



Molecular testing of the São Francisco River as an ecological filter for the Brazilian large-eyed stingray *Hypanus marianae* (Dasyatidae, Myliobatiformes)

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Abstract Ecological niche modeling (ENM) provides information on the potential environmental barriers to a species that can be tested in phylogeographic studies. A previous ENM analysis of the benthic coastal stingray *Hypanus marianae* revealed a low suitability area for its occurrence at the São Francisco River (SFR) mouth, the fourth largest river flowing into Southwestern Atlantic. Hence, phylogeographic analyses were used to test the hypothesis of

two populations: one north and another south of SFR outflow. We sampled 109 specimens in six localities throughout the species' geographic distribution and sequenced mitochondrial (*cytb*) and nuclear (*rag1*) markers. Our analyses corroborated the existence of two groups ($\Phi_{ST}=0.68$, $P<0.0001$) within *H. marianae*, partially agreeing with the ENM results. The commonest mitochondrial haplotype (H2) was shared among almost all localities, except Salvador, where all individuals shared the same and unique haplotype. This group is restricted to a shallow bay area close to SFR, as predicted by the ENM. However, its plume was not effective in isolating a continental island 55 km off the Brazilian coast. While the broad north

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group is protected in a few Marine Protected Areas, our results suggest that the restricted southern one deserves to be managed specifically.

Keywords Marine barriers · Genetic structure · Ecological isolation · Distribution pattern · Elasmobranch

Introduction

Understanding which indicators are involved in the dispersion and distribution of a species is paramount to rational management and conservation. In this context, ecological niche modeling (ENM) can help identifying areas of low habitat suitability that may serve as potential environmental barriers for dispersal, with direct consequences for genetic structuring (Alvarado-Serrano & Knowles, 2014). However, this approach has been barely explored in chondrichthyans, with no more than five ENMs published studies within the Southwestern Atlantic region (Dambach & Rödder, 2011; Sequeira et al., 2014; Costa et al., 2017; Coelho et al., 2020; Melo-Merino et al., 2020; Petean et al., 2020).

Even though chondrichthyans have diversified reproductive modes (Nakaya et al., 2020), all of them lack pelagic eggs or larvae stages, making their dispersion a result of adults' movement (Le Port & Lavery, 2012). Therefore, their mobility could be a good mechanism of gene flow that could be evaluated (Slatkin, 1987), however, it may lead to an underestimation of the intraspecific genetic differentiation (Sandoval-Castillo & Rocha-Olivares, 2011). In dasyatis stingrays, mainly composed by coastal benthic species, a smaller dispersal potential is expected in comparison to pelagic mobulids (Graham et al., 2012; Jaïne et al., 2014; Setyawan et al., 2018) and sharks that, overall, have higher mobility (Braccini et al., 2017).

Stingrays are an excellent group to investigate the relative importance of environmental barriers for marine dispersal, such as depth, habitat preference, and spatial fidelity (Le Port & Lavery, 2012). For example, river discharges may act as barriers for populations, as in *Hypanus berthalutzae* Petean et al., 2020 (Petean et al., 2020), small areas could enhance philopatric segregations of batoids (Flowers et al., 2016), and water temperature changes may induce

metabolic adjustments, as in *Taeniura lymma* (Forskål, 1775) (Dabruzzi et al., 2013).

The Brazilian large-eyed stingray, *Hypanus marianae* (Gomes et al., 2000) is a small-sized tropical species, endemic to the Southwestern Atlantic, inhabiting reef-associated areas. Besides its strong association with reef environments (Gomes et al., 2000; Rosa et al., 2000), *H. marianae* also occurs in surrounding connected areas, such as seagrass beds and sandy bottom near the beach (Costa et al., 2015). Its distribution is restricted to a 3,000 km coastal area from Maranhão to Bahia states (00° 52' S and 17° 58' S, respectively) and the species is absent in Oceanic islands (Gomes et al., 2000). This suggests limited dispersal capacities, given that suitable reef habitats are available in the oceanic islands off the Brazilian coast (Costa et al., 2017). These features, together with the discontinuity of reef areas along the coast (Castro & Pires, 2001; Figueiredo et al., 2008), may influence the gene flow of *H. marianae*. Recent studies, both on elasmobranchs as on evolutionary drivers, have shown that environmental differences help understanding population structure, in addition to geographic distances (Sexton et al., 2014; Wang & Bradburd, 2014; Domingues et al., 2017; Rizo-Fuentes et al., 2020). However, only a handful were done with the Southwestern Atlantic marine batoids (Sales et al., 2019; Cruz et al., 2021).

