Phylogeography and genetic diversity of pantropical spotted dolphin (*Stenella attenuata*) and other delphinids in the Caribbean

Daniel Esteban Marin Munoz

Thesis for the title of: Magister in Biological sciences

Director:
Ph.D. Susana Caballero Gaitan

Co - director:
Ph.D. Jeremy Kiszka

Research area:
Genetics and Conservation Biology
Research laboratory:
Laboratorio de ecología molecular de vertebrados acuáticos (LEMVA)

Universidad de los Andes
Department of Biological Sciences
Bogota, Colombia
2024

Chapter I

Phylogeography and genetic diversity of pantropical spotted dolphin (*Stenella attenuata*) in the Caribbean Sea and Atlantic Ocean: Evidence for significant population structure

Daniel Marin [1*], Jeremy J. Kiszka¶[2], Susana Caballero¶[1]

[1] Departamento de Ciencias Biológicas, Universidad de los Andes, Laboratorio de Ecología Molecular de Vertebrados Acuáticos - LEMVA, Bogota, Colombia.

[2] Department of Biological Sciences, Florida International University, Florida, United States of America.

***Corresponding author:**

de.marin@uniandes.edu.co (DM)

Abstract

The pantropical spotted dolphin (*Stenella attenuata*) is a cetacean species with a broad distribution encompassing tropical waters. However, this species can exhibit geographic variations in coloration and morphology. Such is the case for the subspecies present in the Eastern Pacific, *S. attenuata graffmani*, with higher proportions of spots compared to populations in territories such as Hawaii. On the other hand, *S. attenuata* exhibits considerable abundance compared to other cetaceans in Caribbean regions such as the Gulf of Mexico and in Eastern Pacific regions [1–3]. Recent studies suggest that this species can have genetic structure associated to geographic location [4–7]. Here we assessed the population structure and genetic diversity of this species in the Caribbean, comparing samples originating from different geographic locations, including Guadeloupe (French West Indies), Saint Vincent and the Grenadines (SVG), Curaçao, Dominican Republic, Bahamas, Belize,

Florida (USS) and Gulf of Mexico. Sequences from the mitochondrial gene control region (CR) and 13 microsatellites were used to test the genetic population structure of individuals sampled across the region were compared with other sequences obtained from the genetic database GenBank. Significant genetic differentiation was determined between spotted dolphins from our main sampling locations, particularly SVG, Guadeloupe and other parts of the Western Atlantic. These results also show that the populations from SVG (where dolphins are locally hunted) present significant isolation from other populations sampled in the Caribbean. Also, individuals from Guadeloupe exhibit connectivity with populations from other islands in the Caribbean and the Western Atlantic. The results obtained in this study can provide new insights for the understanding of population dynamics of the pantropical spotted dolphin and can be used as a baseline for local conservation initiatives in the Caribbean.

Key words: control region (CR), conservation genetics, mitochondrial DNA, population structure, *Stenella attenuata*, stock.

Introduction

Genetic analyses can provide information related to population size, genetic diversity, natural history, and population structure. Such aspects are crucial in directing conservation and management decision for taxa that exhibits high dispersal potential, complex and elusive behavior, and harvesting [8–10]. The understanding of population structure in cetaceans is highly important to identify "management stocks" in different species, this concept refers to populations from the same species that present significant differentiation in some genetic markers, such as mitochondrial DNA. These genetic changes involve different conservation approaches in order to preserve genetic diversity. [11–13]. Population declines of different cetacean species, such as members of the family Delphinidae, has been of scientific interest in the past 30 decades because of bycatch in the purse-

seine fishery for yellow fin tuna in the Eastern tropical Pacific Ocean (ETP). This problem had considerable scientific studies and management actions in conservation of endangered species and promoted recovery of these cetaceans in the Pacific [14,15]. Nevertheless, scientific research of the population status of dolphins in the Caribbean and Atlantic Ocean is scarce and studies suggest that some populations maybe already substantially depleted or may have been eliminated due to the absence of genetic data and surveys [16,17]. In recent years, genetic studies have assumed a significant role in providing information for management strategies in conservation as a response for the threats to cetaceans worldwide [3,11,12].

The pantropical spotted dolphin (*Stenella attenuata*) is a widely distributed cetacean species inhabiting all tropical and subtropical waters. It can differ from the Atlantic spotted dolphin *Stenella frontalis* in external coloration and size, being *S. attenuata* a smaller dolphin [3]. However, phenotypic variations in coloration and morphology are observed across different regions. For instance, the subspecies documented in the Eastern Pacific, *S. attenuata graffmani*, exhibits a higher prevalence of spots compared to populations of conspecifics in other territories, such as Hawaii [18]. Additionally, *S. attenuata* exhibits considerable abundance compared to other cetaceans in regions like the Caribbean, such as the Gulf of Mexico, and the Eastern Pacific regions [1–3].

A primary threat to *S. attenuata* comes from incidental fishing or "bycatch", particularly associated with commercial tuna fisheries in the Eastern Pacific since the 1960s. Despite conservation measures, evidence of population recovery remains scant even after a three-decade period [19]. The most recent assessment by the IUCN in 2018 categorizes this species as of Least Concern (LC), with its population trend designated as "unknown" [20].

Although genetic differentiation in widely distributed marine species can be strongly reduced by the absence of geographical barriers and the homogeneity from pelagic ecosystems, reproductive

isolation may arise due to population settlement and adaptation to local conditions. This can induce genetic differentiation, and therefore, speciation [6]. Such is the case of the subspecies *Stenella attenuata graffmani*, distributed along the eastern Pacific coast, and *Stenella attenuata attenuata*, whose distributions occupy pelagic zones of the eastern Pacific, Indian Ocean, and western Pacific [18]. These two subspecies are currently recognized, and are included as geographical management units in the eastern Pacific, including the northeastern offshore, western–southern offshore, and coastal stocks [21]. The northeastern offshore and coastal stocks are listed as ‘‘Depleted’’ under the U.S. Marine Mammal Protection Act.

Conservation efforts for the populations of *S. attenuata* in the Caribbean remain low and reflects the absence of management unit designation in the Atlantic. This is strongly associated with the lack of information related to distribution, abundance, population structure and hunting of small cetaceans in the region. Some reports position the pantropical spotted dolphin as the most common species in the Caribbean [16,22]. Additionally, there is evidence of targeted hunting in some of the Caribbean islands, particularly Saint Vincent and the Grenadines, and the repercussions of this practice remain unknown [3,23].

There´s still a prevalence of reliance upon marine mammals as a food source in human societies, being the odontocetes the most popular in the Eastern Caribbean. Additionally, dolphin products are highly contaminated with mercury and represent a potential risk in health due to consumption [23]. Saint Vincent and the Grenadines (SVG) is a small independent Eastern Caribbean country that exhibits traditional and artisanal whaling practices that represent a potential risk for the genetic and population structure of this cetaceans in the Caribbean [24]. In this scenario, the repercussions of this practice as an additional threat for the population are unknown but points important considerations for the standardization of the genetic structure and conservation policies for *Stenella attenuata* and other small cetaceans in the Eastern Caribbean.

Genetic divergence for the pantropical spotted dolphin has been found in the Eastern Pacific using mitochondrial control region (CR) and microsatellites, evidencing population structure of both subspecies and management units in the Pacific and some islands such as Hawaii [5,6]. Additionally, recent studies using genome wide SNP analyses support these results [7,19]. Nevertheless, there are no studies on the genetic structure using genomic or mitochondrial DNA for *S. attenuata* in the Caribbean. Therefore, there is no information to support potential similarities between the patterns showed for the coastal and pelagic subspecies in the Pacific or the consequences for dolphin hunting in some of the Caribbean Islands such as Saint Vincent and the Grenadines.

The primary aim of this study was to provide initial insights related to the genetic diversity and population structure of *S. attenuata* in the Caribbean, working with continental (Belize, Florida US., Gulf of Mexico) and oceanic locations (Guadeloupe, Saint Vincent and the Grenadines, Curaçao, Dominican Republic and Bahamas), by analyzing fragments of the mitochondrial gene control region (CR) and microsatellites used in genetic studies for other cetacean species [25–30] . We confirmed species identity by barcoding and comparing the mitochondrial sequences present in the NCBI genetic database GenBank. Finally, we discuss our results as they relate to the potential of the existence of an independent stock of *S. attenuata* in SVG, and subsequently, for management implications [31–34].

Materials and Methods

Study area

Coastal and oceanic location across the Eastern Caribbean were assessed in this study. Particularly, the locations of Belize, Florida (US), Gulf of Mexico, and Gulf of Tribuga (COL) as coastal and the islands of Guadeloupe, Saint Vincent and the Grenadines, Antigua, and Barbuda, Curaçao,

Dominican Republic and Bahamas as oceanic. Some of these islands are geographically close to each other and present similar threads for cetacean species, such as artisanal fishing [3,20].

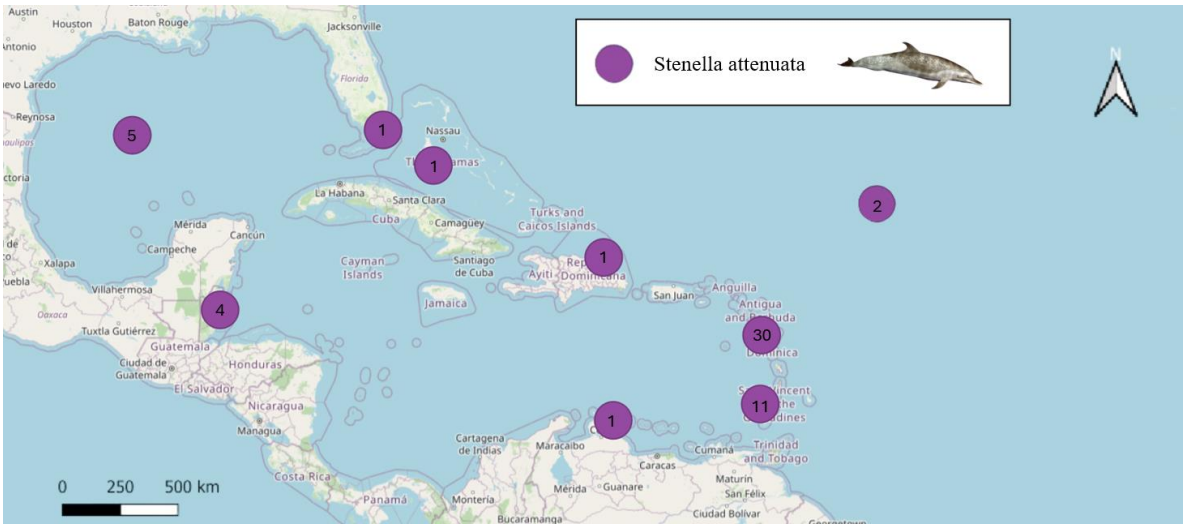

Fig 1. Map of sample locations from the Caribbean Islands in the Atlantic. The purple circles indicate the sampling site and the number of samples from each location. Map developed with QGIS.org (2024).

Sampling and DNA extraction

The samples obtained in each location were collected between May and September 2022. 11 samples were collected in Saint Vincent and the Grenadines (SVG) from artisanal whaling operations. Additionally, 46 samples were collected in the other locations (strandings, biopsies): Belize (4), Florida (1), Gulf of Mexico (5), Guadeloupe (30), Curaçao (1), Dominican Republic (1), Bahamas (1) and 2 samples from pelagic locations in the Atlantic NE (2). Additionally, one sample from the Pacific Coast of Colombia (Gulf of Tribugá) was included for comparison. Individuals were morphologically identified, and samples were labeled with the species determination for further genetic validation. DNA was extracted for a total of 57 samples for *Stenella attenuata* using the DNeasy Blood & Tissue Kit from QIAGEN (see manufacturer instructions).

Mt DNA CR amplification and sequencing

The mitochondrial control region (mtDNA CR) (approximately 500 bp) was amplified (n=57), using the primers Dlp1.5 (5'-TCACCCAAAGCTGRARTTCTA-3') and Dlp5 (5'-CCATCGWGATGTCTTATTTAAGRGGAA-3'), and conditions from Caballero et al. [35]. Successfully amplified PCR products were cleaned using magnetic beads and sequenced.

Microsatellite amplification and sequencing

Samples from *Stenella attenuata* were genotyped at up to 13 microsatellite loci, including previously published primers used for other cetacean and delphinid species: AAT44, MK3, MK5, MK6, MK8, MK9, KWM12, GT211, GT6, GT48, GT142, GT575, GT51 [27,28,36–38]. The loci were separated in three groups for amplification. The three multiplexes had the same conditions and consisted of 2 µL of genomic DNA, and 10 µL of Master Mix for PCR reaction, included both primers for each microsatellite, in a 12 µL reaction with following PCR profile: 93°C for 2 min followed by 15 cycles of 92°C for 30 s, 55°C for 45 s and 72°C for 50 s followed by another set of 20 cycles of 89°C for 30 s, 55°C for 45 s and 72°C for 50 s, with a final extension of 72°C for 15 min. All loci were run in Microchecker [39] to identify null alleles, missed genotyping and stutter bands.

