Haplotype distribution and connectivity of the white sea urchin *Tripneustes ventricosus* across the Brazilian biogeographic province

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Sea urchins play important roles in marine ecosystems as key herbivores and some species have wide geographic range. The Atlantic white sea urchin *Tripneustes ventricosus* is abundant in many rock reefs of the eastern and western Atlantic, and may be found in high densities in Atolls and Archipelagos. Despite the importance of sea urchins in insular ecosystems, there is no study evaluating the genetic structure and the origin of the white sea urchin in isolated ecosystems. Such information is crucial to understand the connectivity and genetic diversity of these populations from the tropical Atlantic provinces. To evaluate the origin of the white sea urchin in Fernando de Noronha Archipelago and the genetic features of this population, we conducted studies on the population structure of the white sea urchin using mitochondrial DNA (COI), in two regions within the Brazilian biogeographic province and compared with other regions in the Atlantic. The white sea urchin from Fernando de Noronha was found to be genetically distinct, with F_{ST} ranging from 0.3 to 0.9 from other populations in Atlantic. The sharing of haplotypes between the Brazilian coast and the archipelago suggests that insular species derived from the Brazilian coast, rather than the East Atlantic. Moreover, all other Atlantic populations were genetically isolated, with low genetic diversity being a common characteristic among them (ranging from 0.0011 to 0.0022). The low connectivity found within populations might be related to the presence of soft barriers among the Brazilian biogeographic province. The low nucleotide diversity may also suggest that *T. ventricosus* may have undergone bottleneck processes at some stage of their evolution. This study has important implications on the geographic distribution, population structure and gene flow of the white sea urchin among the Atlantic regions. Further studies should evaluate the biological and ecological aspects of the species in both insular and continental marine ecosystems.

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Abstract

Sea urchins play important roles in marine ecosystems as key herbivores and some species have wide geographic ranges. The Atlantic white sea urchin *Tripneustes ventricosus* is abundant in many rock reefs of the eastern and western Atlantic, and may be found in high densities in Atolls and Archipelagos. Despite the importance of sea urchins in insular ecosystems, there are no studies evaluating the genetic structure or origin of the white sea urchin in isolated ecosystems. Such information is crucial to understanding the connectivity and genetic diversity of these populations from the tropical Atlantic provinces. To evaluate the origin of the white sea urchin in Fernando de Noronha Archipelago and the genetic features of this population, we conducted studies on the population structure of the white sea urchin using mitochondrial DNA (COI), in two regions within the Brazilian biogeographic province and compared them with other regions in the Atlantic. The white sea urchin from Fernando de Noronha was found to be genetically distinct, with F_{ST} ranging from 0.3 to 0.9, from other populations in the Atlantic. The sharing of haplotypes between the Brazilian coast and the archipelago suggests that insular species derived from the Brazilian coast, rather than the East Atlantic. Moreover, all other Atlantic populations were genetically isolated, with low genetic diversity being a common characteristic among them (ranging from 0.0011 to 0.0022). The low connectivity between populations of the Brazilian biogeographic province might be attributed to the presence of soft barriers. In addition, the low nucleotide diversity may have arisen from a bottleneck event, but may also be the signature of a selective event on the gene locus studied herein. This study has important implications on the geographic distribution, population structure and gene flow of the white sea urchin among the Atlantic regions. Further studies should evaluate the biological and ecological aspects of the species in both insular and continental marine ecosystems.

Introduction

Ecosystems are characterized by a range of species interactions and habitat complexities. A singular change in the dynamic of the ecosystem may result in drastic changes in both species interaction and habitat complexity. Sea urchins play important roles controlling algal growth on rocky reefs ecosystems, and their outbreaks may cause severe impacts on the existing communities, particularly in isolated ecosystems (Eklof et al. 2008; Eklof et al. 2009; McClanahan et al. 1996). Outbreaks in isolated communities, such as archipelagos, may occur during/following high inbreeding events, leading to changes in the genetic structure of the population. A rapid demographic expansion (outbreaks) of the white sea urchin in Fernando de Noronha archipelago was detected between 2000 and 2007, threatening the local diversity along reef-associated habitats. Overgrazing has been documented in most parts of the archipelago of Fernando de Noronha, likely changing the biomass of shallow coastal reefs. Such population expansion has no clear explanation other than a lack of predators in the local ecosystem contributing to the species establishing successfully. High densities of the white sea urchin had been previously recorded in the archipelago (Matthews 1972), but there is no record for outbreaks of that species along the Brazilian coast or anywhere else in the Atlantic. The population structure of the white sea urchin across the Brazilian biogeographic province is unknown and historical cycles of outbreak events may have affected the population structure and the connectivity of the species.