The ENM can indicate areas of low habitat suitability and putative barriers that could be influencing the genetic structure of a species (Knowles et al., 2007). In the Tropical Western Atlantic, the São Francisco River (SFR) outflow is a known barrier of dispersal for marine organisms that usually do not tolerate the low salinity and turbidity increase related to the input of freshwater (Cunha et al., 2014; Pinheiro et al., 2018). A previous ENM study indicated depth, salinity, and temperature as ecological drivers for *H. marianae* occurrence (Costa et al., 2017). This study also indicated a decrease in its habitat suitability at the SFR estuary, indicating that this area may represent an ecological filter for the species dispersal, leading to the existence of distinct populations at the north and south of this estuarine area (Costa et al., 2017). Furthermore, considering that *H. marianae* is a tropical species, it is possible that the population south of SFR may result from a demographic expansion, according to the sea currents and global warming since the last glacial maximum (LGM).

During the glacial cycles, the sea level was lower (up to 130 m), reducing the continental shelf area and freshwater riverine outflow, while in post-glacial periods the continental shelf increased, allowing the colonization of southern areas and population expansion of marine taxa (Ludt & Rocha, 2015).

Defining population structure can help outlining effective conservation measures for this poorly known species (MMA, 2014), which is currently classified as ‘endangered’ according to IUCN criteria, as a consequence of its overexploitation and the decline in habitat quality (Pollom et al., 2020). Therefore, we aimed to test whether the SFR mouth may represent a barrier to gene flow among populations of *H. marianae*, using mtDNA and nuclear data. We hypothesize that

different genetic lineages will be present north and south of SFR mouth, in accordance to the ENM proposed by Costa et al. (2017). We also expect population expansion signals and lower molecular diversity at its southern distribution.

Materials and methods

Data collection

We collected 109 tissue samples of *H. marianae* from six localities throughout its entire known distribution range, two of these south of SFR: Abrolhos and Salvador (Fig. 1). Locations were selected based on

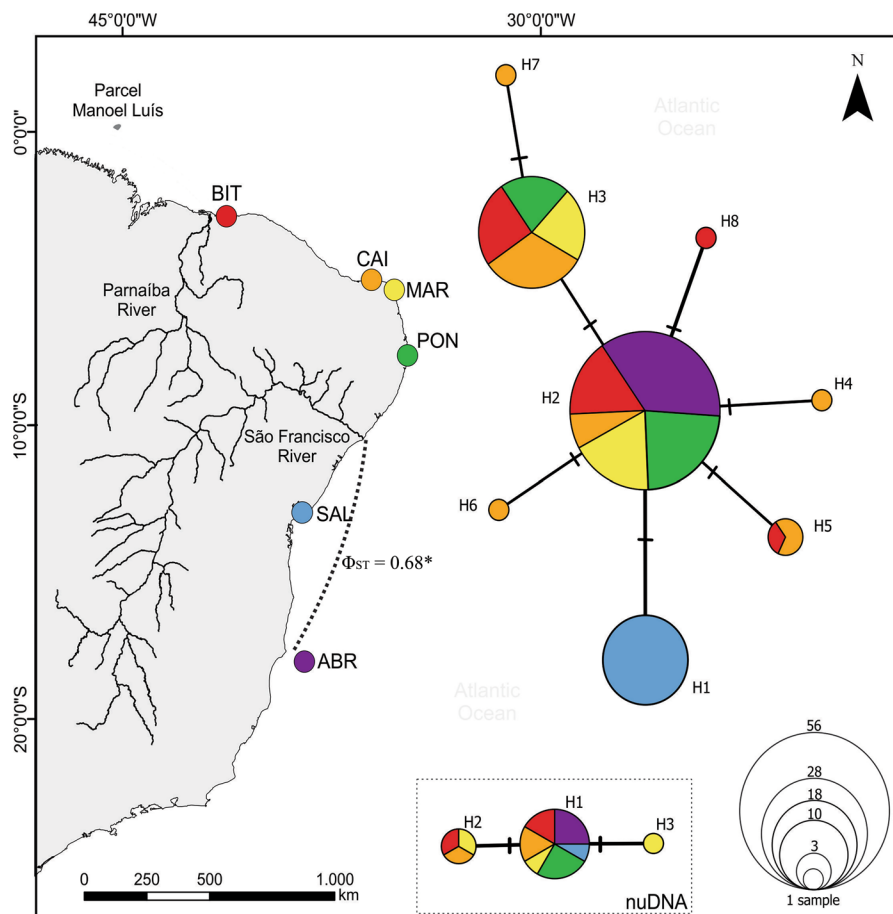


Fig. 1 Sampling sites of the tropical stingray *Hypanus marianae* at the Brazilian coast, indicating the main hydrographic basins of Parnaíba and São Francisco Rivers, including mitochondrial and nuclear (in detail) DNA haplotype networks