Data analysis

MtDNA CR sequence analysis

Sequences were manually edited and aligned using Geneious Prime (www.geneious.com). To detect potential new haplotypes among the new samples included in this study, comparisons with previously defined haplotypes for each of the species analyzed were made with the haplotypes

(sequences) available from Genbank. New haplotypes were defined using MacClade [40]. Haplotype networks were constructed using the Median Joining Network (MJN) from the software PopART [41]. Test of population differentiation was made using an analysis of molecular variance (AMOVA) as implemented in Arlequin [42] based on conventional F_{ST} and Φ_{ST} statistics, using 10,000 random permutations. Haplotype (h) and nucleotide (π) diversity calculations were performed in the program Arlequin Vs. 3.5 [42].

Microsatellite analysis

Nuclear genetic diversity was evaluated by determining the total number of alleles (NA) and the average number of alleles per locus. Expected and observed heterozygosity (HE and HO), Hardy–Weinberg equilibrium and population differentiation tests were conducted in Arlequin v.3.5.1. Genetic differentiation among geographic locations was tested using pairwise F_{ST} values calculated in Arlequin v.3.5.1. Average genetic diversity (AGD), defined as the gene diversity over all loci in each population, was calculated in Arlequin v.3.5.1. Patterns of genetic structure were also evaluated for microsatellite markers using STRUCTURE v. 2.3 [43]. This software also evaluates different K values using population ancestry models. The most likely K was chosen using the Evanno Method, looking at ΔK by calculating the second order rate of change of LnP (D) between the values of K [44]. Fifteen replicates, with the "loc prior" criterion (i.e., using geographic locations as priors), were run in STRUCTURE and results were visualized in Structure Harvester [45].

Results

Mt DNA sequences were compared against *Stenella attenuata* Mt DNA CR sequences available in the NCBI database GenBank by using BLAST.

mtDNA Control Region (CR)

A fragment of 420 bp of the CR gene was obtained from 42 samples of *Stenella attenuata* from the following locations: Saint Vincent and the Grenadines (11), Belize (2), Florida (1), Gulf of Mexico (4), Gulf of Tribuga (1), Guadeloupe (20), Curaçao (1), Dominican Republic (1) and Bahamas (1). Samples that did not showed amplification were removed from the mtDNA analysis. All sequences obtained from the samples were manually aligned and compared with a set of 148 sequences from Stenella attenuata stored in GenBank. We identified 148 distinct haplotypes. Unique haplotypes from the locations of Saint Vincent and the Grenadines (H1, H2, H4, H70, H20, H21 H138, H55, H56, H103) were identified. H1 showed 12 mutational steps from a unique haplotype from the location of Guadeloupe; H4 exhibited 9 changes from H3, a central American haplotype. Most of these unique haplotypes were identified in only one individual. Nevertheless, this was not the case for H103, which was present in more than one individual. Some haplotypes were shared with more than one location, where Eastern Tropical Pacific (ETP), Guadeloupe and Central America were the most common; indicating maternal genetic flow between these locations. Other location that exhibited unique haplotypes in the network was Guadeloupe (H125, H8, H73, H74, H57, H53). Haplotypes like H125 and H8 were present in more than one individual. Guadeloupe exhibits a slightly gene flow with only central American locations, like the Gulf of Mexico, Florida and Saint Vincent and the Grenadines. H119 was the most common and probably the most ancestral haplotype, which included all the localities in the study and most of the localities from GenBank. 129 variable sites were identified (Table 1). The haplotype network obtained from PopART by the Median Joining Network method (MJN) showed non differentiated groups or clades but exhibited the presence of unique haplotypes in the locations of Saint Vincent and the Grenadines and Guadalupe suggesting genetic flow between similar locations in the Caribbean (Fig 3) meaning that these populations may stay constantly in the Caribbean and have low connectivity with other localities in the Atlantic and other oceans. For the statistical analyses we compared samples in 4

groups: Saint Vincent and the Grenadines (SVG), Guadeloupe (GU), Atlantic and the Caribbean (ATL) and Eastern Tropical Pacific (ETP). Overall haplotype diversity was h= 0.871. Nucleotide diversity (π) was low for GU, ATL and ETP with similar values close to 0.01%. SVG exhibited a higher value of 0.04% (Table 2).

Table 1. fragment of the complete dataset for 129 variable sites over 420 bp of the mitochondrial CR gene determining 148 haplotypes for *S. attenuata*. Some unique haplotypes for the locations in the study are shown.

Variable sites									
Haplotypes	68	84	86	87	93	94	95	96	99
H1	C	T	T	C	T	T	C	C	T
H2	A	.	.	T	C	A	C	A	C
H3	.	.	.	T	C	A	C	A	C
H4	.	C	.	T	C	A	C	A	C
H5	.	.	.	T	.	.	C	A	.
H6	.	.	.	.	.	.	C	A	.
H7	.	.	.	.	.	.	C	A	.
H8	.	.	.	.	.	.	C	A	.
H19	.	.	.	T	.	.	C	A	.
H20	.	.	.	T	.	.	C	A	.
H21	.	.	.	T	.	.	C	A	.
H52	.	.	.	T	.	.	C	A	.
H53	.	.	.	T	.	.	C	A	.
H54	.	.	.	T	.	.	C	A	.
H55	.	.	.	T	.	.	C	A	.
H56	.	.	.	T	.	.	C	A	.
H70	.	.	C	T	.	.	C	A	.
H71	.	.	.	T	.	.	C	A	.
H72	.	.	.	T	.	.	C	A	.
H73	.	.	.	T	.	.	C	A	.
H74	.	.	.	T	.	.	C	A	.
H138	.	.	.	T	.	.	C	A	.
H139	.	.	.	T	.	.	C	A	.
H140	.	.	.	T	.	.	C	A	.
H141	.	.	.	T	.	.	C	A	.
H142	.	.	.	T	.	.	C	A	.
H143	.	.	.	T	.	.	C	A	.
H144	.	.	.	T	.	.	C	A	.
H145	.	.	.	T	.	.	C	A	.
H146	.	.	.	T	.	.	C	A	.

H147	.	.	.	T	.	.	C	A	.
H148	.	.	.	T	.	.	C	A	.

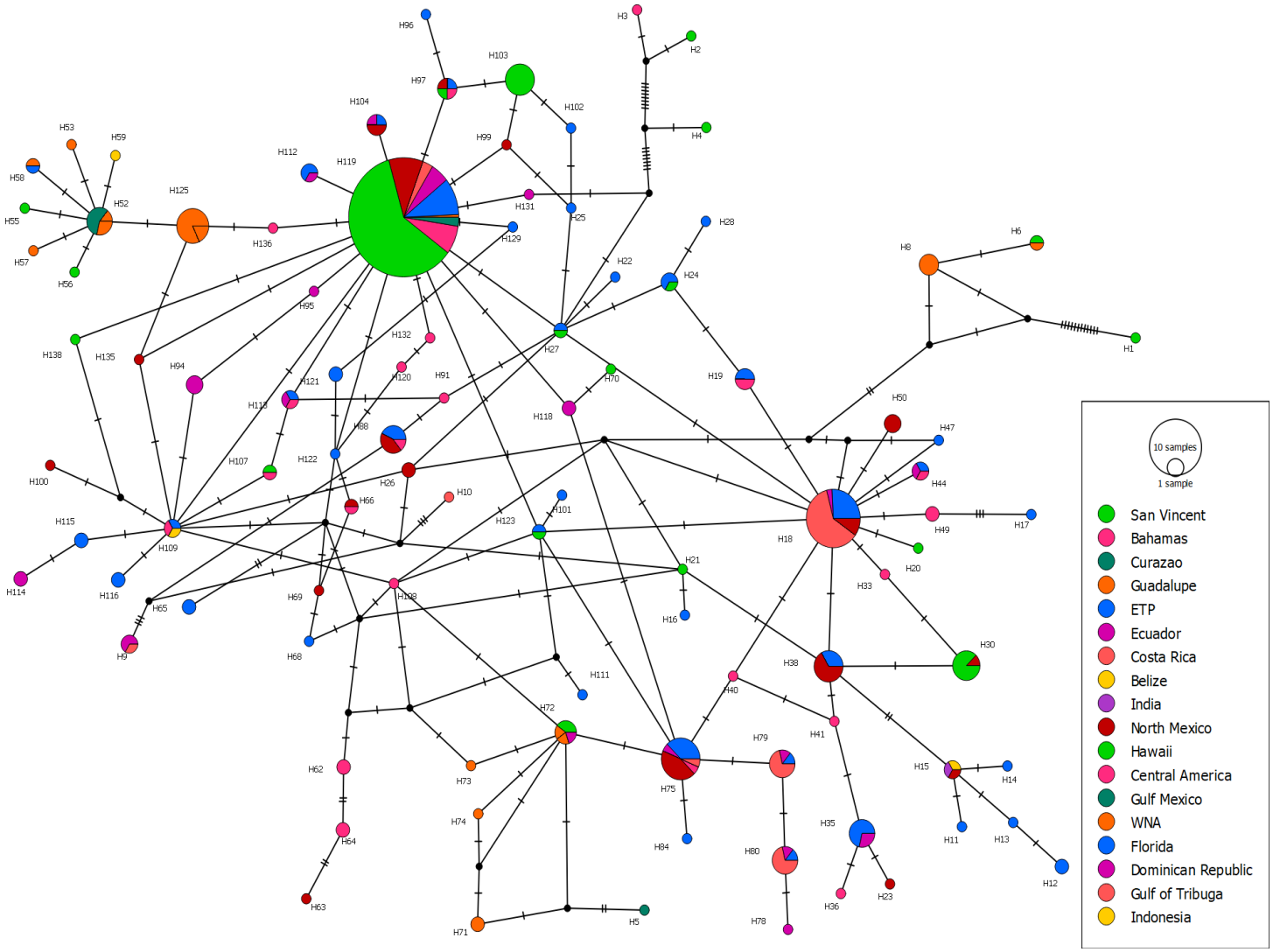

Fig 2. Haplotypic network of the mtDNA Control Region (CR) for *S. attenuata* Circles are sized proportional to haplotype frequency and color coded for location. Black dots represent haplotypes that are either extinct or not detected. Short lines across network represent one mutational change between nodes. Numbers with the letter H on each node indicates de corresponding haplotype number.

Pairwise estimates of F_{ST} values (Table 2) exhibited significant differentiation between all four populations listed. SVG presented higher values for F_{ST} with ATL and ETP; showing high variation rates between these site regardless the close geographical location of SVG and the rest of the Caribbean and the Atlantic. Additionally, GU and SVG had no significant pairwise F_{ST} . This may indicate similarities between the individual in both locations and the presence of potential current or historical gene flow between both islands.

Table 2. Pairwise F_{ST} values (above diagonal) and Φ_{ST} values (below diagonal) for the CR gene from Caribbean and Pacific populations of *S. attenuata* Saint Vincent and the Grenadines (SVG), Guadeloupe (GU), localities from the Atlantic and Caribbean (ATL) and localities from Genbank such as Eastern Tropical Pacific (ETP).

Φ_{ST} \ F_{ST}	SVG	GU	ETP	ECU	CRA	NMX	HWII	CAM	GFMX	WNA
SVG	h=1.0000 π=0.042789	**0.12177****	0.00945	0.01491	**0.17498*****	**0.05184***	**0.41684*****	0.03	**0.13896*****	0.00021
GU	0.08812	h= 0.7737 π=0.017822	**0.10424*****	**0.12149*****	**0.26236*****	**0.14138*****	**0.44121*****	**0.13391*****	**0.25*****	0.03229
ETP	**0.24383*****	**0.16379*****	h=0.9832 π=0.015430	0.00588	**0.13652*****	**0.0127*****	**0.22455*****	**0.0294*****	**0.11785*****	**0.0216****
ECU	**0.17632*****	**0.14575*****	**0.02726****	h=0.9729 π=0.015962	**0.14808*****	**0.05122*****	**0.34717*****	**0.03581*****	**0.13278*****	**0.02740***
CRA	**0.26662*****	**0.25951*****	**0.09002*****	**0.12578*****	h=0.7076 π= 0.011385	**0.18641*****	**0.18641*****	**0.03581*****	**0.13278*****	**0.02740*****
NMX	**0.24705*****	**0.18043*****	-0.00569	**0.05468*****	**0.11157*****	h=0.9090 π=0.013327	**0.19417*****	**0.06345*****	**0.16567****	**0.06355*****
HWII	**0.55451*****	**0.37064*****	**0.13501*****	**0.24710*****	**0.46174*****	**0.17293*****	h=0.4175 π=0.004741	**0.35404*****	**0.51132*****	**0.42147*****
CAM	**0.23631*****	**0.14253*****	**0.02011*****	**0.05290*****	**0.13502*****	0.02047	**0.16054*****	h=0.9462 π=0.012694	**0.14628*****	**0.04217****
GFMX	**0.16778****	0.12076	**0.20398*****	**0.18489*****	**0.43274*****	**0.25741*****	**0.41502*****	**0.21838*****	h=0.7143 π=0.007898	**0.10838***
WNA	**0.11444***	0.02927	**0.19545*****	**0.18215*****	**0.37029*****	**0.23542*****	**0.47761*****	**0.20827*****	**0.04448*****	h=0.9722 π=0.009749

Significant P values at <0.005*, <0.002 and < 0.001*****

The statistics derived from the AMOVA showed significant genetic differentiation between all four groups in the analysis (Φ_{ST}: 0.22210. P< 0.001)

Microsatellite analyses

After the determination of null alleles by Microchecker [39], two loci were removed from the original 13 microsatellites, leaving 11 usable loci for 56 samples (Table 3). Structure was performed under the admixture model because the populations are likely to have a common ancestor. K value was determined using Structure Harvester under the Evanno method (K=3). Arlequin tests were conducted using geographical and biological population units. In both scenarios, significant genetic structure was found between samples from Saint Vicent and the Grenadines, and the other two population units determined.

