



Low Genetic Diversity of the Endangered Franciscana (*Pontoporia blainvillei*) in Its Northernmost, Isolated Population (FMAIa, Espírito Santo, Brazil)

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The franciscana, *Pontoporia blainvillei*, is the most endangered small cetacean in the Southwestern Atlantic Ocean, occurring from Itaúnas, Espírito Santo, Brazil to Chubut province, Argentina. This area is divided into four Franciscana Management Areas (FMA). The northern portion of this species distribution is not continuous and a previous genetic study using mitochondrial DNA (mtDNA) separated it into FMAIa (Espírito Santo state) and FMAIb (North of Rio de Janeiro state). In order to increase the information about this population we expanded the sample number and evaluated mitochondrial and nuclear DNA diversity. Samples of 68 franciscanas found stranded on beaches from 2005 to 2020 were analyzed. Analyses included 350 bp of the mtDNA control region (D-loop) and 12 microsatellite loci. We identified three control region haplotypes in FMAIa, two of them not previously observed in this population, one being a new haplotype. Haplotype and nucleotide diversities were 0.0408 and 0.00012 respectively, the lowest reported for all FMAs analyzed until now. The Neutrality tests were not significant and Mismatch Distribution analysis did not reject the hypothesis of population expansion. One of the microsatellite loci was monomorphic, and for the other loci, two to nine alleles were identified, with expected heterozygosities ranging from 0.306 to 0.801. No substructure was revealed and effective population size (N_e) was estimated in 117.9 individuals. Even with an increased sample size, the high mitochondrial genetic homogeneity suggested for the population in a previous study was confirmed. Among six loci previously analyzed in other franciscana populations, five showed the lowest observed heterozygosities for the Espírito Santo population. The novel microsatellite data also showed low genetic diversity and could not reject the hypothesis of a single, panmitic population along the coast of Espírito Santo. This species has been intensively impacted in the last years by incidental capture during fishing activities and habitat degradation, caused by

pollution, coastal development and environmental disasters in FMAIa. Considering that this population is small, isolated, and with low levels of genetic diversity, we reinforce the necessity of different conservation actions, focusing mainly on the reduction of bycatch of this species in the region.

Keywords: cetacean, conservation, threatened, Franciscana Management Area, microsatellites, Pontoporiidae, D-loop

INTRODUCTION

Pontoporia blainvillei (Gervais and D'orbigny, 1844), known as toninha or franciscana, is a small cetacean and the only member of the Pontoporiidae family. It is considered one of the smallest cetaceans and has as main characteristic a long beak and a bulky head (Crespo, 2018). This species inhabits estuarine regions and most records are in turbid waters up to 30 m deep (Di Benedetto and Ramos, 2001; ICMBIO, 2010).

Franciscanas are endemic to the southwestern coast of the Atlantic Ocean and occur from Itaúnas, Espírito Santo, Brazil, to Chubut province, in Argentina (Siciliano, 1994; Crespo et al., 1998; Bastida et al., 2007). The northern part of the species distribution, in Brazil, is not continuous. There are two gaps, one going from Santa Cruz, Espírito Santo to São Francisco de Itabapoana, Rio de Janeiro, and another from Armação de Búzios, Rio de Janeiro to Piraquara de Dentro, Rio de Janeiro. These gaps can occur due to depth, temperature, and water transparency (Siciliano et al., 2002; ICMBIO, 2010; Do Amaral et al., 2018).

Considering data about abundance, parasitology, growth, genetics, and morphology, four management areas, denominated “Franciscana Management Areas” (FMA) were proposed: FMAI, coast of Espírito Santo and Rio de Janeiro; FMAII, coast of São Paulo to Santa Catarina; FMAIII, coast of Rio Grande do Sul to Uruguay; and FMAIV, coast of Argentina (Secchi et al., 2003). The number and limits between FMAs have been refined using genetic data. Analyses of the mitochondrial marker D-loop, showed genetic structure between the two areas of occurrence within FMAI, and a new subdivision was proposed: FMAIa, Espírito Santo and FMAIb, north of Rio de Janeiro state (Cunha et al., 2014). These two FMA should be considered an Evolutionarily Significant Unit (ESUs). Therefore, the Espírito Santo state harbors the northernmost franciscana population, which is geographically isolated and genetically different from populations further southward, of which FMAIb is the closest both geographically and genetically (Cunha et al., 2014).

The franciscana is considered the most endangered small cetacean species in the western south Atlantic and its status of conservation is “Vulnerable” according with the International Union for the Conservation of Nature’s Red List of Threatened species (Zerbini et al., 2017). In Brazil, *Pontoporia blainvillei* is classified as “Critically Endangered,” according the Red Book of the Brazilian Fauna Threatened of Extinction and the List of Endangered Fauna and Flora list of Espírito Santo (ICMBIO, 2018; MCT, 2019).

The biggest risk factors for franciscanas are accidental catches in fishing (ICMBIO, 2010). In addition, franciscana populations are more vulnerable to environmental degradation processes

since it is a costal species, with limited distribution and narrow preferential habitats. The FMAIa population shows an aggravating factor, by being an isolated population with probably a small number of individuals (ICMBIO, 2010).

