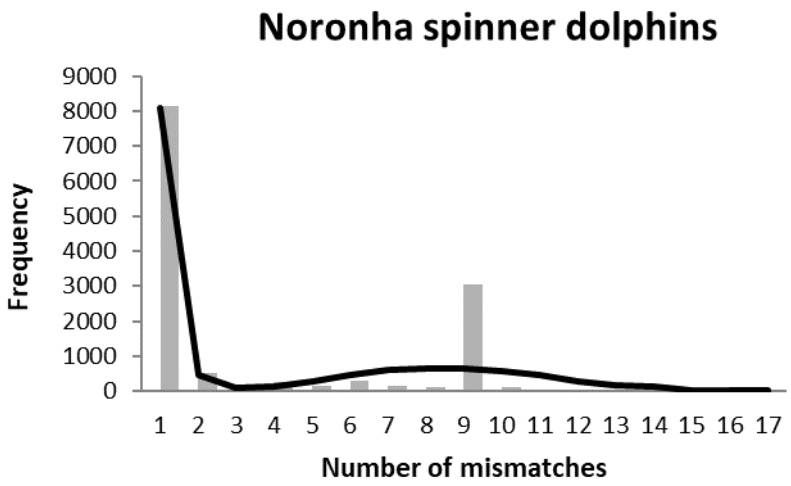

Figure 5 Mismatch distribution of Noronha spinner dolphins. Observed and expected distributions under spatial expansion model are shown with bars and lines, respectively.

Worldwide S. longirostris Genetic Diversity, Population Structure and Phylogeography

To investigate the phylogeographic relationship of Noronha spinner dolphins to other populations worldwide, we analyzed a total of 1055 samples representing 14 regional populations (Supplementary Appendix 2), 162 samples from Noronha spinner dolphins and 893 samples from the other 14 locations. The total length of sequences varied from 374 to 555 bp, allowing a consensus length of 374 bp for all analyses, resolving 158 haplotypes. At the consensus length of 374 bp, the Noronha spinner dolphins showed nine haplotypes (i.e., 2 haplotypes collapsed).

Haplotypic diversity of the 14 populations ranged from (*h:* 0.3455) for Noronha spinner dolphins (despite the large number of specimens) to (*h:* 0.9532) for Mayotte, whereas nucleotide genetic diversity ranged from (*π:* 0.005202) for Hawaiian Islands to (*π:* 0.022361) for Samoa (Table 4). Noronha spinner dolphins showed the second lowest nucleotide diversity (*π:* 0.006109).

Table 4 Samples size and genetic diversities for 374 pb of mtDNA control region: *n*: number of individuals; *k*: number of haplotypes; *h:* haplotype diversity*; π:* nucleotide diversity from the Noronha spinner dolphins. GB: specimens from GenBank.

	mtDNA (374bp)				
Locations	*n*	*k*	*h*	*π*	Source
1. Fernando de Noronha	162	9	0.3455	0.006109	This study
2. Hawaiian Islands	501	19	0.5078	0.005202	GB (Andrews et al. 2010)
3. Society Islands	154	29	0.9227	0.018334	GB (Oremus et al. 2007)
4. Mariana Islands	103	25	0.9280	0.017583	GB (Andrews et al. 2013; Martien et al. 2014)
5. Samoa	32	14	0.9516	0.022361	GB (Andrews et al. 2010; Andrews et al. 2013)
6. Zanzibar	29	12	0.8645	0.018314	GB (Viricel et al. 2016)
7. Mayotte	19	13	0.9532	0.018361	GB (Viricel et al. 2016)
8. La Reunión	16	9	0.9000	0.016846	GB (Viricel et al. 2016)
9. Palmyra	10	7	0.9111	0.010454	GB (Andrews et al. 2013)
10. Philippines	7	6	0.9524	0.021448	GB (Andrews et al. 2013)
11. North Atlantic	7	4	0.7143	0.015873	GB (Andrews et al. 2013)
12. Maldives	6	4	0.8667	0.020251	GB (Andrews et al. 2013)
13. Indonesia	5	4	0.9000	0.0117	GB (Andrews et al. 2013)
14. Taiwan	4	3	0.8333	0.012097	GB (Andrews et al. 2013)
Total	1055	158			

F-statistics of mtDNA control region among spinner dolphins of different locations revealed significant differences among almost all locations and among all ocean basins (Table 5). Significant differentiation was observed between Noronha spinner dolphins and all other locations of the world, the highest value was observed with Indonesia and the lowest with Mariana islands (Figure5). Overall, the highest level of differentiation was observed between Hawaiian Islands and Indonesia and the lowest between Society Islands and Samoa. The AMOVA analysis revealed significant values among locations (F_{ST} = 0.341, $P < 0.001$).

Table 5 Pairwise F_{ST} (lower diagonal) and Φ_{ST} (upper diagonal) for spinner dolphins for mtDNA control region among locations of the world. Statistically significant values are highlighted in gray and have asterisks: *P < 0.05, **P < 0.01, ***P < 0.001.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. F. Noronha	-	0.48621***	0.32150***	0.26314***	0.37976***	0.54041***	0.48208***	0.57196***	0.35414**	0.52800***	0.51118**	0.56241***	0.57204***	0.48233**
2. Hawaiian I.	0.55027***	-	0.43284***	0.41262***	0.48641***	0.64730***	0.58549***	0.67604***	0.48839***	0.65828***	0.64475***	0.55646***	0.64283***	0.56698***
3. Society I.	0.36867***	0.31674***	-	0.03555***	0.01159	0.15201***	0.09727***	0.16807***	0.04758	0.10057*	0.06532	0.09808*	0.10199*	0.06636
4. Mariana I.	0.38928***	0.31688***	0.05648***	-	0.01092	0.17286***	0.10718***	0.18671***	0.01125	0.12766*	0.08688*	0.12612*	0.14201**	0.05307
5. Samoa	0.44747***	0.33555***	0.03981***	0.03969***	-	0.13473***	0.07941**	0.14850***	0.01062	0.05521	0.05427	0.06910	0.09463*	0.01260
6. Zanzibar	0.48744***	0.37764***	0.10358***	0.10028***	0.09159***	-	-0.00525	0.00387	0.23073***	0.06306	0.05301	0.25913**	0.15190*	0.25018**
7. Mayotte	0.47268***	0.34918***	0.06348***	0.05993**	0.04761***	0.01718	-	0.03214	0.17326**	0.03078	0.00130	0.20320**	0.14038*	0.19568*
8. La Reunión	0.46630***	0.37220***	0.08727***	0.08442***	0.07278***	0.11306***	0.07296***	-	0.28221**	0.06105	0.08967	0.34956***	0.18692*	0.31807**
9. Palmyra	0.51480***	0.37360***	0.07138**	0.06380**	0.04890*	0.11185***	0.06640**	0.09477**	-	0.20105*	0.17736*	0.18247**	0.23940***	0.13024
10. Philippines	0.51673**	0.36950***	0.06352*	0.06199*	0.04806*	0.09877*	0.04715*	0.07671*	0.05589	-	0.06448	0.26795*	0.12873	0.15152
11. N. Atlantic	0.54571***	0.40101***	0.09867**	0.09559**	0.08290**	0.10370*	0.06725*	0.11410**	0.10900*	0.08912*	-	0.14902	0.14593*	0.20749*
12. Maldives	0.54576***	0.39600**	0.08336*	0.08244*	0.06866*	0.12201*	0.06841*	0.10000*	0.09370	0.07190	0.11743	-	0.25044**	0.11948
13. Indonesia	0.58000***	0.44158***	0.15455***	0.15220***	0.14387***	0.19439***	0.14785***	0.17931**	0.18007**	0.16667*	0.21255*	0.20126*	-	0.22814*
14. Taiwan	0.57029**	0.42047**	0.10761**	0.10436*	0.09099**	0.14626**	0.09155*	0.12479*	0.11988	0.09880	0.14805	0.13105	0.23629*	-

The haplotype network among spinner dolphin of different locations around the world emphasizes the divergence and distinctiveness of the H1 and H2 haplotypes in Noronha showing that these dolphins are more differentiated than other island populations in the Pacific and Indian Oceans (Figure 5). H1 encompasses the majority of Noronha spinner dolphins with other haplotypes (H3, H4, H5, H7, H9) represented by one or two specimens around it. H1 and H2 are closest to other haplotypes from geographically very distant locations

than each other. The H2, H6 and H8 are very distant from all other haplotypes from Fernando de Noronha, at least nine mutation steps of separation (Fig.5). The haplotype network also showed no phylogeographic signal among the locations of the world, and that Noronha spinner dolphins share two haplotypes with other island populations of the world: the H1 shared with one sample from La Reunión and H8 represented by one sample from Noronha, three samples from Zanzibar, one sample from Mayotte and two samples from Maldives (Figure 6).

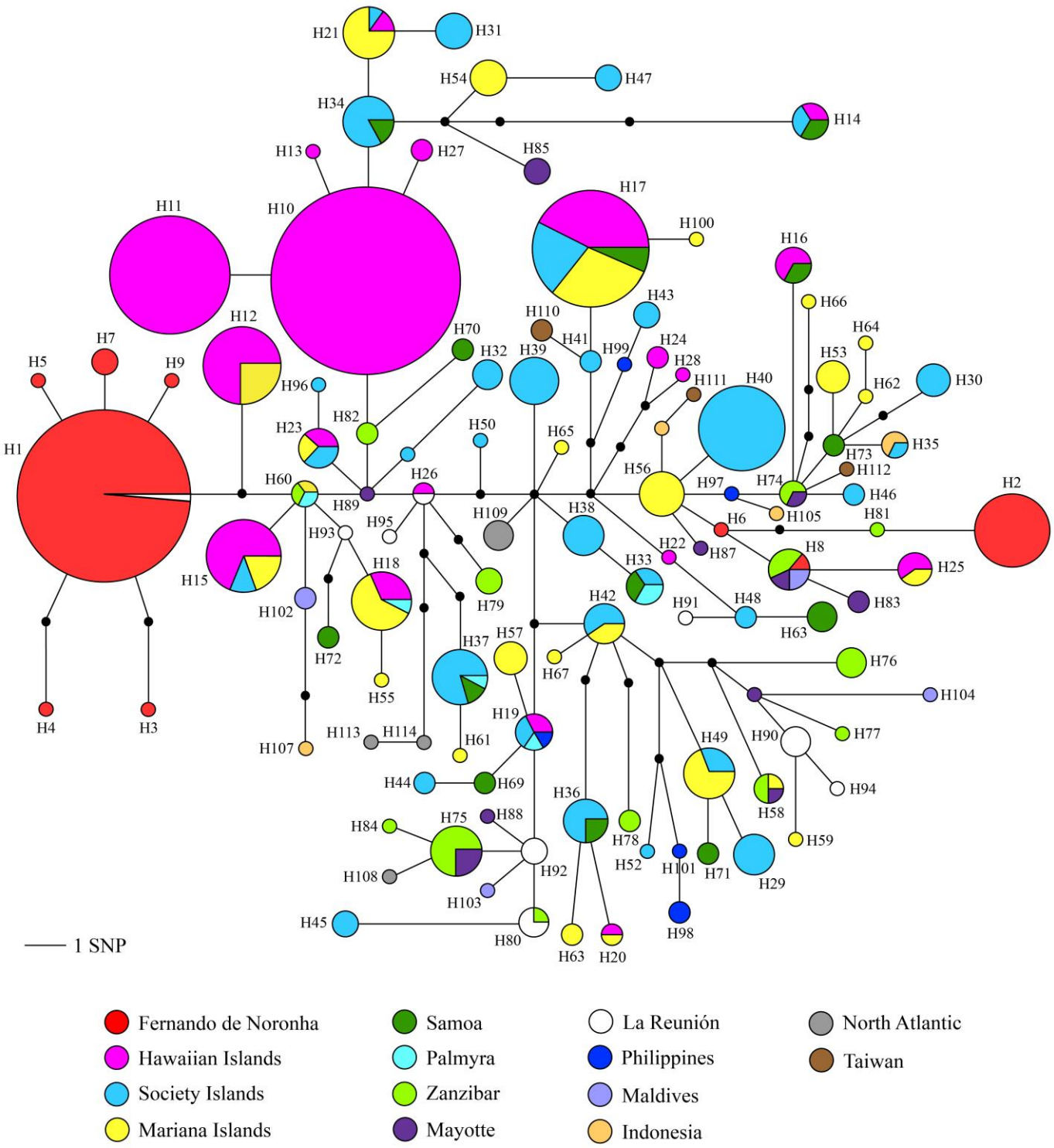

Figure 6 Median-Joining network of mtDNA control region haplotypes. Each circle corresponds to a haplotype, and its size is proportional to its frequency in the population. Black circles indicate potential intermediate haplotypes not sampled. Different colors represent the geographic sampling locations of specimens with those haplotypes. Lengths of lines connecting haplotypes are proportional to the number Single Nucleotide Polymorphism (SNP) differences between haplotypes.

Discussion

Understanding the basic biology of a species, including the degree of genetic variability, as well as the spatial and temporal aspects of its intraspecific population structure is necessary for the implementation of adequate conservation and management strategies. To our knowledge, this is the first study to evaluate the genetic diversity and population structure of spinner dolphins of Atlantic Ocean especially in the Southwest Atlantic Ocean. Here we used mitochondrial DNA (D-loop) and nuclear microsatellite loci to assess the patterns of genetic diversity and population structure of the spinner dolphins of Fernando de Noronha Archipelago, Southwest Atlantic Ocean. Mitochondrial DNA revealed low genetic diversity and high differentiation, in terms of pair-wise F_{ST}, and high isolation from other populations around the world. These findings emphasize spinner dolphin vulnerability to genetic processes, particularly drift in island associated populations, and highlight the importance of conservation concerns for this population face to anthropogenic threats of the tourism industry in the Fernando de Noronha archipelago.

Noronha spinner dolphins revealed the lowest level of mtDNA diversity of any spinner dolphin population around the world (Andrews *et al.* 2010; Andrews *et al.* 2013; Oremus *et al.* 2007; Martien *et al.* 2014; Viricel *et al.* 2016) and even among threatened odontocetes (Lyrholm *et al.* 1996; Hamner *et al.* 2012; Chen *et al.* 2014; Cunha *et al.* 2014). To our knowledge, the only report of a population of dolphins with lower mtDNA diversity is the Hector's dolphin (*Cephalorhynchus hectori*) of the north island of New Zealand (Pichler & Baker 2000). Now recognized as a subspecies, the Māui dolphin currently consists of just a single matrilineal lineage, presented a control region diversity of 0.410.

However, low variability in the mitochondrial DNA control region does not necessarily reflects low levels of heterozygosity in the nuclear genome (Rosel Rojas-Brancho 1999). Indeed, microsatellites revealed moderate to high levels of heterogozity for Noronha spinner dolphins. Low values of mitochondrial genetic diversity were reported for spinner dolphins of the North-western Hawaiian Islands: Kure Atoll (N= 51, *h*= 0.395), Midway Atoll (N= 117, *h* = 0.405), Pearl & Hermes Reef (N=47, *h* = 0.200) (Andrews *et al.* 2010). Small population sizes were estimated for islands by photo-identification studies for Kure (≅120, Karczmarski *et al.* 2005a) and Midway (≅ 260, Karczmarski *et al.* 2005b), and were consistent with the scarcity of resting habitat and foraging habitat in this region. This pattern seems not to be applicable to Noronha spinner dolphins (N=162) which encompasses large numbers of individuals estimated daily by photo-identification studies (≅ 600 individuals, Silva & Silva-Jr 2009). Instead, an alternative explanation would be low levels of immigration driven by social or demographic forces, as it has been seen in the Tahiti spinner dolphin (Oremus *et al*. 2007); or recent demographic bottleneck as demonstrated by significant microsatellite results.

The predominance of one haplotype of Noronha spinner dolphins can be explained by the founder effect. The bimodal curve of the mismatch distribution chart indicates that this population unit has undergone a recent demographic expansion (Slatkin & Hudson 1991; Rogers & Harpending 1992) or through a scale expansion with high levels of migration between neighbors (Excoffier 2004). This analysis is usually multimodal for population in demographic balance, given that it reflects the highly stochastic gene trees. We can hypothesize that Noronha spinner dolphins originally had few mitochondrial

lineages and that the effects of genetic drift would lead to loss of alleles (haplotypes) and the restricted gene flow (promoted mainly by females) between this population unit and the spinner dolphins of other localities of Brazilian coast would not be sufficient to compensate the effects of genetic drift leading to loss of the rarer allele and the maintenance of the most frequent haplotype. However, further analysis are necessary to prove this pattern and the lack of information about spinner dolphins from other localities along the Brazilian coastline limits answers about the maintenance of gene flow between these stocks.

The presence of two distinctive and divergent haplotypes of Noronha spinner dolphin's lineages also suggests colonization of this archipelago by at least two different matrilines. Comparisons among locations of the world indicate genetic isolation of Noronha spinner dolphins from all other spinner dolphin's populations. This is likely due to the geographic isolation of this island from other islands of the Atlantic and Pacific Oceans. The Fernando de Noronha Archipelago encompasses the only islands in the Southwest Atlantic Ocean with resources and rest areas to support a large number of spinner dolphins (Silva & Silva-Jr 2009). Studies describe that spinner dolphins are found in every ocean (Galver 2002; Oremus *et al*. 2007; Andrews *et al*. 2010) encompassing large groups of individuals (Silva Jr 2005; Galver 2002) displaying the ability to travel for long distances and to dispersal between islands and atolls contributing to variations in the levels of disperse and genetic divergence around the world (Martin *et al*. 1990; Würsig *et al*. 1994 a; Würsig *et al*. 1994 b; Wells & Gannon 2005). Our results indicate that these dolphins

followed a mainland-island model of metapopulation dynamic, with pelagic populations representing the ‘mainland’ in the model (Harrison 1991).

Ocean water masses of Southwest Atlantic Ocean also exercise highly significant role on the distribution of spinner dolphins and can have important role in levels gene flow of spinner dolphins in the Brazilian coast. The Brazil current (C28) and the Malvinas Current (C43) are considered as the limit of distribution of this species in southern Brazil and encourage the displacement of these dolphins to the south of the country in hot seasons, in colder seasons these dolphins move to the north of the country (Secchi & Siciliano 1995; Moreno *et al.* 2005).

The vicinities of Pernambuco, where the South Equatorial Current (C27) splits to the north (North Brazil Current, C8) and south (Brazil's current, C28), can be the limit of distribution of *S. longirostris longirostris* between Northeast and Southeast of Brazil. The South Equatorial Current (C27) can encourage the displacement of spinner dolphins of the Fernando de Noronha Archipelago towards the Brazilian coast (Okolodkov 2010). This current can also influence the displacement of dolphins from the coast of Africa and Pacific Ocean to the Brazilian coast explaining the shared haplotypes among Noronha spinner dolphins and La Reunión, Maldives and Zanzibar. Consequences of climate change have also been identified as the causes of habitat changes and alterations in the gene flow of small cetacean population units (Fontaine *et al.* 2007; Taguchi *et al.* 2010).

Some evidence of heterogeneity in the Noronha population was suggested by annual difference in mtDNA frequencies, although not in microsatellite. This could be explained by recent observations suggesting two

different groups of spinner dolphins that appear to use distinct feeding strategies at Fernando de Noronha Archipelago (Silva Jr 2005). Typically, spinner dolphins use the calm waters of the archipelago during the day to rest, play, care for their young and take refuge from sharks and during the night moving to sea to feed (Silva Jr 2005). However, in Fernando de Noronha Arquipelago, observations of the same dolphins coming to the archipelago in several days and the long periods of absence of other dolphins can indicate two distinct feeding strategies: “around the archipelago” (in a radius of up to 5 nautical miles), and “oceanic” (on the slopes of mountains or submerged underwater geological chain of oceanic region adjacent to Fernando de Noronha).

Our analyses provide the first valuable insights into genetic diversity and differentiation of Gray’s spinner dolphins of the Southwest Atlantic Ocean. Our study also presents one of the most complete analyses of mtDNA structure and diversity of spinner dolphins in terms of sample size (893 individuals) and phylogeographic coverage (15 populations) crossing 3 ocean basins.

We showed that Noronha spinner dolphins display low levels of diversity and that the colonization of this archipelago was driven by at least two different matrilineal lines. We also suggest that these dolphins live in societies with strong geographic fidelity greater genetic isolation when comparing to other island populations around the world.

Conservation implications

Our results highlight the importance of the availability of island resources and resting areas for these pelagic dolphins especially in the Southwest Atlantic Ocean. A growing concern is the anthropogenic impacts on the Noronha spinner dolphins generated by the ecotourism industry in the archipelago. Our genetic analyses reinforce that the Noronha spinner dolphins deserves special attention in conservation strategies once high site fidelity at this archipelago was demonstrated, even these islands already occurring in an area that involves the area of Environmental Protection and the Marine National Park of Fernando de Noronha.