Connectivity is a measure of the gene flow between populations within their geographic range (Curley & Gillings 2009; Saenz-Agudelo et al. 2011; Vergara-Chen et al. 2010), but it can also be measured through larval dispersal and estimates of post-larval duration (Selkoe &

Toonen 2011). The analysis of population connectivity of species using planktotrophic larval stage reveals oceanographic or topographic boundaries across the oceans, thus structuring the present marine biogeographic provinces (Coleman et al. 2011a; Coleman et al. 2011b; Cowen et al. 2000; Hellberg 2009; Kohn & Clements 2009). However, population connectivity within a particular biogeographic province is usually assumed to be strong due to the absence of physical barriers. The correlation of geographic range and connectivity is essential to define the spatial limits of marine biogeographic provinces (Briggs & Bowen 2012; Briggs & Bowen 2013).

The Atlantic biogeographic province was previously comprised of the Caribbean and Brazilian areas, due to similar marine fauna and the absence of an obvious physical barrier. However, studies on tropical Atlantic reef fishes based on life history traits (Luiz et al. 2011), geographic distribution (Ferreira et al. 2004; Floeter et al. 2008; Joyeux et al. 2001), and genetic structure (Lima et al. 2005; Rocha et al. 2002; Rocha & Bowen 2008) revealed that the outflow of the Amazon river acts as an important barrier to speciation of coral reef fishes, and therefore, the Brazilian biogeographic province, including the Archipelago of Fernando de Noronha, St. Peter and St. Paul Archipelago, the Rocas atoll, and the Trindade island may consist of a unique province of the Atlantic. Within those locations, a high number of endemic fishes are found (Floeter et al. 2008).

Unlike reef fishes, the species distribution and population connectivity of sea urchins across the Brazilian province is not well known. For example, the white sea urchin *Tripneustes ventricosus*, a key species of the reef benthic community, is found in tropical areas of the Atlantic, but its connectivity across the Atlantic provinces is unknown. Phylogeographic studies have showed that the species is distributed from the Caribbean Sea downwards to south Brazil, the tropical latitudes of the Western African coast, São Tomé Island, and the Brazilian

91 archipelagos (Lessios et al. 2003). The haplotype distribution of the white sea urchin for the
92 Atlantic, including only few specimens from Brazil revealed that the species may have
93 undergone speciation when the Isthmus of Panama emerged, approximately three million years
94 ago (Lessios et al. 2003). Despite the wide Atlantic distribution the genetic structure and
95 population connectivity of this key species are poorly studied within the Brazilian province.

96 An investigation into the haplotype distribution of the white sea urchin within the
97 Brazilian biogeographic province may uncover the genetic structure and connectivity among
98 these populations. Furthermore, the haplotype distribution within and among populations can
99 also shed light on the origin of the white sea urchin in Fernando de Noronha, thus providing
100 important information on the biological and ecological aspects of this species in the region
101 (Lessios et al. 2003; Lessios et al. 2001; Lessios et al. 1999; Zigler & Lessios 2004). This study
102 therefore aims to investigate the haplotype distribution or genetic structure among populations of
103 the Atlantic white sea urchin (*T. ventricosus)*, and to answer whether the subpopulation
104 (subpopulation herein classified as part of the population, but geographically distant) found in
105 Fernando de Noronha Archipelago is genetically more similar to East or West Atlantic
106 populations or, whether it is highly genetically structured. If the subpopulation found in
107 Fernando de Noronha has similar genetic characteristics to either the Brazilian population or to
108 the African population, then it is likely that a mass migration of white sea urchins had recently
109 reached the archipelago. This study will provide important information about the current genetic
110 status of sea urchins in Atlantic and, particularly, about the population found in Fernando de
111 Noronha Archipelago.
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Methods

Sampling data

Four putative populations of *T. ventricosus* from the Atlantic were sampled; the Caribbean region, São Tomé Island and two Brazilian locations - mainland and Fernando de Noronha archipelago (FN). In total, 56 individuals were collected from the Brazilian coast, here represented by Bahia state (13º00'55 S''; 38º28" W), and FN (sample number) (3º51'31 S: 32º24'16'' W) (1200 Km apart). Although white sea urchins are described from other tropical regions in Brazil, they are commonly found along the inshore reefs of Bahia state. Gonad tissue was collected from all individuals and preserved in 95% ethanol. Sampling was carried out under permit number 1840-1, conceded by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) and authorized by the Sistema de Autorização e Informação em Biodiversidade (SISBIO). For other Atlantic regions, we utilized the available sequences from *T. ventricosus* on GenBank (Lessios et al. 2003).