A population that has been reduced in size for a long period of time or has suffered a bottleneck effect, tends to have low genetic diversity, decreasing its evolutionary potential and increasing the possibility of extinction in the face of environmental changes (Frankham et al., 2006). Therefore, the study of genetic diversity in a population like this is extremely important once the loss of diversity is directly related with the reduction of the evolutionary and reproductive potential (Frankham et al., 2006).

Molecular markers of the mitochondrial DNA (matrilineally inherited) such as the control region (D-loop) and of the nuclear DNA (biparentally inherited) such as microsatellite loci largely used in cetacean population genetics studies (Cunha et al., 2014; Gariboldi et al., 2015; Donato et al., 2019; Faria et al., 2020). These molecular markers can be used to access the genetic diversity of a population, to study the differences between the populations of a species and also to determine taxonomic relationships (Frankham et al., 2006). It is important to use mitochondrial and nuclear molecular markers for a better understanding of the populational genetics of a species.

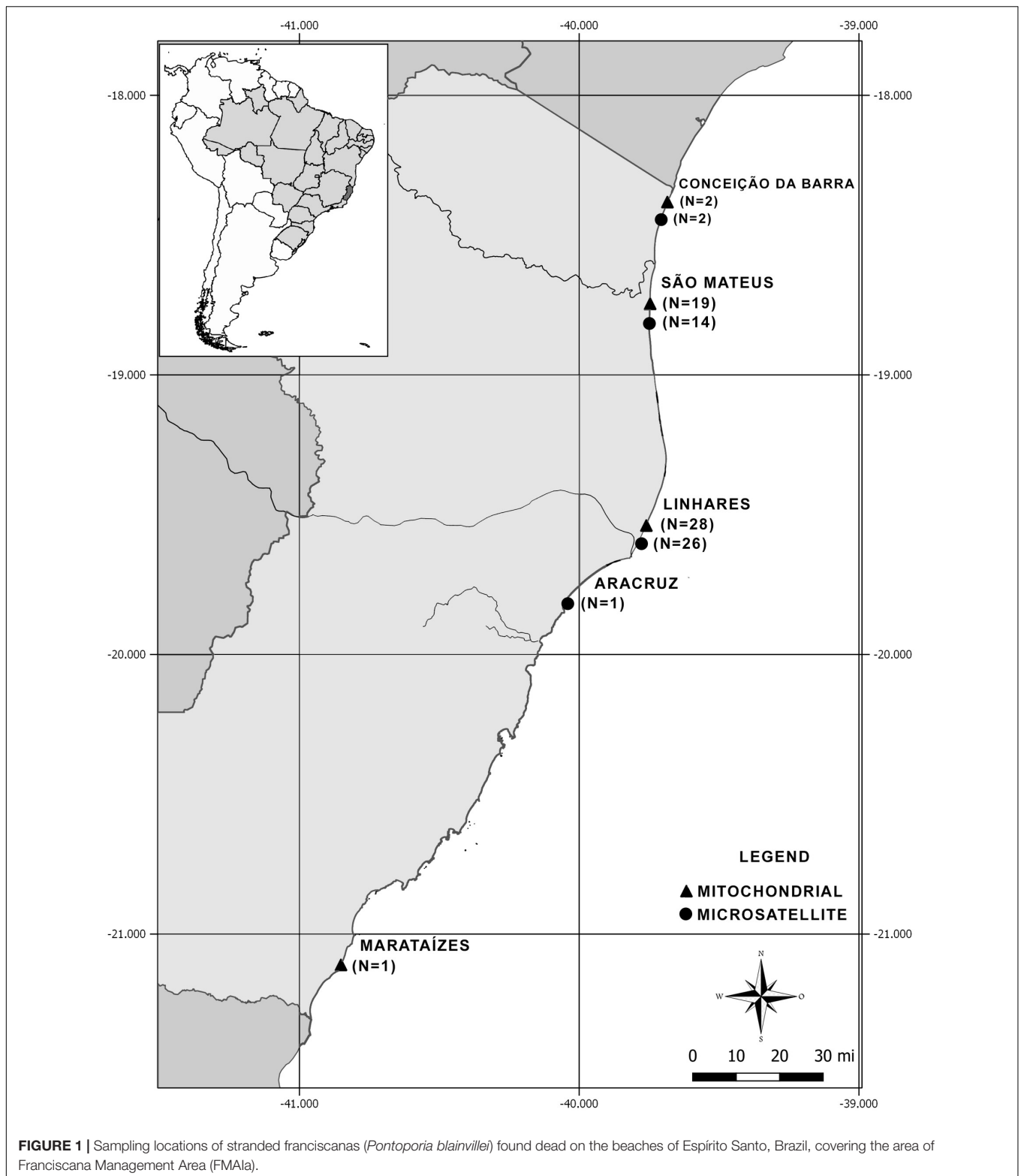
D-loop is a region of mitochondrial DNA that shows some advantageous traits for these types of studies, such as being highly variable, having a small population size by being haploid, and lacking recombination (Frankham et al., 2006). Microsatellites or SSRs (Simple Sequence Repeats) have been extensively used for assessing nuclear DNA polymorphism for different species (Costa-Urrutia et al., 2012; Lima et al., 2017; Faria et al., 2020). They show codominance and high levels of polymorphism with many alleles per locus (Frankham et al., 2006). They have biparental transmission so they can be used to examine the flow of genes mediated by males and females (Oremus et al., 2007).