(BIT Bitupitá, CAI Caiçara, MAR Maracajaú, PON Ponta de Pedras, SAL Salvador, ABR Abrolhos). Dotted line illustrates the potential barrier to gene flow and Φ_{ST} value between Group 1 (BIT + CAI + MAR + PON + ABR) and Group 2 (SAL)

previous information regarding the species' occurrence and capture by artisanal fishermen. All samples were derived from traps, submarine and line fishing, except in Abrolhos Marine National Park. In this locality, specimens were captured by free diving with a circular net, and kept in a tray with water. A small fin clip was removed from their anterolateral disc portion, and the ray was immediately released. The procedure time, from capture, tissue extraction, manipulation, and monitoring recovery state to release was of 5 min, approximately. All collections were done under SISBIO 41086-1 permit of the Instituto Chico Mendes de Conservação da Biodiversidade, and the tissue samples were stored in ethanol 95% and preserved at -10°C at the Universidade Federal do Rio Grande do Norte fish collection.

DNA amplification and sequencing

Genomic DNA was extracted using the GF-1 Nucleic Acid Extraction kit (Vivantis, Malaysia) according to the manufacturer's instructions. Fragments of the mitochondrial (mtDNA) gene cytochrome b (*cytb*) and the nuclear (nuDNA) recombination activating gene 1 (*rag1*) were amplified using the primers GluFish-F and THR-Fish-R (Sevilla et al., 2007) and Rag1For61 and Rag1Rev12 (Puckridge et al., 2013), respectively. Polymerase chain reactions (PCR) were conducted in a final volume of 25 μl , containing 1 μl of each primer (final concentration of 0.4 $\mu\text{M}/\mu\text{l}$), 12.5 μl of Taq DNA Polymerase Master Mix (Ampliqon A/S) (0.2 mM of each dNTP, 1.5 mM of MgCl_2), 8.5 μl of H_2O , and 10–30 ng of DNA (0.4–1.2 ng/ μl).

Cycling parameters for PCR of *rag1* followed Puckridge et al. (2013), while for *cytb* were denaturation for 4 min at 95°C , followed by 36 cycles of 35 s at 94°C for denaturation, 20 s at 52°C for annealing, and 70 s at 72°C for extension, and a final extension step of 5 min at 72°C . To verify if PCR products were ready for sequencing, each sample was run on a 1% agarose gel electrophoresis with GelRed DNA loading dye (Biotium) and observed through UV light. After confirming the reaction had occurred and the DNA molecules had the expected weight by comparing to a 100 bp DNA ladder (ThermoFischer Scientific), PCR products were purified and sequenced in both forward and reverse directions, using Big-Dye chemistry at the Laboratório de Biodiversidade

Molecular of the Universidade Federal do Rio de Janeiro, in a laser-based ABI 3500 (Applied Biosystems) DNA-automated sequencer. Nuclear DNA data were acquired from an initial sub-sampling of three specimens of each locality.

Genetic analyses

All forward and reverse DNA sequences were edited and contigs' assembly were done using the software Geneious 9.0.2 (Kearse et al., 2012), aligned using CLUSTAL W (Thompson et al., 1994) in MEGA 6 (Tamura et al., 2013), and inspected for the presence of stop-codons (Song et al., 2008; Hazkani-Covo et al., 2010). Sequences were deposited in GenBank with access numbers MZ301320–MZ301428 and MZ301429–MZ301444 for *cytb* and *rag1* sequences, respectively. Molecular diversity indices were calculated through the software Arlequin 3.5 (Excoffier & Lischer, 2010), likewise Tajima's D test (Tajima, 1989) and Fu's F_s (Xiao, 1997) to detect deviation from neutrality indicating significant demographic fluctuations. Haplotype networks were generated using the algorithm TCS v.1.21 (Clement et al., 2002) in the software PopArt (Leigh & Bryant, 2015) to observe the genealogical relations and haplotypes' frequencies (Table 1).

The genetic structure was evaluated through GENELAND 4.0.3 (Guillot et al., 2005a, b) under a Bayesian attribution of geographic information to multiloci (mtDNA and nuDNA) molecular analysis to infer the most probable number of populations and the spatial frontiers among such groups without a priori population attribution. Five independent runs were carried out in k values ranging from 1 to 6, in 500,000 iterations, interval of 100, burn-in of 500, and taking into account coordinates' uncertainty given that these stingrays are mobile, and that their occurrence area might be larger than the exact coordinate where they were sampled. The genetic structure found by GENELAND was afterward evaluated by the fixation index (Φ_{ST}) estimative between the putative populations in Arlequin.