DNA sequences

DNA was extracted using Ilustra® Tissue & Cells Mini Spin kit (GE Healthcare) following the manufacturer's instructions. Proteins were precipitated by adding 170 µl of 5 M NaCl and microcentrifuged at 14,000 rpm for 5 min. Supernatant (600 µl) was recovered into a new tube and the DNA precipitated by adding 600 µl of ice-cold absolute ethanol. Tubes were stored at – 20 ºC for 1 h. DNA was then recovered after microcentrifugation (14,000 rpm) for 3

min. Following decanting, remaining ethanol was removed with a micropipette. DNA was suspended in 100 µl of TE buffer (10 mM Tris HCl, pH 7.6;1 mM EDTA) prior to storage at -20 ºC. DNA yield was checked through 1% agarose gel electrophoresis using TBE (90 mM TRIS-borate; 2 mM EDTA). A 680 bp portion of the mitochondrial Cytochrome *c* Oxidase I (COI) gene was amplified using primers TR1f and TR1 (Lessios *et al.* (2003). Polymerase chain reaction (PCR) was carried out with the use of PuReTaq Ready-To-Go PCR Beads (GE Healthcare) plus 0,4 µM of each primer, on 25 µl reactions. PCR conditions were set up as follows: initial denaturing at 94ºC/4min; 40 cycles of 94ºC/60s, 50ºC/30s, 72ºC/60s; final extension at 72ºC/10min. All PCR products were purified with the Wizard® SV Gel and PCR Clean-Up System (Promega) and sent to Sanger sequencing by Macrogen Co. (Korea).

Data analysis

Sequences were edited and assembled with the software MEGA5 (Tamura et al. 2007) and aligned with Clustal W (Larkin et al. 2007). Haplotype and nucleotide diversities were estimated using Arlequin v.3.5 (Schneider & Excoffier 2000), as well as pairwise genetic distances (F_{ST}) between populations. Analysis of Molecular Variance (AMOVA) (Excoffier et al. 1992) based on Kimura 2P was carried out for comparisons of population structure among all four regions. Probability values described for genetic distances are based on $P< 0.005$, also calculated be Arlequin.

Haplotype network was obtained using TCS 1.21 (Clement et al. 2000), showing the relationship of haplotypes for the Atlantic populations of white sea urchins based on parsimony methods.

The demographic histories of the Atlantic populations were also analysed using Tajima's D-test and Fu's Fs test (Arlequin 3.5). Analyses were performed for all populations separately, although FN fauna is known to be part of the Brazilian biogeographic province (Floeter et al. 2008).

Results

Haplotype distribution

A total of 89 sequences of *T. ventricosus* from the Caribbean, São Tomé, Bahia and Fernando de Noronha were analysed. Of 680 bp obtained, we utilized sequences lengths of 557-663bp, in order to fit the Genbank sequences. A total of 24 haplotypes were detected amongst the Atlantic populations of white sea urchin. Fifteen polymorphic sites were observed from Bahia, whereas four polymorphic sites were found within individuals from Fernando de Noronha (data not added in the tables). Two substitutions resulted in protein changes in the Bahia population. The Caribbean and São Tomé populations presented five and four polymorphic sites respectively.

The number of unique haplotypes in each region varied from 3 to 11 haplotypes (Table 1). All regions had a unique group of haplotypes, except for the Brazilian region, which presented both unique and shared haplotypes between the mainland and F. Noronha (Figure 1).

Population structure

All Atlantic populations of *T. ventricosus* showed low nucleotide diversity (mean 0.0014235 +/- 0.001791), while haplotype diversity ranged from 0.4233 to 0.6785) (Table 1). Pairwise values of F_{ST} were all significant ($p<0.005$) and ranged from 0.32 (Bahia vs. F. Noronha) to 0.92 (São Tomé vs. Caribbean) (Table 2). Exact tests of sample differentiation based on haplotype frequencies confirmed the F_{ST} values between populations, with $p< 0.05$ (data not presented).