The present study aimed to evaluate the genetic diversity of the franciscana population from FMAIa, using sequences of the mitochondrial DNA (mtDNA) and twelve microsatellite loci, to increase the sample number and to test the occurrence of a single panmictic population in that area.

MATERIALS AND METHODS

Sampling and DNA Extraction

Of a total of 68 samples, 50 were used in mitochondrial analyses and 43 in microsatellite loci analyses (**Supplementary Table 1**). Skin, liver or muscle samples were obtained from dead stranded franciscanas on the beaches of Espírito Santo coast (**Figure 1** and



Supplementary Table 1). The majority of samples were collected by the teams of Organização Consciência Ambiental (ORCA) and Instituto Baleia Jubarte (IBJ), responsible for assisting stranding of cetaceans and other marine mammals on the Espírito Santo

coast. A few samples were collected by CTA – Serviços em Meio Ambiente, which performs the daily monitoring of Espírito Santo beaches, financed by PETROBRAS, a Brazilian company operating in the oil, natural gas and energy industry.

DNA extraction was performed using the protocols of phenol-chloroform (Sambrook et al., 1989) or saline extraction (Bruford et al., 1992). The mitochondrial DNA control region (D-loop) was amplified with the primers KRAdLp (Andrews et al., 2006) and DLp5 (Pichler et al., 2001).

mtDNA Amplification, Sequencing, and Data Analyses

Polymerase Chain Reactions were performed in 20 μ L total volumes containing 20 ng DNA, Reaction Buffer 10X (Invitrogen), 200 μ M of each dNTP, 2 mM of $MgCl_2$, 0.5 units of Taq DNA polymerase (Invitrogen) and 0.2 μ M of each primer. Cycle conditions were as follows: 95°C for 1 min, followed by 40 cycles of 94°C for 30 s, 54°C for 30 s and 72°C for 30 s, followed by a final extension of 72°C for 15 min.

Amplification products were sequenced in both directions (*forward and reverse*) by outsourced companies. The sequences were edited manually and aligned using the algorithm Muscle of MEGA 7.07 (Kumar et al., 2016). The generated sequences were compared with sequences deposited in GenBank¹.

Arlequin v.3.11 (Excoffier and Lischer, 2010) was used to calculate haplotypic and nucleotide diversities, number of haplotypes, neutrality tests Fu's F_s and Tajima's D , and Mismatch distribution analysis. DnaSP v5 (Librado and Rozas, 2009) was used to identify polymorphic sites. A median joining haplotype network was built using Network (Bandelt et al., 1999).

For comparisons between the populations of Espírito Santo and Rio de Janeiro, sequences from north of Rio de Janeiro ($N = 5$) and south of Rio de Janeiro ($N = 2$) were added. Of these, four from north of Rio de Janeiro were generated in this study; one from north of Rio de Janeiro (Secchi et al., 1998) and two from south of Rio de Janeiro (Cunha et al., 2014) were downloaded from GenBank.

Population structuring among franciscanas from FMAIa was investigated using two putative populations: *Central* ($N = 28$, samples from Linhares) and *North* ($N = 21$, samples from Conceição da Barra and São Mateus). The only sample from south of Espírito Santo (Marataízes) was excluded from this analysis. AMOVA and pairwise F_{ST} were estimated with 10,000 random generations using Arlequin 3.5.2.2 (Excoffier and Lischer, 2010).

Microsatellite Amplification, Genotyping, and Analyses

Twelve microsatellite loci were amplified (Table 1). Polymerase Chain Reactions were performed in 10 μ L total volumes containing around 30 ng DNA, Reaction Buffer 1X (Promega), 200 μ M of each dNTP, 2.0–2.5 mM of $MgCl_2$, 1U of Taq DNA polymerase (Promega) and 0.3 μ M of each primer. Cycle conditions were as follows: 94°C for 4 min, followed by 30 cycles of 92°C for 45 s, T °C for 45 s and 72°C for 45 s, then 8 cycles of 92°C for 45 s, 53 °C for 45 s and 72°C for 45 s, followed by a final extension of 72°C for 30 min. Annealing temperatures for each of the primer pairs are listed in Table 1.

TABLE 1 | Microsatellite loci used to access genetic diversity of franciscana (*Pontoporia blainvillei*). Locus name (Locus), allele range of amplified fragments size (base pairs, bp), fluorescent primer labeling (dye), annealing temperature (T), and original loci development (reference).

Locus	Allele range (bp)	Dye	T (°C)	References
D22	106–118	VIC	60	Shinohara et al. (1997)
EV5Pm	150–166	NED	60	Valsecchi and Amos (1996)
FCB2	161–183	6-FAM	60	Buchanan et al. (1996)
FCB5	121–141	PET	60	Buchanan et al. (1996)
FCB17	171–211	NED	58	Buchanan et al. (1996)
EV76Mn	168–202	6-FAM	54	Valsecchi and Amos (1996)
Pb11	130–154	6-FAM	60	Cunha et al., in press
Pb26	280–322	6-FAM	60	Cunha et al., in press
Pb28	147–209	PET	58	Cunha et al., in press
Pb16	282–308	VIC	60	Cunha et al., in press
Pb19	184–206	VIC	58	Cunha et al., in press
Pb8	287–307	6-FAM	56	Cunha et al., in press

Capillary electrophoresis of PCR products was performed on ABI 3500 sequencer. Geneious version 2020.1.2² (Kearse et al., 2012) software was used to identify fragment sizes. Linkage disequilibrium was evaluated using GENEPOP on the Web (Raymond and Rousset, 1995) and testes of microsatellite loci for the presence of null alleles and/or genotyping errors were performed using the Microchecker 2.2.0.3 (Van Oosterhout et al., 2004).