Based on the ENM proposed by Costa et al. (2017, see Figs. 3 and 4 of such article), in which there is a habitat suitability decrease at SFR mouth, mainly due to its low salinity values, we tested the hypothesis that this outflow acts as an ecological filter for *H. marianae* dispersal. For that, we used an analysis of

Table 1 Number of samples (*N*) and GenBank accession numbers of *Hypanus marianae* from sampling sites along the Brazilian coast

<i>N</i>	Location	State	Latitude	Longitude	GenBank accession numbers	
					<i>cytb</i>	<i>rag1</i>
18	Bitupitá (BIT)	CE	02° 53' 26.53" S	41° 16' 29.02" W	MZ301391–MZ301408	MZ301432–MZ301434
18	Caiçara do Norte (CAI)	RN	05° 03' 43.72" S	36° 03' 8.30" W	MZ301373–MZ301390	MZ301435–MZ301437
16	Maracajaú (MAR)	RN	05° 24' 35.96" S	35° 18' 37.66" W	MZ301357–MZ301372	–
19	Ponta de Pedras (PON)	PE	07° 37' 55.90" S	34° 48' 38.95" W	MZ301338–MZ301356	MZ301441–MZ301443
18	Salvador (SAL)	BA	12° 59' 39.75" S	38° 31' 45.19" W	MZ301320–MZ301337	MZ301444
20	Abrolhos (ABR)	BA	17° 58' 10.55" S	38° 42' 33.44" W	MZ301409–MZ301428	MZ301429–MZ301431

States are Bahia (BA), Ceará (CE), Pernambuco (PE), and Rio Grande do Norte (RN)

molecular variance (AMOVA, Excoffier et al., 1992) using the *cytb* dataset. The AMOVA was performed in Arlequin 3.5 using 10,000 permutations and a significance level of 0.05, which was also used to corroborate the genetic structuring through pairwise Φ_{ST} fixation index between the sampling sites, an analog to Wright's F_{ST} (Cockerham & Weir, 1993).

Results

Cytb sequences (887 bp) from 109 specimens of *H. marianae* presented eight haplotypes in the six sampled localities, with seven polymorphic sites (Online Resource SI Tables 1, 2). *Rag1* sequences (nuDNA) with 878 bp and two polymorphic sites were distributed into three haplotypes (Online Resource

SI Tables 3, 4). According to the results presented below, specimens were divided into two groups: Group 1 (BIT, CAI, MAR, PON, ABR) and Pop 2 (SAL). Both localities southern of the SFR (ABR and SAL) revealed zero genetic diversity at the two analyzed loci (a single haplotype in each for both markers), while in the other four localities it ranged from two (MAR and PON) to six (CAI) haplotypes in *cytb* (Table 2), and from three (MAR) to one (PON) in *rag1* (Online Resource SI Table 3).

Haplotype diversities (*h*) varied from zero (SAL and ABR, both south of the SFR) to 0.71 (CAI), and nucleotide diversity (π) from 0 to 0.12 for the same localities. Considering both groups, these values were 0.531 (*h*) and 0.06 (π) for the broad Group 1, and zero for SAL (Group 2). Furthermore, when analyzing only the two locations south of SFR, both have

Table 2 Genetic diversity indices and neutrality tests using the mitochondrial gene cytochrome b for each locality and population of the Brazilian large-eyed stingray *Hypanus marianae*

Location	<i>N</i>	<i>K</i>	<i>S</i>	<i>h</i>	π * 100	Fu's F_S	Tajima's <i>D</i>
BIT	18	4	3	0.628 ± 0.073	0.082 ± 0.072	−0.841	−0.466
CAI	18	6	5	0.719 ± 0.093	0.120 ± 0.093	−2.255*	−0.845
MAR	16	2	1	0.500 ± 0.074	0.056 ± 0.057	1.253	1.376
PON	19	2	1	0.456 ± 0.085	0.051 ± 0.053	1.193	1.095
ABR	20	1	0	0.000	0.000	0.000	0.000
Group 1	91	7	6	0.531 ± 0.041	0.067 ± 0.060	0.108	0.193
SAL ^{Group 2}	18	1	0	0.000	0.000	0.000	0.000
Total	109	8	7	0.648 ± 0.033	0.090 ± 0.073	−0.108	−0.922

Number of sequences (*N*), haplotypes (*K*), polymorphic sites (*S*), haplotype (*h*), and nucleotide (π) diversities. Group 1 = BIT + CAI + MAR + PON + ABR. Group 2 = SAL

BIT Bitupitá, CAI Caiçara, MAR Maracajaú, PON Ponta de Pedras, ABR Abrolhos, SAL Salvador

*Significant *P* values ($P < 0.05$)

Table 3 Analysis of molecular variance (*cytb*) of the genetic structure of the tropical stingray *Hypanus marianae* based on the São Francisco River as a barrier and according to the GENELAND structure

Composition of groups ($K=2$)	Between groups (Φ_{CT})		Between populations within groups (Φ_{SC})		Within populations (Φ_{ST})	
	VoC	PoV	VoC	PoV	VoC	PoV
	BIT+CAI+MAR+PON/SAL+ABR	0.14488	28.85	0.13246*	26.38	0.22478*
BIT+CAI+MAR+PON+ABR/SAL	0.52277	66.60	0.03737*	4.76	0.22478*	28.64