The populations identified by Structure were: (1) Guadeloupe (n= 30); (2) Dominican Republic/Antigua y Barbuda/Curaçao/Gulf of Mexico/Belize/Bahamas/Colombia/ North Atlantic (n= 16); and (3) Saint Vincent and the Grenadines (n= 10). The three populations were named as follows: Saint Vincent and the Grenadines (SVG), Guadeloupe (GU) and the cluster composed by the other locations was conceived as Caribbean (CAR).

Genetic diversity indexes were obtained for the eleven loci and were represented in the values of: Expected Heterozygosity (H_e), Observed Heterozygosity (H_o) and number of alleles per population (n). Microsatellites like AAT 44 had higher values in the populations of GU and SVG, while presented lower values in CAR. CAR and SVG had one significant locus out of HW equilibrium (MK9), which was the only case in the whole analyses. Additionally, in most of the loci H_e was significantly lower than H_o.

Pairwise population differentiation index F_{ST} was calculated for all sampling locations (Table 4). Pairwise F_{ST} between SVG and the other two populations were significant. This was not the case between GU and CAR. These results suggest current genetic flow between GU and CAR; and genetic isolation in the case of SVG.

As a response to the possible effects caused by having multiple alleles for all the loci, that usually affects the estimate of the F_{ST} value; Jost´s D was also calculated for each loci. This coefficient focuses on the fraction of allelic variation among populations [46].

Table 3. Genetic diversity for 11 nuclear microsatellites tested in the Caribbean.

Locus	GU	CAR	SVG
AAT 44	n = 2	n = 2	n = 3
	Ho = 0.96667	Ho = 0.78571	Ho = 0.90000
	He = 0.50791	He = 0.49471	He = 0.68947
GT6	n = 7	n = 4	n = 6
	Ho = 0.70000	Ho = 0.70000	Ho = 0.75000
	He = 0.75932	He = 0.74737	He = 0.84167
MK5	n = 6	n = 4	n = 4
	Ho = 0.58621	Ho = 0.55556	Ho = 0.55556
	He = 0.61283	He = 0.73856	He = 0.73856
MK6	n = 15	n = 12	n = 6
	Ho = 0.80000	Ho = 0.86667	Ho = 0.50000
	He = 0.86893	He = 0.93563	He = 0.84167
MK9	n = 3	n = 2	n = 1
	Ho = 0.05882	Ho = 0.00000	Ho = 0.00000
	He = 0.43672	He = 0.66667	He = 0.00000
GT211	n = 5	n = 4	n = 4
	Ho = 0.53846	Ho = 0.60000	Ho = 0.71429
	He = 0.59125	He = 0.53333	He = 0.64835
KWM12	n = 10	n = 10	n = 8
	Ho = 0.93333	Ho = 0.80000	Ho = 0.80000
	He = 0.82373	He = 0.84368	He = 0.86842
MK3	n = 8	n = 8	n = 4
	Ho = 0.60714	Ho = 0.58333	Ho = 0.33333

	He = 0.75584	He = 0.79710	He = 0.77273
GT575	n = 9	n = 5	n = 5
	Ho = 0.75862	Ho = 0.80000	Ho = 1.00000
	He = 0.78524	He = 0.75556	He = 0.79167
GT51	n = 8	n = 3	n = 6
	Ho = 0.58824	Ho = 0.50000	Ho = 0.55556
	He = 0.78610	He = 0.83333	He = 0.78431
MK8	n = 7	n = 8	n = 3
	Ho = 0.90000	Ho = 0.92308	Ho = 0.50000
	He = 0.82373	He = 0.87692	He = 0.73158

Fig 5. Barplot of the likelihood (Y-axis) of each individual´s assignment to a particular population unit for K = 3.

Table 4. Pairwise F_{ST} between populations of *S. attenuata* Saint Vincent and the Grenadines (SVG), Guadeloupe (GU), localities from the Atlantic and Caribbean (CAR).

F_{ST} / ΦST	GU	CAR	SVG
GU	-	0.00295	**0.03301*** **
CAR	-0.00286	-	**0.02052*** **
SVG	**0.07245*** **	**0.05769** **	-

Significant P values at <0.005*, <0.002 and < 0.001*****

Table 5. Pairwise Jost´s *D* between populations of *S. attenuata* Saint Vincent and the Grenadines (SVG), Guadeloupe (GU), localities from the Atlantic and Caribbean (CAR). Jost´s *D* values are shown below the diagonal and P values are shown above the diagonal.

Jost´s *D*	**GU**	**CAR**	**SVG**
GU	**-**	0.018	0.001
CAR	**0.079***	**-**	0.001
SVG	**0.534****	**0.431****	**-**

Significant P values at <0.005*, <0.002 and < 0.001*****

Discussion

The present study presents an initial approach for the understanding of genetic structure in populations of *Stenella attenuata* in the Caribbean. Additionally, these results are baseline information that can be helpful in further identifying changes in the population due to anthropogenic (hunting) and biological pressures (competition, resources and social structure), which are highly significant for small cetaceans [47]. In the case of the mitochondrial CR DNA analyses, significant pairwise F_{ST} values (Table 2) were detected when CAR and ETP populations were compared with SVG and GU populations, indicating population structure for SVG and GU. These results can be consistent with previous studies related to genetic structure in small cetaceans, where populations can have significant differentiation among coastal and pelagic populations [5,19,48,49]. The non-significant F_{ST} value resulted from comparisons between SVG and GU could indicate connectivity between both localities in terms of female migration at present or occurring in the past. Another possible explanation for these results could be the time of divergence present in these haplotypes which could be lower in comparison with the CAR and ETP populations. In the case of the microsatellite analyses, the results show a similar story. Pairwise F_{ST} values are highly significant when SVG is compared to CAR and GU (Table 4), showing strong genetic differentiation for the loci in this location. The differences found in these F_{ST} values for SVG and GU can be explained by the inclusion of microsatellites in the analyses, which provide greater

power to detect potential population differences [47]. Additionally, STRUCTURE analyses provided a significant differentiation between SVG with CAR and GU (Figure 5). In this case, the results support a well differentiated cluster composed by the samples from Saint Vincent and the Grenadines and some samples from Guadeloupe. The connectivity and gene flow seems to remain between Guadeloupe and the islands and regions from the Caribbean (Dominican Republic, Antigua y Barbuda, Curaçao, Gulf of Mexico, Belize, and Bahamas). These results support the idea of a well differentiated cluster in the location of SVG, where individuals seem to have a site fidelity in these regions. Previous studies in close related species like *Stenella longirostris* have shown that spotted dolphins can remain in coastal locations due to strong competition or resources availability in Atlantic islands [47]. Genetic diversity analyses for the different loci provided more evidence to support the significant genetic structure results. In the case of the SVG population, most of the loci presented Higher H_o values than H_e values (Table 3). These results can be understood as a reduction in genetic diversity for the microsatellites due likely to population isolation [50].

The case of management units for the conservation of subspecies of *Stenella attenuata* is present in locations in the Pacific, where two supported subspecies: Coastal (*S. a. graffmani*) and pelagic (*S. a. attenuata*). This differentiation has been supported by genetic approaches where population structure is evaluated and helped providing significant information for international conservation management. As a result, both populations are considered different stocks [6,19]. However, the presence of these two subspecies in the Atlantic and Caribbean cannot be confirmed, and this population remains as a single species with at least three management units. Studies in the Atlantic proposed that *S. attenuata* may have a genetic structure similar to the Pacific, where populations differentiate due to coastal and pelagic habits [5]. In this scenario, international fisheries like NOAA, have used this information to create different management stocks for *S. attenuata* in Hawaiian waters. There is evidence that the significance provided by F_{ST} values in the determination of population structure for cetaceans can work as a first approach to declare different stocks and

preserve the genetic diversity [5]. With our study, we consider that the populations of *S. attenuata* in the locations of 1) Saint Vincent and the Grenadines, 2) Guadeloupe and 3) the remaining locations (Dominican Republic/Antigua y Barbuda/Curaçao/Gulf of Mexico/Belize/Bahamas/Colombia/ North Atlantic) should be different management units for this species in the Caribbean.

Previous studies focused on cetacean monitoring have suggested that *S. attenuata* might be one of the most common species around the Caribbean islands such as Guadeloupe[16]. However, studies like Fieldings et al., [24] establish the lack of knowledge related to the structure of these species in the Caribbean and the islands in this region. Specifically, the authors highlight the absence of information focused on cetacean catches in countries like Saint Vincent and the Grenadines, where food products derived from cetaceans are consumed by most of the adult population. SVG and GU can figure as crucial locations for the development of conservation management to provide significant intervention in the reduction of potential threats for the *S. attenuata* populations in the Caribbean and the preservation of the unique stocks present in these locations.

Future studies focused on the understanding of the population structure for *S. attenuata* in the Caribbean should be further developed. In this case, future genetic studies with increased numbers of samples for the locations of Saint Vincent and the Grenadines, Guadeloupe and other island locations could provide new information in the understanding of the population dynamics for this species. It is also important to provide new genetic markers as well as wider genome analysis like SNP´s to develop deeper insights into the genetic flow and genetic differentiation between populations. Additionally, future research should focus on the origin of the individuals and the locations where they were sampled. This information could help in concluding if the differentiation between coastal and pelagic stocks is also present in the Caribbean an provide stronger arguments for the development of new conservation management for *S. attenuata* in the Caribbean.

Chapter II

Mitochondrial genetic diversity and population structure of delphinid species in Saint Vincent and the Grenadines: Implications for their management and conservation in the Caribbean

Daniel Marin [1*], Jeremy J. Kiszka [2], Susana Caballero [1]

[1] Departamento de Ciencias Biológicas, Universidad de los Andes, Laboratorio de Ecología Molecular de Vertebrados Acuáticos - LEMVA, Bogota, Colombia.

[2] Department of Biological Sciences, Florida International University, Florida, United States of America.

***Corresponding author:**

de.marin@uniandes.edu.co (DM)

Abstract

Members of the family Delphinidae exhibit significant population structure associated with geographic locations and complexity in their social behaviors. Such is the case of species like *Peponocephala electra* that presents genetic differentiation, strong social structure and population structure previously reported [51]. Here we assessed the population structure and genetic diversity of different members of the family Delphinidae (*Peponocephala electra, Lagenodelphis hosei, Stenella frontalis, Stenella clymene*) by comparing samples originating from Saint Vincent and the Grenadines with previous reports present in the genetic database GenBank . Sequences from the mitochondrial gene control region (CR) were used to test the genetic population structure of all species. Significant genetic differentiation was determined between delphinids present in SVG and the geographical locations in the Caribbean and Pacific. The results obtained in this study can

provide new insights for the understanding of population dynamics of some strongly elusive species and can be used as a baseline for local conservation initiatives in the Caribbean.

Key words: control region (CR), conservation genetics, mitochondrial DNA, population structure, delphinids, stock.

Introduction

Understanding the levels of genetic diversity and population structure is crucial for the proper implementation of management and conservation strategies [52]. Populations with higher levels of genetic diversity are more able to adapt to a constantly changing environment, exhibiting higher rates of heterozygosity than the populations with lower genetic diversity [53]. In order to respond to these significant items, conservation genetics rises as a potential strategy that focuses on the use of genetic techniques (DNA studies) to address questions ranging from taxonomic units to the establishment of conservation units across populations from different species [54]. These aspects are crucial for the conservation of species such as cetaceans that may have high migration rates, continuous fishing pressure across different territories, and difficulties in approaching due to their elusive behavior [9].

Cetaceans are characterized as highly migratory marine species that present wide geographic ranges with low presence of boundaries for genetic isolation. Nevertheless, populations of different species are able to adapt to local conditions and present genetic differentiation [6]. Such is the case of members of the family Delphinidae, which not only exhibit population structure associated with geographic locations but also the complexity in their social behaviors can significantly influence the degree of reproductive isolation they may present. Recent studies suggest that species like *Peponocephala electra* can have significant genetic differentiation associated with their strong social structure and population structure previously reported [51]. In this scenario, species like the

melon headed whales could present genetic isolation between coastal and pelagic populations due to resources availability and strong competition between individuals from the same and different populations. Similar cases can be found in species like short-finned pilot whales *Globicephala macrorhynchus* and the pygmy killer whales *Feresa attenuata* where there is a evident differentiation in mitochondrial markers between populations; showing significant variations across coastal and pelagic environments[55,56].

Direct hunting and consumption are some of the most significant threads for marine mammal species [57]. Despite the reduction of global consumption, there is evidence that small cetaceans remain endangered as a direct consequence of the increase of direct hunting in these groups. Additionally, the lack of knowledge of population structure for the wide variety of odontocete cetacean species involved in extractive fishing processes for subsistence in Caribbean regions is evident [23]. Some initial studies have been published for a limited number of species, including the bottlenose dolphin *Tursiops truncatus* [58], the Atlantic spotted dolphin *Stenella frontalis* [34,59]. These studies have found significant genetic differentiation for some species (*T. truncatus and G. macrorhynchus*), but for others, evidence of current gene flow within the Caribbean has been found (*Stenella frontalis*), yet significant differentiation between Caribbean populations and those from other areas such as the Madeira and Azores islands [59].