The analysis of molecular variance incorporating the four putative populations of *T. ventricosus* suggested that 78% of the overall genetic variance was observed between populations, with only 22% within populations ($P<0.05$). This suggests that populations of the white sea urchins from the Atlantic are highly genetically structured.

Demographic history

Given the strong genetic structure found in the populations of *T. ventricosus*, we tested the hypotheses of recent expansion or bottleneck events for each region and for the whole Atlantic population. Neutrality tests indicated recent population expansion in the Bahia (D= -2.230; Fu's= -8.717 ($p<0.001$) and Caribbean populations (D= -1.523; Fu's= -2.104 ($p<0.001$). Tajima's D and Fu's F tests suggested a bottleneck event in F. Noronha (D= 0.254; Fu's= 0.414 ($p<0.001$), while São Tomé presented positive and negative values (D= -1.534; Fu's= 0.204 (p-$p<0.001$). However, such results can also indicate a selective event affecting only COI.

Phylogeography

Analyses of haplotype distributions across the geographical range identified four genetically structured populations of the white sea urchin. Haplotypes from the Caribbean population showed a single connection to Bahia population. Bahia presented the highest number of haplotypes, with two haplotypes shared with FN. Following the connections within haplotypes, there was one single haplotype connecting the eastern (São Tomé population) to the western Atlantic, through FN, with six mutations between them (Figure 1).

The population of white sea urchin in FN has never been genetically investigated and four new haplotypes were recorded. The distribution of 15 haplotypes identified in 56 individuals from Bahia and F. Noronha derived from two main haplotypes, here named as HB1 and HF1 (Figure 1). Twenty individuals shared the former and nine individuals, the latter haplotype. The difference between these two main haplotypes is based on a single mutation.

Discussion

Genetic structure and gene flow

The analysis of mtDNA of the species *T. ventricosus* showed high genetic structure across the Atlantic, suggesting that geographic distances of populations within the Brazilian biogeographic province may influence the genetic structure of the species (Lessios et al. 2003). The level of structure found in populations of *T. ventricosus* is higher than other sea urchins from the Atlantic (Lessios et al. 1999), Red Sea (Debenham et al. 2000), Indo-Pacific (Lessios et al. 2003) or even when populations from two oceans are compared (Duran et al. 2004). The eastern Atlantic clade (São Tomé) was the most distant population (F_{ST} = 0.92) from all other regions, and the lack of genetic similarity with Fernando de Noronha specimens is likely due to mid-Atlantic Barrier separating both islands, with waters as deep as 5000 meters. This mid-Atlantic barrier is also a constraint for Atlantic reef fishes (Floeter et al. 2008; Joyeux et al. 2001; Luiz et al. 2011), and sea urchins of the genus *Diadema* (Lessios et al. 2001).

The spatial distribution of marine organisms is greatly influenced by the presence of geographical and environmental barriers (Briggs & Bowen 2012). A better knowledge of the occurrence of marine barriers has contributed to the understanding of how biogeographic provinces may determine the spatial arrangement of marine diversity on a global scale (Avise 2000; Briggs & Bowen 2012; Mayr 1954; Mayr 1982). Atlantic provinces are delineated by five major barriers; the mid-Atlantic Barrier (MAB), the Terminal Tethyan Event (TTE), the outflow of Orinoco-Amazon plume (OAP), The Isthmus of Panama (IOP) and the Benguela Current (BC) (Floeter et al. 2008). A common feature of these biogeographic provinces is the role of constraining or impeding species connectivity, ensuing speciation processes (Miglietta et al. 2011; Palumbi 1994; Rocha & Bowen 2008).