BIT Bitupitá, *CAI* Caiçara, *MAR* Maracajaú, *PON* Ponta de Pedras, *SAL* Salvador, *ABR* Abrolhos, *VoC* variance of components, *PoV* percentage of variation

*Significant P values (≤ 0.05)

Table 4 Pairwise fixation index (Φ_{ST}) using the mitochondrial cytochrome b gene between sampling sites of *Hypanus marianae*

	BIT	CAI	MAR	PON	SAL
CAI	-0.007				
MAR	-0.054	0.005			
PON	-0.036	0.049	-0.047		
SAL	0.758*	0.709*	0.831*	0.822*	
ABR	0.289*	0.376*	0.403*	0.285*	1*

BIT Bitupitá, *CAI* Caiçara, *MAR* Maracajaú, *PON* Ponta de Pedras, *SAL* Salvador, *ABR* Abrolhos

*Significant P values (≤ 0.05)

low genetic diversity, agreeing with the expectations of higher molecular diversity indices at the north than at the south. Even though neutrality tests of BIT and CAI were negative, only Fu's F_S was significant for CAI, suggesting no significant deviations from demographic stability (Table 2), even though the *cytb* haplotype network was star-shaped. This network showed two widely shared haplotypes among specimens from the northern locations (BIT, CAI, MAR, and PON), with four exclusive haplotypes in CAI ($n=3$) and BIT ($n=1$) (Fig. 1). While seven haplotypes were found north of the SFR, only two were present at the south, one in each locality. ABR shared the commonest haplotype with northern locations, whereas stingrays from SAL had a single haplotype that was not shared with any other locality.

GENELAND analysis supported two genetically distinct groups ($K=2$) with high posterior probability. SAL was found to belong to an isolated group with a posterior attribution probability of 90%, whereas the other five sampling locations had posterior probabilities of attribution to that group of only 10% (Fig. 2).

ABR, which is also situated south of the SFR, grouped with the localities north of the drainage. The estimated Φ_{ST} value between these two groups was 0.68 ($P < 0.0001$).

The genetic structure among sampling localities of *H. marianae* separated by the SFR (BIT+CAI+MAR+PON/SAL+ABR), calculated through an AMOVA, explained 28.9% of the genetic variation, but it was not significant (at $\alpha=0.05$). The hypothesis regarding the GENELAND results, considering only SAL as a separated group explained 66.6%, but also not significant (Table 3).

The pairwise Φ_{ST} analyses showed a significant genetic differentiation between SAL and ABR, as well as between these and the other locations. It also does not reject panmixia among the locations north of the SFR (BIT+CAI+MAR+PON) (Table 4).

Discussion

Phylogeography and the ecological filters of niche modeling

We used a phylogeographic analysis to test the hypothesis raised by the ENM of the Brazilian large-eyed stingray by (Costa et al., 2017) in which the SFR freshwater outflow could be the main force driving the species' genetic structure. The study essentially corroborates the population structure proposed by Costa et al. (2017), except for a group from a continental archipelago south of the SFR which, nonetheless, grouped with the northern individuals. There is a slight difference between the genetic structure and the environmental barriers suggested by the ENM of the species. In other words, a Bayesian assignment