Total number of alleles (N_a), observed (H_o) and expected (H_e) heterozygosity, polymorphic information content (PIC), allelic richness (R_a) and inbreeding coefficient (F_{IS}) were calculated using Arlequin 3.5.2.2 (Excoffier and Lischer, 2010) and Fstat v.2.9.3.2 (Goudet, 2001). Effective population size (N_e) 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), we used a P_{crit} value of 0.05 and 95% confidence intervals to reduce the potential bias for low frequency alleles (Do et al., 2014). A test for past events of population bottleneck based on allele frequencies using the software Bottleneck 1.2.02 was performed (Cornuet and Luikart, 1996); the one-tailed Wilcoxon sign-rank test for heterozygote excess was used under the stepwise mutational model (SMM) (Ohta and Kimura, 1973) and the two-phase model (TPM; 30 variations, mutational model 70 gradual, 10,000 interactions) (DiRenzo et al., 1994). A probability level of 0.05 was considered significant (Piry et al., 1999).

Tests for the existence of population structuring using microsatellite markers in franciscanas from FMAIa were also performed by using two putative populations: *Central* ($N = 27$), samples from Linhares e Aracruz, *North* ($N = 16$), samples from Conceição da Barra e São Mateus. The only sample from south of Espírito Santo (Marataízes) was excluded from this analysis. AMOVA and pairwise F_{ST} , based on number of different alleles, were estimated with 10,000 random generations using Arlequin 3.5.2.2 (Excoffier and Lischer, 2010).

¹www.ncbi.nlm.nih.gov/Genbank

²http://www.geneious.com

TABLE 2 | Variable sites of 350 bp of the mitochondrial DNA control region (D-loop) of franciscana (*Pontoporia blainvillei*) determining three haplotypes and their respective frequencies.

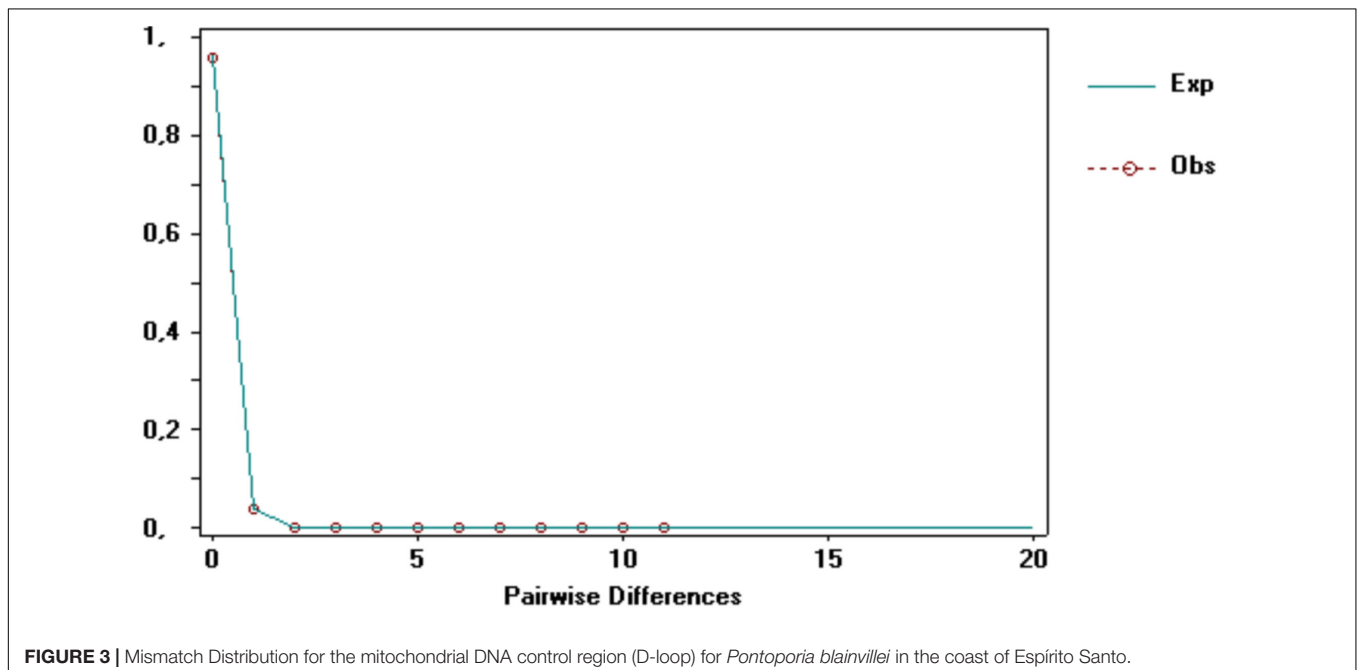
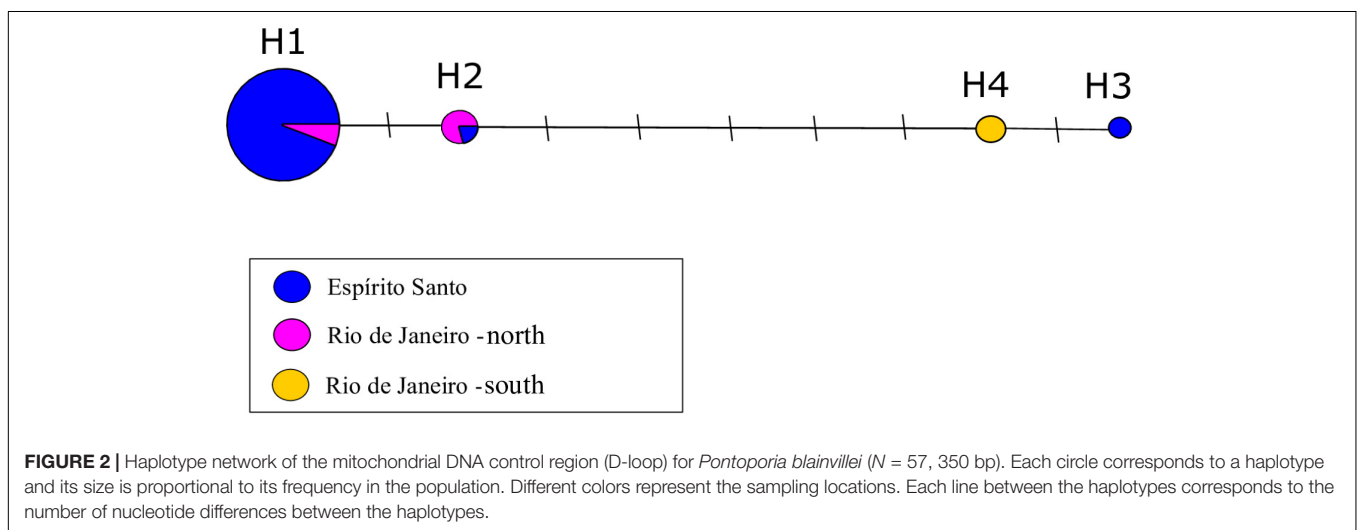
Haplotype	Variable sites							Absolute frequency
	221	257	263	270	275	313	345	
H1	A	G	A	C	A	G	C	48
H2	–	A	–	–	–	–	–	1
H3	G	A	G	T	G	A	T	1