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Supporting information

Appendix 1 Specimens of *Stenella longirostris* from the Fernando de Noronha Archipelago analysed. Number of specimens, sampling year, sex and D-loop haplotypes (fragment lengh).

Sample	Sampling year	Sex	D-loop Haplotype (413 bp)	D-loop Haplotype (374 bp)
Slo_01	2004	M	NOR1	H1
Slo_02	2006	M	NOR2	H2
Slo_03	2006	M	NOR1	H1
Slo_04	2006	M	NOR2	H2
Slo_05	2006	M	NOR3	H3
Slo_06	2006	M	NOR1	H1
Slo_07	2006	M	NOR4	H4
Slo_08	2006	F	NOR1	H1
Slo_09	2006	F	NOR1	H1
Slo_10	2006	M	NOR1	H1
Slo_11	2006	M	NOR1	H1
Slo_12	2006	M	NOR1	H1
Slo_13	2006	M	NOR1	H1
Slo_14	2006	M	NOR1	H1
Slo_15	2006	F	NOR1	H1
Slo_16	2006	M	NOR1	H1
Slo_17	2006	F	NOR2	H2
Slo_18	2006	-	NOR1	H1
Slo_19	2006	M	NOR5	H1
Slo_20	2006	M	NOR2	H2
Slo_21	2006	F	NOR1	H1
Slo_22	2006	-	NOR1	H1
Slo_23	2006	M	NOR1	H1
Slo_24	2006	F	NOR1	H1
Slo_25	2006	M	NOR1	H1
Slo_26	2006	M	NOR1	H1
Slo_27	2006	-	NOR6	H6
Slo_28	2006	-	NOR1	H1
Slo_29	2006	-	NOR2	H2
Slo_30	2006	-	NOR2	H2
Slo_31	2006	F	NOR1	H1
Slo_32	2006	M	NOR2	H2
Slo_33	2006	M	NOR1	H1
Slo_34	2006	M	NOR7	H1
Slo_35	2006	M	NOR2	H2
Slo_36	2006	M	NOR1	H1

Sample	Sampling year	Sex	D-loop Haplotype (413 bp)	D-loop Haplotype (374 bp)
Slo_37	2009	F	NOR1	H1
Slo_38	2009	F	NOR1	H1
Slo_39	2009	F	NOR1	H1
Slo_40	2009	F	NOR8	H7
Slo_41	2009	F	NOR1	H1
Slo_42	2009	M	NOR1	H1
Slo_43	2009	M	NOR1	H1
Slo_44	2009	-	NOR1	H1
Slo_45	2009	M	NOR1	H1
Slo_46	2009	F	NOR1	H1
Slo_47	2009	F	NOR1	H1
Slo_48	2009	F	NOR1	H1
Slo_49	2009	M	NOR1	H1
Slo_50	2009	F	NOR1	H1
Slo_51	2009	F	NOR1	H1
Slo_52	2009	M	NOR2	H2
Slo_53	2009	F	NOR1	H1
Slo_54	2009	F	NOR1	H1
Slo_55	2009	M	NOR1	H1
Slo_56	2009	M	NOR1	H1
Slo_57	2009	-	NOR1	H1
Slo_58	2009	M	NOR1	H1
Slo_59	2009	M	NOR1	H1
Slo_60	2009	M	NOR1	H1
Slo_61	2009	M	NOR2	H2
Slo_62	2009	F	NOR1	H1
Slo_63	2009	M	NOR1	H1
Slo_64	2009	M	NOR1	H1
Slo_65	2009	M	NOR2	H2
Slo_66	2009	M	NOR1	H1
Slo_67	2009	F	NOR1	H1
Slo_68	2009	F	NOR1	H1
Slo_69	2009	F	NOR1	H1
Slo_70	2009	M	NOR2	H2
Slo_71	2009	-	NOR2	H2
Slo_72	2009	M	NOR1	H1
Slo_73	2009	M	NOR2	H2
Slo_74	2009	F	NOR1	H1
Slo_75	2009	M	NOR1	H1
Slo_76	2009	M	NOR2	H2
Slo_77	2009	F	NOR1	H1
Slo_78	2009	F	NOR1	H1

Sample	Sampling year	Sex	D-loop Haplotype (413 bp)	D-loop Haplotype (374 bp)
Slo_79	2009	M	NOR1	H1
Slo_80	2009	M	NOR1	H1
Slo_81	2009	M	NOR9	H1
Slo_82	2009	F	NOR1	H1
Slo_83	2009	F	NOR2	H2
Slo_84	2009	M	NOR1	H1
Slo_85	2009	M	NOR1	H1
Slo_86	2009	M	NOR9	H1
Slo_87	2009	F	NOR1	H1
Slo_88	2009	-	NOR1	H1
Slo_89	2009	M	NOR1	H1
Slo_90	2009	M	NOR1	H1
Slo_91	2009	M	NOR1	H1
Slo_92	2009	M	NOR1	H1
Slo_93	2009	M	NOR1	H1
Slo_94	2009	M	NOR1	H1
Slo_95	2009	M	NOR1	H1
Slo_96	2009	M	NOR2	H2
Slo_97	2009	F	NOR1	H1
Slo_98	2009	F	NOR1	H1
Slo_99	2009	-	NOR1	H1
Slo_100	2009	-	NOR2	H2
Slo_101	2009	M	NOR2	H2
Slo_102	2009	M	NOR1	H1
Slo_103	2009	F	NOR2	H2
Slo_104	2012	M	NOR1	H1
Slo_105	2012	M	NOR1	H1
Slo_106	2012	M	NOR1	H1
Slo_107	2012	F	NOR1	H1
Slo_108	2012	M	NOR1	H1
Slo_109	2012	-	NOR1	H1
Slo_110	2012	M	NOR1	H1
Slo_111	2012	M	NOR1	H1
Slo_112	2012	M	NOR1	H1
Slo_113	2012	F	NOR1	H1
Slo_114	2012	F	NOR1	H1
Slo_115	2012	-	NOR1	H1
Slo_116	2012	F	NOR1	H1
Slo_117	2012	M	NOR2	H2
Slo_118	2012	F	NOR1	H1
Slo_119	2012	-	NOR2	H2
Slo_120	2012	F	NOR1	H1

Sample	Sampling year	Sex	D-loop Haplotype (413 bp)	D-loop Haplotype (374 bp)
Slo_121	2012	M	NOR2	H2
Slo_122	2012	F	NOR1	H1
Slo_123	2012	F	NOR1	H1
Slo_124	2012	M	NOR1	H1
Slo_125	2012	-	NOR1	H1
Slo_126	2012	M	NOR1	H1
Slo_127	2012	M	NOR1	H1
Slo_128	2012	-	NOR1	H1
Slo_129	2012	F	NOR1	H1
Slo_130	2012	M	NOR1	H1
Slo_131	2012	M	NOR1	H1
Slo_132	2012	F	NOR1	H1
Slo_133	2012	M	NOR1	H1
Slo_134	2012	-	NOR10	H8
Slo_135	2012	M	NOR1	H1
Slo_136	2012	F	NOR1	H1
Slo_137	2012	-	NOR1	H1
Slo_138	2012	-	NOR11	H9
Slo_139	2012	M	NOR8	H7
Slo_140	2012	F	NOR1	H1
Slo_141	2012	M	NOR1	H1
Slo_142	2012	-	NOR1	H1
Slo_143	2012	F	NOR1	H1
Slo_144	2012	M	NOR1	H1
Slo_145	2012	-	NOR1	H1
Slo_146	2012	-	NOR1	H1
Slo_147	2012	F	NOR1	H1
Slo_148	2012	M	NOR1	H1
Slo_149	2012	-	NOR1	H1
Slo_150	2012	M	NOR1	H1
Slo_151	2012	M	NOR1	H1
Slo_152	2012	-	NOR2	H2
Slo_153	2012	M	NOR1	H1
Slo_154	2012	F	NOR1	H1
Slo_155	2012	F	NOR1	H1
Slo_156	2012	F	NOR1	H1
Slo_157	2012	F	NOR8	H7
Slo_158	2012	F	NOR1	H1
Slo_159	2012	M	NOR1	H1
Slo_160	2012	M	NOR1	H1
Slo_161	2012	F	NOR1	H1
Slo_162	2012	F	NOR1	H1

Appendix 2 Haploypes from GenBank used in this study. Source, GenBank accession number, haplotypes codes of the original papers and of the present study, and, geographic localizations with number of individuals.

Source (Paper)	GenBank acession	Haplotype code (Original Paper)	Haplotype code (This Study)	Geographic localization (nº of individuals)
Andrews *et al.* 2010	GU253256	HW01	H 10	Hawaii (344)
Andrews *et al.* 2010	GU253257	HW02	H 11	Hawaii (63)
Andrews *et al.* 2010	GU253258	HW03	H 12	Hawaii (20)
Andrews *et al.* 2010	GU253259	HW04	H 13	Hawaii (1)
Andrews *et al.* 2010	GU253260	HW05	H 14	Hawaii (2), Samoa (1)
Andrews *et al.* 2010	GU253261	HW06	H 15	Hawaii (18)
Andrews *et al.* 2010	GU253262	HW07	H 16	Hawaii (4), Samoa (2)
Andrews *et al.* 2010	GU253263	HW08	H 17	Hawaii (26), Samoa (2)
Andrews *et al.* 2010	GU253264	HW09	H 18	Hawaii (5)
Andrews *et al.* 2010	GU253265	HW10	H 19	Hawaii (2)
Andrews *et al.* 2010	GU253266	HW11	H 20	Hawaii (1)
Andrews *et al.* 2010	GU253267	HW12	H 21	Hawaii (2)
Andrews *et al.* 2010	GU253268	HW13	H 22	Hawaii (1)
Andrews *et al.* 2010	GU253269	HW14	H 23	Hawaii (3)
Andrews *et al.* 2010	GU253270	HW15	H 24	Hawaii (2)
Andrews *et al.* 2010	GU253271	HW16	H 25	Hawaii (3)
Andrews *et al.* 2010	GU253272	HW17	H 26	Hawaii (1)
Andrews *et al.* 2010	GU253273	HW18	H 27	Hawaii (2)
Andrews *et al.* 2010	GU253274	HW19	H 28	Hawaii (1)
Andrews *et al.* 2010	GU253275	HW20	H 68	Samoa (2)
Andrews *et al.* 2010	GU253276	HW21	H 69	Samoa (1)
Andrews *et al.* 2010	GU253277	HW22	H 70	Samoa (1)
Andrews *et al.* 2010	GU253278	HW23	H 36	Samoa (1)
Andrews *et al.* 2010	GU253279	HW24	H 34	Samoa (1)
Andrews *et al.* 2010	GU253280	HW25	H 43	Samoa (1)
Andrews *et al.* 2010	GU253281	HW26	H 33	Samoa (1)
Andrews *et al.* 2010	GU253282	HW27	H 37	Samoa (1)
Andrews *et al.* 2010	GU253283	HW28	H 71	Samoa (1)
Andrews *et al.* 2010	GU253284	HW29	H 72	Samoa (1)
Andrews *et al.* 2013	KC160999	StLo003	H 15	Guam (1), Saipam (2), Samoa (2)
Andrews *et al.* 2013	KC161018	StLo022	H 60	Palmyra (1)
Andrews *et al.* 2013	KC161021	StLo025	H 74	Zanzibar (2)
Andrews *et al.* 2013	KC161037	StLo041	H 8	Maldives (2), Zanzibar (2)
Andrews *et al.* 2013	KC161038	StLo042	H 14	American Samoa (1)
Andrews *et al.* 2013	KC161041	StLo045	H 108	Gulf of mexico (1)
Andrews *et al.* 2013	KC161042	StLo046	H 109	Gulf of mexico (4)
Andrews *et al.* 2013	KC161043	StLo047	H 62	Guam (1)
Andrews *et al.* 2013	KC161044	StLo048	H 63	Guam (1)
Andrews *et al.* 2013	KC161045	StLo049	H 57	Guam (1)
Andrews *et al.* 2013	KC161046	StLo050	H 54	Guam (1), Saipan (1)

Source (Paper)	GenBank acession	Haplotype code (Original Paper)	Haplotype code (This Study)	Geographic localization (nº of individuals)
Andrews *et al.* 2013	KC161047	StLo051	H 105	Indonesia (1)
Andrews *et al.* 2013	KC161048	StLo052	H 35	Indonesia (2)
Andrews *et al.* 2013	KC161049	StLo053	H 106	Indonesia (1)
Andrews *et al.* 2013	KC161050	StLo054	H 107	Indonesia (1)
Andrews *et al.* 2013	KC161051	StLo055	H 102	Maldives (2)
Andrews *et al.* 2013	KC161052	StLo056	H 103	Maldives (1)
Andrews *et al.* 2013	KC161053	StLo057	H 104	Maldives (1)
Andrews *et al.* 2013	KC161055	StLo059	H 113	North atlantic (1)
Andrews *et al.* 2013	KC161056	StLo060	H 114	North atlantic (1)
Andrews *et al.* 2013	KC161057	StLo061	H 18	Saipam (1), Palmyra (1)
Andrews *et al.* 2013	KC161058	StLo062	H 19	Palmyra (1), Philippines (1)
Andrews *et al.* 2013	KC161059	StLo063	H 97	Philippines (1)
Andrews *et al.* 2013	KC161060	StLo064	H 98	Philippines (2)
Andrews *et al.* 2013	KC161061	StLo065	H 99	Philippines (1)
Andrews *et al.* 2013	KC161062	StLo066	H 100	Philippines (1)
Andrews *et al.* 2013	KC161063	StLo067	H 101	Philippines (1)
Andrews *et al.* 2013	KC161064	StLo068	H 23	Palmyra (3)
Andrews *et al.* 2013	KC161065	StLo069	H 96	Palmyra (1)
Andrews *et al.* 2013	KC161066	StLo070	H 37	Palmyra (1), American Samoa (1)
Andrews *et al.* 2013	KC161067	StLo071	H 33	American Samoa (1), Palmyra (2)
Andrews *et al.* 2013	KC161068	StLo072	H 68	American Samoa (2)
Andrews *et al.* 2013	KC161069	StLo073	H 69	American Samoa (1)
Andrews *et al.* 2013	KC161070	StLo074	H 70	American Samoa (1)
Andrews *et al.* 2013	KC161071	StLo075	H 73	American Samoa (2)
Andrews *et al.* 2013	KC161072	StLo076	H 36	American Samoa (1)
Andrews *et al.* 2013	KC161073	StLo077	H 34	American Samoa (1)
Andrews *et al.* 2013	KC161074	StLo078	H 43	American Samoa (1)
Andrews *et al.* 2013	KC161075	StLo079	H 71	American Samoa (1)
Andrews *et al.* 2013	KC161076	StLo080	H 72	American Samoa (1)
Andrews *et al.* 2013	KC161077	StLo081	H 25	Saipan (1)
Andrews *et al.* 2013	KC161096	StLo100	H 110	Taiwan (2)
Andrews *et al.* 2013	KC161097	StLo101	H 111	Taiwan (1)
Andrews *et al.* 2013	KC161098	StLo102	H 112	Taiwan (1)
Andrews *et al.* 2013	KC161113	StLo117	H 75	Zanzibar (2)
Andrews *et al.* 2013	KC161114	StLo118	H 76	Zanzibar (2)
Andrews *et al.* 2013	KC161115	StLo119	H 77	Zanzibar (1)
Oremus *et al.* 2007	EF558737	Slo02FP01	H 29	Society Islands: Moorea (7), Tahiti (1)
Oremus *et al.* 2007	EF558738	Slo02FP02	H 30	Society Islands: Moorea (5)
Oremus *et al.* 2007	EF558739	Slo02FP03	H 21	Society Islands: Moorea (1)
Oremus *et al.* 2007	EF558740	Slo02FP04	H 31	Society Islands: Moorea (5), Huahine (1)
Oremus *et al.* 2007	EF558741	Slo02FP05	H 32	Society Islands: Moorea (1), Huahine (2), Raiatea-Tahaa (1)
Oremus *et al.* 2007	EF558742	Slo02FP06	H 33	Society Islands: Moorea (2)
Oremus *et al.* 2007	EF558743	Slo02FP07	H 34	Society Islands: Moorea (5), Huahine (2), Bora Bora (2), Raiatea-Tahaa (2)
Oremus *et al.* 2007	EF558744	Slo02FP08	H 35	Society Islands: Moorea (1)
Oremus *et al.* 2007	EF558745	Slo02FP09	H 36	Society Islands: Moorea (3), Bora Bora (1), Raiatea-Tahaa (3)

Source (Paper)	GenBank acession	Haplotype code (Original Paper)	Haplotype code (This Study)	Geographic localization (nº of individuals)
Oremus *et al.* 2007	EF558746	Slo02FP11	H 17	Society Islands: Moorea (4), Tahiti (8), Huahine (1)
Oremus *et al.* 2007	EF558747	Slo02FP15	H 37	Society Islands: Moorea (7), Bora Bora (1), Raiatea-Tahaa (4)
Oremus *et al.* 2007	EF558748	Slo02FP20	H 38	Society Islands: Moorea (6), Raiatea-Tahaa (1)
Oremus *et al.* 2007	EF558749	Slo02FP22	H 39	Society Islands: Moorea (11)
Oremus *et al.* 2007	EF558750	Slo02FP27	H 40	Society Islands: Moorea (7), Tahiti (24), Bora Bora (1), Raiatea-Tahaa (1)
Oremus *et al.* 2007	EF558751	Slo02FP36	H 19	Society Islands: Moorea (1), Raiatea-Tahaa (1)
Oremus *et al.* 2007	EF558752	Slo02FP38	H 14	Society Islands: Moorea (2)
Oremus *et al.* 2007	EF558753	Slo02FP45	H 41	Society Islands: Moorea (1)
Oremus *et al.* 2007	EF558754	Slo02FP49	H 42	Society Islands: Moorea (1), Raiatea-Tahaa (1)
Oremus *et al.* 2007	EF558755	Slo03FP18	H 43	Society Islands: Bora Bora (1)
Oremus *et al.* 2007	EF558756	Slo03FP26	H 44	Society Islands: Huahine (2)
Oremus *et al.* 2007	EF558757	Slo03FP32	H 15	Society Islands: Huahine (3)
Oremus *et al.* 2007	EF558758	Slo03FP33	H 41	Society Islands: Huahine (1)
Oremus *et al.* 2007	EF558759	Slo03FP34	H 45	Society Islands: Huahine (2), Raiatea-Tahaa (1)
Oremus *et al.* 2007	EF558760	Slo03FP37	H 46	Society Islands: Huahine (2)
Oremus *et al.* 2007	EF558761	Slo03FP41	H 47	Society Islands: Huahine (1), Raiatea-Tahaa (2)
Oremus *et al.* 2007	EF558762	Slo04FP59	H 48	Society Islands: Raiatea-Tahaa (1), Tahiti (1)
Oremus *et al.* 2007	EF558763	Slo04FP70	H 49	Society Islands: Raiatea-Tahaa (1), Nuku Hiva (3)
Oremus *et al.* 2007	EF558764	Slo04FP78	H 50	Society Islands: Nuku Hiva (1)
Oremus *et al.* 2007	EF558765	Slo04FP79	H 51	Society Islands: Nuku Hiva (1)
Oremus *et al.* 2007	EF558766	Slo04FP82	H 52	Society Islands: Nuku Hiva (1)
Oremus *et al.* 2007	EF558767	Slo04FP83	H 42	Society Islands: Nuku Hiva (2)
Martien *et al.* 2014	KY457781	H1	H 64	Guam (1)
Martien *et al.* 2014	KY457782	H2	H 53	3-Islands (4), Rota (1)
Martien *et al.* 2014	KY457783	H3	H 63	Guam (1)
Martien *et al.* 2014	KY457784	H4	H 17	3-Islands (9), Rota (2), Guam (4)
Martien *et al.* 2014	KY457785	H5	H 17	3-Islands (2), Guam (2)
Martien *et al.* 2014	KY457786	H6	H 18	3-Islands (8), Rota (1)
Martien *et al.* 2014	KY457787	H7	H 55	3-Islands (1)
Martien *et al.* 2014	KY457788	H8	H 15	3-Islands (3), Guam (2)
Martien *et al.* 2014	KY457789	H9	H 65	Guam (1)
Martien *et al.* 2014	KY457790	H10	H 56	3-Islands (8), Guam (1)
Martien *et al.* 2014	KY457791	H11	H 12	3-Islands (3), Rota (2), Guam (2)
Martien *et al.* 2014	KY457792	H12	H 21	3-Islands (4), Rota (1), Guam (4)
Martien *et al.* 2014	KY457793	H13	H 25	3-Islands (1)
Martien *et al.* 2014	KY457794	H14	H 61	Rota (1)
Martien *et al.* 2014	KY457795	H15	H 57	3-Islands (3), Guam (1)
Martien *et al.* 2014	KY457796	H16	H 49	3-Islands (3), Rota (2), Guam (4)
Martien *et al.* 2014	KY457797	H17	H 42	3-Islands (2), Rota (1)
Martien *et al.* 2014	KY457798	H18	H 58	3-Islands (1)
Martien *et al.* 2014	KY457799	H19	H 59	3-Islands (1)
Martien *et al.* 2014	KY457800	H20	H 66	Guam (1)
Martien *et al.* 2014	KY457801	H21	H 67	Guam (1)
Martien *et al.* 2014	KY457802	H22	H 23	3-Islands (2)
Martien *et al.* 2014	KY457803	H23	H 60	3-Islands (1)