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242 Despite the high genetic structure observed, the reasons for such characteristic may vary
243 from one population to another. In the Caribbean, the low levels of genetic diversity (both in
244 terms of haplotype number and nucleotide diversity) found in white sea urchins (individuals
245 from Panama, Belize, Guatemala and Bahamas) suggest a fast expansion after a severe reduction
246 in population density. The causes for a severe reduction in the Caribbean is unknown, however,
247 with the closure of the Isthmus of Panama, the population of sea urchin became isolated from the
248 Pacific population. Habitat conditions also changed, thus likely affecting the population density
249 (Haug & Tiedemann 1998). Furthermore, White sea urchin is considered a gourmet item in the
250 Caribbean regions, with exploitation since more than a century ago likely causing a large
251 population decline (Scheibling & Mladenov 1987). However, the limitation of this work can only
252 suggest when a population expansion may have taken place, but not when genetic changes
253 occurred. According to Lessios et al. (2001), speciation of the Caribbean species from the Pacific
254 sister species *Tripneustes gratilla* occurred around 3mya, which coincides with the closure of the
255 Isthmus. It is also important to mention that this study is based on a single locus, COI, therefore,
256 analysis of nuclear markers would generate a more robust discussion of population expansion.
257 Biogeographic barriers isolating the Caribbean from the Eastern Pacific (Isthmus of Panama) and
258 from South America (Amazon outflow) may also have limited gene flow exchanges between
259 these other regions. Conversely, the highest nucleotide diversity found in the white sea urchin
260 from Bahia may result from the fact that sea urchins have never been through major changes in
261 habitats or, at least not as drastic as the changes in the Caribbean. This species is observed in
262 tropical and subtropical waters along the coast, mainly found in subtidal rocky reefs, but further

ecological and biological studies are needed to investigate the spatial distribution of the species along the Brazilian coast.

Sea urchin from Fernando de Noronha

In the Fernando de Noronha Archipelago, the white sea urchins have recently been overpopulating the inshore reef, but a previous survey of the benthic community in the 1980s described sea urchin presence as scarce throughout the islands (Eston et al. 1986). However, anecdotal information from Matthews (1972) indicates large densities of *T. ventricosus* at least in Santo Antonio Bay, on the main island.

As sea urchin outbreak events seem to be common, it is likely that there is a natural cycle in the population density of white sea urchins. In this case, anthropogenic impacts would not be the main cause of the current overpopulation, even though it might contribute to the intensity of the event. For example, the demographic growth of the white sea urchin in F. Noronha could be a consequence of a drastic reduction in the population of a natural sea urchin predator, the king helmet *Cassis* spp., once abundant on the island (Matthews 1972).

The population of white sea urchins presented F_{ST} index = 0.32 when compared to the Bahia population, and there were only three haplotypes found in this insular subpopulation. These features suggest that the occurrence of white sea urchin in Fernando de Noronha is not a sporadic event involving the species migrating from somewhere else for a period of time, but rather the white sea urchins of the island having unique genetic characteristics not present in other regions of Atlantic. Even though our methods do not clarify when sea urchins have reached

the island, the haplotype distribution observed between the island and the Brazilian mainland suggests that the insular population has more likely originated from Brazil than from East Africa.

What could explain such differences in genetic structure in the Brazilian region, since Fernando de Noronha is only 1200 km away from Bahia? It is likely that the Equatorial current that flows westwards may limit the connectivity of marine organisms between the Brazilian coast and FN. There is, however, a possible migration path via the Equatorial Under-current, which carries water at a depth around 60 m from the West Atlantic towards the Archipelago. This is also a likely explanation of the fact that the reef fish assemblages of St. Peter & St. Paul archipelago, in the middle of the Atlantic, are more similar to the Brazilian than to the African fish assemblage (Floeter et al. 2008; Joyeux et al. 2001). Further studies on white sea urchin larval behaviour and physiological requirements may help us understand its dispersal capabilities.

Processes driving the high genetic structure of the white sea urchin populations may be greatly influenced by oceanographic soft barriers (e.g. upwelling, change in water temperature or depth, etc) across the Atlantic ocean, thus causing low connectivity among the populations. In addition, climate changes and anthropogenic impacts (nutrient enrichment, lack of predators, water temperature) could modify the rates of inbreeding among the populations (Eklof et al. 2008; McClanahan 1995; Norderhaug & Christie 2009; Sivertsen 2006; Tewfik et al. 2005).

In conclusion, the location of Fernando de Noronha Archipelago as well as the haplotype distribution observed in the island, revealed its importance to the distributional range of *T. ventricosus* across Atlantic biogeographic provinces. Haplotype network, F_{ST} index, and average pairwise differences between populations suggest that sea urchins from Fernando de Noronha originated from the Brazilian mainland population. This suggestion, however, is based solely on

one locus (COI mitochondrial DNA). Further studies incorporating other loci may be done to support our results.