A Bayesian clustering analysis was performed to estimate the most probable number of populations in 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 10,000 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 and VonHoldt, 2012) (available at³), a web-based program for collating results generated by the program Structure 2.3.2 (Earl and VonHoldt, 2012).

Analyses of genetic structuring were performed using three different combinations of loci: 10 loci (EV5Pm, FCB17, Pb11, Pb26, Pb16, Pb19, D22, FCB5, EV76Mn, and PB28); 8 loci

³<http://taylor0.biology.ucla.edu/structureHarvester/>



(EV5Pm, FCB17, Pb11, Pb26, Pb16 e Pb19, D22, and Pb28); and 6 loci (EV5Pm, FCB17, Pb11, Pb26, Pb16, and Pb19).

RESULTS

mtDNA

Sequences 350 bp-long were obtained for all 50 franciscanas and their identity (as *Pontoporia blainvillei*) were confirmed with 99–100% homology. A total of seven variable sites were identified (Table 2), representing three haplotypes. BLAST searches revealed that one of them was new MW248737. Haplotype H1 was the most frequent and was found in 48 of the 50 individuals. H2 was found in only one individual from Guriri, São Mateus. H3 was found in only one individual from Marataízes, an area within the species distribution gap. This latter haplotype is six mutational steps different from H2 and seven mutational steps different from H1.

The Median-Joining network identified four haplotypes: H1, the most representative haplotype, shared by 48 individuals from Espírito Santo and one individual from north of Rio de Janeiro; H2, shared among four individuals from north of Rio de Janeiro and one individual from Espírito Santo; H3, an exclusive haplotype, represented by the individual from Marataízes and never reported for this species; H4, represented by two individuals from south of Rio de Janeiro. H3, found in Marataízes, is closer to the haplotypes of south of Rio de Janeiro than to the haplotypes of Espírito Santo. Given that, this individual that was found stranded in the beach of Marataízes (Espírito Santo) probably does not belong to Espírito Santo and was excluded from later population analyses in that region (Figure 2).

For the Espírito Santo population, the haplotype and nucleotide diversities were low, 0.0408 and 0.00012, respectively. The neutrality tests were not significant (Fu's $F_s = -1.619$, $P = 0.045$; Tajima's $D = -1.104$, $P = 0.126$). The Mismatch Distribution analysis showed that the distribution of the differences between the pairs of sequences is not statistically different from that expected in a population expansion scenario ($P_{Ssd} = 0.100$; $P_{Raggedness} = 0.920$; Figure 3).

Genetic structuring tests using mtDNA showed no statistically significant F_{ST} values: $F_{ST} = 0.01408$, $P = 0.435$. AMOVA results showed that the highest levels of differentiation were

within populations (locations) and no detectable structure was identified (Table 3).

Microsatellite Loci

Forty-three franciscanas were genotyped. The diversity at 12 microsatellite loci was moderate, with from two to nine alleles, and expected heterozygosities ranging from 0.306 to 0.801 (Table 4). One locus was monomorphic (Pb8), four exhibited possible presence of null alleles with significant deviation from Hardy-Weinberg equilibrium (D22, FCB5, EV76Mn, and Pb28). None of the loci pairs was in linkage disequilibrium (Table 4).