Source (Paper)	GenBank acession	Haplotype code (Original Paper)	Haplotype code (This Study)	Geographic localization (nº of individuals)
Martien *et al.* 2014	KY457804	H24	H 20	Guam (1)
Viricel *et al.* 2016	KX905105	Hap1	H 75	Mayotte (2), Zanzibar (8)
Viricel *et al.* 2016	KX905106	Hap2	H 58	Mayotte (1), Zanzibar (2)
Viricel *et al.* 2016	KX905107	Hap3	H 78	Zanzibar (2)
Viricel *et al.* 2016	KX905108	Hap4	H 60	Zanzibar (1)
Viricel *et al.* 2016	KX905109	Hap5	H 8	Mayotte (1), Zanzibar (1)
Viricel *et al.* 2016	KX905110	Hap6	H 79	Zanzibar (3)
Viricel *et al.* 2016	KX905111	Hap7	H 80	La Reunión (3), Zanzibar (1)
Viricel *et al.* 2016	KX905112	Hap8	H 81	Zanzibar (1)
Viricel *et al.* 2016	KX905113	Hap9	H 82	Zanzibar (1)
Viricel *et al.* 2016	KX905114	Hap11	H 83	Mayotte (2)
Viricel *et al.* 2016	KX905115	Hap12	H 84	Mayotte (1)
Viricel *et al.* 2016	KX905116	Hap13	H 85	Mayotte (3)
Viricel *et al.* 2016	KX905117	Hap14	H 74	Mayotte (1)
Viricel *et al.* 2016	KX905118	Hap15	H 86	Mayotte (1)
Viricel *et al.* 2016	KX905119	Hap16	H 87	Mayotte (1)
Viricel *et al.* 2016	KX905120	Hap17	H 75	Mayotte (1)
Viricel *et al.* 2016	KX905121	Hap18	H 76	Mayotte (2)
Viricel *et al.* 2016	KX905122	Hap19	H 88	Mayotte (1)
Viricel *et al.* 2016	KX905123	Hap20	H 89	Mayotte (1)
Viricel *et al.* 2016	KX905124	Hap22	H 89	Mayotte (1)
Viricel *et al.* 2016	KX905125	Hap23	H 90	La Reunión (4)
Viricel *et al.* 2016	KX905126	Hap24	H 91	La Reunión (1)
Viricel *et al.* 2016	KX905127	Hap25	H 92	La Reunión (3)
Viricel *et al.* 2016	KX905128	Hap26	H 93	La Reunión (1)
Viricel *et al.* 2016	KX905129	Hap27	H 94	La Reunión (1)
Viricel *et al.* 2016	KX905130	Hap28	H 95	La Reunión (1)
Viricel *et al.* 2016	KX905131	Hap29	H 26	La Reunión (1)
Viricel *et al.* 2016	KX905132	Hap30	H 1	La Reunión (1)

Capítulo 3

Global genetic differentiation of spinner dolphins (Stenella longirostris, Gray 1828)

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Em preparação

Global genetic differentiation of spinner dolphins (*Stenella longirostris,* Gray 1828)

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Abstract

Spinner dolphins have a circumtropical distribution in all three oceans (Atlantic, Pacific and Indian) presenting significant levels of genetic structure and diversity within island-associated groups, particularly in the Pacific Ocean. However, little is known about global structuring patterns especially incorporating samples from the Southwest Atlantic Ocean. In this study we evaluated the genetic diversity and genetic structuring within spinner dolphin's locations, among locations and among ocean basins. We evaluated mitochondrial DNA control region of 1138 specimens from fifteen different locations within the three oceans, including published records, and, genotypes from 12 nuclear microsatellite loci for a subset of 415 specimens from eight different locations of the Atlantic and Pacific oceans. F-statistic analyses revealed differentiation among locations and ocean basins, and, in particular, high differentiation for Hawaiian Islands and the Fernando de Noronha Archipelago. Low levels of differentiation was observed between locations of the Atlantic and Indian oceans. Network analysis did not show strong phylogeographic signal among ocean basins and Bayesian clustering analysis displayed five clusters for spinner dolphins. Our results showed two groups in the Southwest Atlantic Ocean, one formed by Island-associated dolphins of the Fernando de Noronha Archipelago and another composed by Non-island-associated dolphins of the Brazilian Coast. We also demonstrated that spinner dolphins' populations of western South Atlantic Ocean are differentiated from other locations, especially from the Pacific Ocean. These results suggest a metapopulation dynamic with periodic long-distance dispersal or interchange, and local colonization and site fidelity of suitable island habitats. We also highlight that special attention to conservation strategies should be given to the populations of spinner dolphins that display strong site fidelity to island - associated groups, like those of the Fernando de Noronha Archipelago and the Hawaiian Archipelago.

Keywords: Atlantic Ocean, Delphinidae, genetic structuring, Indian Ocean, Pacific Ocean, population genetics.

Introduction

The investigation about the genetic diversity and population structure is essential for understanding the ecological and evolutionary processes of the species and this information is very important to develop effective conservation strategies. In marine species that are distributed along continuous environments with no conspicuous barriers to dispersal, that are highly mobile and that show the ability to travel for long distances, it is expected that high connectivity among populations will lead to persistent gene flow and little genetic differentiation, especially in geographically distant populations (Palumbi 1996).

The study of connectivity and gene flow in marine environments organisms is challenging once there is no evident geographical barriers like in terrestrial environments and there are generally organisms with large populations that regularly struggle genetic structure resulting in low statistical power to detect genetic differentiation (Amaral *et al.* 2012). However, in the absence of geographical barriers, other environmental variables like ocean currents, cold water masses at higher latitudes, open-ocean expanses, upwelling, variation in sea surface temperature and salinity can limit gene flow in marine species (Bowen *et al.* 2001; Banks *et al*. 2007; Banks *et al.* 2010; Selkoe *et al.* 2010).

Cetaceans are highly mobile marine mammals that can travel for long distances probably increasing the tendency to population stability via gene flow (Leslie & Morin 2018). For these animals, structuring among populations may be the result of dispersal restrictions or conformation to the social group (Enguiarte *et al.* 2007). Certainly, water surface temperature, behavioural specializations to optimize access to resources, social structure, and historical

processes are determining factors in the structuring of dolphin's populations (Hoelzel 1998; Hoelzel *et al.* 2002).

Several studies showed that many dolphins species present significant population genetic structure (Pimper et al. 2010; Martien et al. 2012; Amaral *et al.* 2012). *Stenella longirostris* (Cetacea, Delphinidae), are one such species that show significant genetic differentiation among populations in a number of locations: Hawaii (Andrews *et al.* 2010), the Society Islands of French Polynesia (Oremus *et al.* 2007) and Indian Ocean islands (Zanzibar, Mayotte and La Reunión) (Viricel *et al.* 2016). Popularly known as spinner dolphins due to aerobatics, this species has a wide distribution, occurring in tropical and subtropical waters of the Atlantic, Pacific and Indian Oceans. They can form large groups away from the coast or near atolls and archipelagos (Norris *et al.* 1994). The most recent estimate suggests that there are over half a million spinner dolphins around the world (Galver 2002). The Gray's spinner subspecies *(Stenella longirostris longirostris)* is preferentially found around volcanic islands, such as those of Hawaii, French Polynesia and Fernando de Noronha Archipelago. In waters adjacent to the Brazilian coast, Gray's spinner dolphins occur mainly in tropical waters between 170 and 2,700m depth, being very common in the Fernando de Noronha Archipelago (Moreno *et al.* 2005) forming groups of 600 individuals daily (Silva & Silva-Jr 2009). Their distribution may be related to environmental variables that influence the segregation of preferred prey (Cañadas *et al.* 2002; Ballance *et al.* 2006).

Population genetic studies performed until now have demonstrated significant genetic differentiation among populations inhabiting the same archipelagos of the same ocean basins for spinner dolphins. Spinner dolphins

from Society Islands, Pacific Ocean, displayed high genetic structure among geographic close islands, attributed to habitat fidelity (especially by females), with restricted gene flow promoted by males (Oremus *et al.* 2007). Genetic isolation was also reported for Hawaiian spinner dolphins, Pacific Ocean, mainly of waters adjacent to the island of Kona (largest island) in relation to remaining of the archipelago, also attributed to site fidelity due to the availability of rest and food areas (Andrews *et al.* 2010). Spinner dolphins of Indian Ocean showed significant differentiation among populations of three islands (Zanzibar, Mayotte and La Reunión) attributed to geographic isolation and availability of habitats to rest and socialize (Viricel *et al.* 2016).

The first study of spinner dolphins of Atlantic Ocean evaluated the Noronha spinner dolphins, inhabiting the Fernando de Noronha Archipelago, Southwest Atlantic Ocean. Analysis of microsatellites and D-loop revealed no genetic structuring, low genetic diversity, two common exclusive haplotypes and high mtDNA differentiation with Pacific and Atlantic oceans populations. These analyses showed that these dolphins are more differentiated than other island populations suggesting that they compose a stable society with strong site fidelity (Faria *et al. in prep*).

Using a broader sampling our aim is to expand upon recent studies evaluating genetic structure among spinner dolphins of the Atlantic, Pacific and Indian oceans. We increased the locations of the Southwest Atlantic Ocean evaluated, besides specimens of the Fernando de Noronha Archipelago we include specimens surveyed along the Brazilian coast, and, include specimens from North of Atlantic, Pacific and Indian oceans. We analyzed the genetic diversity of the locations within the ocean basins and tested the null hypothesis

of a single Southwest Atlantic Ocean population, and, the hypothesis of three genetically differentiated ocean basins populations.

Materials and methods

Sample collection and DNA extraction

We used samples of Atlantic, Pacific and Indian oceans (Table 1, Figure 1, Supplementary Appendix 1, 2, 3).

Table 1 Number of specimens (N) of spinner dolphins sampled for each ocean basin and for each location according to the molecular marker analyzed, and, sampling method used**.**

Ocean Basin/ Location	Abreviations	Microsatellites (N)	D-loop (N)	Sampling
Atlantic Ocean		59	252	
1. Fernando de Noronha	FN	12	199	Skin swabbing
2. Brazilian Coast	BC	47	46	
a. Ceará	CE	3	4	Stranding
b. Rio Grande do Norte	RN	1	1	Stranding
c. Pernambuco	PE	2	2	Stranding
d. Espírito Santo	ES	3	3	Stranding
e. Rio de Janeiro	RJ	26	25	Biopsy
f. Paraná	PR	1	1	Biopsy
g. São Paulo	SP	5	5	Biopsy
h. Santa Catarina	SC	5	5	Biopsy
i. Rio Grande do sul	RS	-	1	Biopsy
North Atlantic Ocean		-	7	
3. Nort Atlantic	NA	-	5	Biopsy
4. Gulf of Mexico	GM	-	2	Biopsy
Pacific Ocean		356	811	
5. Hawaiian Islands	HW	75	501	Biopsy
6. Society Islands	SO	161	154	Biopsy
7. Mariana Islands	MARI	-	103	Biopsy
8. Samoa	SA	37	32	Biopsy
9. Palmyra	PA	-	10	Biopsy
10. Philippines	PH	-	7	Biopsy
11. Taiwan	TA	-	4	Biopsy
12. Marquesas Islands	MARQ	51	-	Biopsy
13. New Caledonia	NC	14	-	Biopsy
14. Tuvalu	TU	18	-	Biopsy
Indian Ocean		-	75	
15. Zanzibar	ZA	-	29	Stranding
16. Mayotte	MAYO	-	19	Biopsy
17. La Reunión	LR	-	16	Biopsy
18. Maldives	MALD	-	6	Biopsy
19. Indonesia	IN	-	5	Biopsy
Total		415	1138	

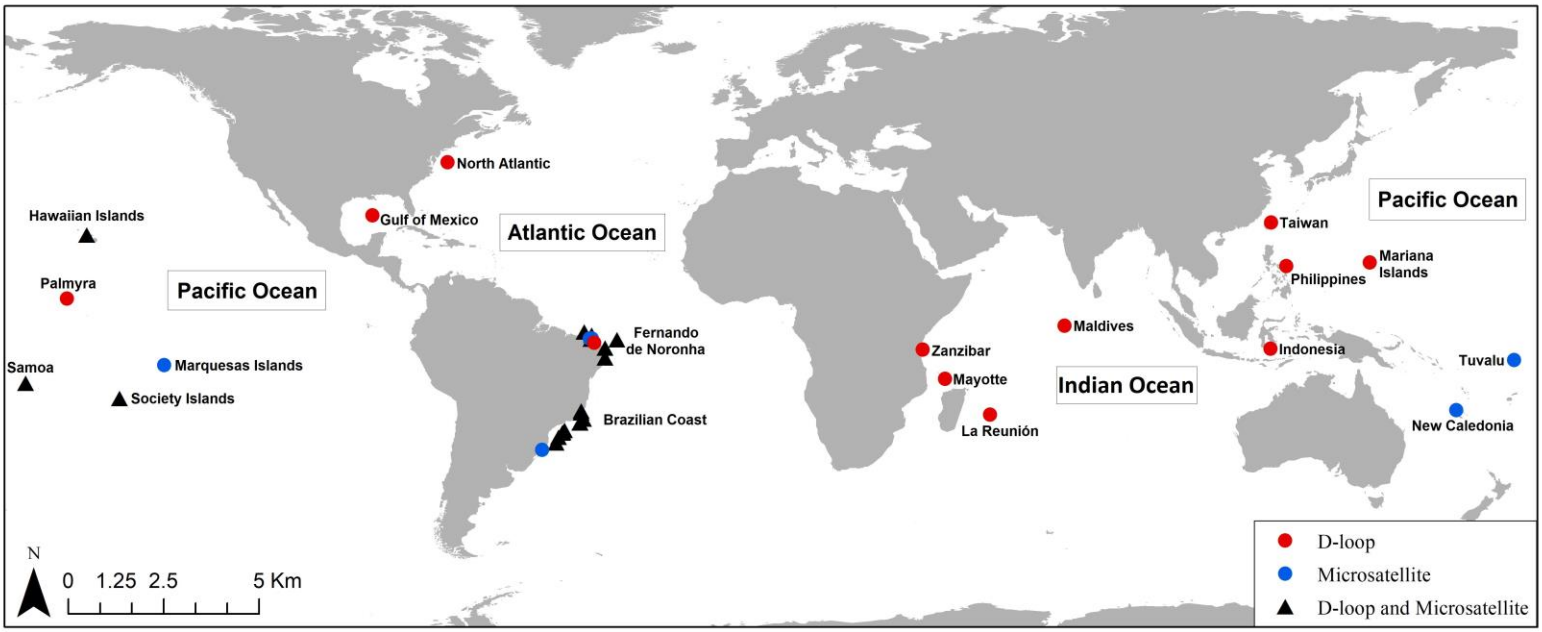

Figure 1 Oceanic locations sampled. Map showing sampling locations for spinner dolphins analyzed in this study.

From Southwest Atlantic Ocean we used samples of Island-associated dolphins of the Fernando de Noronha Archipelago and of Non-island-associated dolphins of the Brazilian Coast (Table 1, Figure 1, Supplementary Appendix 1). Samples of skin were obtained through the swabbing method (Harlin *et al.* 1999; Farro *et al.* 2008a) or biopsy method (Krutzen *et al.* 2002) of free - ranging animals, and, samples of muscle were obtained from stranded animals found in the beaches. Tissue samples were stored in 70% alcohol.

Two DNA extraction protocols were used in order to obtain sufficient DNA of Southwest Atlantic Ocean specimens: skin samples were extracted with 5% Chelex resin (SIGMA) according to manufacturer's instructions; muscle, liver and heart samples were extracted following the protocol in saline solution (Bruford *et al.* 1992, adapted by David Vieites).

We also reconstructed haplotypes from a total 159 sequences available in GenBank from five published papers (www.ncbi.nlm.nhi.gov/Genbank)

representing 893 specimens of Gray's spinner subspecies of 13 different regions of the world (Table 1, Figure 1, Supplementary Appendix 3).

mtDNA sequences and analyses

Polymerase chain reactions (PCRs) amplified a portion of the 5' mtDNA control region with the following primers: KRAdLp 1.5 t-pro (Andrews *et al.* 2006) and dLp5 (Pichler *et al*. 2001). Polymerase Chain Reactions (PCR) were performed in 12,5 µl total volumes containing 1X Reaction Buffer, 200 µM of each dNTP, 2.0 mM $MgCl_2$, 0.5 units of *Taq* DNA polymerase (INVITROGEN) and 0.2 µM of each primer. Cycle conditions were as follows: 95ºC for 1 min; followed by 40 cycles of 94ºC for 30 s, 54 ºC for 30 s, and 72 ºC for 30 s; followed by a final 72ºC extension for 15 min. Amplification products were sequenced in both directions, with an ABI 310 automated sequencer (Applied Biosystems), and the sequences were aligned and edited manually using the algorithm Muscle of MEGA 6.06 (Tamura *et al*. 2013).

The number of haplotypes, nucleotide (π) and haplotypic (h) diversities were calculated using Arlequin 3.5.2.2 (Excoffier & Lischer 2010). In order to investigate population structure among locations and oceans basins, genealogical relationships between D-loop haplotypes were examined using the Median-Joining analysis through Network 4.5 (Bandelt *et al.* 1999; http: www.fluxus-enginering.com), the MP option was used to reduce the complexity (Bandelt *et al.* 1999).

Genetic differentiation was assessed through pairwise F-statistics and AMOVA using Arlequin 3.5.2.2 (Excoffier & Lischer 2010). Two different F-statistics were used: a measure that incorporates mtDNA sequence divergence

(Φ_{ST}) using haplotype differences, and, a measure based on haplotype frequencies (F_{ST}). Pairwise F_{ST} and Φ_{ST} were estimated with 10 000 random generations of significance.

Microsatellite loci and analysis

Twelve microsatellite loci were amplified (Table 2) by PCR carried out as follow: 10 µl total volumes containing 1X Reaction Buffer, 0.2 mM of each dNTP, 1.5 mM $MgCl_2$, 0.5 units of Platinum Taq and 0.4 µM of each primer. Cycle conditions were as follows: 94ºC for 3 min; followed by 35 cycles of 94ºC for 30 s, annealing temperature for 30 s, and 72 ºC for 30 s; followed by a final 72ºC extension for 15 minutes. Capillary electrophoresis of PCR products was performed on ABI 3730 sequencer (Applied Biosystems). Fragment sizes were identified using GeneMapper software 5.0 (Applied Biosystems).

Table 2 Microsatellite loci used. Locus name (Locus), annealing temperature (T°C), motif repeat and Reference.

Locus	Annealing temp (°C)	Motif repeat	Reference
415-416	45°C	GT or GA dinucleotide	Amos *et al.* 1993
AAT44	50°C	AAT12	Caldwell *et al.* 2002
EV1	50°C	(AC)13(TC)12	Valescchi & Amos 1996
EV94	50°C	(TC)6[...](AC)20	Valescchi & Amos 1996
GATA98	50°C	GATAn	Palsbøll *et al.* 1997
GT575	55°C	GTn	Bérubé *et al.* 2000
GT6	50°C	CA18	Caldwell *et al.* 2002
KWM12	50°C	?	Hoelzel *et al.* 1998b
MK5	50°C	(TG)13CT(TG)2CA(TG)2(TA)2(TG)4	Krützen *et al.* 2001
MK6	50°C	GT17	Krützen *et al.* 2001
PPHO131	55°C	CA13	Rosel *et al.* 1999
PPHO142	55°C	CA22	Rosel *et al.* 1999

The Hardy-Weinberg Equilibrium and linkage disequilibrium were tested using GENEPOP on the Web (Raymond & Rousset 1995; Rousset 2008). Null

alleles, large allele dropout and scoring errors due to stutter peaks were evaluated using Microchecker 2.2.0.3 (Van Oosterhout *et al.* 2004), with Bonferroni correction. Potential duplicate samples were searched using Cervus 3.0.3 (Kalinowski *et al.* 2007) by comparing their genotypes.