Fernando de Noronha is an important marine protected area and its marine biodiversity is composed of many endemic species (Floeter et al. 2008). Sea urchin outbreaks could cause profound effects on the marine benthic composition and its associated fauna. Therefore, it is important to further investigate the physiological aspects of the white sea urchin, as well as the environmental conditions along the archipelago, such as sea water temperature, nutrient enrichment and the population dynamics of sea urchin predators. Monitoring programs to evaluate the demographic expansion of the sea urchin would help to put in place efficient measures to protect the diversity of the coastal rocky reef ecosystem.

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317 **Table 1.** Sample size (N), number of haplotypes (H), haplotype diversity (*h*) and nucleotide
318 diversity (π) of the *Tripneustes ventricosus* populations.
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Locations	N	H	*h*	π
Caribbean	25	5	0.4233	0.001113
Bahia	38	12	0.6785	0.001966
F. Noronha	18	4	0.6340	0.002265
São Tomé	8	3	0.4643	0.001795

321 **Table 2**. Population pairwise F_{ST} for *Tripneustes ventricosus*. (*) indicates significance at $p <$

322 0.05.

Locations	1	2	3	4
1. Caribbean	-			
2. Bahia	0.770*	-		
3. F.Noronha	0.800*	0.323*	-	
4. São Tomé	0.919*	0.867*	0.839*	-

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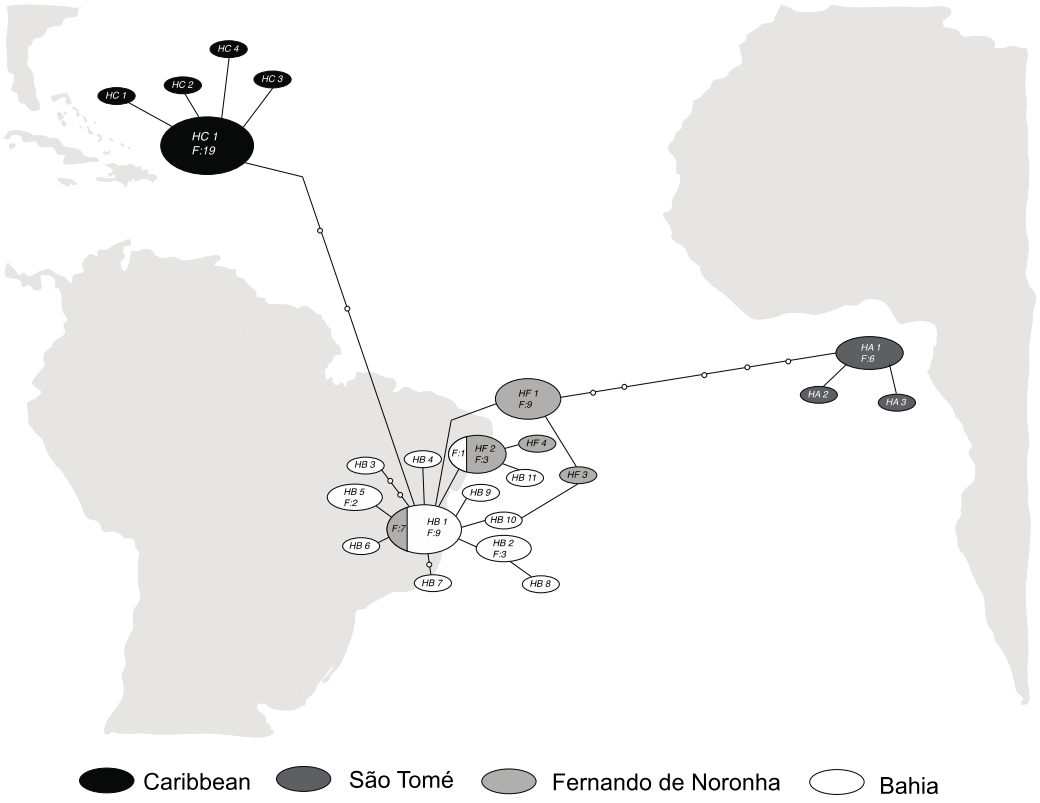
Figures

Figure 1. Haplotype distribution of the white sea urchin populations in the Atlantic. Frequency (F) is given in the circles. The white circles represent mutations between the haplotypes. HC (Haplotypes Caribbean); HA (Haplotypes Africa); HB (Haplotypes Bahia); HF (Haplotypes FN). F indicates frequency of sequences with same haplotype. The largest haplotype circle in each group indicates the region where samples were taken.



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