TABLE 4 | Genetic diversity of franciscana for twelve microsatellite loci.

Loci	K	Ho	He	PIC	Ra	FIS
D22	3	0.069	0.306	0.282	2.930	0.775 (0.004)*
EV5Pm	6	0.674	0.705	0.646	5.229	0.045 (0.425)
FCB2	2	0.666	0.514	0.374	2.000	-0.308 (0.970)
FCB5	5	0.157	0.520	0.481	4.325	0.700 (0.004)*
FCB17	8	0.525	0.620	0.582	6.059	0.155 (0.070)
EV76Mn	9	0.433	0.801	0.759	7.743	0.463 (0.004)*
Pb11	2	0.757	0.477	0.360	2.000	-0.600 (1.000)
Pb26	2	0.500	0.383	0.305	2.000	-0.313 (1.000)
Pb28	2	0.181	0.402	0.318	2.000	0.552 (0.004)*
Pb16	3	0.478	0.633	0.544	3.000	0.250 (0.108)
Pb19	2	1	0.509	0.375	2.000	-1.000 (1.000)
Pb8	-	-	-	-	-	-

k, total number of alleles; *HO*, observed heterozygosity; *He*, expected heterozygosity; *PIC*, polymorphic information content; *Ra*, allelic richness; *FIS*, inbreeding coefficient. *Statistically significant values with Bonferroni Correction for *FIS* ($\alpha = 0.00417$).

TABLE 5 | AMOVA statistics of franciscana for three different combination of microsatellite loci.

Source of variation	Degrees of freedom	Sum of squares	Variance components	% variation
10 loci				
Among populations	1	0.641	0.00332 Va	0.65
Within populations	84	42.394	0.50469 Vb	99.35
Total	85	43.035	0.50801	
F_{ST}	0.00654			
<i>P</i> -value	0.4125			
8 loci				
Among populations	1	0.641	0.00332 Va	0.65
Within populations	84	42.394	0.50469 Vb	99.35
Total	85	43.035	0.50801	
F_{ST}	0.00654			
<i>P</i> -value	0.41881			
6 loci				
Among populations	1	0.576	0.00548 Va	1.54
Within populations	84	29.424	0.35029 Vb	98.46
Total	85	30.000	0.35577	
F_{ST}	0.01542			
<i>P</i> -value	0.17356			

TABLE 3 | AMOVA statistics for franciscana (*Pontoporia blainvillei*) of FMA1a for 350 bp of mtDNA control region (D-loop).

MtDNA				
Source of variation	Degrees of freedom	Sum of squares	Variance components	% variation
Among population	1	0.027	0.00029 Va	1.41
Within population	47	0.952	0.02026 Vb	98.59
Total	48	0.980	0.02055	
F_{ST}	0.01408			
<i>P</i> -value	0.43471			

Effective population size (N_e) for franciscanas of FMAIa was estimated to be 117.9 (95% CI: 27.8– ∞ ; harmonic mean sample size: 25.6). The Wilcoxon one-tailed test revealed that the franciscanas did not go through a bottleneck event based on the TPM mutational model ($P = 0.12012$), and SMM model ($P = 0.51709$).

The genetic structuring into two putative populations (*Central* and *North*) tested using three different combinations of loci showed no statistically significant F_{ST} values: 10 loci, pairwise $F_{ST} = 0.006$ ($P = 0.415$); 8 loci, pairwise $F_{ST} = 0.001$ ($P = 0.411$); 6 loci, pairwise $F_{ST} = 0.001$ ($P = 0.406$). AMOVA results showed that the highest levels of differentiation were within putative populations and no detectable structure was identified (Table 5).

The Bayesian clustering analysis did not detect structuring using the same combination of loci. The combination of 10 loci showed the clearest results. Delta K indicated $K = 2$ as the most likely scenario, but the greatest posterior probability was for $K = 1$. The analysis of Q for $K = 2$ showed all individuals to have similarly mixed ancestries (Figure 4 and Supplementary Figures 1, 2). These results thus reinforce the presence of just one population of franciscanas throughout FMAIa.

DISCUSSION

This is the first microscale study about *Pontoporia blainvillei* from northern Espírito Santo (FMAIa). In this study, we increased the number of samples evaluated using the mitochondrial marker ($N = 50$) in relation to the macroscale study developed by Cunha et al. (2014), and presented novel nuclear DNA data, providing important information that can assist in conservation strategies for this isolated population.

With increased sampling it was possible to detect a new haplotype for the population of Espírito Santo (FMAIa), not described by Cunha et al. (2014). Three haplotypes were found, including the new one, present in an individual who was found stranded in Marataizes, ES, an area known to be a gap in the species distribution. This haplotype showed many mutational steps from the most frequent haplotype in FMAIa, so this animal may have been caught by a fishing boat elsewhere and discarded in Espírito Santo. This would be unsurprising since incidental catch is one of the main threat factors for the species throughout its distribution.