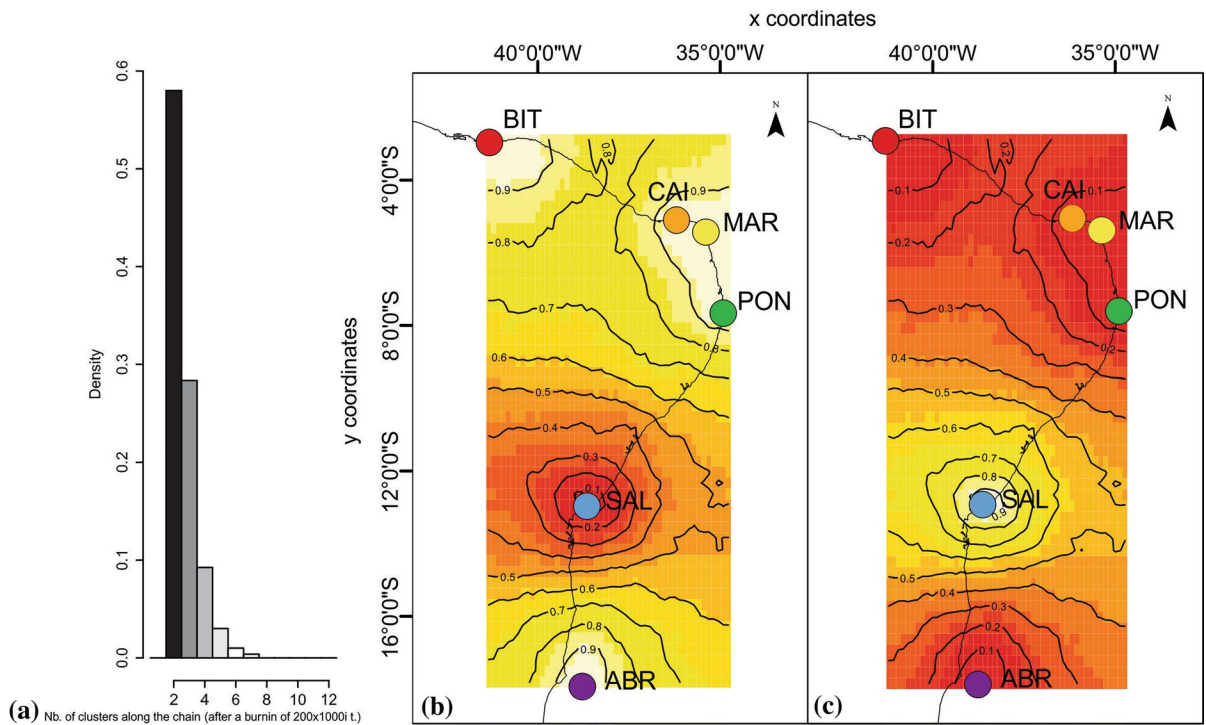


Fig. 2 Population structure of the stingray *Hypanus marianae* indicates the highest probability partitioning in two populations (a). In each map, isoclines represent the posterior probabilities of a group of localities belonging to the same genetic

cluster (locality abbreviations as in Tables 1, 2). **b** Map with a broad population encompassing BIT, CAI, MAR, PON, and ABR, and **c** with a unique population in SAL

analysis suggested a grouping of two populations, with Group 1 composed of all sampling sites north of the SFR, together with ABR, a continental archipelago south of the drainage, and Group 2 composed only of SAL in the Todos os Santos Bay, in Bahia State, south of the SFR. The population structure of *H. marianae* along a relatively short geographical range is generally unusual for marine elasmobranchs, however, it has also been noticed in other restricted-range elasmobranch species (with less than 15° of latitude) in similar coastal benthic habitats (Sandoval-Castillo et al., 2004; Smith et al., 2009).

The apparent connectivity between ABR and other locations of Group 1 could be either the result of incomplete lineage sorting, or current gene flow among ABR and the other localities (Madison & Knowles, 2006), indicating that the effect of the SFR is not strong enough to act on continental islands. In the first scenario, there could be a retention of ancestral polymorphisms (Zhou et al., 2017) and in the second, ongoing migration

(Mallet, 2001). Both hypotheses should be tested by the usage of more variable molecular markers and algorithms. Even though the multiloci Bayesian attribution of individuals to genetically differentiated groups by GENELAND and haplotype networks of both markers suggest the existence of two groups with ABR belonging to Group 1, pairwise Φ_{ST} between this location and others are significant, suggesting the structuration of three groups (BIT + CAI + MAR + PON/SAL/ABR). However, this apparent structure should be addressed in future studies, using highly polymorphic nuclear markers such as microsatellites or genomic single-nucleotide polymorphisms (SNPs), to evaluate males' role in this species distribution and populations' structure. Indeed, only one haplotype of each mitochondrial and nuclear markers were found in SAL and in ABR, leading to a null haplotype diversity, and probably biasing the Φ_{ST} , further suggesting the *rag1* nuclear marker might be too conserved to detect population structure.

The Abrolhos reefs, located about 55 km offshore, are under the influence of the Brazil Current flowing to the south. It could thus be possible that the plume of the SFR is strong enough to isolate the coastal SAL group, but not enough to act as a barrier to *H. marianae* further away from the coast along the broad and shallow continental shelf in the Abrolhos Bank (Alberoni et al., 2020). This would explain why the ABR sample is less differentiated from the northern groups than the SAL sample. The width of the continental shelf in front of SFR estuary varies from 20 to 40 km and its depth from 50 to 70 m, with a muddy bottom (Knoppers et al., 2006). Besides, since its main dispersal path is towards the Southwest, the farther from the SFR mouth, the higher the salinity gets (Knoppers et al., 2006), which could be a suitable corridor for specimens flowing among Group 1 locations (northern locations and ABR), avoiding SAL. These stingrays may have developed life-history strategies to avoid areas under the influence of freshwater, which maybe explains its north limit coinciding with the Parnaíba River Estuary, the last of a series of sedimentary Amazonian rivers, but also occurring in the Parcel Manoel Luís, a reef area about 80 km off the coast (Gomes et al., 2000; Motta et al., 2009; Cavalcante et al., 2020; Escobar et al., 2020; Dominguez & Guimarães, 2021).