Total number of alleles (K), mean number of alleles (Nam), number of private alleles (Nap), observed (Ho) and expected (He) heterozygosity, genetic diversity (Dg) and inbreeding coefficient (FIS) were calculated per microsatellite locus and per location using Fstat v.2.9.3.2 (Goudet 2001) and Arlequin 3.5.2.2 (Excoffier & Lischer 2010). The software Arlequin 3.5.2.2 was also used to calculate pairwise F-statistics and AMOVA. Pairwise F_{ST} and Φ_{ST} were estimated with 10 000 random generations of significance.

A Bayesian approach to identify the number of populations (K) present in the dataset was used to investigate population structure among locations as implemented in the software Structure 2.3.2 (Pritchard *et al.* 2000). The admixture model without prior population information and a correlated allele frequencies model were used. The burn-in length was set at 10^4 steps, followed by 500.000 repetitions of Markov Chain Simulation and Monte Carlo (MCMC). Ten independent executions were performed for each value of K varying between one and ten to test the consistency of estimates of P (XK). The number of clusters that best fit in the data was assessed and visualized using Structure Harvester (Earl & VonHoldt 2012) (available at http://taylor0.biology.ucla.edu/structureHarvester/), a web-based program for collating results generated by the program Structure 2.3.2 (Pritchard *et al.* 2000). The maximum loglikelihood values from all runs corresponding to each given K were checked for consistency and averaged (Amaral *et al.* 2012;

Pritchard *et al.* 2000). The K with the highest averaged maximum log-likelihood was considered the most likely number of clusters that better explains our dataset (Amaral *et al.* 2012; Pritchard *et al.* 2000).

Results

mtDNA - Genetic diversity and population structure

D-loop analyses encompassed a total of 1138 samples representing 15 locations. The total length of sequences varied from 374 to 555 bp, allowing a consensus length of 374 bp for all analyses, revealing a total of 129 haplotypes and 83 variable sites. For the Southwest Atlantic Ocean samples, we found a total of 33 haplotypes, 12 specific of the Fernando de Noronha Archipelago and 21 specific of the Brazilian Coast, three haplotypes were shared between them.

Both haplotype and nucleotide diversities were lower at Fernando de Noronha Archipelago than any other location, with exception of Hawaiian Islands that, showed slightly smaller nucleotide diversity and North Atlantic (possible due to the low number of samples analyzed). For the other locations, including Brazilian Coast, haplotype diversities were significantly higher ($P<0.05$), however, the same pattern was not observed for nucleotide diversities. Haplotype and nucleotide diversities were higher for Indian Ocean despite the low number of samples compared to other ocean basins. Taking into consideration the differences in sample sizes between Indian and Atlantic oceans proportionally a lot more haplotypes were observed for the first one (29 haplotypes) than for the second one (32 haplotypes) (Table 3).

Table 3 Mitochondrial (mtDNA) control region diversity statistics for spinner dolphins sampled at each location: *n*: number of individuals; *k*: number of haplotypes; *h:* haplotype diversity*; π:* nucleotide diversity**.**

	mtDNA (374bp)				
Ocean basin and location	*n*	*K*	*h*	*π*	Source
Atlantic Ocean	252	32	0.5806	0.011008	-
1.Fernando de Noronha	199	12	0.3398	0.005684	This Study
2.Brazilian Coast	46	21	0.9498	0.018885	This Study
3.North Atlantic	7	4	0.7143	0.015873	GB (Andrews et al. 2013)
Pacific Ocean	811	82	0.8158	0.013443	-
4.Hawaiian Islands	501	19	0.5078	0.005202	GB (Andrews et al. 2010)
5.Society Islands	154	29	0.9227	0.018334	GB (Oremus et al. 2007)
6.Mariana Islands	103	25	0.9280	0.017583	GB (Andrews et al. 2013; Martien et al. 2014)
7.Samoa	32	14	0.9516	0.022361	GB (Andrews et al. 2010; Andrews et al. 2013)
8.Palmyra	10	7	0.9111	0.010454	GB (Andrews et al. 2013)
9.Philippines	7	6	0.9524	0.021448	GB (Andrews et al. 2013)
10.Taiwan	4	3	0.8333	0.012097	GB (Andrews et al. 2013)
Indian Ocean	75	29	0.9758	0.018850	-
11.Zanzibar	29	12	0.8645	0.018314	GB (Viricel et al. 2016)
12.Mayotte	19	13	0.9532	0.018361	GB (Viricel et al. 2016)
13.La Reunión	16	9	0.9000	0.016846	GB (Viricel et al. 2016)
14.Maldives	6	4	0.8667	0.020251	GB (Andrews et al. 2013)
15.Indonesia	5	4	0.9000	0.0117	GB (Andrews et al. 2013)

The mtDNA haplotype network showed notable different patterns between Southwest Atlantic Ocean locations, Fernando de Noronha (Island-associated dolphins) and Brazilian Coast (Non-island-associated dolphins). Taking into considerations the differences in sample sizes between these two locations (Fernando de Noronha N=199, Brazilian Coast N=46), proportionally far more haplotypes were observed in Brazilian Coast (21 haplotypes) than in Fernando de Noronha Archipelago (12 haplotypes). Only one haplotype was shared exclusively between these two locations (H12). The dataset from Fernando de Noronha Archipelago was dominated by one haplotypes (H1), this haplotype was shared among Brazilian Coast (one specimen) and La Reunión (one specimen). One haplotype of Fernando de Noronha Archipelago (H3) was at least fifteen mutational steps distant from all other haplotypes of this location. Fernando de Noronha Archipelago and Brazilian Coast shared another

haplotype between themselves and with Zanzibar, Maldives and Mayotte (H9). Most haplotypes from Brazilian Coast were scattered throughout the network, two haplotypes were shared with North Atlantic (H15 and H17) and one haplotype was shared with Hawaiian Islands (H28). However, the network showed no evidence for phylogeographic structuring of mtDNA lineages among ocean basins (Atlantic, Pacific and Indian) (Figure 2).

F-statistics showed significant levels of differentiation among all locations and among all ocean basins (Table 4). The highest level of differentiation was found between Hawaiian Islands and La Reunión, and, the lowest between Society Islands and Mariana Islands. Significant differentiation between Southwest Atlantic Ocean locations, Fernando de Noronha Archipelago and Brazilian Coast was observed. Significant differentiation between Fernando de Noronha Archipelago and other locations of Pacific and Indian oceans was observed. Brazilian Coast showed no significant differentiation with Maldives and North Atlantic. F-statistics revealed significant differentiation among oceans basins: Atlantic Ocean X Pacific Ocean (F_{ST} = 0.28347; Φ_{ST} = 0.16671; P < 0.001); Atlantic Ocean X Indian Ocean (F_{ST} = 0.11243; Φ_{ST} = 0.22484; P < 0.001); Pacific Ocean X Indian Ocean (F_{ST} = 0.24584; Φ_{ST} = 0.27018; P < 0.001). AMOVA analysis revealed significant values among locations (F_{ST} = 0.34040, P < 0.001). Hierarchical AMOVA among ocean basins showed that the highest levels of differentiation were within the locations (F_{CT} = 0.13850, P < 0.10644; F_{SC} = 0.28285, P < 0.0000; F_{ST} = 0.38218, P < 0.0000).

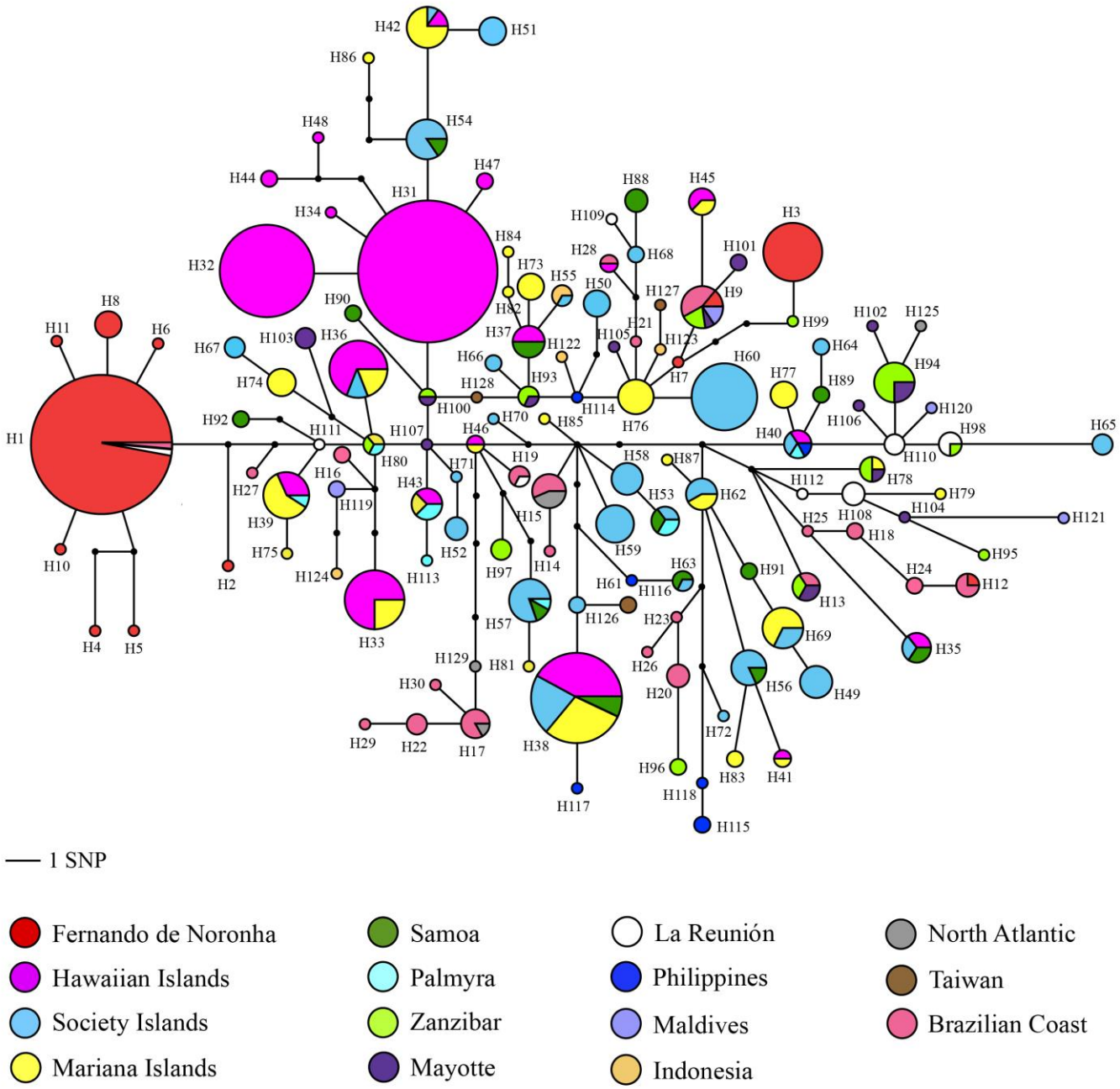

Figure 2 Median-Joining network of mtDNA control region haplotypes. Each circle corresponds to a haplotype, and its size is proportional to its frequency in the population. Black circles Indicate potential intermediate haplotypes not sampled. Different colors represent the geographic sampling regions of specimens with those haplotypes. Lengths of lines connecting haplotypes are proportional to the number Single Nucleotide Polymorphism (SNP) differences between haplotypes.

Table 4 Pairwise F_{ST} (lower diagonal) and Φ_{ST} (upper diagonal) for spinner dolphins for mtDNA control region among locations of the Atlantic, Pacific and Indian oceans.. Statistically significant values are highlighted in gray and have asterisks: *P < 0.05, **P < 0.01, ***P < 0.001. FN (Fernando de Noronha); BC (Brazilian Coast); NA (North Atlantic); HW (Hawaiian Islands); SO (Society Islands); MARQ (Marquesas Islands); SA (Samoa); PA (Palmyra); PH (Philippines); TA (Taiwan); ZA (Zanzibar); MAYO (Mayotte); LR (Lareunión); MALD (Maldives); IN (Indonesia).

	1.FN	2.BC	3.NA	4.HW	5.SO	6.MA	7.SA	8.PA	9.PH	10.TA	11.ZA	12.MAY	13.LR	14.MAL	15.IN
1.FN	-	0.49497***	0.59349***	0.49935***	0.34276***	0.28117***	0.41123***	0.37367***	0.55115***	0.50813**	0.56612***	0.50850***	0.59474***	0.54029***	0.59018***
2.BC	0.42978***	-	0.00672	0.58765***	0.11159***	0.15099***	0.11563***	0.19364***	0.10556*	0.18538**	0.09153***	0.08823***	0.11954**	0.10997*	0.20912**
3.NA	0.58715***	0.07596*	-	0.65043***	0.10199*	0.14201**	0.10187*	0.23940**	0.12873	0.22814*	0.15190*	0.14038*	0.18692*	0.14593*	0.25044**
4.HW	0.55587***	0.33607***	0.44158***	-	0.43667***	0.41723***	0.49535***	0.49680***	0.66575***	0.57575***	0.65365***	0.59274***	0.68272***	0.65236***	0.56448***
5.SO	0.38336***	0.06446***	0.15455***	0.31674***	-	0.03555***	0.00975	0.04758*	0.10057*	0.06636	0.15201***	0.09727***	0.16807***	0.06532	0.09808*
6.MARQ	0.40503***	0.06148***	0.15220***	0.31688***	0.05648***	-	0.00855	0.01125	0.12766*	0.05307	0.17286***	0.10718***	0.18671***	0.08688*	0.12612*
7.SA	0.46495***	0.05329***	0.14840***	0.33856***	0.04377***	0.04366***	-	0.01306	0.06263	0.01989	0.13847***	0.08259**	0.15454***	0.06046	0.07153
8.PA	0.52427***	0.06726**	0.18007**	0.37360***	0.07138**	0.06380**	0.05336*	-	0.20105*	0.13024	0.23073***	0.17326**	0.28221**	0.17736*	0.18247**
9.PH	0.52556***	0.04913*	0.16667**	0.36950***	0.06352*	0.06199*	0.05267*	0.05589	-	0.15152	0.06306	0.03078	0.06105	0.06448	0.26795*
10.TA	0.57734***	0.09147*	0.23629*	0.42047**	0.10761**	0.10436*	0.09598*	0.11988	0.09880	-	0.25018**	0.19568*	0.31807**	0.20749*	0.11948*
11.ZA	0.49986***	0.07636***	0.19439***	0.37764***	0.10358***	0.10028***	0.09565***	0.11185***	0.09877*	0.14626**	-	-0.00525	0.00387	0.05301	0.25913**
12.MAYO	0.48467***	0.03758**	0.14785***	0.34918***	0.06348***	0.05993***	0.05178***	0.06640**	0.04715*	0.09155*	0.01718	-	0.03214	0.00130	0.20320**
13.LR	0.47711***	0.06950***	0.17931***	0.37220***	0.08727***	0.08442***	0.07700***	0.09477***	0.07671*	0.12479*	0.11306***	0.07296***	-	0.08967	0.34956***
14.MALD	0.55289***	0.04287	0.21255*	0.40101***	0.09867**	0.09559**	0.08758**	0.10900*	0.08912*	0.14805	0.10370*	0.06725*	0.11410*	-	0.14902
15.IN	0.55352***	0.06946*	0.20126*	0.39600***	0.08336*	0.08244*	0.07349**	0.09370	0.07190	0.13105	0.12201*	0.06841*	0.10000*	0.11743	-

Microsatellites - Genetic diversity and population structure

In total 415 spinner dolphin's specimens of eight different locations were genotyped at 12 microsatellite loci. Identification of matching genotypes revealed that four individuals from the Brazilian Coast had been re-sampled once, of these only one copy of the genotype was retained for subsequent analyses. No microsatellite loci demonstrated Linkage Disequilibrium, however, some deviated from Hardy-Weinberg Equilibrium for some locations (Table 5).

Levels of genetic diversity were high for most locations, given by mean number of alleles, allelic richness, observed and expected heterozygosity. Pacific Ocean showed higher number of alleles and allelic richness, but, lower observed and expected heterozygosity and genetic diversity than Atlantic Ocean. Significant F_{IS} values were obtained for New Caledonia and Samoa,

which can be due to the low number of samples or to population sub-structure (i.e. Wahlund effect), and for Pacific Ocean (Table 6).

Table 5 Test of the deviation of allele frequencies from expected in HWE. N: number of individuals. FN (Fernando de Noronha); BC (Brazilian Coast); SO (Society Islands); HW (Hawaiian Islands); MARQ (Marquesas Islands); NC (New Caledonia); SA (Samoa); TU (Tuvalu).

Loci	HWE p-value							
	FN (N=12)	BC (N=47)	SO (N=161)	HW (N=75)	MARQ (N=51)	NC (N=14)	SA (N=37)	TU (N=18)
415416	0.502	0.688	0.360	-	0.964	0.077	0.715	0.089
AAT44	0.293	0.486	0.372	0.309	0.977	1	0.453	0.001*
EV1	0.003*	0.269	0.005*	0.050	0.714	0.906	0.199	0.448
EV94	0.065	0.149	0.028*	0.229	0.238	0.326	0.463	0.961
GATA98	0.663	0.634	0.843	0.093	0.598	0.028*	0.179	0.264
GT575	0.108	0.016*	0.057	0.141	0.274	0.208	0.004*	0.712
GT6	0.469	0.082	0.644	0.179	0.416	0.495	0.109	0.556
KWM12a	0.541	0.377	0.077	0.106	0.339	0.328	0.681	-
MK5	0.173	0.398	0.456	0.359	0.273	0.334	0.488	0.115
MK6	0.474	0.793	0.131	0.069	0.343	0.929	0.469	0.017*
PPHO131	0.913	0.272	0.027*	0.148	0.181	0.134	0.368	0.827
PPHO142	0.217	0.008*	0.000*	0.983	0.008*	0.240	0.010*	0.187

Table 6 Microsatellite diversity statistics for spinner dolphins sampled at each location of the Pacific and Atlantic oceans. *N*: number of individuals; *Na*: mean number of alleles; *Ra*: allelic richness H_o: observed heterozygosity; H_e: expected heterozygosity; *Dg*: genetic diversity; F_{IS}: inbreeding coefficient. (*) Statistically significant values P < 0.05.

Loci	*k*	H_o	H_e	PIC	*Ra*	*Dg*	F(Null)	P(HWE)
415416	14	0.792	0.853	0.836	14.000	0.853	+0.0366	0.853
AAT44	13	0.751	0.793	0.761	12.427	0.793	+0.028	0.793
EV1	28	0.797	0.905	0.896	26.998	0.905	+0.064	0.905
EV94	21	0.846	0.897	0.888	20.900	0.897	+0.029	0.897
GATA98	12	0.764	0.813	0.786	11.940	0.813	+0.031	0.813
GT575	11	0.728	0.822	0.800	10.544	0.822	+0.060	0.822
GT6	12	0.736	0.788	0.758	11.568	0.788	+0.033	0.788
KWM12a	14	0.824	0.834	0.813	13.637	0.834	+0.007	0.835
MK5	16	0.804	0.847	0.830	15.447	0.848	+0.028	0.847
MK6	22	0.825	0.905	0.896	21.464	0.905	+0.047	0.904
PPHO131	21	0.838	0.896	0.887	20.740	0.897	+0.032	0.896
PPHO142	17	0.777	0.788	0.759	16.286	0.788	+0.001	0.788

F-statistics showed significant levels of differentiation among all locations and ocean basins (Table 7). The highest level of differentiation was found between Hawaiian Islands and Fernando de Noronha and the lowest between Marquesas Islands and Tuvalu. Brazilian Coast (Non-island-associated

dolphins) and Fernando de Noronha Archipelago (Island-associated dolphins) showed moderate levels of differentiation between themselves and with all other locations of the Pacific Ocean (Table 7). F-statistics revealed significant level of differentiation between Atlantic and Pacific oceans (Φ_{ST} = 0.02282; P < 0.05, P < 0.01, P < 0.001).

Table 7 *F*-statistics for spinner dolphins among locations of the Pacific and Atlantic oceans for 12 microsatellite loci. Statistically significant values have asterisks: *P < 0.05, **P < 0.01, ***P < 0.001.