Conservation efforts for the maintenance of cetacean species across the Caribbean are still low. The principal cause is the absence of information related to abundance, and genetic structure for highly threatened cetaceans in the Caribbean region. This is the case of some of the islands present in this region, such as Saint Vincent and the Grenadines (SVG) [3,23]. This territory exhibits strong whaling operations directed to small cetaceans in the main island, where species like pilot whales (*G. macrorhynchus*), killer whales (*Orcinus orca*) and other members of the family Delphinidae are targeted for human consumption [23]. The main risk of these practices is the absence of information

related to the impact that they induce in the populations of cetaceans and the changes in the genetic diversity across the Caribbean. This also creates future repercussions in the local communities that depend on these activities.

The primary aim of this study was to provide preliminary insights related to the mitochondrial genetic diversity and population structure of four small cetaceans in the Caribbean: *Stenella frontalis, Peponocephala electra, Lagenodelphis hosei* and *Stenella clymene;* This was done by analyzing fragments of the mitochondrial control region (CR) (500 bps). We confirmed species identity by barcoding and comparing the mitochondrial sequences present in the NCBI genetic database GenBank. Finally, we discuss our results as initial information related to the mitochondrial genetic diversity for these cetacean species and the opportunity to provide new insights for future conservation management in SVG and the Caribbean.

Materials and Methods

Study area

The SVG island an Eastern Caribbean country conceived as an archipelago due to the presence of five small, inhabited islands connected to the main one (Saint Vincent). This location possesses a strong whaling culture developed by artisanal fishermen for local human consumption [24].

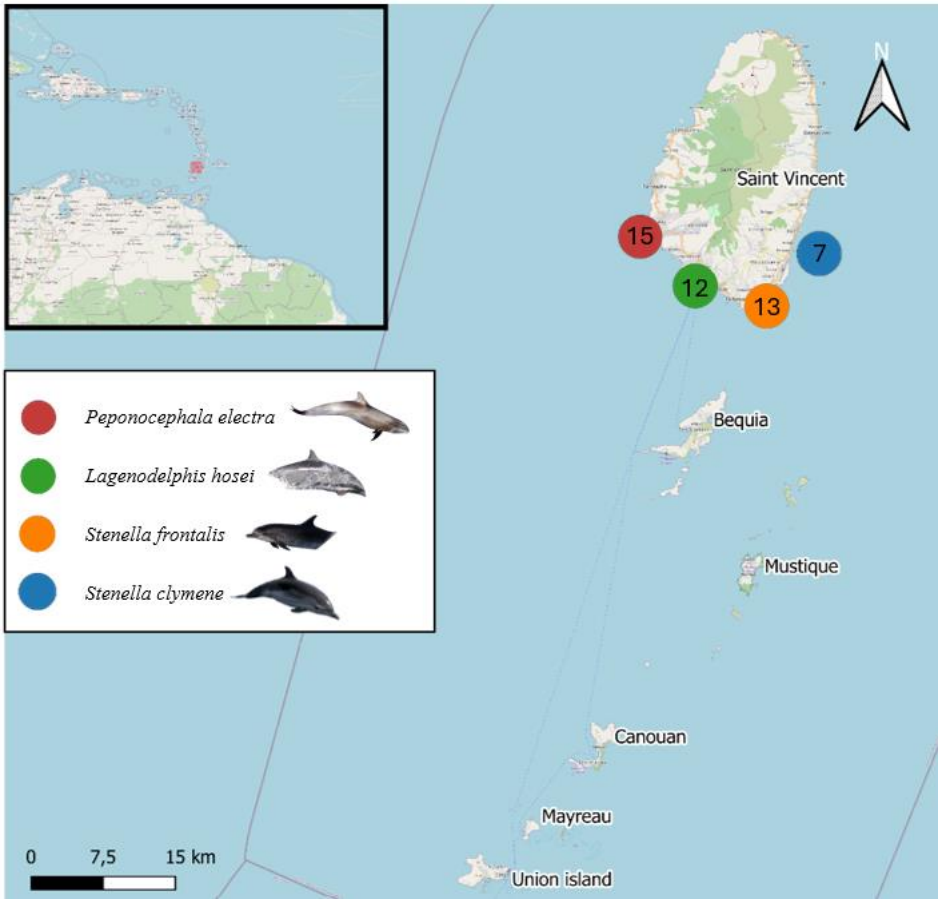

Fig 1. Map of sample locations by species from SVG. The colored circles indicate the sampling site and the number of samples for each species. Map developed with QGIS.org (2024).

Sampling and DNA extraction

The samples obtained in SVG were collected between June 2022 and August 2022. Samples were collected in Saint Vincent and the Grenadines (SVG) from artisanal fishing or strandings for different cetacean species as follows: *Peponocephala electra* (n=15), *Lagenodelphis hosei* (n=12), *Stenella frontalis* (n=13), *Stenella clymene* (n=7) (Fig 1). Individuals were morphologically identified, and samples were labeled with the species identification for further genetic validation. DNA was extracted for all samples (n=47) using the DNeasy Blood & Tissue Kit from QIAGEN (see manufacturer instructions).

Mt DNA CR amplification and sequencing

The mitochondrial control region (mtDNA CR) (approximately 500 bp) was amplified using the primers and conditions from Caballero et al. [35]. Successfully amplified PCR products were cleaned using magnetic beads and sequenced.

Data analysis

MtDNA CR sequence analysis

Sequences were manually edited and aligned using Geneious Prime (www.geneious.com). To detect potential new haplotypes among the samples included in this study, comparisons with previously defined haplotypes for each of the species analyzed were made with the haplotypes (sequences) available from Genbank. New haplotypes were defined using MacClade [40]. Haplotype networks were constructed using the Median Joining Network (MJN) from the software PopART [41]. Test of population differentiation were conducted using an analysis of molecular variance (AMOVA) as implemented in Arlequin [42] based on conventional F_{ST} and Φ_{ST} statistics, using 10,000 random permutations. Haplotype (h) and nucleotide (π) diversity calculations were performed in the software Arlequin Vs. 3.5 [42].

Results

Mt DNA sequences were compared against Mt DNA CR sequences for each species available in the NCBI database GenBank by using BLAST.

mtDNA Control Region (CR)

A fragment between 340 bp and 400 bp of the CR gene was obtained from 47 samples of *Peponocephala electra, Lagenodelphis hosei, Stenella frontalis* and *Stenella clymene* from Saint Vincent and the Grenadines. Samples that did not showed amplification were removed from the mtDNA analysis. All sequences obtained from the samples were manually aligned and compared with sequences from the same mitochondrial marker stored in GenBank.

Peponocephala electra

A total of 67 sequences from mtDNA CR of 420 bp (including samples from GenBank and locations) were obtained and analyzed. We identified 26 distinct haplotypes. Unique haplotypes from the locations of Saint Vincent and the Grenadines (H1, H3, H6) were identified (Figure 2). H3 showed two mutational changes from a possible ancestral haplotype present in SVG and shared by other territories in the Caribbean, such as Bahamas and Puerto Rico and H1 showed 10 mutational changes from the same haplotype. Most of these unique haplotypes were identified in only one individual. H2 was present in individuals from different locations across the Caribbean, showing a potential ancestral haplotype from the Caribbean and Atlantic Ocean. Additionally, this could indicate current or historical maternal genetic flow between these locations. In the case of the Pacific populations, H14 and H9 were predominant in abundance for different locations. Like in the case of H2, this may indicate potential ancestral haplotypes for the Pacific populations. The haplotype network obtained from PopART by the Median Joining Network method (MJN) showed slightly differentiated groups, separating haplotypes from the Caribbean in a particular cluster. In the case of the locations from the Pacific, genetic structure could be present by the prevalence of two nodes composed by samples from central Pacific. Current or historical maternal genetic flow

may be present between some populations from the Atlantic (Hawaii, Brazil, Western North Atlantic) with localities in the Pacific (Mediterranean, Central Pacific), this can be confirmed in the haplotypes shared by these locations, like H9, H14 and H13 (Figure 2). Overall haplotype diversity was $h = 0.8116$. Nucleotide diversity (π) remained in the ranges between 0.004% (ETP) to 0.01% (SVG) (Table 2).

Table 1. Pairwise F_{ST} values (above diagonal) and Φ_{ST} values (below diagonal) for the CR gene from Caribbean and Pacific populations of *P. electra* Saint Vincent and the Grenadines (SVG), localities from the Atlantic and Caribbean (ATL) and localities from Genbank such as Eastern Tropical Pacific (TP).

ΦST \ F_{ST}	SVG	BAHM	NP	HWII	WNA	INDO	CEP	SP
SVG	h=1.0000 π=0.042789	**0.51183*** **	0.00637	**0.12627*** **	-0.08942	**0.35484*** **	**0.37069*** **	**0.26775*** **
BAHM	**0.3949*** **	h= 0.7737 π=0.017822	**0.83981** **	**0.36554*** **	**0.68789** **	**0.72892*** **	**0.5179*** **	**0.56696*** **
NP	-0.03741	**0.83981** **	h=0.9832 π=0.015430	**0.24832*** **	0	0.70732	**0.51376*** **	**0.47988*** **
HWII	**0.35274*** **	**0.45453*** **	0.17214	h=0.9729 π=0.015962	0.10397	**0.23043*** **	**0.23025*** **	**0.11338*** **
WNA	-0.04432	**0.80187*** **	0	**0.20998***	h=0.7076 π= 0.011385	0.42857	**0.39658*** **	0.27826
INDO	**0.25085***	**0.77095*** **	0.83099	**0.44277*** **	**0.72691***	h=0.9090 π=0.013327	**0.34817*** **	**0.34644*** **
CEP	**0.41886*** **	**0.43235*** **	0.14887	**0.1455*** **	0.20425	**0.37236*** **	h=0.4175 π=0.004741	**0.21397*** **
SP	**0.20096*** **	**0.66973*** **	0.23567	0.0088	0.2486	**0.53976*** **	**0.11339***	h=0.9462 π=0.012694

Significant P values at <0.005*, <0.002 and < 0.001*****

The statistics derived from the AMOVA showed significant genetic differentiation between all four groups in the analysis (Φ_{ST}: 0.26732, $p < 0.001$)

Table 2. fragment of the complete dataset for 35 variable sites over 430 bp of the mitochondrial CR gene determining 26 haplotypes for *P. electra*. Some unique haplotypes for the locations in the study are shown.

Variable sites								
Haplotypes	25	28	33	72	74	84	89	96
H1	A	A	C	T	C	C	C	C
H2	.	.	A	.	T	T	T	.
H3	C	.	.	.	.	.	.	.
H4	.	.	.	.	.	.	.	.
H5	.	.	.	.	.	.	.	.
H6	.	.	.	.	.	.	C	.
H7	.	.	.	C	.	.	.	.
H8	.	.	.	.	.	.	.	.
H9	.	.	.	.	.	.	.	.
H10	.	.	.	.	.	.	.	.
H11	.	.	.	.	.	.	.	T
H12	.	.	.	.	.	.	.	.
H13	.	.	.	.	.	.	.	.
H14	.	.	.	.	.	.	.	.
H15	.	.	.	C	.	.	.	.
H16	.	.	.	.	.	.	.	.
H17	.	.	.	.	.	.	.	.
H18	.	.	.	.	.	.	.	.
H19	.	.	.	.	.	.	.	.
H20	.	.	.	.	.	.	.	.
H21	.	.	.	.	.	.	.	.
H22	.	.	.	.	.	.	.	.
H23	.	.	.	.	.	.	.	.
H24	.	.	.	.	.	.	.	.
H25	.	G	.	.	.	.	.	.
H26	.	.	.	.	.	.	.	.