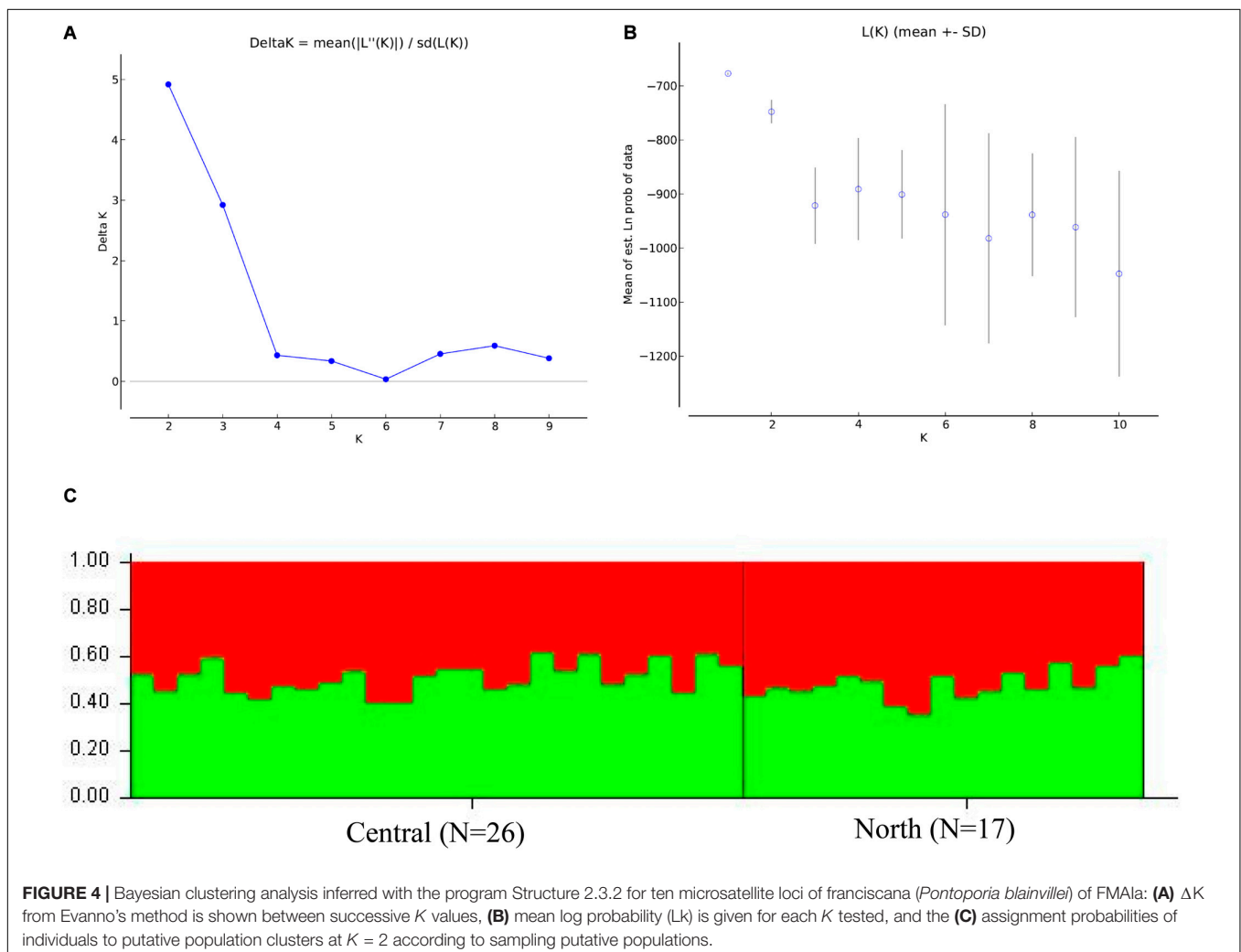


FIGURE 4 | Bayesian clustering analysis inferred with the program Structure 2.3.2 for ten microsatellite loci of franciscana (*Pontoporia blainvillei*) of FMAIa: **(A)** ΔK from Evanno's method is shown between successive K values, **(B)** mean log probability (L_k) is given for each K tested, and the **(C)** assignment probabilities of individuals to putative population clusters at $K = 2$ according to sampling putative populations.

The genetic diversity both at mitochondrial DNA and microsatellite loci was lower than already reported for *P. blainvillei* in other FMAs. Studies carried out with franciscanas from other FMAs found values ranging from 0.476 to 1.00 for haplotypic diversity (Table 6) and 0.0010 to 0.014 for nucleotide diversity. For microsatellite analyses, five out of six loci showed lower diversity in FMAIa than in previous studies (Mendez et al., 2007, 2010; Costa-Urrutia et al., 2012; Cunha et al., 2014; Gariboldi et al., 2015).

The results presented here follow the same pattern reported in a macroscale study, which reported an increasing gradient of haplotypic diversity from the south to the north of the species distribution (Cunha et al., 2014).

When compared with other species of dolphins, threatened or not, franciscanas from FMAIa still have one of the lowest haplotype genetic diversity found (Rosel and Rojas-Bracho, 1999; Chivers et al., 2002; Parsons et al., 2002; Caballero et al., 2010; Hamner et al., 2012; Faria et al., 2020; Table 6).

The paucity of genetic variation may suggest that the situation of this endangered species in Espírito Santo is worrying. According to Frankham et al. (2006) species with small populations or that have suffered bottleneck events have low genetic diversity and suffer from accelerated endogamy, which leads to less reproductive and evolutionary potential. However,

despite the low genetic variability seem in franciscanas from FMAIa, Fu (Fs), and Tajima (D) analyses were not significant, and mismatch distribution analysis did not discard the hypothesis of population expansion, while specific analysis with microsatellite data also did not detect signs of bottleneck events.