	1	2	3	4	5	6	7	8
1. Fernando de Noronha	-							
2. Brazilian Coast	0.050***	-						
3. Society Islands	0.046***	0.042***	-					
4. Hawaiian Islands	0.109***	0.075***	0.059***	-				
5. Marquesas Islands	0.034***	0.019***	0.023***	0.073***	-			
6. New Caledonia	0.049**	0.030***	0.035***	0.066***	0.023**	-		
7. Samoa	0.057***	0.022***	0.026***	0.048***	0.019***	0.045***	-	
8. Tuvalu	0.047**	0.024***	0.015**	0.072***	0.011*	0.031**	0.016*	-

Hierarquical AMOVA between Atlantic and Pacific oceans revealed that the highest levels of differentiation were obtained within the locations (F_{CT} = 0.00285, P < 0.32218; F_{SC} = 0.04227, P < 0.0000; F_{ST} = 0.04500, P < 0.0000).

We did three different Bayesian clustering analysis: 1) incorporating all the locations of Atlantic and Pacific oceans revealing the highest average posterior probability at K= 3, although variance was relatively high for K=4 and k=5 (Figure 3); 2) using only the samples of Southwest Atlantic Ocean (FN and BC), revealing two population clusters (K=2), although one specimen of Fernando de Noronha Archipelago showed to be closer related to the Brazilian Coast cluster (Figure 4); 3) excluding Society Islands because of the high levels of structuring among these islands (Oremus *et al.* 2008), revealing the highest average posterior probability occurred at k=4 (Figure 5). Based on these results, we considered K = 5 as the most probable value among all locations.

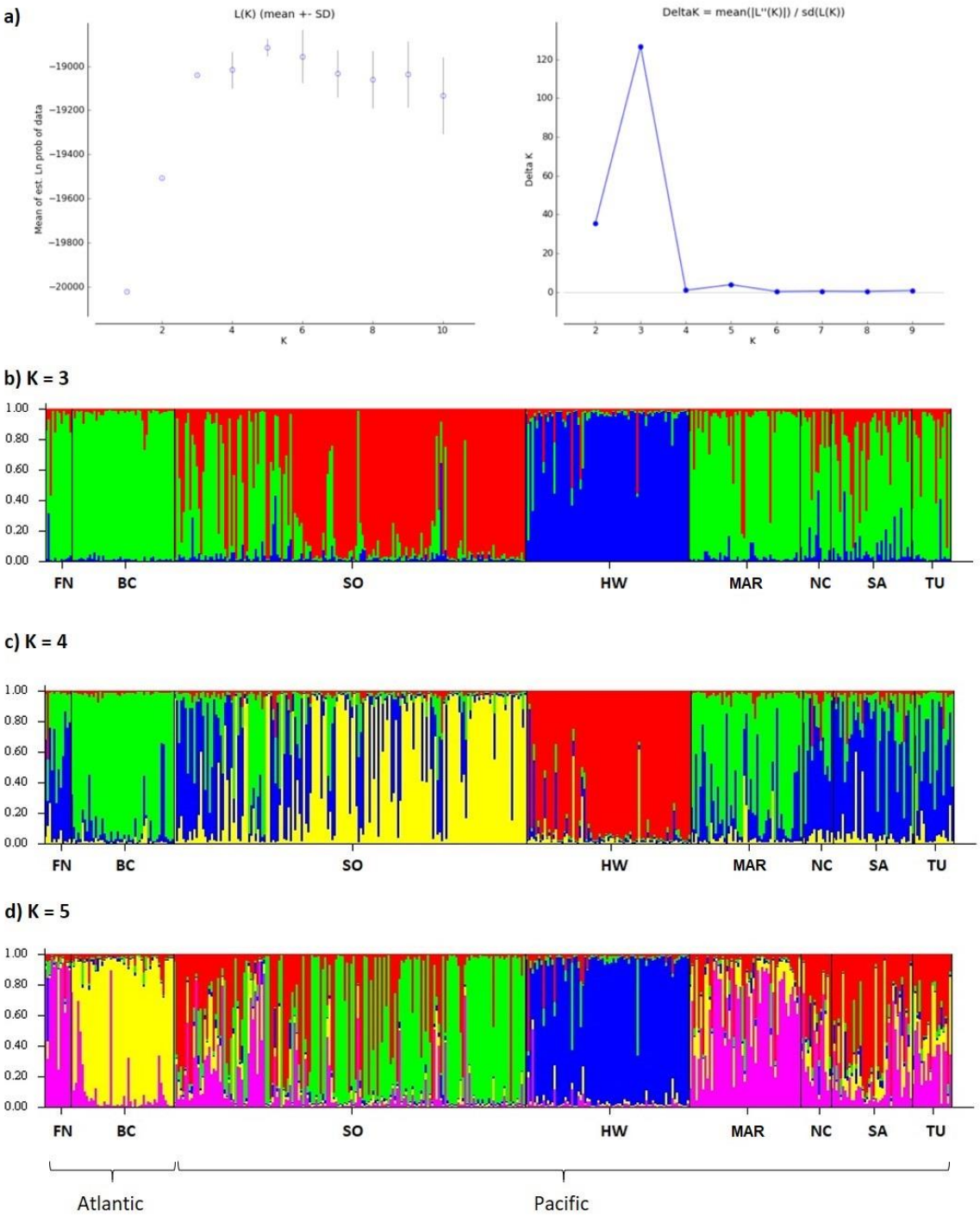

Figure 3 Assignment probabilities of individuals to putative population clusters: (a) mean log probability and Delta K; (b) K = 3; (c) K = 4; and (d) K = 5. Locations and oceans basins where individuals were sampled are indicated below the graph. Abbreviations: FN - Fernando de Noronha Archipelago (Island-associated dolphins); BC - Brazilian Coast (Non-island-associated dolphins); SO - Society Islands; HW - Hawaiian Islands; MAR - Marquesas Islands; NC - New Caledonia; SA - Samoa; TU - Tuvalu.

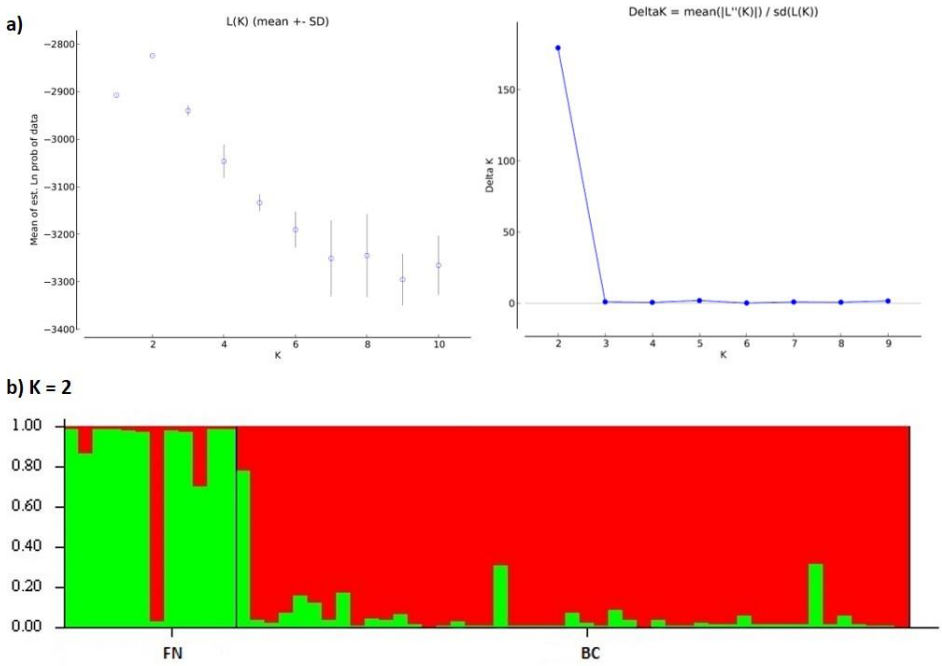

Figure 4 Assignment probabilities of individuals to putative population clusters for Southwest Atlantic Ocean locations: (a) mean log probability and Delta K; (b) K = 2. Abbreviations: FN - Fernando de Noronha Archipelago (Island-associated dolphins); BC - Brazilian Coast (Non-island-associated dolphins).

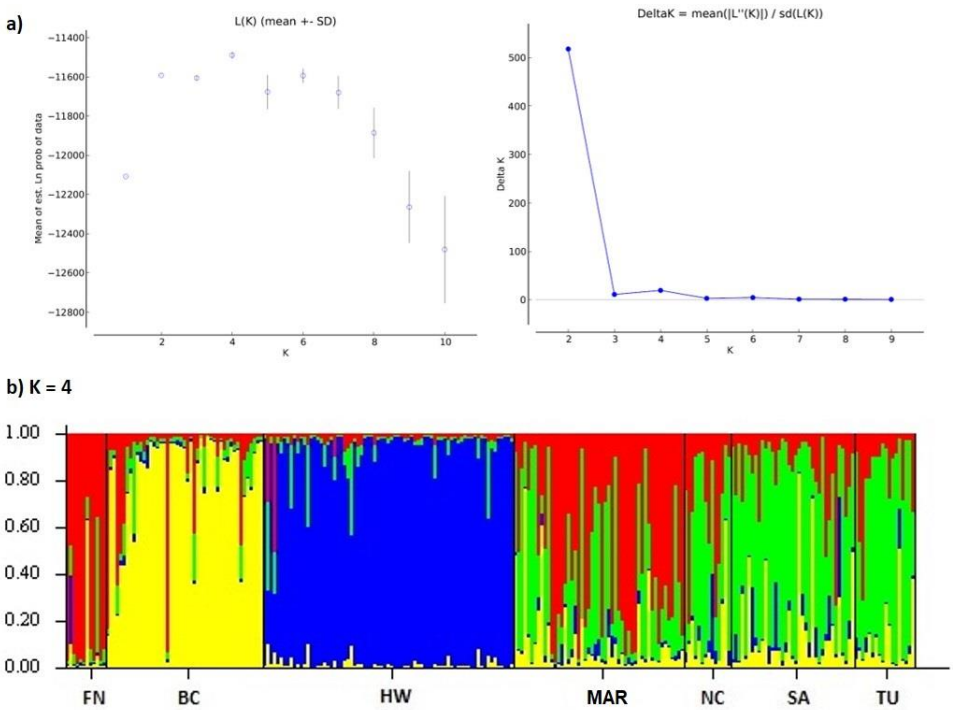

Figure 5 Assignment probabilities of individuals to putative population clusters for analysis without Society Islands: (a) mean log probability and Delta K; (b) K = 4. Abbreviations: FN - Fernando de Noronha Archipelago (Island-associated dolphins); BC - Brazilian Coast (Non-island-associated dolphins); SO - Society Islands; HW - Hawaiian Islands; MAR - Marquesas Islands; NC - New Caledonia; SA - Samoa; TU - Tuvalu.

Discussion

Genetic differentiation and diversity within Southwest Atlantic Ocean

We found significant levels of differentiation between the two locations of Southwest Atlantic Ocean analyzed, Brazilian Coast (Non-island-associated dolphins) and Fernando de Noronha Archipelago (Island-associated dolphins), at microsatellite loci and D-loop mtDNA demonstrating that there are two different populations of spinner dolphins in the coast of Brazil.

The high differentiation of Noronha spinner dolphins compared to other locations around the world was already demonstrated in previously study using only samples from living animals surveyed by the skin swabbing method and was attributed to high site fidelity driven by low levels of immigration caused by social or demographic forces (Faria *et al. in prep*). In the present study we increased the sample number to almost 200 animals of Fernando de Noronha Archipelago including samples from stranded animals and we confirm that Noronha spinner dolphins are high differentiated from the other locations around the world and even from Brazilian Coast and presents the lowest level of diversity overall. Brazilian Coast, even with less samples than Fernando de Noronha Archipelago, demonstrated high diversity level.

Genetic structuring of spinner dolphins of Southwest Atlantic Ocean can be explained by availability of resources and social structure (Andrews *et al.* 2010). Although resting areas and prey abundance have not been studied to Fernando de Noronha Archipelago and Brazilian Coast these variables can influence these two populations. Fernando de Noronha is considered a natural refuge of the spinner dolphins encompassing large groups of 600 individuals daily, being the only island of Southwest Atlantic Ocean with the amount of

resting areas and resource to house high number of animals and keep these animals in the archipelago vicinities (Silva & Silva-Jr 2009). Indeed, it is known that islands show high environmental suitability (70%-80%) for these dolphins (Amaral *et a*l. 2015). Brazilian Coast, otherwise, it is considered to show low levels of productivity with small islands highly distant one of another (Secchi & Siciliano 1995; Moreno *et al.* 2005; Amaral *et al.* 2015). Therefore, locations surveyed around Brazilian Coast, likely display fewer resources and rest areas as well small populations (Greenwood 1980; Johnson & Gaines 1990; Pusey & Wolf 1996).

Social structure may be another factor influencing structuring in Southwest Atlantic Ocean as reported by Norris and Schilt (1988) to spinner dolphins in Hawaii, that show segregation in *schools*. It is noteworthy that all genetic population studies of spinner dolphins known until now attributed site fidelity to ecological and social aspects, considered as main reason for the isolation between populations, even in groups geographically near (Oremus *et al.* 2007; Andrews *et al.* 2010).

The Brazilian superficial marine currents can display some influence in the distributions of spinner dolphins through the coast of Brazil. The currents are directed from Fernando de Noronha Archipelago to the Brazilian Coast favoring the displacement of the spinner dolphins from the archipelago to the continent, but, making difficult the countercurrent movement of animals towards the archipelago. The influence of marine currents was used by Kingston et al. (2009) to assign the endemism *S. clymene* and *S. frontalis* in the Atlantic Ocean because of the movement of the currents around the Cape of Good Hope,

which may have been unfavorable output of these species in the Atlantic Ocean.

We also demonstrated that Southwest Atlantic Ocean locations are differentiated from other locations, especially from the Pacific Ocean. Leslie & Morin (2018) demonstrated deepest divergence between the Atlantic Ocean specimens and all other lineages of Pacific and Indian Ocean, but, they did not include specimens of the Southwest Ocean. We reinforce the distinctiveness of the Southwest Atlantic Ocean.

Genetic differentiation among Atlantic, Pacific and Indian ocean basins

We detected significant levels of differentiation among locations of the same ocean basin. Within the Atlantic Ocean, we detected some shared haplotypes among Brazilian Coast, Fernando de Noronha Archipelago and North Atlantic, but, we also detected high level of differentiation among them, and so, these locations could not compose a solely Atlantic Ocean population or lineage. Leslie & Morin (2018) indicated a single Atlantic Ocean lineage based solely in specimens of North Atlantic Ocean, here, we can see the distinctiveness of southwest specimens.

Within the Pacific Ocean, here composed by ten locations, we found moderate to high levels of differentiation among each other. Low levels of differentiation were detected using microsatellites only between Tuvalu X Marquesas Islands. Hawaiian Islands composed a highly differentiated location compared to all locations of the Pacific Ocean and overall, and, the geographic isolation might be the better explanation (Andrews *et al.* 2010, 2013).

Within Indian Ocean, here composed of five locations and analyzed only with d-loop, we detected significant differentiation among all locations as demonstrated by Viricel *et al.* (2016). Here we included the Indonesia location belonging to the Indian Ocean and we found significant levels of differentiation among the other four locations of this ocean, however, some lack of differentiation was detected between Indonesia and other three locations of the Pacific Ocean (Philippines, Taiwan, and Palmyra) and one shared haplotype was detected with Society islands. Therefore, Indonesia spinner dolphins are more closely related to the Pacific Ocean populations than to Indian Ocean populations probably due to the high numbers of islands of the Pacific Ocean near to Indonesia and the little geographic distance among them enabling the displacement. It is also possible that reduced availability resources in smaller islands forces dolphins to move to other islands, and therefore gene flow of dolphins among these areas can be more common (Andrews *et al.* 2010). These results corroborate that the spinner dolphins display high ability to disperse among locations geographically distant contributing to variations in the levels of genetic divergence around the world (Martin *et al.* 1990; Würsig *et al.* 1994 a; Würsig *et al*. 1994 b; Wells & Gannon 2005; Leslie & Morin 2018).

We found high and significant degrees of genetic divergence across large geographic scales, among different oceans basins: among Atlantic, Pacific and Indian using D-loop mtDNA; and, between Atlantic and Pacific oceans using microsatellite loci. Atlantic and Pacific oceans displayed the highest level of differentiation, similar results were also found by Andrews *et al.* (2013) using only samples of North Atlantic, without specimens of Southwest Atlantic.

Atlantic and Indian oceans displayed the lowest level of differentiation among ocean basins. We also found lack of genetic differentiation among Brazilian Coast (Southwest Atlantic Ocean) and Maldives (Indic Ocean); shared haplotypes among Southwest Atlantic Ocean locations and La Reunión, and, Zanzibar, Mayotte and Maldives. These findings agree with the hypothesis of Davies (1963) that expected that Atlantic specimens were more closely related to the western Indian Ocean specimens. Differently, Leslie & Morin (2018) found the Atlantic Ocean specimens phylogenetically positioned outside the clade including Tanzania specimens, here referred as Zanzibar specimens. Therefore, such evidences may indicate that Atlantic was secondarily colonized by specimens from different populations of other oceans, probably of the Indian Ocean, possibly through the southern Africa gate (Perrin 2007).

We also found, in addition to the two clusters cited above, three other clusters encompassing the Pacific Ocean locations: one cluster for Hawaii, another for Society islands, and, another for Samoa + Tuvalu + New Caledonia. Society Islands displayed some mixing specimens with the third cluster. We also found several shared haplotypes among the locations of this ocean basin. Leslie & Morin (2018) found an Indo-Western Pacific Ocean group and suggest that the Indo- Pacific boundary is not a barrier to genetic mixing.

Final considerations

The present study is one of the most conclusive analyses of genetic structure and diversity of spinner dolphins in terms of sample sizes, 1138 specimens analyzed with d-loop and 415 specimens analyzed with microsatellites, and geographic coverage of 19 locations in total encompassing

all three spinner dolphin distribution (Atlantic, Pacific and Indian ocean basins). Besides, this is the first global study that include specimens of the Southwest Atlantic Ocean.

Our results demonstrated that spinner dolphins are structured in two populations in the Southwest Atlantic Ocean, an Island-associated dolphins population of Fernando de Noronha Archipelago, and, Non-island-associated dolphin population of the Brazilian Coast. Comparisons among locations demonstrated that spinner dolphins are differentiated and show high site fidelity. The great distances among locations of the Pacific Ocean it is not a barrier to de displacement of spinner dolphins that can travel for long distances searching for resources and rest areas. The similar pattern was observed for the connection between Atlantic and Indian oceans trough the southern Africa. Fernando de Noronha Archipelago and Hawaiian Archipelago deserves special attention in the light of conservation strategies once they house populations highly differentiated from all others around the world and presented low levels of genetic diversity. Future studies could be developed to clarify the connection between Atlantic and Indian Oceans as did Leslie & Morin (2018) including samples of South Atlantic Ocean.

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Supporting Information

Appendix 1 Specimens of the coast of Brazil used in this study. Number of samples, sample identity, location, sampling method and molecular marker analyzed for each specimen.