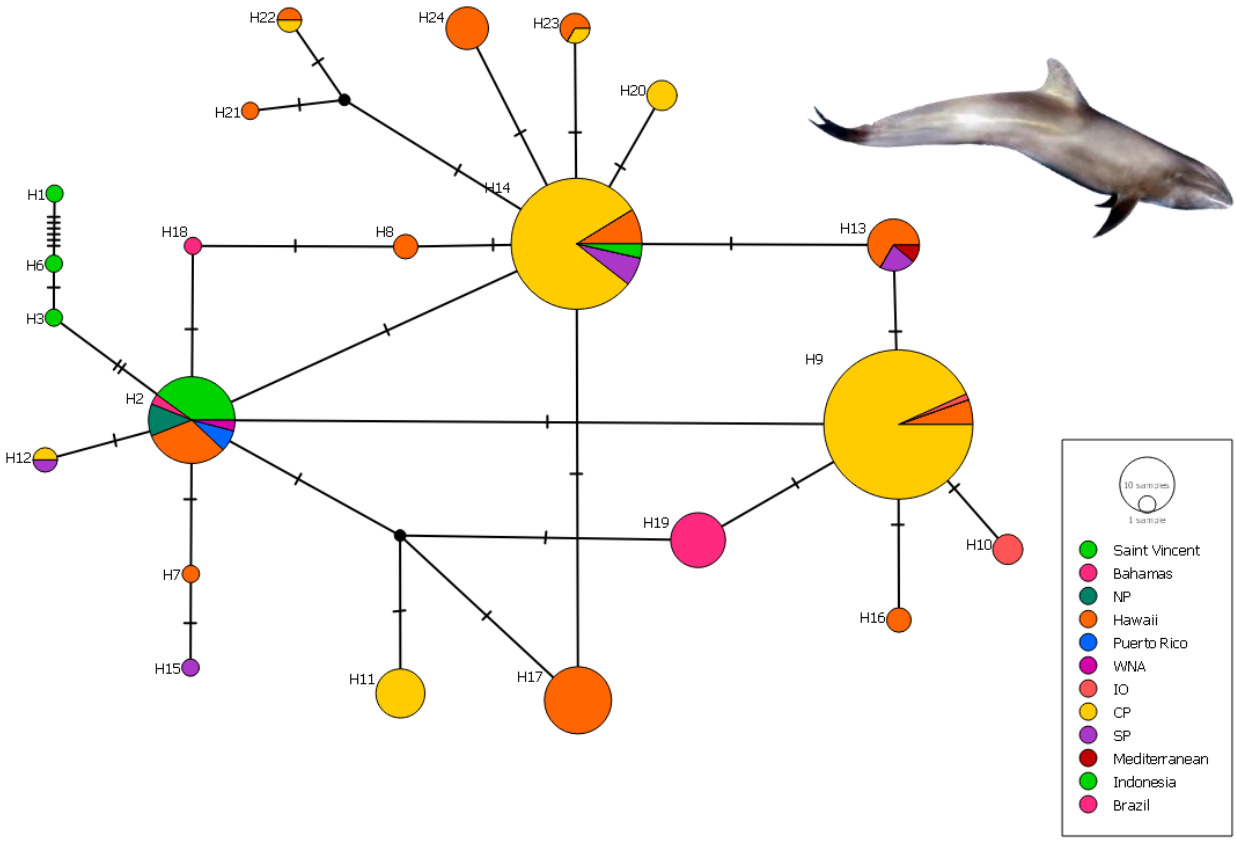

Fig 2. Haplotypic network of the mtDNA Control Region (CR) for *P. electra* Circles are sized proportional to haplotype frequency and color coded for location. Black dots represent haplotypes that are hypothesized to exist but were not detected. Short lines across network represent one mutational change between nodes. Numbers with the letter H on each node indicates de corresponding haplotype number. (NP: North Pacific, WNA: Western North Atlantic, CP: Central Pacific, SP: South Pacific).

Lagenodelphis hosei

A total of 112 sequences from mtDNA CR (420 bp) (including samples from GenBank and other locations) were obtained and analyzed. 42 variable sites were identified (Table 3). 54 haplotypes were identified between the samples from Saint Vincent and the samples from GenBank. Unique haplotypes from the locations of Saint Vincent and the Grenadines (H2, H3, H48, H49, H50) were identified. H2 showed six mutational steps from H49, another unique haplotype from the region that was present in more than one individual. H51 was a haplotype shared by locations of Saint Vincent and Japan. Most of these unique haplotypes were identified in only one individual. H19, H42 were haplotypes shared by locations from the Pacific (Taiwan, Philippines) and the Caribbean (Saint Vincent and the Grenadines and Puerto Rico). The haplotype network shows a significant separation by five mutational steps of some haplotypes from Japan, Taiwan, and most of the samples from Saint Vincent and the Grenadines from another set of haplotypes from Japan and Taiwan. This may indicate genetic variation between populations of *L. hosei* in Japan and Taiwan. The haplotype network obtained from PopART by the Median Joining Network method (MJN) showed two differentiated groups or clades between populations from the Pacific Ocean (Japan, Taiwan) and suggested historical or current gene flow between one of these clades with specific regions in the Atlantic, like Puerto Rico and Saint Vincent and the Grenadines (Figure 3). This means that there could be genetic differentiation in populations of *L. hosei* in Japan and Taiwan. It also shows likely past connectivity between Caribbean and Pacific populations. For the statistical analyses we compared samples in three groups: Saint Vincent and the Grenadines (SVG), Atlantic and the Caribbean (ATL) and Tropical Pacific (TP). Overall haplotype diversity was $h = 0.9666$. Nucleotide diversity (π) was low for all three populations with values around 0.01%. Pairwise F_{ST} values were significant between individuals from SVG and ATL; but were not significantly different from the populations from the Pacific (Table 4).

Table 3. fragment of the complete dataset for 42 variable sites over 430 bp of the mitochondrial CR gene determining 53 haplotypes for *L. hosei*. Some unique haplotypes for the locations in the study are shown.

Variable sites																
Haplotypes	25	26	27	49	78	99	102	108	130	141	151	197	198	227	238	241
H1	T	C	A	G	A	A	T	.	.	.	T	C	C	T	T	A
H2	.	.	.	A	G	.	.	.	.	.	.	.	T	.	C	.
H3	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.
H4	C	T	C	.	.	.	.	.	.	.	.	.	.	.	.	.
H5	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
H6	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
H7	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
H8	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.
H9	.	.	.	A	.	.	.	T	.	.	.	.	T	.	.	.
H10	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
H11	.	.	.	A	.	.	.	T	.	.	.	.	T	.	.	.
H12	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.
H13	.	.	.	.	.	.	.	.	G	.	.	.	T	.	.	.
H14	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.
H15	.	.	.	.	.	.	C	.	.	.	G	T	.	.	.	.
H16	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.
H17	.	.	.	.	.	.	.	.	.	G	.	.	T	.	.	.
H18	.	.	.	A	.	.	.	T	.	.	.	.	T	.	.	.
H19	.	.	.	A	G	.	.	.	.	.	.	.	T	.	C	.
H20	.	.	.	.	.	.	C	.	.	.	.	.	T	.	.	.
H21	.	.	.	A	G	G	.	.	.	.	.	.	T	.	C	.
H22	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	G
H23	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.
H24	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H25	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.
H26	.	.	.	A	G	G	.	.	.	.	.	.	T	.	C	.
H27	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.
H28	.	.	.	A	G	.	.	.	.	.	.	.	T	.	C	.
H29	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.
H30	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
H31	.	.	.	A	.	.	.	T	.	.	.	.	T	.	.	.
H32	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.
H33	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
H34	.	.	.	A	G	G	.	.	.	.	.	.	T	.	C	.
H35	.	.	.	A	G	.	.	.	.	.	.	.	T	.	C	.

H36	.	.	.	.	.	.	C	.	.	.	.	.	.	.	C	.
H37	.	.	.	A	.	.	.	T	.	.	.	.	T	.	.	.
H38	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
H39	.	.	.	A	.	.	.	T	.	.	.	.	T	.	C	.
H40	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.
H41	.	.	.	A	.	.	.	T	.	.	.	.	T	.	.	.
H42	.	.	.	A	.	.	.	T	.	.	.	.	T	.	.	.
H43	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H44	.	.	.	A	G	.	.	.	.	.	.	.	T	.	C	.
H45	.	.	.	A	.	.	.	T	.	.	.	.	T	.	.	.
H46	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H47	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H48	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.
H49	.	.	.	A	G	.	.	.	.	.	.	.	T	.	C	.
H50	.	.	.	A	.	.	.	T	.	.	.	.	T	.	.	.
H51	.	.	.	A	G	.	.	.	.	.	.	.	T	.	C	.
H52	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.
H53	.	.	.	A	.	.	.	T	.	.	.	.	T	.	.	.

Table 4. Pairwise F_{ST} values (above diagonal) and Φ_{ST} values (below diagonal) for the CR gene from Caribbean and Pacific populations of *L. hosei* Saint Vincent and the Grenadines (SVG) , localities from the Atlantic and Caribbean (ATL) and localities from Genbank such as Eastern Tropical Pacific (ETP).

ΦST \ F_{ST}	SVG	JPN	PHLP	HWII	WNA	PR
SVG	h=1.0000 π=0.042789	**0.0391*** **	0.02877	0.02913	**0.12023***	0.02703
JPN	**0.08635*** **	h= 0.7737 π=0.017822	**0.03024***	0.01279	**0.13687*** **	0.0065
PHLP	-0.01383	0.02871	h=0.9832 π=0.015430	0.02812	**0.15773*** **	-0.02564
HWII	-0.05003	-0.08511	-0.10388	h=0.9729 π=0.015962	0.18644	0
WNA	0.10404	-0.02614	-0.03143	-0.10053	h=0.7076 π= 0.011385	0.16667
PR	0.07081	0.05436	-0.00075	-0.11087	-0.14493	h=0.9090 π=0.013327

Significant P values at <0.005*, <0.002 and < 0.001*****

The statistics derived from the AMOVA showed significant genetic differentiation between all four groups in the analysis (Φ_{ST}: 0.02678, p< 0.005)

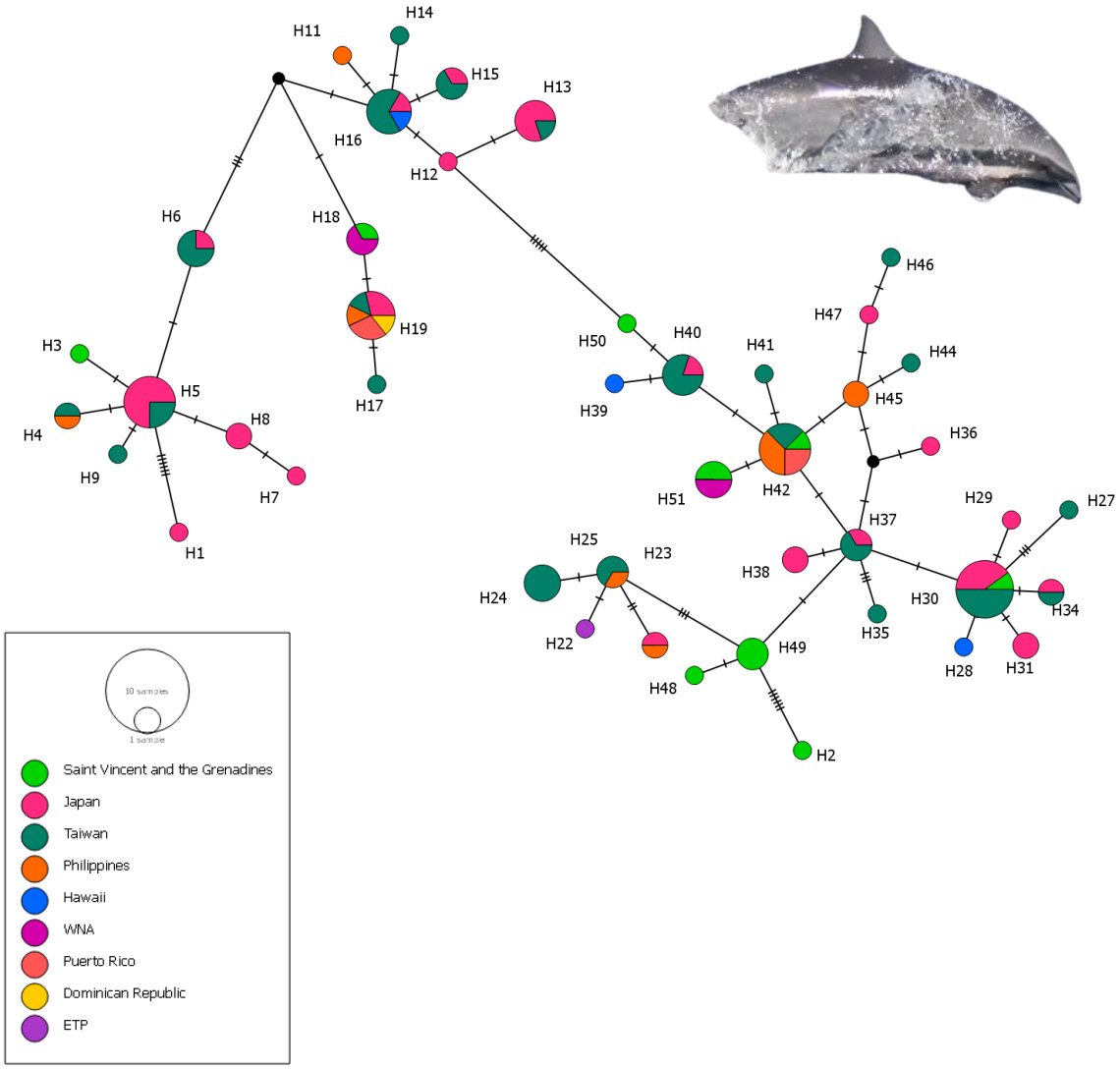

Fig 3. Haplotypic network of the mtDNA Control Region (CR) for *L. hosei* Circles are sized proportional to haplotype frequency and color coded for location. Black dots represent haplotypes that are hypothesized to exist but were not detected. Short lines across network represent 1 mutational change between nodes. Numbers with the letter H on each node indicates de corresponding haplotype number. (WNA: Western North Atlantic, ETP: Eastern Tropical Pacific).

Stenella frontalis

A total of 154 sequences from mtDNA CR (420 bp) (including samples from GenBank and other locations) were obtained and analyzed. 75 haplotypes were identified between the samples from Saint Vincent and the samples from GenBank. (Figure 4) Genetic differentiation between samples from Caribbean/Pacific and SVG was present in the network with 11 mutational steps for unique haplotypes (H1, H2, H3, H11) from SVG and one haplotype shared with Uruguay and Portugal (H10). In general, the network might not have a particular genetic differentiation between most of the population analyzed. Additionally, haplotype H6 was the most frequent in all the samples and was present in most of the populations in the analyses; this may indicate that this haplotype is the most ancestral in the network. Overall haplotype diversity was h= 0.9235. Nucleotide diversity (π) was 0.05% for SVG and 0.013% for ATL. Pairwise F_{ST} values were significant between SVG and ATL, indicating highly significant differentiation between both populations (Table 3).