Assuming the isolation of SAL and that the pattern of genetic diversity decreases latitudinally, we can infer that the isolated haplotype of this group could be the consequence of a recent founder event or allele fixation by genic drift (Matute, 2013). Apparently, *H. marianae* had recently colonized Todos os Santos Bay as the lineage present in this area (SAL) presents one mutational step in relation to the commonest *cytb* haplotype. For the last 2 million years, during the Quaternary, there were many eustatic variations of the sea level, with very few intervals of high sea level, as the one we currently live in. Besides, over the last 500,000 years, with an increase on the amplitude of sea level variations, its lowest point reached -45 m, close the current continental shelf break, which increased the erosion in the area due to the exposure of low granulometric sedimentary rocks at Todos os Santos Bay (Dominguez & Bittencourt, 2009). As a consequence, this area is a lowland flooded only during periods of high sea levels, being a transitory feature (Dominguez & Bittencourt, 2009) with a current average depth of 9.8 m (Cirano & Lessa, 2007). The

coastal area south of the SFR mouth is under direct influence of many perennial rivers, such as the Paraguaçu, Contas, and Jequitinhonha (Rosa et al., 2003; Abell et al., 2008; Souza & Knoppers, 2011), which may have resulted in a lower salinity coast, inducing an isolation by environment (IBE) in SAL.

Previous studies have shown IBE (Wang & Bradburd, 2014) as a frequent pattern of population isolation that delimits genetic differentiation and gene flow in marine fishes (Nanninga et al., 2014; Gaither et al., 2015). The IBE model predicts a positive relation between genetic and environmental divergence, with a higher habitat differentiation among populations, decreasing the fitness of dispersing organisms, and leading to divergent selection (Nanninga et al., 2014). Based on that, IBE could explain the genetic structure of *H. marianae*, as observed in a hammerhead shark, *Sphyrna lewini* (Griffith & Smith, 1834) (Duncan et al., 2006) and in the guitarfish genus *Pseudobatos* (Last et al., 2016; Sandoval-Castillo & Beheregaray, 2020). In an IBE scenario, environmental differences can reduce gene flow and enhance local adaptations (Wang, 2013; Sexton et al., 2014). It can be generated by non-random reproduction due to environmental differences (e.g., reproductive timing) and local adaptations as a result of strong selection (Sexton et al., 2014). These environmental differences between both groups of *H. marianae* were shown by the ENM (Costa et al., 2017), which stressed salinity and depth as drivers for the species distribution, including a gap at the SFR mouth (Alberoni et al., 2020).

Studies have been emphasizing the relevance of differential sexual behavior for genetic diversity in elasmobranchs, in which females are usually philopatric, and males tend to have wider migration areas (Corcoran et al., 2013; Tilley et al., 2013; Portnoy et al., 2015; Schwanck et al., 2020). Females of elasmobranchs are usually found in shallow, warmer areas for reproduction purposes, avoiding males that seldomly appear in these regions (Nosal et al., 2013; Schwanck et al., 2020). The pattern observed in *H. marianae* agrees with the hypothesis of female philopatry, given this genetic structure was only observed for the mitochondrial marker. However, the nuclear marker that we used may be too conserved to detect population signature. Future molecular studies should focus on codominant and more variable nuclear markers to verify if there is different behavior between sexes, as observed in *Hypanus americanus*

(Hildebrand & Schroeder, 1928; Schwanck et al., 2020).

Even though genetic diversity indices varied among localities, regardless of sample sizes, results suggest a negative correlation between genetic diversity and latitude. Northern localities (BIT, CAI, MAR, and PON), in smaller latitudes ($2^{\circ} 53'$ to $7^{\circ} 38'$ S), show higher genetic diversity than localities in Southern latitudes (SAL and ABR, $12^{\circ} 59'$ to $17^{\circ} 58'$ S). This negative correlation may have been caused by the colonization times of each group, with northern localities hosting a putative larger and older population (haplotype 2) that could have acted as a center of radiation to SAL and ABR due to a global warming tendency and higher sea levels since the LGM, allowing the colonization of shallow and southern areas. As a consequence, low genetic diversity in higher latitudes (SAL and ABR) may be the result of reduced groups' sizes in peripheral populations (Le Port & Lavery, 2012).

Conservation remarks

Hypanus marianae was recently classified as 'endangered' by the IUCN (Pollom et al., 2020). Despite being a smaller species, it is exposed to pressures of unregulated artisanal and commercial fisheries, especially for consumption along the Brazilian Northeastern coast (Costa et al., 2015). The species is also casually explored for the ornamental fish trade in specific locations of Bahia (Pollom et al., 2020). Its exploration should not be underestimated given that stingray landings have surpassed sharks' for the last three decades in the world (Dulvy et al., 2014) and experts suspect that *H. marianae* has suffered a 50–79% reduction of its global population over the past three generations (Pollom et al., 2020).