Sample	Sample ID.	Localization	Sampling	D-loop	Microsatellite
1	Slo_01	Fernando de Noronha/PE	Stranding	-	Ok
2	Slo_02	Fernando de Noronha/PE	Stranding	Ok	Ok
3	Slo_04	Fernando de Noronha/PE	Stranding	Ok	Ok
4	Slo_05	Fernando de Noronha/PE	Stranding	Ok	-
5	Slo_06	Fernando de Noronha/PE	Stranding	Ok	Ok
6	Slo_08	Fernando de Noronha/PE	Stranding	Ok	Ok
7	Slo_09	Fernando de Noronha/PE	Stranding	Ok	Ok
8	Slo_10	Fernando de Noronha/PE	Stranding	-	Ok
9	Slo_11	Fernando de Noronha/PE	Stranding	Ok	-
10	Slo_12	Fernando de Noronha/PE	Stranding	Ok	-
11	Slo_13	Fernando de Noronha/PE	Stranding	Ok	Ok
12	Slo_14	Fernando de Noronha/PE	Stranding	Ok	-
13	Slo_15	Fernando de Noronha/PE	Stranding	Ok	Ok

Sample	Sample ID.	Localization	Sampling	D-loop	Microsatellite
14	Slo_16	Fernando de Noronha/PE	Stranding	Ok	-
15	Slo_17	Fernando de Noronha/PE	Stranding	Ok	Ok
16	Slo_18	Fernando de Noronha/PE	Stranding	Ok	-
17	Slo_19	Fernando de Noronha/PE	Stranding	Ok	Ok
18	Slo_20	Fernando de Noronha/PE	Stranding	Ok	-
19	Slo_21	Fernando de Noronha/PE	Stranding	Ok	Ok
20	Slo_22	Fernando de Noronha/PE	Stranding	Ok	-
21	Slo_62	Fernando de Noronha/PE	Skin swabbing	Ok	-
22	Slo_63	Fernando de Noronha/PE	Skin swabbing	Ok	-
23	Slo_64	Fernando de Noronha/PE	Skin swabbing	Ok	-
24	Slo_65	Fernando de Noronha/PE	Skin swabbing	Ok	-
25	Slo_66	Fernando de Noronha/PE	Skin swabbing	Ok	-
26	Slo_67	Fernando de Noronha/PE	Skin swabbing	Ok	-
27	Slo_69	Fernando de Noronha/PE	Skin swabbing	Ok	-
28	Slo_70	Fernando de Noronha/PE	Skin swabbing	Ok	-
29	Slo_71	Fernando de Noronha/PE	Skin swabbing	Ok	-
30	Slo_72	Fernando de Noronha/PE	Skin swabbing	Ok	-
31	Slo_74	Fernando de Noronha/PE	Skin swabbing	Ok	-
32	Slo_76	Fernando de Noronha/PE	Skin swabbing	Ok	-
33	Slo_81	Fernando de Noronha/PE	Skin swabbing	Ok	-
34	Slo_82	Fernando de Noronha/PE	Skin swabbing	Ok	-
35	Slo_84	Fernando de Noronha/PE	Skin swabbing	Ok	-
36	Slo_85	Fernando de Noronha/PE	Skin swabbing	Ok	-
37	Slo_87	Fernando de Noronha/PE	Skin swabbing	Ok	-
38	Slo_88	Fernando de Noronha/PE	Skin swabbing	Ok	-
39	Slo_92	Fernando de Noronha/PE	Skin swabbing	Ok	-
40	Slo_93	Fernando de Noronha/PE	Skin swabbing	Ok	-
41	Slo_95	Fernando de Noronha/PE	Skin swabbing	Ok	-
42	Slo_96	Fernando de Noronha/PE	Skin swabbing	Ok	-
43	Slo_97	Fernando de Noronha/PE	Skin swabbing	Ok	-
44	Slo_98	Fernando de Noronha/PE	Skin swabbing	Ok	-
45	Slo_102	Fernando de Noronha/PE	Skin swabbing	Ok	-
46	Slo_104	Fernando de Noronha/PE	Skin swabbing	Ok	-
47	Slo_105	Fernando de Noronha/PE	Skin swabbing	Ok	-
48	Slo_108	Fernando de Noronha/PE	Skin swabbing	Ok	-
49	Slo_113	Fernando de Noronha/PE	Skin swabbing	Ok	-
50	Slo_116	Fernando de Noronha/PE	Skin swabbing	Ok	-
51	Slo_117	Fernando de Noronha/PE	Skin swabbing	Ok	-
52	Slo_119	Fernando de Noronha/PE	Skin swabbing	Ok	-
53	Slo_121	Fernando de Noronha/PE	Skin swabbing	Ok	-
54	Slo_122	Fernando de Noronha/PE	Skin swabbing	Ok	-
55	Slo_123	Fernando de Noronha/PE	Skin swabbing	Ok	-
56	Slo_124	Fernando de Noronha/PE	Skin swabbing	Ok	-
57	Slo_128	Fernando de Noronha/PE	Skin swabbing	Ok	-
58	Slo_133	Fernando de Noronha/PE	Skin swabbing	Ok	-

Sample	Sample ID.	Localization	Sampling	D-loop	Microsatellite
59	Slo_134	Fernando de Noronha/PE	Skin swabbing	Ok	-
60	Slo_136	Fernando de Noronha/PE	Skin swabbing	Ok	-
61	Slo_137	Fernando de Noronha/PE	Skin swabbing	Ok	-
62	Slo_139	Fernando de Noronha/PE	Skin swabbing	Ok	-
63	Slo_141	Fernando de Noronha/PE	Skin swabbing	Ok	-
64	Slo_144	Fernando de Noronha/PE	Skin swabbing	Ok	-
65	Slo_147	Fernando de Noronha/PE	Skin swabbing	Ok	-
66	Slo_148	Fernando de Noronha/PE	Skin swabbing	Ok	-
67	Slo_149	Fernando de Noronha/PE	Skin swabbing	Ok	-
68	Slo_150	Fernando de Noronha/PE	Skin swabbing	Ok	-
69	Slo_151	Fernando de Noronha/PE	Skin swabbing	Ok	-
70	Slo_152	Fernando de Noronha/PE	Skin swabbing	Ok	-
71	Slo_154	Fernando de Noronha/PE	Skin swabbing	Ok	-
72	Slo_157	Fernando de Noronha/PE	Skin swabbing	Ok	-
73	Slo_158	Fernando de Noronha/PE	Skin swabbing	Ok	-
74	Slo_159	Fernando de Noronha/PE	Skin swabbing	Ok	-
75	Slo_160	Fernando de Noronha/PE	Skin swabbing	Ok	-
76	Slo_161	Fernando de Noronha/PE	Skin swabbing	Ok	-
77	Slo_162	Fernando de Noronha/PE	Skin swabbing	Ok	-
78	Slo_164	Fernando de Noronha/PE	Skin swabbing	Ok	-
79	Slo_166	Fernando de Noronha/PE	Skin swabbing	Ok	-
80	Slo_168	Fernando de Noronha/PE	Skin swabbing	Ok	-
81	Slo_169	Fernando de Noronha/PE	Skin swabbing	Ok	-
82	Slo_171	Fernando de Noronha/PE	Skin swabbing	Ok	-
83	Slo_172	Fernando de Noronha/PE	Skin swabbing	Ok	-
84	Slo_174	Fernando de Noronha/PE	Skin swabbing	Ok	-
85	Slo_175	Fernando de Noronha/PE	Skin swabbing	Ok	-
86	Slo_177	Fernando de Noronha/PE	Skin swabbing	Ok	-
87	Slo_178	Fernando de Noronha/PE	Skin swabbing	Ok	-
88	Slo_179	Fernando de Noronha/PE	Skin swabbing	Ok	-
89	Slo_180	Fernando de Noronha/PE	Skin swabbing	Ok	-
90	Slo_182	Fernando de Noronha/PE	Skin swabbing	Ok	-
91	Slo_183	Fernando de Noronha/PE	Skin swabbing	Ok	-
92	Slo_184	Fernando de Noronha/PE	Skin swabbing	Ok	-
93	Slo_186	Fernando de Noronha/PE	Skin swabbing	Ok	-
94	Slo_188	Fernando de Noronha/PE	Skin swabbing	Ok	-
95	Slo_189	Fernando de Noronha/PE	Skin swabbing	Ok	-
96	Slo_190	Fernando de Noronha/PE	Skin swabbing	Ok	-
97	Slo_191	Fernando de Noronha/PE	Skin swabbing	Ok	-
98	Slo_192	Fernando de Noronha/PE	Skin swabbing	Ok	-
99	Slo_193	Fernando de Noronha/PE	Skin swabbing	Ok	-
100	Slo_194	Fernando de Noronha/PE	Skin swabbing	Ok	-
101	Slo_195	Fernando de Noronha/PE	Skin swabbing	Ok	-
102	Slo_197	Fernando de Noronha/PE	Skin swabbing	Ok	-
103	Slo_198	Fernando de Noronha/PE	Skin swabbing	Ok	-

Sample	Sample ID.	Localization	Sampling	D-loop	Microsatellite
104	Slo_200	Fernando de Noronha/PE	Skin swabbing	Ok	-
105	Slo_201	Fernando de Noronha/PE	Skin swabbing	Ok	-
106	Slo_202	Fernando de Noronha/PE	Skin swabbing	Ok	-
107	Slo_203	Fernando de Noronha/PE	Skin swabbing	Ok	-
108	Slo_204	Fernando de Noronha/PE	Skin swabbing	Ok	-
109	Slo_205	Fernando de Noronha/PE	Skin swabbing	Ok	-
110	Slo_206	Fernando de Noronha/PE	Skin swabbing	Ok	-
111	Slo_208	Fernando de Noronha/PE	Skin swabbing	Ok	-
112	Slo_209	Fernando de Noronha/PE	Skin swabbing	Ok	-
113	Slo_210	Fernando de Noronha/PE	Skin swabbing	Ok	-
114	Slo_214	Fernando de Noronha/PE	Skin swabbing	Ok	-
115	Slo_215	Fernando de Noronha/PE	Skin swabbing	Ok	-
116	Slo_217	Fernando de Noronha/PE	Skin swabbing	Ok	-
117	Slo_218	Fernando de Noronha/PE	Skin swabbing	Ok	-
118	Slo_219	Fernando de Noronha/PE	Skin swabbing	Ok	-
119	Slo_220	Fernando de Noronha/PE	Skin swabbing	Ok	-
120	Slo_221	Fernando de Noronha/PE	Skin swabbing	Ok	-
121	Slo_223	Fernando de Noronha/PE	Skin swabbing	Ok	-
122	Slo_224	Fernando de Noronha/PE	Skin swabbing	Ok	-
123	Slo_225	Fernando de Noronha/PE	Skin swabbing	Ok	-
124	Slo_227	Fernando de Noronha/PE	Skin swabbing	Ok	-
125	Slo_228	Fernando de Noronha/PE	Skin swabbing	Ok	-
126	Slo_229	Fernando de Noronha/PE	Skin swabbing	Ok	-
127	Slo_230	Fernando de Noronha/PE	Skin swabbing	Ok	-
128	Slo_231	Fernando de Noronha/PE	Skin swabbing	Ok	-
129	Slo_232	Fernando de Noronha/PE	Skin swabbing	Ok	-
130	Slo_233	Fernando de Noronha/PE	Skin swabbing	Ok	-
131	Slo_234	Fernando de Noronha/PE	Skin swabbing	Ok	-
132	Slo_235	Fernando de Noronha/PE	Skin swabbing	Ok	-
133	Slo_236	Fernando de Noronha/PE	Skin swabbing	Ok	-
134	Slo_238	Fernando de Noronha/PE	Skin swabbing	Ok	-
135	Slo_239	Fernando de Noronha/PE	Skin swabbing	Ok	-
136	Slo_240	Fernando de Noronha/PE	Skin swabbing	Ok	-
137	Slo_241	Fernando de Noronha/PE	Skin swabbing	Ok	-
138	Slo_242	Fernando de Noronha/PE	Skin swabbing	Ok	-
139	Slo_243	Fernando de Noronha/PE	Skin swabbing	Ok	-
140	Slo_245	Fernando de Noronha/PE	Skin swabbing	Ok	-
141	Slo_246	Fernando de Noronha/PE	Skin swabbing	Ok	-
142	Slo_249	Fernando de Noronha/PE	Skin swabbing	Ok	-
143	Slo_254	Fernando de Noronha/PE	Skin swabbing	Ok	-
144	Slo_257	Fernando de Noronha/PE	Skin swabbing	Ok	-
145	Slo_258	Fernando de Noronha/PE	Skin swabbing	Ok	-
146	Slo_261	Fernando de Noronha/PE	Skin swabbing	Ok	-
147	Slo_264	Fernando de Noronha/PE	Skin swabbing	Ok	-
148	Slo_265	Fernando de Noronha/PE	Skin swabbing	Ok	-

Sample	Sample ID.	Localization	Sampling	D-loop	Microsatellite
149	Slo_267	Fernando de Noronha/PE	Skin swabbing	Ok	-
150	Slo_268	Fernando de Noronha/PE	Skin swabbing	Ok	-
151	Slo_269	Fernando de Noronha/PE	Skin swabbing	Ok	-
152	Slo_272	Fernando de Noronha/PE	Skin swabbing	Ok	-
153	Slo_276	Fernando de Noronha/PE	Skin swabbing	Ok	-
154	Slo_277	Fernando de Noronha/PE	Skin swabbing	Ok	-
155	Slo_278	Fernando de Noronha/PE	Skin swabbing	Ok	-
156	Slo_280	Fernando de Noronha/PE	Skin swabbing	Ok	-
157	Slo_281	Fernando de Noronha/PE	Skin swabbing	Ok	-
158	Slo_284	Fernando de Noronha/PE	Skin swabbing	Ok	-
159	Slo_286	Fernando de Noronha/PE	Skin swabbing	Ok	-
160	Slo_288	Fernando de Noronha/PE	Skin swabbing	Ok	-
161	Slo_290	Fernando de Noronha/PE	Skin swabbing	Ok	-
162	Slo_293	Fernando de Noronha/PE	Skin swabbing	Ok	-
163	Slo_298	Fernando de Noronha/PE	Skin swabbing	Ok	-
164	Slo_315	Fernando de Noronha/PE	Skin swabbing	Ok	-
165	Slo_316	Fernando de Noronha/PE	Skin swabbing	Ok	-
166	Slo_317	Fernando de Noronha/PE	Skin swabbing	Ok	-
167	Slo_319	Fernando de Noronha/PE	Skin swabbing	Ok	-
168	Slo_320	Fernando de Noronha/PE	Skin swabbing	Ok	-
169	Slo_321	Fernando de Noronha/PE	Skin swabbing	Ok	-
170	Slo_322	Fernando de Noronha/PE	Skin swabbing	Ok	-
171	Slo_324	Fernando de Noronha/PE	Skin swabbing	Ok	-
172	Slo_325	Fernando de Noronha/PE	Skin swabbing	Ok	-
173	Slo_330	Fernando de Noronha/PE	Skin swabbing	Ok	-
174	Slo_331	Fernando de Noronha/PE	Skin swabbing	Ok	-
175	Slo_332	Fernando de Noronha/PE	Skin swabbing	Ok	-
176	Slo_333	Fernando de Noronha/PE	Skin swabbing	Ok	-
177	Slo_339	Fernando de Noronha/PE	Skin swabbing	Ok	-
178	Slo_340	Fernando de Noronha/PE	Skin swabbing	Ok	-
179	Slo_342	Fernando de Noronha/PE	Skin swabbing	Ok	-
180	Slo_348	Fernando de Noronha/PE	Skin swabbing	Ok	-
181	Slo_349	Fernando de Noronha/PE	Skin swabbing	Ok	-
182	Slo_353	Fernando de Noronha/PE	Skin swabbing	Ok	-
183	Slo_356	Fernando de Noronha/PE	Skin swabbing	Ok	-
184	Slo_358	Fernando de Noronha/PE	Skin swabbing	Ok	-
185	Slo_364	Fernando de Noronha/PE	Skin swabbing	Ok	-
186	Slo_367	Fernando de Noronha/PE	Skin swabbing	Ok	-
187	Slo_368	Fernando de Noronha/PE	Skin swabbing	Ok	-
188	Slo_369	Fernando de Noronha/PE	Skin swabbing	Ok	-
189	Slo_370	Fernando de Noronha/PE	Skin swabbing	Ok	-
190	Slo_371	Fernando de Noronha/PE	Skin swabbing	Ok	-
191	Slo_373	Fernando de Noronha/PE	Skin swabbing	Ok	-
192	Slo_376	Fernando de Noronha/PE	Skin swabbing	Ok	-
193	Slo_379	Fernando de Noronha/PE	Skin swabbing	Ok	-

Sample	Sample ID.	Localization	Sampling	D-loop	Microsatellite
194	Slo_380	Fernando de Noronha/PE	Skin swabbing	Ok	-
195	Slo_384	Fernando de Noronha/PE	Skin swabbing	Ok	-
196	Slo_392	Fernando de Noronha/PE	Skin swabbing	Ok	-
197	Slo_393	Fernando de Noronha/PE	Skin swabbing	Ok	-
198	Slo_394	Fernando de Noronha/PE	Skin swabbing	Ok	-
199	Slo_403	Fernando de Noronha/PE	Skin swabbing	Ok	-
200	Slo_408	Fernando de Noronha/PE	Skin swabbing	Ok	-
201	Slo_411	Fernando de Noronha/PE	Skin swabbing	Ok	-
202	Slo_413	Rio Grande do Norte (RN)	Stranding	Ok	Ok
203	Slo_414	Ceará (CE)	Stranding	Ok	Ok
204	Slo_415	Ceará (CE)	Stranding	Ok	-
205	Slo_416	Ceará (CE)	Stranding	-	Ok
206	Slo_417	Ceará (CE)	Stranding	Ok	Ok
207	Slo_420	Ceará (CE)	Stranding	-	Ok
208	Slo_418	Pernambuco (PE)	Stranding	Ok	Ok
209	Slo_419	Pernambuco (PE)	Stranding	Ok	Ok
210	Slo_421	Espírito Santo (ES)	Stranding	Ok	Ok
211	Slo_422	Espírito Santo (ES)	Stranding	Ok	Ok
212	Slo_423	Espírito Santo (ES)	Stranding	Ok	Ok
213	Slo_424	Rio de Janeiro (RJ)	Skin Biopsy	Ok	Ok
214	Slo_425	Rio de Janeiro (RJ)	Skin Biopsy	Ok	Ok
215	Slo_426	Rio de Janeiro (RJ)	Skin Biopsy	Ok	Ok
216	Slo_427	Rio de Janeiro (RJ)	Skin Biopsy	Ok	Ok
217	Slo_428	Rio de Janeiro (RJ)	Skin Biopsy	Ok	Ok
218	Slo_429	Rio de Janeiro (RJ)	Skin Biopsy	Ok	Ok
219	Slo_430	Rio de Janeiro (RJ)	Skin Biopsy	Ok	Ok
220	Slo_431	Rio de Janeiro (RJ)	Skin Biopsy	Ok	Ok
221	Slo_432	Rio de Janeiro (RJ)	Skin Biopsy	Ok	Ok
222	Slo_433	Rio de Janeiro (RJ)	Skin Biopsy	Ok	-
223	Slo_434	Rio de Janeiro (RJ)	Skin Biopsy	Ok	Ok
224	Slo_435	Rio de Janeiro (RJ)	Skin Biopsy	Ok	Ok
225	Slo_436	Rio de Janeiro (RJ)	Skin Biopsy	Ok	Ok
226	Slo_437	Rio de Janeiro (RJ)	Skin Biopsy	Ok	Ok
227	Slo_438	Rio de Janeiro (RJ)	Skin Biopsy	Ok	Ok
228	Slo_439	Rio de Janeiro (RJ)	Skin Biopsy	Ok	Ok
229	Slo_440	Rio de Janeiro (RJ)	Skin Biopsy	Ok	Ok
230	Slo_441	Rio de Janeiro (RJ)	Skin Biopsy	Ok	Ok
231	Slo_442	Rio de Janeiro (RJ)	Skin Biopsy	Ok	Ok
232	Slo_443	Rio de Janeiro (RJ)	Skin Biopsy	Ok	Ok
233	Slo_444	Rio de Janeiro (RJ)	Skin Biopsy	Ok	Ok
234	Slo_445	Rio de Janeiro (RJ)	Skin Biopsy	Ok	Ok
235	Slo_446	Rio de Janeiro (RJ)	Skin Biopsy	Ok	Ok
236	Slo_447	Rio de Janeiro (RJ)	Skin Biopsy	Ok	Ok
237	Slo_448	Rio de Janeiro (RJ)	Skin Biopsy	Ok	Ok
238	Slo_449	Rio de Janeiro (RJ)	Skin Biopsy	Ok	Ok

Sample	Sample ID.	Localization	Sampling	D-loop	Microsatellite
239	Slo_450	São Paulo (SP)	Skin Biopsy	Ok	Ok
240	Slo_451	Paraná (PR)	Skin Biopsy	Ok	Ok
241	Slo_452	Paraná (PR)	Skin Biopsy	Ok	Ok
242	Slo_453	Paraná (PR)	Skin Biopsy	Ok	Ok
243	Slo_454	Paraná (PR)	Skin Biopsy	Ok	Ok
244	Slo_455	Paraná (PR)	Skin Biopsy	Ok	Ok
245	Slo_456	Santa Catarina (SC)	Skin Biopsy	Ok	Ok
246	Slo_457	Santa Catarina (SC)	Skin Biopsy	Ok	Ok
247	Slo_458	Santa Catarina (SC)	Skin Biopsy	Ok	Ok
248	Slo_460	Santa Catarina (SC)	Skin Biopsy	Ok	Ok
249	Slo_461	Santa Catarina (SC)	Skin Biopsy	Ok	Ok
250	Slo_462	Rio Grande do Sul (RS)	Stranding	-	Ok

Appendix 2 Number of specimens of the Paficfic Ocean used in this study genotyped for 12 microsatellite loci, sample identity, island location, location and sampling method.