Table 5. fragment of the complete dataset for 53 variable sites over 430 bp of the mitochondrial CR gene determining 75 haplotypes for *S. frontalis* Some unique haplotypes for the locations in the study are shown.

Variable sites								
Haplotypes	49	56	64	78	86	88	97	98
H1	T	A	G	T	C	T	T	T
H2	.	.	.	.	.	.	.	.
H3	.	.	.	.	.	.	.	.
H4	.	.	.	.	.	.	C	A
H5	G	.	.	.	G	.	C	A
H6	.	.	.	.	.	.	C	A
H7	.	.	.	.	.	.	C	A
H8	.	.	.	G	.	.	C	A
H9	.	.	.	.	.	.	C	A
H10	.	.	.	.	.	.	.	.
H11	.	.	.	.	.	.	.	.
H12	.	.	.	.	.	.	C	A
H13	.	.	.	.	.	.	C	A
H14	.	.	.	.	.	.	C	A

H15	.	.	.	.	.	.	C	A
H16	.	G	.	G	.	.	C	A
H17	.	G	.	.	.	.	C	A
H18	.	.	.	G	.	.	C	A
H19	.	.	A	.	.	C	T	A
H20	.	.	.	.	.	.	C	A
H21	.	.	.	.	.	.	C	A
H22	.	.	.	.	.	.	C	A
H23	.	.	.	.	.	.	C	A
H24	.	.	.	.	.	.	C	A
H25	.	.	.	.	.	.	C	A
H26	.	.	.	.	.	.	C	A
H27	.	.	.	.	.	.	C	A
H28	.	.	.	.	.	.	C	A
H29	.	.	.	.	.	.	C	A
H30	.	.	.	.	.	.	C	A
H31	.	.	.	.	.	.	C	A
H32	.	.	.	.	.	.	C	A
H33	.	.	.	.	.	.	C	A
H34	.	.	.	.	.	.	C	A
H35	.	.	.	.	.	.	C	A
H36	.	.	.	.	.	.	C	A
H37	.	.	.	.	.	.	C	A
H38	.	.	.	.	.	.	C	A
H39	.	.	.	.	.	.	C	A

Table 6. Pairwise F_{ST} values (above diagonal)) and Φ_{ST} values (below diagonal) for the CR gene from Caribbean and Pacific populations of *S. frontalis* Saint Vincent and the Grenadines (SVG), localities from the Atlantic and Caribbean (ATL).

ΦST \ F_{ST}	SVG	WNA	UR	GMX	PORT	SPN
SVG	h=1.0000 π=0.042789	0.0155	**0.16551*** **	0.01181	0.0101	**0.12312***
WNA	**0.37128*** **	h= 0.7737 π=0.017822	**0.16483*** **	**0.01522** **	**0.05055*** **	**0.117*** **
UR	**0.46284*** **	**0.06751*** **	h=0.9832 π=0.015430	**0.15313*** **	**0.2456*** **	**0.33979*** **
GMX	**0.30468*** **	**0.03561***	**0.07302*** **	h=0.9729 π=0.015962	**0.04501*** **	**0.1225** **
PORT	0.13851	**0.06214***	**0.24421*** **	**0.13459*** **	h=0.7076 π= 0.011385	**0.17197***
SPN	0.06718	0.09692	0.30363	0.10773	0.00678	h=0.9090 π=0.013327

Significant P values at <0.005*, <0.002 and < 0.001*****

The statistics derived from the AMOVA showed significant genetic differentiation between all four groups in the analysis (Φ_{ST}: 0.16709, P< 0.001)

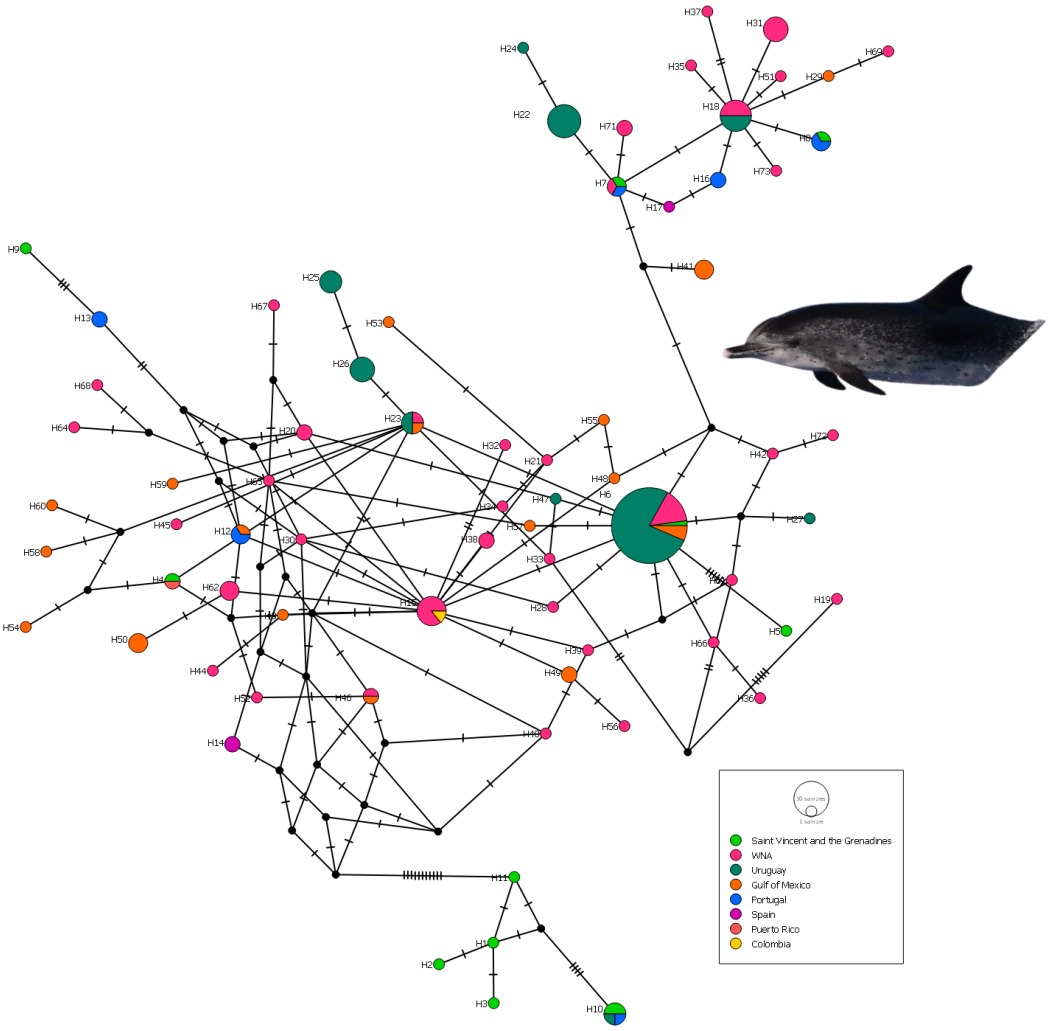

Fig 4. Haplotypic network of the mtDNA Control Region (CR) for *S. frontalis* Circles are sized proportional to haplotype frequency and color coded for location. Black dots represent haplotypes that are likely extinct or not detected. Short lines across network represent 1 mutational change between nodes. Numbers with the letter H on each node indicates de corresponding haplotype number. (WNA: Western North Atlantic).

Stenella clymene

A total of 21 sequences from mtDNA CR (420 bp) (including samples from GenBank and locations) were obtained and analyzed. Most of the samples from Saint Vincent (SV) were identified as new haplotypes for this location and were different from the sequences in GenBank from Western North Atlantic (WNA). One

haplotype was shared in samples from Saint Vincent and WNA (Figure 5). 6 unique haplotypes were identified in the analyses (H1-H7). Haplotypes H6 and H3 presented four and nine mutational changes with other nodes from the network, suggesting genetic isolation in both cases. Because of the low number of samples obtained from *S. clymene*, all haplotypes were present in only one individual. This scenario causes that potential ancestral and predominant haplotypes in the Caribbean population remain unknown. Increasing the sample size might provide better understanding in the genetic structure for this species. The haplotypes for SVG apparently show no genetic isolation from the samples of WNA. Overall haplotype diversity was h= 0.9952. Nucleotide diversity (π) was 0.03% for SVG and 0.022% for ATL. Pairwise F_{ST} values were significant between SVG and ATL, indicating significant differentiation between both populations (Table 4).

Table 4. Pairwise F_{ST} values (above diagonal)) and Φ_{ST} values (below diagonal) for the CR gene from Caribbean populations of *S. clymene* Saint Vincent and the Grenadines (SVG) and localities from the Atlantic and Caribbean (ATL).

Φ_{ST} \ F_{ST}	**SVG**	**WNA**
SVG	h=1.0000 π=0.042789	-0.01031
WNA	**0.08360***	h= 0.7737 π=0.017822

Significant P values at <0.005*, <0.002 and < 0.001*****

The statistics derived from the AMOVA were not computed or significant due to the lower number of samples for comparison.

Table 5. fragment of the complete dataset for 43 variable sites over 430 bp of the mitochondrial CR gene determining 20 haplotypes for *S. clymene* Some unique haplotypes for the locations in the study are shown.

Variable sites								
Haplotypes	45	59	71	75	85	92	99	101
H1	C	G	A	A	A	T	C	C
H2	.	.	.	.	.	.	.	.
H3	T	A	.	.	G	.	T	.
H4	.	.	.	.	.	.	.	.
H5	T	.	.	.	G	.	.	.
H6	.	.	.	.	.	.	.	.
H7	.	.	.	.	.	.	.	.
H8	.	.	.	.	.	.	.	.
H9	.	.	.	.	.	.	.	.
H10	.	.	.	.	.	.	.	.
H11	.	.	.	.	.	.	.	T
H12	.	.	.	.	.	.	.	.
H13	T	.	.	.	G	.	.	.
H14	.	.	.	.	.	.	.	.
H15	.	.	.	.	.	.	.	.
H16	.	.	T	C	.	.	.	.
H17	.	.	.	.	.	.	.	.
H18	.	.	.	C	.	.	.	.
H19	.	.	.	.	.	.	.	.
H20	T	.	.	.	G	A	.	.

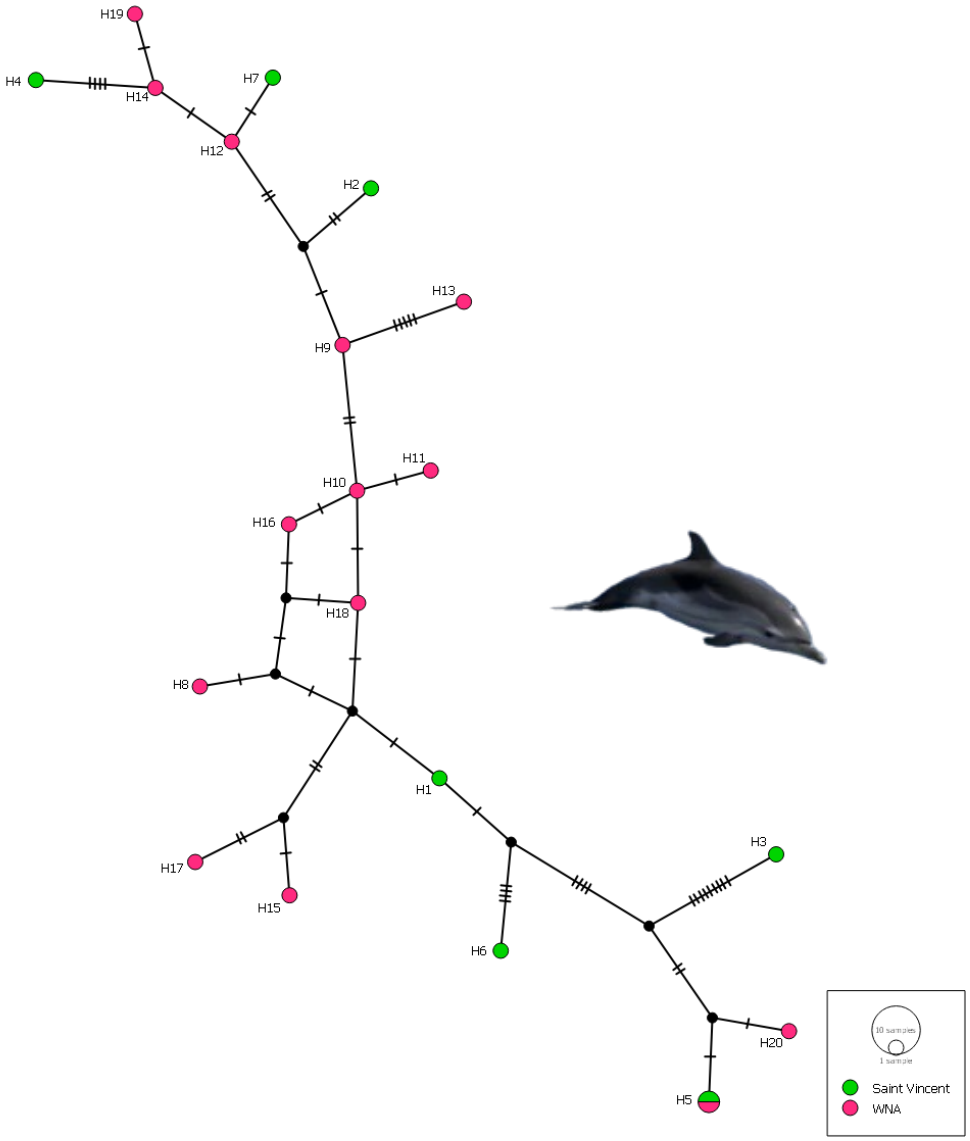

Fig 5. Haplotypic network of the mtDNA Control Region (CR) for *S. clymene* Circles are sized proportional to haplotype frequency and color coded for location. Black dots represent haplotypes that are likely extinct or not detected. Short lines across network represent 1 mutational change between nodes. Numbers with the letter H on each node indicates de corresponding haplotype number. (WNA: Western North Atlantic).