Implications for Management and Conservation

The conservation status and viability of the franciscana population in Espírito Santo is a known concern (Danilewicz et al., 2012). Franciscans were not seen more than 13 km from the coast (19 m depth), which suggests that in FMAIa they have more coastal habits than in other FMAs, and the size of this population is believed to be small (Danilewicz et al., 2012). In this study effective population size was estimated to be 117.9 for the franciscanas in FMAIa. Reduction in population size directly results in the loss of genetic diversity, which is already observed for this population. The loss of genetic diversity and the reduction of population size are factors considered to be vortices of extinction (Frankham et al., 2006).

Habitat degradation has been considered a critical factor for the conservation of coastal cetaceans. According to Reeves et al. (2003), coastal cetaceans are much more threatened than ocean species due to the intense use of these environments. Anthropogenic actions are the main threats for these species (ICMBIO, 2010). In the case of *Pontoporia blainvillei*, as with other coastal small cetaceans, interaction with fisheries plays a major role (Crespo et al., 1994; Capozzo et al., 2007; Negri et al., 2012).

Besides the mortality caused by accidental captures, franciscanas from Espírito Santo also suffered with extreme habitat degradation caused by the Fundão mining dam rupture, which occurred in November 2015 in the city of Mariana, MG, Brazil, was considered the biggest environmental disaster in South America (Marta-Almeida et al., 2016). After 14 months, 74 million tons of mining sediment were launched into the Doce River until reaching the Atlantic Ocean (Magris et al., 2019). The coastal area that immediately received the largest amount of trace-elements after the dam burst was the south side of the Doce river mouth (Magris et al., 2019), a region in FMAIa known to be important for franciscanas.

The presence of ore tailings in the franciscana habitat can affect their survival over time in several ways. High levels of trace-element accumulation in plankton, marine shrimp, and fishes were found after the disaster (RRDM, 2019). Cetaceans are top-of-the-chain animals, being highly affected by the bioaccumulation of chemicals in the food chain (Torre et al., 2012). A study comparing the concentration of trace-elements in the organs of franciscanas from the Espírito Santo coast before and after the disaster are being concluded (Manhães et al., in prep).

The franciscana population in Espírito Santo (FMAIa) is the smallest. It is geographically and genetically isolated and shows low genetic diversity. All of these characteristics enhance the vulnerability of this population, making even more important and urgent the development of conservation strategies in FMAIa, such as the creation of MPA (Marine Protected Areas)

TABLE 6 | Comparison of *Pontoporia blainvillei* haplotypic diversity between management units along species distribution and comparison with other small cetacean species.

Species	Management Units/Locality	Haplotype diversity	References
<i>Pontoporia blainvillei</i>	FMAIa	0.041	This study
	FMAIb	0.667	Cunha et al., 2014
	FMAIIa	0.786	Cunha et al., 2014
	FMAIIb	0.476–0.743	Cunha et al., 2014
	FMAIIIa	0.860	Costa-Urrutia et al., 2012
	FMAIIIb	1	Costa-Urrutia et al., 2012
	FMAIVa	0.770–0.833	Mendez et al., 2010
	FMAIVb	0.778–0.860	Mendez et al., 2010
	FMAIVc	0.615–0.860	Mendez et al., 2010
<i>Phocoena phocoena</i>	Eastern North Pacific	0.876	Chivers et al., 2002
<i>Cephalorhynchus hectori</i>	New Zealand	0.846	Hamner et al., 2012
<i>Tursiops truncatus</i>	United Kingdom	0.697	Parsons et al., 2002
<i>Stenella longirostris</i>	Fernando de Noronha	0.374	Faria et al., 2020
<i>Sotalia guianensis</i>	Brazilian coast	0.284	Caballero et al., 2010
<i>Phocoena sinus</i>	Mexico	0	Rosel and Rojas-Bracho, 1999
<i>Cephalorhynchus hectori maui</i>	New Zealand	0	Hamner et al., 2012

or maintenance/reinforcement of a fisheries exclusion zone in the impacted area. To avoid human intoxication, fishing has been prohibited in the Rio Doce mouth since the disaster. That measure could impact franciscanas positively, but it is uncertain to what extent this prohibition has been strictly followed. Thus, it is also worth mentioning the importance of monitoring accidental captures in FMAIa in the short, medium and long term.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are publicly available. This data can be found here: <https://www.ncbi.nlm.nih.gov/genbank/> with sequence accession number is MW248737.

ETHICS STATEMENT

Ethical review and approval was not required for the animal study because the animals come from carcasses found stranded on beaches.

AUTHOR CONTRIBUTIONS

VO: formal analysis of mtDNA, writing-original draft, writing-review and editing, conceptualization, discussion, and investigation and validation. DF: formal analysis of microsatellites, writing the microsatellites part, review and editing, conceptualization, and discussion. HC: writing-review and editing, conceptualization, discussion, and funding acquisition. TS: microsatellite laboratory part and writing-review. AC and LB: resources and writing-review and editing. MF: extraction of some samples and writing-review and editing.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2020.608276/full#supplementary-material>

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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