Sample	Sample ID.	Island Localization	Localization	Sampling Method
1	Slo03FP16	Leeward -BoraBora (French Polynesia Islands-SEPAC)	Society islands	Biopsy
2	Slo03FP17	Leeward -BoraBora (French Polynesia Islands-SEPAC)	Society islands	Biopsy
3	Slo03FP18	Leeward -BoraBora (French Polynesia Islands-SEPAC)	Society islands	Biopsy
4	Slo03FP19	Leeward -BoraBora (French Polynesia Islands-SEPAC)	Society islands	Biopsy
5	Slo03FP20	Leeward -BoraBora (French Polynesia Islands-SEPAC)	Society islands	Biopsy
6	Slo03FP21	Leeward -BoraBora (French Polynesia Islands-SEPAC)	Society islands	Biopsy
7	Slo03FP26	Leeward -Huahine (French Polynesia Islands-SEPAC)	Society islands	Biopsy
8	Slo03FP27	Leeward -Huahine (French Polynesia Islands-SEPAC)	Society islands	Biopsy
9	Slo03FP28	Leeward -Huahine (French Polynesia Islands-SEPAC)	Society islands	Biopsy
10	Slo03FP29	Leeward -Huahine (French Polynesia Islands-SEPAC)	Society islands	Biopsy
11	Slo03FP31	Leeward -Huahine (French Polynesia Islands-SEPAC)	Society islands	Biopsy
12	Slo03FP33	Leeward -Huahine (French Polynesia Islands-SEPAC)	Society islands	Biopsy
13	Slo03FP34	Leeward -Huahine (French Polynesia Islands-SEPAC)	Society islands	Biopsy
14	Slo03FP35	Leeward -Huahine (French Polynesia Islands-SEPAC)	Society islands	Biopsy
15	Slo03FP36	Leeward -Huahine (French Polynesia Islands-SEPAC)	Society islands	Biopsy
16	Slo03FP37	Leeward -Huahine (French Polynesia Islands-SEPAC)	Society islands	Biopsy
17	Slo03FP39	Leeward -Huahine (French Polynesia Islands-SEPAC)	Society islands	Biopsy
18	Slo03FP40	Leeward -Huahine (French Polynesia Islands-SEPAC)	Society islands	Biopsy
19	Slo03FP41	Leeward -Huahine (French Polynesia Islands-SEPAC)	Society islands	Biopsy
20	Slo03FP42	Leeward -Huahine (French Polynesia Islands-SEPAC)	Society islands	Biopsy
21	Slo03FP22	Leeward - Tahaa-Raiatea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
22	Slo03FP23	Leeward - Tahaa-Raiatea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
23	Slo04FP63	Leeward - Tahaa-Raiatea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
24	Slo04FP64	Leeward - Tahaa-Raiatea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
25	Slo04FP65	Leeward - Tahaa-Raiatea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
26	Slo04FP66	Leeward - Tahaa-Raiatea (French Polynesia Islands-SEPAC)	Society islands	Biopsy

Sample	Sample ID.	Island Localization	Localization	Sampling Method
27	Slo04FP67	Leeward - Tahaa-Raiatea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
28	Slo04FP69	Leeward - Tahaa-Raiatea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
29	Slo04FP70	Leeward - Tahaa-Raiatea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
30	Slo04FP71	Leeward - Tahaa-Raiatea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
31	Slo04FP72	Leeward - Tahaa-Raiatea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
32	Slo04FP73	Leeward - Tahaa-Raiatea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
33	Slo04FP74	Leeward - Tahaa-Raiatea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
34	Slo04FP75	Leeward - Tahaa-Raiatea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
35	Slo04FP78	Leeward - Tahaa-Raiatea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
36	Slo04FP79	Leeward - Tahaa-Raiatea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
37	Slo04FP80	Leeward - Tahaa-Raiatea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
38	Slo04FP81	Leeward - Tahaa-Raiatea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
39	Slo04FP82	Leeward - Tahaa-Raiatea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
40	Slo04FP83	Leeward - Tahaa-Raiatea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
41	Slo04FP84	Leeward - Tahaa-Raiatea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
42	Slo04FP85	Leeward - Tahaa-Raiatea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
43	Slo02FP01	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
44	Slo02FP02	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
45	Slo02FP03	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
46	Slo02FP04	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
47	Slo02FP05	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
48	Slo02FP06	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
49	Slo02FP08	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
50	Slo02FP10	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
51	Slo02FP11	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
52	Slo02FP12	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
53	Slo02FP13	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
54	Slo02FP14	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
55	Slo02FP15	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
56	Slo02FP16	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
57	Slo02FP17	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
58	Slo02FP19	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
59	Slo02FP20	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
60	Slo02FP22	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
61	Slo02FP23	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
62	Slo02FP24	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
63	Slo02FP25	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
64	Slo02FP26	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
65	Slo02FP27	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
66	Slo02FP28	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
67	Slo02FP29	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
68	Slo02FP30	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
69	Slo02FP31	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
70	Slo02FP33	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
71	Slo02FP34	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy

Sample	Sample ID.	Island Localization	Localization	Sampling Method
72	Slo02FP35	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
73	Slo02FP36	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
74	Slo02FP37	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
75	Slo02FP38	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
76	Slo02FP39	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
77	Slo02FP40	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
78	Slo02FP42	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
79	Slo02FP43	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
80	Slo02FP44	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
81	Slo02FP45	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
82	Slo02FP49	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
83	Slo02FP51	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
84	Slo02FP52	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
85	Slo02FP53	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
86	Slo02FP54	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
87	Slo02FP55	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
88	Slo02FP56	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
89	Slo02FP57	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
90	Slo02FP58	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
91	Slo02FP59	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
92	Slo02FP60	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
93	Slo02FP61	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
94	Slo02FP63	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
95	Slo02FP64	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
96	Slo03FP08	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
97	Slo03FP09	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
98	Slo03FP10	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
99	Slo03FP11	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
100	Slo03FP12	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
101	Slo03FP13	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
102	Slo03FP14	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
103	Slo03FP15	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
104	Slo04FP01	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
105	Slo04FP03	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
106	Slo04FP04	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
107	Slo04FP05	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
108	Slo04FP06	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
109	Slo04FP07	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
110	Slo04FP08	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
111	Slo04FP09	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
112	Slo04FP10	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
113	Slo04FP11	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
114	Slo04FP13	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
115	Slo04FP14	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
116	Slo04FP15	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy

Sample	Sample ID.	Island Localization	Localization	Sampling Method
117	Slo04FP18	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
118	Slo04FP19	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
119	Slo04FP20	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
120	Slo04FP21	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
121	Slo04FP23	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
122	Slo04FP24	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
123	Slo04FP25	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
124	Slo04FP28	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
125	Slo04FP31	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
126	Slo04FP32	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
127	Slo04FP34	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
128	Slo04FP35	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
129	Slo04FP36	Windward-Moorea (French Polynesia Islands-SEPAC)	Society islands	Biopsy
130	Slo03FP44	Windward-Tahiti (French Polynesia Islands-SEPAC)	Society islands	Biopsy
131	Slo03FP45	Windward-Tahiti (French Polynesia Islands-SEPAC)	Society islands	Biopsy
132	Slo03FP46	Windward-Tahiti (French Polynesia Islands-SEPAC)	Society islands	Biopsy
133	Slo03FP47	Windward-Tahiti (French Polynesia Islands-SEPAC)	Society islands	Biopsy
134	Slo03FP48	Windward-Tahiti (French Polynesia Islands-SEPAC)	Society islands	Biopsy
135	Slo03FP49	Windward-Tahiti (French Polynesia Islands-SEPAC)	Society islands	Biopsy
136	Slo03FP50	Windward-Tahiti (French Polynesia Islands-SEPAC)	Society islands	Biopsy
137	Slo03FP51	Windward-Tahiti (French Polynesia Islands-SEPAC)	Society islands	Biopsy
138	Slo04FP37	Windward-Tahiti (French Polynesia Islands-SEPAC)	Society islands	Biopsy
139	Slo04FP38	Windward-Tahiti (French Polynesia Islands-SEPAC)	Society islands	Biopsy
140	Slo04FP40	Windward-Tahiti (French Polynesia Islands-SEPAC)	Society islands	Biopsy
141	Slo04FP41	Windward-Tahiti (French Polynesia Islands-SEPAC)	Society islands	Biopsy
142	Slo04FP42	Windward-Tahiti (French Polynesia Islands-SEPAC)	Society islands	Biopsy
143	Slo04FP43	Windward-Tahiti (French Polynesia Islands-SEPAC)	Society islands	Biopsy
144	Slo04FP44	Windward-Tahiti (French Polynesia Islands-SEPAC)	Society islands	Biopsy
145	Slo04FP45	Windward-Tahiti (French Polynesia Islands-SEPAC)	Society islands	Biopsy
146	Slo04FP46	Windward-Tahiti (French Polynesia Islands-SEPAC)	Society islands	Biopsy
147	Slo04FP47	Windward-Tahiti (French Polynesia Islands-SEPAC)	Society islands	Biopsy
148	Slo04FP48	Windward-Tahiti (French Polynesia Islands-SEPAC)	Society islands	Biopsy
149	Slo04FP49	Windward-Tahiti (French Polynesia Islands-SEPAC)	Society islands	Biopsy
150	Slo04FP50	Windward-Tahiti (French Polynesia Islands-SEPAC)	Society islands	Biopsy
151	Slo04FP51	Windward-Tahiti (French Polynesia Islands-SEPAC)	Society islands	Biopsy
152	Slo04FP52	Windward-Tahiti (French Polynesia Islands-SEPAC)	Society islands	Biopsy
153	Slo04FP53	Windward-Tahiti (French Polynesia Islands-SEPAC)	Society islands	Biopsy
154	Slo04FP54	Windward-Tahiti (French Polynesia Islands-SEPAC)	Society islands	Biopsy
155	Slo04FP55	Windward-Tahiti (French Polynesia Islands-SEPAC)	Society islands	Biopsy
156	Slo04FP56	Windward-Tahiti (French Polynesia Islands-SEPAC)	Society islands	Biopsy
157	Slo04FP57	Windward-Tahiti (French Polynesia Islands-SEPAC)	Society islands	Biopsy
158	Slo04FP58	Windward-Tahiti (French Polynesia Islands-SEPAC)	Society islands	Biopsy
159	Slo04FP59	Windward-Tahiti (French Polynesia Islands-SEPAC)	Society islands	Biopsy
160	Slo04FP61	Windward-Tahiti (French Polynesia Islands-SEPAC)	Society islands	Biopsy
161	Slo04FP62	Windward-Tahiti (French Polynesia Islands-SEPAC)	Society islands	Biopsy

Sample	Sample ID.	Island Localization	Localization	Sampling Method
162	SLONBI13	Big Island	Hawaiian Islands	Biopsy
163	SLONBI14	Big Island	Hawaiian Islands	Biopsy
164	SLONBI19	Big Island	Hawaiian Islands	Biopsy
165	SLONBI20	Big Island	Hawaiian Islands	Biopsy
166	SLONBI21	Big Island	Hawaiian Islands	Biopsy
167	SLONBI22	Big Island	Hawaiian Islands	Biopsy
168	SLONBI24	Big Island	Hawaiian Islands	Biopsy
169	SLONBI28	Big Island	Hawaiian Islands	Biopsy
170	SLONBI31	Big Island	Hawaiian Islands	Biopsy
171	SLONBI32	Big Island	Hawaiian Islands	Biopsy
172	SLONBI37	Big Island	Hawaiian Islands	Biopsy
173	SLONBI40	Big Island	Hawaiian Islands	Biopsy
174	SLONBI44	Big Island	Hawaiian Islands	Biopsy
175	SLONBI51	Big Island	Hawaiian Islands	Biopsy
176	SLONBI56	Big Island	Hawaiian Islands	Biopsy
177	SLONBI60	Big Island	Hawaiian Islands	Biopsy
178	SLONBI62	Big Island	Hawaiian Islands	Biopsy
179	SLONBI63	Big Island	Hawaiian Islands	Biopsy
180	SLONMU22	Maui	Hawaiian Islands	Biopsy
181	SLONMU23	Maui	Hawaiian Islands	Biopsy
182	SLONMU24	Maui	Hawaiian Islands	Biopsy
183	SLONMU25	Maui	Hawaiian Islands	Biopsy
184	SLONMU27	Maui	Hawaiian Islands	Biopsy
185	SLONMU29	Maui	Hawaiian Islands	Biopsy
186	SLONMU31	Maui	Hawaiian Islands	Biopsy
187	SLONMU32	Maui	Hawaiian Islands	Biopsy
188	SLONMU33	Maui	Hawaiian Islands	Biopsy
189	SLONMU35	Maui	Hawaiian Islands	Biopsy
190	SLONMU36	Maui	Hawaiian Islands	Biopsy
191	SLONMU37	Maui	Hawaiian Islands	Biopsy
192	SLONMU39	Maui	Hawaiian Islands	Biopsy
193	SLONMU40	Maui	Hawaiian Islands	Biopsy
194	SLONMU41	Maui	Hawaiian Islands	Biopsy
195	SLONMU46	Maui	Hawaiian Islands	Biopsy
196	SLONMU50	Maui	Hawaiian Islands	Biopsy
197	SLONMU55	Maui	Hawaiian Islands	Biopsy
198	SLONMU60	Maui	Hawaiian Islands	Biopsy
199	SLONNU01	Niihau	Hawaiian Islands	Biopsy
200	SLONNU02	Niihau	Hawaiian Islands	Biopsy
201	SLONNU04	Niihau	Hawaiian Islands	Biopsy
202	SLONNU05	Niihau	Hawaiian Islands	Biopsy
203	SLONNU06	Niihau	Hawaiian Islands	Biopsy
204	SLONNU07	Niihau	Hawaiian Islands	Biopsy
205	SLONNU08	Niihau	Hawaiian Islands	Biopsy
206	SLONNU09	Niihau	Hawaiian Islands	Biopsy

Sample	Sample ID.	Island Localization	Localization	Sampling Method
207	SLONNU11	Niihau	Hawaiian Islands	Biopsy
208	SLONNU12	Niihau	Hawaiian Islands	Biopsy
209	SLONNU13	Niihau	Hawaiian Islands	Biopsy
210	SLONNU14	Niihau	Hawaiian Islands	Biopsy
211	SLONNU15	Niihau	Hawaiian Islands	Biopsy
212	SLONNU16	Niihau	Hawaiian Islands	Biopsy
213	SLONNU17	Niihau	Hawaiian Islands	Biopsy
214	SLONNU18	Niihau	Hawaiian Islands	Biopsy
215	SLONNU19	Niihau	Hawaiian Islands	Biopsy
216	SLONNU20	Niihau	Hawaiian Islands	Biopsy
217	SLONNU22	Niihau	Hawaiian Islands	Biopsy
218	SLONPH31	Pearl & Hermes	Hawaiian Islands	Biopsy
219	SLONPH32	Pearl & Hermes	Hawaiian Islands	Biopsy
220	SLONPH33	Pearl & Hermes	Hawaiian Islands	Biopsy
221	SLONPH34	Pearl & Hermes	Hawaiian Islands	Biopsy
222	SLONPH35	Pearl & Hermes	Hawaiian Islands	Biopsy
223	SLONPH36	Pearl & Hermes	Hawaiian Islands	Biopsy
224	SLONPH37	Pearl & Hermes	Hawaiian Islands	Biopsy
225	SLONPH38	Pearl & Hermes	Hawaiian Islands	Biopsy
226	SLONPH39	Pearl & Hermes	Hawaiian Islands	Biopsy
227	SLONPH40	Pearl & Hermes	Hawaiian Islands	Biopsy
228	SLONPH41	Pearl & Hermes	Hawaiian Islands	Biopsy
229	SLONPH42	Pearl & Hermes	Hawaiian Islands	Biopsy
230	SLONPH44	Pearl & Hermes	Hawaiian Islands	Biopsy
231	SLONPH45	Pearl & Hermes	Hawaiian Islands	Biopsy
232	SLONPH46	Pearl & Hermes	Hawaiian Islands	Biopsy
233	SLONPH47	Pearl & Hermes	Hawaiian Islands	Biopsy
234	SLONPH48	Pearl & Hermes	Hawaiian Islands	Biopsy
235	SLONPH49	Pearl & Hermes	Hawaiian Islands	Biopsy
236	SLONPH51	Pearl & Hermes	Hawaiian Islands	Biopsy
237	Slo12MA001	Marquesas-Hiva Ao (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
238	Slo12MA002	Marquesas-Hiva Ao (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
239	Slo12MA008	Marquesas-Hiva Ao (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
240	Slo12MA009	Marquesas-Hiva Ao (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
241	Slo12MA010	Marquesas-Hiva Ao (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
242	Slo12MA011	Marquesas-Hiva Ao (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
243	Slo12MA012	Marquesas-Hiva Ao (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
244	Slo12MA013	Marquesas-Hiva Ao (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
245	Slo12MA014	Marquesas-Hiva Ao (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
246	Slo12MA017	Marquesas-Hiva Ao (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
247	Slo12MA018	Marquesas-Hiva Ao (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
248	Slo12MA019	Marquesas-Hiva Ao (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
249	Slo12MA020	Marquesas-Hiva Ao (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
250	Slo12MA029	Marquesas-Nuku Hiva (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
251	Slo12MA030	Marquesas-Nuku Hiva (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy

Sample	Sample ID.	Island Localization	Localization	Sampling Method
252	Slo12MA031	Marquesas-Nuku Hiva (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
253	Slo12MA032	Marquesas-Nuku Hiva (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
254	Slo12MA033	Marquesas-Nuku Hiva (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
255	Slo12MA034	Marquesas-Nuku Hiva (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
256	Slo12MA035	Marquesas-Nuku Hiva (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
257	Slo12MA036	Marquesas-Nuku Hiva (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
258	Slo12MA037	Marquesas-Nuku Hiva (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
259	Slo12MA038	Marquesas-Nuku Hiva (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
260	Slo12MA039	Marquesas-Nuku Hiva (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
261	Slo12MA040	Marquesas-Nuku Hiva (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
262	Slo12MA041	Marquesas-Nuku Hiva (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
263	Slo12MA042	Marquesas-Nuku Hiva (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
264	Slo12MA043	Marquesas-Nuku Hiva (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
265	Slo12MA044	Marquesas-Nuku Hiva (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
266	Slo12MA045	Marquesas-Nuku Hiva (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
267	Slo12MA046	Marquesas-Nuku Hiva (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
268	Slo12MA047	Marquesas-Nuku Hiva (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
269	Slo12MA048	Marquesas-Nuku Hiva (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
270	Slo12MA050	Marquesas-Nuku Hiva (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
271	Slo12MA051	Marquesas-Nuku Hiva (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
272	Slo12MA052	Marquesas-Nuku Hiva (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
273	Slo12MA053	Marquesas-Nuku Hiva (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
274	Slo12MA004	Marquesas-Tahuata (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
275	Slo12MA005	Marquesas-Tahuata (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
276	Slo12MA006	Marquesas-Tahuata (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
277	Slo12MA007	Marquesas-Tahuata (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
278	Slo12MA015	Marquesas-Tahuata (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
279	Slo12MA016	Marquesas-Tahuata (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
280	Slo12MA021	Marquesas-Ua Huka (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
281	Slo12MA022	Marquesas-Ua Huka (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
282	Slo12MA023	Marquesas-Ua Huka (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
283	Slo12MA024	Marquesas-Ua Pou (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
284	Slo12MA025	Marquesas-Ua Pou (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
285	Slo12MA026	Marquesas-Ua Pou (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
286	Slo12MA027	Marquesas-Ua Pou (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
287	Slo12MA028	Marquesas-Ua Pou (Marquesas Islands - SEPAC)	Marquesas Islands	Biopsy
288	NC08-005	New Caledonia (SWPAC)	New Caledonia	Biopsy
289	NC08-006	New Caledonia (SWPAC)	New Caledonia	Biopsy
290	NC08-007	New Caledonia (SWPAC)	New Caledonia	Biopsy
291	NC08-008	New Caledonia (SWPAC)	New Caledonia	Biopsy
292	NC08-018	New Caledonia (SWPAC)	New Caledonia	Biopsy
293	NC08-019	New Caledonia (SWPAC)	New Caledonia	Biopsy
294	NC08-020	New Caledonia (SWPAC)	New Caledonia	Biopsy
295	NC08-030	New Caledonia (SWPAC)	New Caledonia	Biopsy
296	NC08-031	New Caledonia (SWPAC)	New Caledonia	Biopsy