Discussion

Peponocephala electra

Previous studies suggested that strong differentiation between populations might be found in *Peponocephala electra*, like other closely related species. The scenario found in this study suggests high likelihood of past or present connectivity between most of the populations from the Pacific and the Atlantic. The results mentioned are similar to the data obtained by Martien et al., [51]. Nevertheless, in the case of the data obtained from Saint Vincent and the Grenadines, the results show a strong genetic differentiation between haplotypes from SVG and the rest of the Atlantic and Pacific; this is also supported by unique haplotypes from the region with significant mutational changes from potentially ancestral Atlantic nodes (H2) (Figure 2). Comparing these results with the conclusions proposed by previous authors, we might consider the hypothesis of a more complex structure for populations of *P. electra* in the Atlantic and the Caribbean islands. A possible explanation for the difference in our results is that suggested by Martien et al., [51] in which the Atlantic region could have a low sample number which could explain their results in the haplotypic network. Additionally, our network suggests the presence of potential ancestral haplotypes (H14, H2) present in different locations from the Pacific and the Atlantic. Because of the mt DNA origin, these nodes could provide information associated to ancestral divergence between populations. F_{ST} values were significant between all three populations, being the comparisons of SVG with ATL and ETP the most significant (Table 1). These results suggest that there is an internal genetic divergence in the Atlantic, showing possible local establishment for populations of *P. electra*. Additionally, this information could be consistent with previous studies in small cetaceans that propose scenarios for high competition over food resources between species and populations from the same species [47]. In this case, small locations could present small populations competing over fewer resources, which could lead to genetic isolation across island regions. It is important to clarify that this study only used mt DNA CR as a genetic marker. This only source of information could provide a fragment of the whole story of the genetic isolation in this species, being associated with maternal gene flow and previous events of divergence. Nuclear information could bring more insights for more recent

events of genetic isolation and migration of both males and females in the population. This conclusion could also be an explanation for the differences in our results with previous studies that used microsatellite information[51].

Lagenodelphis hosei

Previous studies focused on population and demographic surveys propose that, despite the low sample size found in Atlantic regions, there is a significant increase in the numbers for locations like Madeira and Azores archipelagos. As a conclusion for these reports, researchers consider the Fraser´s dolphin a vagrant species in the Caribbean region [60]. Additionally, information related to their social behavior and geographical distribution consider pots composed by 50 individuals with small habitat usage [60]. In terms of genetic structure, the lack of information for the populations in the Atlantic complicates the understanding of genetic flow between populations in the different Caribbean locations. The only information associated with genetic structure for this species is focused on the Pacific regions, being Japan and Taiwan the most studied locations in the analyses[61]. The study proposes genetic isolation for different populations in the pacific region. Some of the explanations for this genetic isolation are associated with the low range of distribution for some populations due to resources availability and competition [61].

Our results suggest that there may be some past or current gene flow between Pacific populations such as Japan and Taiwan with the Caribbean regions. Nevertheless, our haplotype network shows a significant differentiation between haplotypes from Japan and Taiwan, showing that this likely gene flow between Pacific and Atlantic could be present with some of the genetically differentiated populations in the Pacific, while others remain isolated. The F_{ST} values show significant genetic isolation between populations from the Atlantic and SVG. Additionally, results suggest connectivity between SVG and the Pacific region. Some of these results could be explained by ancestral gene flow associated with the mt DNA CR. In this case, more nuclear information could provide new insights to understand current genetic flow and isolation between these three populations.

Stenella frontalis

Our genetic diversity analyses and haplotypic network (Figure 4) suggest that there might not be a strong genetic structure for populations of *S. frontalis* in the Caribbean. These results are supported by the number of connections between haplotypes in the network. These results differ from previous studies that suggest that there is a strong genetic differentiation between different locations across the Atlantic [34]. A possible explanation for the differences in results could be the number of samples obtained in the study and the sampling location information. In this case, our analysis used sequences from GenBank; but in some scenarios, most of the samples didn't have the specific geographic location in which these were collected. As a result of this lack of geographic information, most of the Atlantic samples were identified as a WNA. Nevertheless, the samples obtained from Saint Vincent and the Grenadines were separated from the other haplotypes by 11 mutational changes, showing significant genetic differentiation in terms of mt DNA. These results are also supported by significant F_{ST} value when SVG and ATL were compared.

The strong genetic differentiation is consistent with previous studies that suggest the presence of an oceanic population with genetic differentiation with more coastal populations [34]. Do Amaral et al., [34] identified unique haplotypes from the location of Bahamas in the Caribbean, which presented differentiation from all populations conceived in the study. Additionally, they propose a possible explanation for these strong differences associated with the philopatry in dolphins in regions like Bahamas [62,63]. Associated with these results found in an island location in the Caribbean region for *S. frontalis*, we suggest that there may be a similar scenario associated with female philopatry in SVG.

Stenella clymene

The first study for the population structure for *S. clymene* in the Caribbean was published in 2017 and provided important information for the population dynamics for this species in the Caribbean [64]. In the article, the authors conclude that there might be three well defined populations in the Atlantic (North Atlantic, South Atlantic and Gulf of Mexico). The results that supported these conclusions were related to significant genetic isolation y mt DNA CR and Cytochrome *b*. This was also supported by phylogenetic analyses. In this study, we did not find significant genetic structure in the Atlantic region. This could be explained by the number of sequences used for the analyses and the possible lack of information associated to the sampling locations. All the data from the Atlantic was conceived as WNA. However, genetic diversity estimates provide information associated with the possible presence of a genetic differentiation in the samples from SVG when compared with the samples from WNA. In this case, more data should be provided to understand the exact tendencies of the genetic structure for this species in the Caribbean.

In synthesis, the present study provides new insights for the genetic structure in different species in the Caribbean. Specifically, bring new information for Saint Vincent and the Grenadines, a particular location that hasn't been studied and could have a highly significant importance for conservation of different cetacean species due to the genetic isolation present in the region.

References

1. Ballance, L. T., & Pitman, R. L. (1998). CETACEANS OF THE WESTERN TROPICAL INDIAN OCEAN: DISTRIBUTION, RELATIVE ABUNDANCE, AND COMPARISONS WITH CETACEAN COMMUNITIES OF TWO OTHER TROPICAL ECOSYSTEMS. *Marine Mammal Science*, *14*(3), 429-459. https://doi.org/10.1111/j.1748-7692.1998.tb00736.x

2. Jefferson, T. A., & Schiro, A. J. (1997). Distribution of cetaceans in the offshore Gulf of Mexico. *Mammal Review*, *27*(1), 27-50. https://doi.org/10.1111/j.1365-2907.1997.tb00371.x

3. Perrin, W. F. (2001). Stenella attenuata. *Mammalian Species*, *683*, 1-8. https://doi.org/10.1644/1545-1410(2001)683<0001:SA>2.0.CO;2

4. Yao, C.-J., Yamada, T. K., Chen, Y.-J., & Chou, L.-S. (2008). Cranial Variation in the Pantropical Spotted Dolphin, Stenella attenuata, in the Pacific Ocean. *Zoological Science*, *25*(12), 1234-1246. https://doi.org/10.2108/zsj.25.1234

5. Courbis, S., Baird, R. W., Cipriano, F., & Duffield, D. (2014). Multiple Populations of Pantropical Spotted Dolphins in Hawaiian Waters. *Journal of Heredity*, *105*(5), 627-641. https://doi.org/10.1093/jhered/esu046

6. Escorza-Treviño, S., Archer, F. I., Rosales, M., Lang, A., & Dizon, A. E. (2005). Genetic differentiation and intraspecific structure of Eastern Tropical Pacific spotted dolphins, Stenella attenuata, revealed by DNA analyses. *Conservation Genetics*, *6*(4), 587-600. https://doi.org/10.1007/s10592-005-9013-9

7. Leslie, M. S., Archer, F. I., & Morin, P. A. (2019). Mitogenomic differentiation in spinner (Stenella longirostris) and pantropical spotted dolphins (S. attenuata) from the eastern

tropical Pacific Ocean. *Marine Mammal Science*, *35*(2), 522-551. https://doi.org/10.1111/mms.12545

8. Andrews, K. (2014). Population Genetics in the Conservation of Cetaceans and Primates. En J. Yamagiwa & L. Karczmarski (Eds.), *Primates and Cetaceans* (pp. 289-308). Springer Japan. https://doi.org/10.1007/978-4-431-54523-1_15

9. De Oliveira, L. R., Loizaga De Castro, R., Cárdenas-Alayza, S., & Bonatto, S. L. (2012). Conservation genetics of South American aquatic mammals: An overview of gene diversity, population structure, phylogeography, non-invasive methods and forensics. *Mammal Review*, *42*(4), 275-303. https://doi.org/10.1111/j.1365-2907.2011.00201.x

10. Hoelzel, A. R. (1992). Conservation genetics of whales and dolphins. *Molecular Ecology*, *1*(2), 119-125. https://doi.org/10.1111/j.1365-294X.1992.tb00163.x

11. Waples, R. (1998). Separating the wheat from the chaff: Patterns of genetic differentiation in high gene flow species. *Journal of Heredity*, *89*(5), 438-450. https://doi.org/10.1093/jhered/89.5.438

12. Taylor, B. L., & Dizon, A. E. (1996). The Need to Estimate Power to Link Genetics and Demography for Conservation. *Conservation Biology*, *10*(2), 661-664. https://doi.org/10.1046/j.1523-1739.1996.10020661.x

13. Caballero, S., Hollatz, C., Rodríguez, S., Trujillo, F., & Baker, C. S. (2018). Population Structure of Riverine and Coastal Dolphins Sotalia fluviatilis and Sotalia guianensis: Patterns of Nuclear and Mitochondrial Diversity and Implications for Conservation. *Journal of Heredity*. https://doi.org/10.1093/jhered/esy049

14. Gerrodette, T., & Forcada, J. (2005). Non-recovery of two spotted and spinner dolphin populations in the eastern tropical Pacific Ocean. *Marine Ecology Progress Series*, *291*, 1-21. https://doi.org/10.3354/meps291001

15. Hedley, C. (2010). The 1998 Agreement on the International Dolphin Conservation Program: Recent Developments in the Tuna-Dolphin Controversy in the Eastern Pacific Ocean. *Ocean Development & International Law*. https://doi.org/10.1080/00908320150502203

16. Boisseau, O., Leaper, R., & Moscrop, A. (2006). Observations of small cetaceans in the Eastern Caribbean. *2006*.

17. Ortiz-Wolford, J. S., Corona-Figueroa, M. F., Machuca Coronado, O. H., & Giró Petersen, A. (2022). Cetacean sightings in the Caribbean Sea of Guatemala. *Latin American Journal of Aquatic Mammals*. https://doi.org/10.5597/lajam00279

18. Perrin, W. F. (2009). Pantropical Spotted Dolphin: Stenella attenuata. En W. F. Perrin, B. Würsig, & J. G. M. Thewissen (Eds.), *Encyclopedia of Marine Mammals (Second Edition)* (pp. 819-821). Academic Press. https://doi.org/10.1016/B978-0-12-373553-9.00192-9

19. Leslie, M. S., & Morin, P. A. (2016). Using Genome-Wide SNPs to Detect Structure in High-Diversity and Low-Divergence Populations of Severely Impacted Eastern Tropical Pacific Spinner (Stenella longirostris) and Pantropical Spotted Dolphins (S. attenuata). *Frontiers in Marine Science, 3*. https://www.frontiersin.org/articles/10.3389/fmars.2016.00253

20. Kiszka, J., & Braulik, G. (2018). IUCN Red List of Threatened Species: Stenella attenuata. *IUCN Red List of Threatened Species*. https://www.iucnredlist.org/en

21. Dizon, A. E., Perrin, W. F., & Akin, P. A. (s. f.). Stocks of Dolphins (Stenella spp. And Delphinus delphis) in the Eastern Tropical Pacific: A Phylogeographic Classification. *1994*.

22. Vidal, O. (1994). Cetaceans and gillnet fisheries in Mexico, Central America and the Wider Caribbean: A preliminary review. *1994*.