Oliveira et al. (2021) showed that *Hypanus guttatus* (Bloch & Schneider, 1801) suffered an alarming demographic decline of 11.83% per year due to fishing capture in Northeastern Brazil, especially in Rio Grande do Norte and Alagoas states. These areas also encompass the distribution of *H. marianae* and, even though specimens of the northern group are widely distributed throughout almost the totality of the species' occurrence area, its threatened status raises concerns regarding the population condition in the near future.

The Abrolhos Archipelago is the largest and richest coral reef system in South Atlantic (Floeter et al., 2001; Leão et al., 2003) and about 5% of its area is protected by the Abrolhos National Marine Park (Bruce et al., 2012) ensuring, to a certain extent, protection to the species, but most of the population remains in areas under exploitation and the known loss of habitat in urbanized coastal regions. Currently, there are approximately 10 marine protected areas (MPA) in Northeastern Brazil (Costa et al., 2015) but their role in preserving the genetic diversity is poorly known. MPAs should work as a connected network of individuals' flow among distinct localities (Crooks, 2006), helping to keep the population resilience (Fernandes et al., 2012). For *H. marianae*, Costa et al. (2015) have already highlighted, the relevance of MPAs in species' management and conservation, which is corroborated by the presence of a single population from Ceará to Bahia.

Local conservation measures are well-described for an MPA in Rio Grande do Norte State by Costa et al. (2015). The authors elucidated that reef-associated environments are crucial for *H. marianae*, such as seagrass beds and some areas close to the beach. Seagrass beds appeared to be the preferred habitat of adult females, while immature individuals were concentrated along the sandy bottom near the beach (Costa et al., 2015). The species' low fecundity (Pollom et al., 2020) maximizes the importance of these environments. Therefore, in addition to reef areas, it is important to locally map seagrass beds and coastal areas where these immature stingrays occur. Once characterized the importance of at least three different marine ecosystems such as reefs, seagrass beds, and shallow sand bottoms, we suggest an integrated conservation management. Such ecosystems are connected and subjected to constant habitat loss due to known threats and impacts as disorderly occupation of the coast, coastal erosion, and climate change. Thus, the establishment of new MPAs covering such habitats seems to be a useful tool for this species' protection.

Specifically, Salvador's coast, where the unique haplotype 1 is located, is an urban area without any MPA, therefore most susceptible to the impacts of fishing or habitat loss. Thus, precise protection measures should be evaluated to keep this exclusive genetic stock. Considering that the capture of the species is occasional and without economic relevance,

the prohibition of its capture and trade can be considered a good alternative with low social impact. Locally fishing quotas and size class restrictions for *H. marianae* would probably be beneficial, despite agency and enforcement difficulties. It is also important to highlight that, unfortunately, over the last years the Brazilian Red List of Fishes (MMA, 2014) has been legally invalidated based on no scientific arguments, mainly due to pressure from part of the Brazilian government (Ministry of Agriculture, Livestock and Supply) and the industrial fishing sector (Ceretta et al., 2020).

To control this stingray's catch, we suggest its inclusion on Appendix II of CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) to guarantee a sustainable and traceable fishery of this species. Even though CITES regulates mostly the international trade, its Convention Article XIV, on the 'Effect on Domestic Legislation and International Conventions', states that if a species is in an Appendix, it does not affect national laws, which could be even more restrictive than those proposed by the Convention (Contracting States, 1973). Therefore, more effort and investments in governmental actions, such as permanent programs of environmental education and inspection, are necessary for the conservation of this endangered species.

Conclusion

Overall, the genetic structure pattern of *H. marianae* partially validates our hypothesis based on its ENM provided by Costa et al. (2017), which suggested the SFR as a putative barrier, resulting in at least two groups situated on both sides of its estuary; however, without an evident allopatric barrier to gene flow. The combination of a narrow continental shelf southward of SAL and the freshwater outflow of perennial rivers might support this population structure, which seems to be better explained by an IBE, than by limited mobility. Besides, there is a tendency towards a lower genetic diversity southwards and a possible historical population expansion on the same direction indicated by a low haplotype diversity south of the SFR.

Studies using more variable molecular markers, such as microsatellites or genomic SNPs, can elucidate the role of males and detect fine-structure patterns. Furthermore, additional samplings close to the

SFR estuary will be important to understand more accurately the consequences of the freshwater outflow as a barrier to gene flow in this coastal species. It may also clarify the restrictive factors to the group from Todos os Santos Bay, as well as help in the conservation measures of this isolated lineage which should include integrated protection of the diversity of ecosystems used by the species such as reefs, algae beds, and sandy shallow bottom near the beach.

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Data availability The *cytb* and *rag1* sequences have been submitted to the GenBank databases under accession number MZ301320–MZ301428 and MZ301429–MZ301444, respectively.

Declarations

Conflict of interest The authors declare that there are no conflicts of interest.

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