Sample	Sample ID.	Island Localization	Localization	Sampling Method
297	NC08-032	New Caledonia (SWPAC)	New Caledonia	Biopsy
298	NC08-057	New Caledonia (SWPAC)	New Caledonia	Biopsy
299	NC08-069	New Caledonia (SWPAC)	New Caledonia	Biopsy
300	NC08-070	New Caledonia (SWPAC)	New Caledonia	Biopsy
301	Slo03NC02	New Caledonia (SWPAC)	New Caledonia	Biopsy
302	Slo03Sa01	Samoa (SWPAC)	Samoa	Biopsy
303	Slo03Sa02	Samoa (SWPAC)	Samoa	Biopsy
304	Slo03Sa03	Samoa (SWPAC)	Samoa	Biopsy
305	Slo07Sa01	Samoa (SWPAC)	Samoa	Biopsy
306	Slo07Sa02	Samoa (SWPAC)	Samoa	Biopsy
307	Slo07Sa03	Samoa (SWPAC)	Samoa	Biopsy
308	Slo07Sa06	Samoa (SWPAC)	Samoa	Biopsy
309	Slo07Sa07	Samoa (SWPAC)	Samoa	Biopsy
310	Slo07Sa08	Samoa (SWPAC)	Samoa	Biopsy
311	Slo07Sa09	Samoa (SWPAC)	Samoa	Biopsy
312	Slo07Sa10	Samoa (SWPAC)	Samoa	Biopsy
313	Slo07Sa11	Samoa (SWPAC)	Samoa	Biopsy
314	Slo11AS001	Samoa (SWPAC)	Samoa	Biopsy
315	Slo12SA001	Samoa (SWPAC)	Samoa	Biopsy
316	Slo12SA002	Samoa (SWPAC)	Samoa	Biopsy
317	Slo12SA003	Samoa (SWPAC)	Samoa	Biopsy
318	Slo12SA009	Samoa (SWPAC)	Samoa	Biopsy
319	Slo12SA015	Samoa (SWPAC)	Samoa	Biopsy
320	Slo12SA017	Samoa (SWPAC)	Samoa	Biopsy
321	Slo12SA018	Samoa (SWPAC)	Samoa	Biopsy
322	Slo12SA019	Samoa (SWPAC)	Samoa	Biopsy
323	Slo12SA020	Samoa (SWPAC)	Samoa	Biopsy
324	Slo12SA022	Samoa (SWPAC)	Samoa	Biopsy
325	Slo12SA023	Samoa (SWPAC)	Samoa	Biopsy
326	Slo12SA024	Samoa (SWPAC)	Samoa	Biopsy
327	SLONSM03	American Samoa	Samoa	Biopsy
328	SLONSM04	American Samoa	Samoa	Biopsy
329	SLONSM05	American Samoa	Samoa	Biopsy
330	SLONSM06	American Samoa	Samoa	Biopsy
331	SLONSM07	American Samoa	Samoa	Biopsy
332	SLONSM09	American Samoa	Samoa	Biopsy
333	SLONSM10	American Samoa	Samoa	Biopsy
334	SLONSM11	American Samoa	Samoa	Biopsy
335	SLONSM12	American Samoa	Samoa	Biopsy
336	SLONSM14	American Samoa	Samoa	Biopsy
337	SLONSM15	American Samoa	Samoa	Biopsy
338	SLONSM16	American Samoa	Samoa	Biopsy
339	Slo07TU01	Tuvalu (SWPAC)	Tuvalu	Biopsy
340	Slo07TU03	Tuvalu (SWPAC)	Tuvalu	Biopsy
341	Slo07TU04	Tuvalu (SWPAC)	Tuvalu	Biopsy

Sample	Sample ID.	Island Localization	Localization	Sampling Method
342	Slo07TU05	Tuvalu (SWPAC)	Tuvalu	Biopsy
343	Slo07TU06	Tuvalu (SWPAC)	Tuvalu	Biopsy
344	Slo07TU07	Tuvalu (SWPAC)	Tuvalu	Biopsy
345	Slo07TU08	Tuvalu (SWPAC)	Tuvalu	Biopsy
346	Slo07TU09	Tuvalu (SWPAC)	Tuvalu	Biopsy
347	Slo07TU10	Tuvalu (SWPAC)	Tuvalu	Biopsy
348	Slo07TU11	Tuvalu (SWPAC)	Tuvalu	Biopsy
349	Slo07TU12	Tuvalu (SWPAC)	Tuvalu	Biopsy
350	Slo07TU13	Tuvalu (SWPAC)	Tuvalu	Biopsy
351	Slo07TU15	Tuvalu (SWPAC)	Tuvalu	Biopsy
352	Slo07TU16	Tuvalu (SWPAC)	Tuvalu	Biopsy
353	Slo07TU17	Tuvalu (SWPAC)	Tuvalu	Biopsy
354	Slo07TU18	Tuvalu (SWPAC)	Tuvalu	Biopsy
355	Slo07TU19	Tuvalu (SWPAC)	Tuvalu	Biopsy
356	Slo07TU20	Tuvalu (SWPAC)	Tuvalu	Biopsy

Appendix 3 Haploypes of D-loop from GenBank used in this study. Source, GenBank accession number, haplotypes codes of the original papers and of the present study, and, geographic localizations with number of individuals.

Source (Paper)	GenBank acession	Haplotype code (Original Paper)	Haplotype code (This Study)	Geographic localization (nº of individuals)
Andrews *et al.* 2010	GU253256	HW01	H 10	Hawaii (344)
Andrews *et al.* 2010	GU253257	HW02	H 11	Hawaii (63)
Andrews *et al.* 2010	GU253258	HW03	H 12	Hawaii (20)
Andrews *et al.* 2010	GU253259	HW04	H 13	Hawaii (1)
Andrews *et al.* 2010	GU253260	HW05	H 14	Hawaii (2), Samoa (1)
Andrews *et al.* 2010	GU253261	HW06	H 15	Hawaii (18)
Andrews *et al.* 2010	GU253262	HW07	H 16	Hawaii (4), Samoa (2)
Andrews *et al.* 2010	GU253263	HW08	H 17	Hawaii (26), Samoa (2)
Andrews *et al.* 2010	GU253264	HW09	H 18	Hawaii (5)
Andrews *et al.* 2010	GU253265	HW10	H 19	Hawaii (2)
Andrews *et al.* 2010	GU253266	HW11	H 20	Hawaii (1)
Andrews *et al.* 2010	GU253267	HW12	H 21	Hawaii (2)
Andrews *et al.* 2010	GU253268	HW13	H 22	Hawaii (1)
Andrews *et al.* 2010	GU253269	HW14	H 23	Hawaii (3)
Andrews *et al.* 2010	GU253270	HW15	H 24	Hawaii (2)
Andrews *et al.* 2010	GU253271	HW16	H 25	Hawaii (3)
Andrews *et al.* 2010	GU253272	HW17	H 26	Hawaii (1)
Andrews *et al.* 2010	GU253273	HW18	H 27	Hawaii (2)
Andrews *et al.* 2010	GU253274	HW19	H 28	Hawaii (1)
Andrews *et al.* 2010	GU253275	HW20	H 68	Samoa (2)
Andrews *et al.* 2010	GU253276	HW21	H 69	Samoa (1)
Andrews *et al.* 2010	GU253277	HW22	H 70	Samoa (1)
Andrews *et al.* 2010	GU253278	HW23	H 36	Samoa (1)
Andrews *et al.* 2010	GU253279	HW24	H 34	Samoa (1)

Source (Paper)	GenBank acession	Haplotype code (Original Paper)	Haplotype code (This Study)	Geographic localization (nº of individuals)
Andrews *et al.* 2010	GU253280	HW25	H 43	Samoa (1)
Andrews *et al.* 2010	GU253281	HW26	H 33	Samoa (1)
Andrews *et al.* 2010	GU253282	HW27	H 37	Samoa (1)
Andrews *et al.* 2010	GU253283	HW28	H 71	Samoa (1)
Andrews *et al.* 2010	GU253284	HW29	H 72	Samoa (1)
Andrews *et al.* 2013	KC160999	StLo003	H 15	Guam (1), Saipam (2), Samoa (2)
Andrews *et al.* 2013	KC161018	StLo022	H 60	Palmyra (1)
Andrews *et al.* 2013	KC161021	StLo025	H 74	Zanzibar (2)
Andrews *et al.* 2013	KC161037	StLo041	H 8	Maldives (2), Zanzibar (2)
Andrews *et al.* 2013	KC161038	StLo042	H 14	American Samoa (1)
Andrews *et al.* 2013	KC161041	StLo045	H 108	Gulf of mexico (1)
Andrews *et al.* 2013	KC161042	StLo046	H 109	Gulf of mexico (4)
Andrews *et al.* 2013	KC161043	StLo047	H 62	Guam (1)
Andrews *et al.* 2013	KC161044	StLo048	H 63	Guam (1)
Andrews *et al.* 2013	KC161045	StLo049	H 57	Guam (1)
Andrews *et al.* 2013	KC161046	StLo050	H 54	Guam (1), Saipan (1)
Andrews *et al.* 2013	KC161047	StLo051	H 105	Indonesia (1)
Andrews *et al.* 2013	KC161048	StLo052	H 35	Indonesia (2)
Andrews *et al.* 2013	KC161049	StLo053	H 106	Indonesia (1)
Andrews *et al.* 2013	KC161050	StLo054	H 107	Indonesia (1)
Andrews *et al.* 2013	KC161051	StLo055	H 102	Maldives (2)
Andrews *et al.* 2013	KC161052	StLo056	H 103	Maldives (1)
Andrews *et al. 2013*	KC161053	StLo057	H 104	Maldives (1)
Andrews *et al.* 2013	KC161055	StLo059	H 113	North Atlantic (1)
Andrews *et al.* 2013	KC161056	StLo060	H 114	North Atlantic (1)
Andrews *et al.* 2013	KC161057	StLo061	H 18	Saipam (1), Palmyra (1)
Andrews *et al.* 2013	KC161058	StLo062	H 19	Palmyra (1), Philippines (1)
Andrews *et al.* 2013	KC161059	StLo063	H 97	Philippines (1)
Andrews *et al.* 2013	KC161060	StLo064	H 98	Philippines (2)
Andrews *et al.* 2013	KC161061	StLo065	H 99	Philippines (1)
Andrews *et al.* 2013	KC161062	StLo066	H 100	Philippines (1)
Andrews *et al.* 2013	KC161063	StLo067	H 101	Philippines (1)
Andrews *et al.* 2013	KC161064	StLo068	H 23	Palmyra (3)
Andrews *et al.* 2013	KC161065	StLo069	H 96	Palmyra (1)
Andrews *et al.* 2013	KC161066	StLo070	H 37	Palmyra (1), American Samoa (1)
Andrews *et al.* 2013	KC161067	StLo071	H 33	American Samoa (1), Palmyra (2)
Andrews *et al.* 2013	KC161068	StLo072	H 68	American Samoa (2)
Andrews *et al.* 2013	KC161069	StLo073	H 69	American Samoa (1)
Andrews *et al.* 2013	KC161070	StLo074	H 70	American Samoa (1)
Andrews *et al.* 2013	KC161071	StLo075	H 73	American Samoa (2)
Andrews *et al.* 2013	KC161072	StLo076	H 36	American Samoa (1)
Andrews *et al.* 2013	KC161073	StLo077	H 34	American Samoa (1)
Andrews *et al.* 2013	KC161074	StLo078	H 43	American Samoa (1)
Andrews *et al.* 2013	KC161075	StLo079	H 71	American Samoa (1)
Andrews *et al.* 2013	KC161076	StLo080	H 72	American Samoa (1)
Andrews *et al.* 2013	KC161077	StLo081	H 25	Saipan (1)
Andrews *et al.* 2013	KC161096	StLo100	H 110	Taiwan (2)
Andrews *et al.* 2013	KC161097	StLo101	H 111	Taiwan (1)
Andrews *et al.* 2013	KC161098	StLo102	H 112	Taiwan (1)
Andrews *et al.* 2013	KC161113	StLo117	H 75	Zanzibar (2)
Andrews *et al.* 2013	KC161114	StLo118	H 76	Zanzibar (2)

Source (Paper)	GenBank acession	Haplotype code (Original Paper)	Haplotype code (This Study)	Geographic localization (nº of individuals)
Andrews *et al.* 2013	KC161115	StLo119	H 77	Zanzibar (1)
Oremus *et al.* 2007	EF558737	Slo02FP01	H 29	Society Islands: Moorea (7), Tahiti (1)
Oremus *et al.* 2007	EF558738	Slo02FP02	H 30	Society Islands: Moorea (5)
Oremus *et al.* 2007	EF558739	Slo02FP03	H 21	Society Islands: Moorea (1)
Oremus *et al.* 2007	EF558740	Slo02FP04	H 31	Society Islands: Moorea (5), Huahine (1)
Oremus *et al.* 2007	EF558741	Slo02FP05	H 32	Society Islands: Moorea (1), Huahine (2), Raiatea-Tahaa (1)
Oremus *et al.* 2007	EF558742	Slo02FP06	H 33	Society Islands: Moorea (2)
Oremus *et al.* 2007	EF558743	Slo02FP07	H 34	Society Islands: Moorea (5), Huahine (2), Bora Bora (2), Raiatea-Tahaa (2)
Oremus *et al.* 2007	EF558744	Slo02FP08	H 35	Society Islands: Moorea (1)
Oremus *et al.* 2007	EF558745	Slo02FP09	H 36	Society Islands: Moorea (3), Bora Bora (1), Raiatea-Tahaa (3)
Oremus *et al.* 2007	EF558746	Slo02FP11	H 17	Society Islands: Moorea (4), Tahiti (8), Huahine (1)
Oremus *et al.* 2007	EF558747	Slo02FP15	H 37	Society Islands: Moorea (7), Bora Bora (1), Raiatea-Tahaa (4)
Oremus *et al.* 2007	EF558748	Slo02FP20	H 38	Society Islands: Moorea (6), Raiatea-Tahaa (1)
Oremus *et al.* 2007	EF558749	Slo02FP22	H 39	Society Islands: Moorea (11)
Oremus *et al.* 2007	EF558750	Slo02FP27	H 40	Society Islands: Moorea (7), Tahiti (24), Bora Bora (1), Raiatea-Tahaa (1)
Oremus *et al.* 2007	EF558751	Slo02FP36	H 19	Society Islands: Moorea (1), Raiatea-Tahaa (1)
Oremus *et al.* 2007	EF558752	Slo02FP38	H 14	Society Islands: Moorea (2)
Oremus *et al.* 2007	EF558753	Slo02FP45	H 41	Society Islands: Moorea (1)
Oremus *et al.* 2007	EF558754	Slo02FP49	H 42	Society Islands: Moorea (1), Raiatea-Tahaa (1)
Oremus *et al.* 2007	EF558755	Slo03FP18	H 43	Society Islands: Bora Bora (1)
Oremus *et al.* 2007	EF558756	Slo03FP26	H 44	Society Islands: Huahine (2)
Oremus *et al.* 2007	EF558757	Slo03FP32	H 15	Society Islands: Huahine (3)
Oremus *et al.* 2007	EF558758	Slo03FP33	H 41	Society Islands: Huahine (1)
Oremus *et al.* 2007	EF558759	Slo03FP34	H 45	Society Islands: Huahine (2), Raiatea-Tahaa (1)
Oremus *et al.* 2007	EF558760	Slo03FP37	H 46	Society Islands: Huahine (2)
Oremus *et al.* 2007	EF558761	Slo03FP41	H 47	Society Islands: Huahine (1), Raiatea-Tahaa (2)
Oremus *et al.* 2007	EF558762	Slo04FP59	H 48	Society Islands: Raiatea-Tahaa(1), Tahiti (1)
Oremus *et al.* 2007	EF558763	Slo04FP70	H 49	Society Islands: Raiatea-Tahaa (1), Nuku Hiva (3)
Oremus *et al. 2007*	EF558764	Slo04FP78	H 50	Society Islands: Nuku Hiva (1)
Oremus *et al.* 2007	EF558765	Slo04FP79	H 51	Society Islands: Nuku Hiva (1)
Oremus *et al.* 2007	EF558766	Slo04FP82	H 52	Society Islands: Nuku Hiva (1)
Oremus *et al.* 2007	EF558767	Slo04FP83	H 42	Society Islands: Nuku Hiva (2)
Martien *et al.* 2014	KY457781	H1	H 64	Guam (1)
Martien *et al.* 2014	KY457782	H2	H 53	3-Islands (4),Rota (1)
Martien *et al.* 2014	KY457783	H3	H 63	Guam (1)
Martien *et al.* 2014	KY457784	H4	H 17	3-Islands (9),Rota (2), Guam (4)
Martien *et al.* 2014	KY457785	H5	H 17	3-Islands (2), Guam (2)
Martien *et al.* 2014	KY457786	H6	H 18	3-Islands (8),Rota (1)
Martien *et al.* 2014	KY457787	H7	H 55	3-Islands (1)
Martien *et al.* 2014	KY457788	H8	H 15	3-Islands (3), Guam (2)
Martien *et al.* 2014	KY457789	H9	H 65	Guam (1)
Martien *et al.* 2014	KY457790	H10	H 56	3-Islands (8), Guam (1)
Martien *et al.* 2014	KY457791	H11	H 12	3-Islands (3), Rota (2), Guam (2)
Martien *et al.* 2014	KY457792	H12	H 21	3-Islands (4), Rota (1), Guam (4)
Martien *et al.* 2014	KY457793	H13	H 25	3-Islands (1)
Martien *et al.* 2014	KY457794	H14	H 61	Rota (1)
Martien *et al.* 2014	KY457795	H15	H 57	3-Islands (3), Guam (1)
Martien *et al.* 2014	KY457796	H16	H 49	3-Islands (3), Rota (2), Guam (4)
Martien *et al.* 2014	KY457797	H17	H 42	3-Islands (2), Rota (1)
Martien *et al.* 2014	KY457798	H18	H 58	3-Islands (1)
Martien *et al.* 2014	KY457799	H19	H 59	3-Islands (1)

Source (Paper)	GenBank acession	Haplotype code (Original Paper)	Haplotype code (This Study)	Geographic localization (nº of individuals)
Martien *et al.* 2014	KY457800	H20	H 66	Guam (1)
Martien *et al.* 2014	KY457801	H21	H 67	Guam (1)
Martien *et al.* 2014	KY457802	H22	H 23	3-Islands (2)
Martien *et al.* 2014	KY457803	H23	H 60	3-Islands (1)
Martien *et al.* 2014	KY457804	H24	H 20	Guam (1)
Viricel *et al.* 2016	KX905105	Hap1	H 75	Mayotte (2), Zanzibar (8)
Viricel *et al.* 2016	KX905106	Hap2	H 58	Mayotte (1), Zanzibar (2)
Viricel *et al.* 2016	KX905107	Hap3	H 78	Zanzibar (2)
Viricel *et al.* 2016	KX905108	Hap4	H 60	Zanzibar (1)
Viricel *et al.* 2016	KX905109	Hap5	H 8	Mayotte (1), Zanzibar (1)
Viricel *et al.* 2016	KX905110	Hap6	H 79	Zanzibar (3)
Viricel *et al.* 2016	KX905111	Hap7	H 80	La Reunión (3), Zanzibar (1)
Viricel *et al.* 2016	KX905112	Hap8	H 81	Zanzibar (1)
Viricel *et al.* 2016	KX905113	Hap9	H 82	Zanzibar (1)
Viricel *et al.* 2016	KX905114	Hap11	H 83	Mayotte (2)
Viricel *et al.* 2016	KX905115	Hap12	H 84	Mayotte (1)
Viricel *et al.* 2016	KX905116	Hap13	H 85	Mayotte (3)
Viricel *et al.* 2016	KX905117	Hap14	H 74	Mayotte (1)
Viricel *et al.* 2016	KX905118	Hap15	H 86	Mayotte (1)
Viricel *et al.* 2016	KX905119	Hap16	H 87	Mayotte (1)
Viricel *et al.* 2016	KX905120	Hap17	H 75	Mayotte (1)
Viricel *et al.* 2016	KX905121	Hap18	H 76	Mayotte (2)
Viricel *et al.* 2016	KX905122	Hap19	H 88	Mayotte (1)
Viricel *et al.* 2016	KX905123	Hap20	H 89	Mayotte (1)
Viricel *et al.* 2016	KX905124	Hap22	H 89	Mayotte (1)
Viricel *et al.* 2016	KX905125	Hap23	H 90	La Reunión (4)
Viricel *et al.* 2016	KX905126	Hap24	H 91	La Reunión (1)
Viricel *et al.* 2016	KX905127	Hap25	H 92	La Reunión (3)
Viricel *et al.* 2016	KX905128	Hap26	H 93	La Reunión (1)
Viricel *et al.* 2016	KX905129	Hap27	H 94	La Reunión (1)
Viricel *et al.* 2016	KX905130	Hap28	H 95	La Reunión (1)
Viricel *et al.* 2016	KX905131	Hap29	H 26	La Reunión (1)
Viricel *et al.* 2016	KX905132	Hap30	H 1	La Reunión (1)