23. Fielding, R., & Kiszka, J. J. (2021). Artisanal and Aboriginal Subsistence Whaling in Saint Vincent and the Grenadines (Eastern Caribbean): History, Catch Characteristics, and Needs for Research and Management. *Frontiers in Marine Science, 8*. https://www.frontiersin.org/articles/10.3389/fmars.2021.668597

24. Fielding, R. (2022). Whalers in "A Post-Whaling World": Sustainable Conservation of Marine Mammals and Sustainable Development of Whaling Communities—With a Case Study from the Eastern Caribbean. *Sustainability*, *14*(14), 8782. https://doi.org/10.3390/su14148782

25. Tardy, C., Planes, S., Jung, J.-L., Ody, D., & Boissin, E. (2020). Characterization of 25 new microsatellite markers for the fin whale (Balaenoptera physalus) and cross-species amplification in other cetaceans. *Molecular Biology Reports*, *47*(9), 6983-6996. https://doi.org/10.1007/s11033-020-05757-0

26. Krützen, M., Valsecchi, E., Connor, R. C., & Sherwin, W. B. (2001). Characterization of microsatellite loci in *Tursiops aduncus*. *Molecular Ecology Notes*, *1*(3), 170-172. https://doi.org/10.1046/j.1471-8278.2001.00065.x

27. Rosel, P. E., France, S. C., Wang, J. Y., & Kocher, T. D. (1999). Genetic structure of harbour porpoise Phocoena phocoena populations in the northwest Atlantic based on mitochondrial and nuclear markers. *Molecular Ecology*, *8*(s1), S41-S54. https://doi.org/10.1046/j.1365-294X.1999.00758.x

28. Valsecchi, E., & Amos, W. (1996). Microsatellite markers for the study of cetacean populations. *Molecular Ecology*, *5*(1), 151-156. https://doi.org/10.1111/j.1365-294X.1996.tb00301.x

29. Dewoody, J., Nason, J. D., & Hipkins, V. D. (2006). Mitigating scoring errors in microsatellite data from wild populations. *Molecular Ecology Notes*, *6*(4), 951-957. https://doi.org/10.1111/j.1471-8286.2006.01449.x

30. Green, M. L., Herzing, D. L., & Baldwin, J. D. (2011). Reproductive success of male Atlantic spotted dolphins (Stenella frontalis) revealed by noninvasive genetic analysis of paternity. *Canadian Journal of Zoology*, *89*(3), 239-253. https://doi.org/10.1139/Z10-111

31. Loizaga, R., Caballero, S., Cunha, H. A., Gravena, W., Herrerra-Trujillo, O., Lopes, F., Milmann, L., Ott, P. H., Pérez-Alvarez, M. J., Túnez, J. I., Durante, C. A., & Oliveira, L. R. de. (2023). Latin American aquatic mammals: An overview of 12 years focusing on

molecular techniques applied to conservation. *Latin American Journal of Aquatic Mammals*, *18*(1), Article 1. https://doi.org/10.5597/lajam00303

32. Piboon, P., Poommouang, A., Buddhachat, K., Kaewmong, P., Kittiwattanawong, K., & Nganvongpanit, K. (2022). The first study of genetic diversity and population structure of Indo- Pacific bottlenose dolphin (Tursiops aduncus) and pantropical spotted dolphin (Stenella attenuata) in the Thai Andaman Sea based on ISSR. *Veterinary World*, 2004-2011. https://doi.org/10.14202/vetworld.2022.2004-2011

33. Faria, D. M., Steel, D., Baker, C. S., Da Silva, J. M., De Meirelles, A. C. O., Souto, L. R. A., Siciliano, S., Barbosa, L. A., Secchi, E., Di Tullio, J. C., De Oliveira, L. R., Ott, P. H., & Farro, A. P. C. (2022). Mitochondrial diversity and inter-specific phylogeny among dolphins of the genus Stenella in the Southwest Atlantic Ocean. *PLOS ONE*, *17*(7), e0270690. https://doi.org/10.1371/journal.pone.0270690

34. Do Amaral, K. B., Barragán-Barrera, D. C., Mesa-Gutiérrez, R. A., Farías-Curtidor, N., Caballero Gaitán, S. J., Méndez-Fernandez, P., Santos, M. C. O., Rinaldi, C., Rinaldi, R., Siciliano, S., Martín, V., Carrillo, M., De Meirelles, A. C. O., Franco-Trecu, V., Fagundes, N. J. R., Moreno, I. B., Lacey Knowles, L., & Amaral, A. R. (2021). Seascape Genetics of the Atlantic Spotted Dolphin (*Stenella frontalis*) Based on Mitochondrial DNA. *Journal of Heredity*, *112*(7), 646-662. https://doi.org/10.1093/jhered/esab050

35. Caballero, S., Trujillo, F., Vianna, J. A., Barrios-Garrido, H., Montiel, M. G., Beltrán-Pedreros, S., Marmontel, M., Santos, M. C., Rossi-Santos, M., Santos, F. R., & Baker, C. S. (2007). Taxonomic Status of the Genus Sotalia: Species Level Ranking for "Tucuxi" (sotalia Fluviatilis) and "Costero" (sotalia Guianensis) Dolphins. *Marine Mammal Science*, *23*(2), 358-386. https://doi.org/10.1111/j.1748-7692.2007.00110.x

36. Krützen, M., Valsecchi, E., Connor, R. C., & Sherwin, W. B. (2001). Characterization of microsatellite loci in Tursiops aduncus. *Molecular Ecology Notes*, *1*(3), 170-172. https://doi.org/10.1046/j.1471-8278.2001.00065.x

37. Caldwell, M., Gaines, M. S., & Hughes, C. R. (2002). Eight polymorphic microsatellite loci for bottlenose dolphin and other cetacean species. *Molecular Ecology Notes*, *2*(4), 393-395. https://doi.org/10.1046/j.1471-8286.2002.00270.x

38. Cunha, H. A., & Watts, P. C. (2007). Twelve microsatellite loci for marine and riverine tucuxi dolphins (Sotalia guianensis and Sotalia fluviatilis). *Molecular Ecology Notes*, *7*(6), 1229-1231. https://doi.org/10.1111/j.1471-8286.2007.01839.x

39. Van Oosterhout, C., Hutchinson, W. F., Wills, D. P. M., & Shipley, P. (2004). MICRO - CHECKER: Software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, *4*(3), 535-538. https://doi.org/10.1111/j.1471-8286.2004.00684.x

40. Maddison, D. R., & Maddison, W. P. (2000). To order or for more information contact Sinauer Associates, Inc., Sunderland, Massachu- setts 01375 U.S.A, or on the web at http://sinauer.com. *2000*.

41. Leigh, J. W., & Bryant, D. (2015). POPART: Full-feature software for haplotype network construction. *Methods in Ecology and Evolution*, *6*(9), 1110-1116. https://doi.org/10.1111/2041-210X.12410

42. Excoffier, L., & Lischer, H. E. L. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, *10*(3), 564-567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

43. Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of Population Structure Using Multilocus Genotype Data. *Genetics*, *155*(2), 945-959. https://doi.org/10.1093/genetics/155.2.945

44. Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software structure: A simulation study. *Molecular Ecology*, *14*(8), 2611-2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

45. Earl, D. A., & vonHoldt, B. M. (2012). STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, *4*(2), 359-361. https://doi.org/10.1007/s12686-011-9548-7

46. Meirmans, P. G., & Hedrick, P. W. (2011). Assessing population structure: FST and related measures. *Molecular Ecology Resources*, *11*(1), 5-18. https://doi.org/10.1111/j.1755-0998.2010.02927.x

47. Andrews, K. R., Karczmarski, L., Au, W. W. L., Rickards, S. H., Vanderlip, C. A., Bowen, B. W., Gordon Grau, E., & Toonen, R. J. (2010). Rolling stones and stable homes: Social structure, habitat diversity and population genetics of the Hawaiian spinner dolphin (*Stenella longirostris*): GENETIC STRUCTURE OF HAWAIIAN SPINNER DOLPHIN. *Molecular Ecology*, *19*(4), 732-748. https://doi.org/10.1111/j.1365-294X.2010.04521.x

48. Oremus, M., Poole, M., Steel, D., & Baker, C. (2007). Isolation and interchange among insular spinner dolphin communities in the South Pacific revealed by individual identification and genetic diversity. *Marine Ecology Progress Series*, *336*, 275-289. https://doi.org/10.3354/meps336275

49. Parsons, K. M., Durban, J. W., Claridge, D. E., Herzing, D. L., Balcomb, K. C., & Noble, L. R. (2006). POPULATION GENETIC STRUCTURE OF COASTAL BOTTLENOSE DOLPHINS (*TURSIOPS TRUNCATUS*) IN THE NORTHERN BAHAMAS. *Marine Mammal Science*, *22*(2), 276-298. https://doi.org/10.1111/j.1748-7692.2006.00019.x

50. Waldick, R. C., Kraus, S., Brown, M., & White, B. N. (2002). Evaluating the effects of historic bottleneck events: An assessment of microsatellite variability in the endangered, North Atlantic right whale. *Molecular Ecology*, *11*(11), 2241-2249. https://doi.org/10.1046/j.1365-294X.2002.01605.x

51. Martien, K., Hancock-Hanser, B., Baird, R., Kiszka, J., Aschettino, J., Oremus, M., & Hill, M. (2017). Unexpected patterns of global population structure in melon-headed whales

Peponocephala electra. *Marine Ecology Progress Series, 577*, 205-220. https://doi.org/10.3354/meps12203

52. Allendorf, F. W. (2017). Genetics and the conservation of natural populations: Allozymes to genomes. *Molecular Ecology*, *26*(2), 420-430. https://doi.org/10.1111/mec.13948

53. Frankham, R. (2005). Stress and adaptation in conservation genetics. *Journal of Evolutionary Biology*, *18*(4), 750-755. https://doi.org/10.1111/j.1420-9101.2005.00885.x

54. Funk, W. C., McKay, J. K., Hohenlohe, P. A., & Allendorf, F. W. (2012). Harnessing genomics for delineating conservation units. *Trends in Ecology & Evolution*, *27*(9), 489-496. https://doi.org/10.1016/j.tree.2012.05.012

55. McSweeney, D. J., Baird, R. W., Mahaffy, S. D., Webster, D. L., & Schorr, G. S. (2009). Site fidelity and association patterns of a rare species: Pygmy killer whales (*Feresa attenuata*) in the main Hawaiian Islands. *Marine Mammal Science*, *25*(3), 557-572. https://doi.org/10.1111/j.1748-7692.2008.00267.x

56. Amos, B., Schlötterer, C., & Tautz, D. (1993). Social Structure of Pilot Whales Revealed by Analytical DNA Profiling. *Science*, *260*(5108), 670-672. https://doi.org/10.1126/science.8480176

57. Crespo, E. A., Schiavini, A. C. M., García, N. A., Franco-Trecu, V., Goodall, R. N. P., Rodríguez, D., Stenghel Morgante, J., & De Oliveira, L. R. (2015). Status, population trend and genetic structure of South American fur seals, *Arctocephalus australis* , in southwestern Atlantic waters. *Marine Mammal Science*, *31*(3), 866-890. https://doi.org/10.1111/mms.12199

58. Caballero, S., Islas-Villanueva, V., Tezanos-Pinto, G., Duchene, S., Delgado-Estrella, A., Sanchez-Okrucky, R., & Mignucci-Giannoni, A. A. (2012). Phylogeography, genetic diversity and population structure of common bottlenose dolphins in the W ider C aribbean inferred from analyses of mitochondrial DNA control region sequences and microsatellite

loci: Conservation and management implications. *Animal Conservation*, *15*(1), 95-112. https://doi.org/10.1111/j.1469-1795.2011.00493.x

59. Caballero, S., De O. Santos, M. C., Sanches, A., & Mignucci-Giannoni, A. A. (2013). Initial description of the phylogeography, population structure and genetic diversity of Atlantic spotted dolphins from Brazil and the Caribbean, inferred from analyses of mitochondrial and nuclear DNA. *Biochemical Systematics and Ecology*, *48*, 263-270. https://doi.org/10.1016/j.bse.2012.12.016

60. Gomes-Pereira, J. N., Marques, R., Cruz, M. J., & Martins, A. (2013). The little-known Fraser's dolpin Lagenodelphis hosei in the North Atlantic: New records and a review of distribution. *Marine Biodiversity*, *43*(4), 321-332. https://doi.org/10.1007/s12526-013-0159-2

61. Chen, I. (2016). Population genetics of Risso's dolphins (Grampus griseus), Fraser's dolphins (Lagenodelphis hosei) and bottlenose dolphins (Tursiops spp.) in the North Pacific Ocean. *2016*.

62. Elliser, C. R., & Herzing, D. L. (2014). Long-term social structure of a resident community of Atlantic spotted dolphins, *Stenella Frontalis* , in the Bahamas 1991–2002. *Marine Mammal Science*, *30*(1), 308-328. https://doi.org/10.1111/mms.12039

63. Herzing, D. L. (1997). THE LIFE HISTORY OF FREE-RANGING ATLANTIC SPOTTED DOLPHINS *(STENELLA FRONTALIS):* AGE CLASSES, COLOR PHASES, AND FEMALE REPRODUCTION. *Marine Mammal Science*, *13*(4), 576-595. https://doi.org/10.1111/j.1748-7692.1997.tb00085.x

64. Nara, L., De Meirelles, A. C. O., Souto, L. R. A., Silva-Jr, J. M., & Farro, A. P. C. (2017). An Initial Population Structure and Genetic Diversity Analysis for Stenella clymene (Gray, 1850): Evidence of Differentiation Between the North and South Atlantic Ocean. *Aquatic Mammals*, *43*(5), 507-516. https://doi.org/10.1578/AM.43.5.2017.507