Lat. Am. J. Aquat. Res., 46(2): 355-363, 2018
DOI: 10.3856/vol46-issue2-fulltext-11

Research Article

Genetic variation and population homogeneity of the sea star *Coscinasterias tenuispina* (Forcipulatida: Asteroidea) on the coast of Rio de Janeiro, Brazil

Carmen Pazoto[1], Carlos Ventura[2], Michelle Duarte[1] & Edson Silva[1]
[1]Departamento de Biologia Marinha, Instituto de Biologia
Universidade Federal Fluminense, Rio de Janeiro, Brazil
[2]Departamento de Invertebrados, Universidade Federal do Rio de Janeiro
Museu Nacional, Rio de Janeiro, Brazil
Corresponding author: Michelle Duarte (michellerezendeduarte@yahoo.com.br)

ABSTRACT. Genetic variation and population structure of the sea star *Coscinasterias tenuispina* were investigated using allozyme electrophoresis (seven polymorphic loci out of nine sampled) at seven sites on the coast of Rio de Janeiro State. Observed values of heterozygosity's were high (H_{OBS} = 0.31 - 0.53), although similar to those found for others marine invertebrates. Departures from Hardy-Weinberg expectations were found only for one locus in three populations. No evidence of linkage disequilibrium was found among any loci for any population. The genetic identities vary from 0.882 to 0.988. The average θ was low and insignificant statistically (θ = 0.017). Furthermore, Bayesian analysis of population partitioning showed that the highest LnP(D) value was achieved for K = 1. These results do not corroborate any effect by upwelling limiting gene flow among the analyzed populations or the hypothesis that the strong sex ratio imbalance previously recorded to Itaipu population has been maintained by asexual reproduction.

Keywords: *Coscinasterias tenuispina*, echinoderms, reproduction, asexual, gene flow, heterozygosity, allozyme electrophoresis.

INTRODUCTION

The asteriid genus *Coscinasterias* Verrill, 1867 is composed by four species: *C. tenuispina* (Lamarck, 1816), *C. calamaria* (Gray, 1840), *C. acutispina* Stimpson, 1862 and *C. muricata* Verrill, 1870. The genus is monophyletic (Waters & Roy, 2003) and is represented in the Atlantic Ocean by the species *C. calamaria* and *C. tenuispina* and by *C. acutispina* and *C. muricata* in the Indo-Pacific Ocean (Clark & Downey, 1992). *C. tenuispina* is widely distributed, ranging from North Carolina (USA) to Santos (Brazil), including the Bermudas, the Mediterranean Sea, Canary Island, the Azores, Cape Verde, Saint Helena Islands, and Guinea. This sea star is a relatively common species that lives from intertidal to 165 m of depth, usually on hard bottoms (Clark & Downey, 1992).

Larval dispersal seems to be the main mechanism to explain the anti-tropical biogeographic pattern of the genus, absent in equatorial regions but present in both hemispheres. Because of the absence of species in the eastern Pacific, geographic dispersal of the genus is thought to have occurred through the Indian-Atlantic Ocean connections instead of the Pacific-Atlantic Ocean union, before the closure of the Panama Isthmus (Waters & Roy, 2003). Colonization ability is potentially enhanced because sexual reproduction occurs in the genus (Mladenov & Emson, 1990; O´Foighil & Smith, 1995) which may explain the ongoing presence of this species in Bermuda and other distant archipelagos.

Like other species in the genus, *C. tenuispina* is able to reproduce asexually by fission and sexually, producing a planktonic larval stage (Emson & Wilkie, 1980; Mladenov & Burke, 1994; Alves *et al*., 2002). Considering that the egg size of *C. tenuispina* is about 100 µm (C.R.R. Ventura, *unpubl. data*), it probably has a high-dispersal larva like that of the congener species *C. muricata* that can live up to 30 days under laboratory conditions (Baker, 1978).

Some intraspecific variation has been recorded from *C. tenuispina* for both morphological traits (Tortonese, 1982; Clark & Downey, 1992) and molecular data

Corresponding editor: Claudia Bremec

(Waters & Roy, 2003). Such morphological variations among Brazilian, Bermudan, and Mediterranean populations have prompted suggestions that several subspecies occur within *C. tenuispina*. Molecular analyses corroborate such hypotheses especially regarding specimens from Brazil and Mediterranean Sea (Waters & Roy, 2003).

Along the Brazilian coast, *C. tenuispina* has a discontinuous distribution and populations show remarkable differences in abundance, size structure, reproductive effort and sex ratio particularly along the coast of Rio de Janeiro State (Alves *et al*., 2002; Ventura *et al*., 2004). As most of the different sea stars species, *C. tenuispina* is a top predator, thus regulating marine invertebrates communities where occurs, especially in littoral zones. Furthermore, *C. tenuispina* is included in the list of Brazilian under threat species due to entropic impacts in its environment. In this way, the definition of population genetics baselines (Heyden *et al*., 2014) is of surmounting importance. In this study, molecular genetic analyses based on allozyme electrophoresis were performed for seven populations of *C. tenuispina* at a mesogeographic scale (5 to 320 km). Some sample sites were affected by upwelling events (Knoppers *et al*., 2002; Carbonel, 2003) and one population showed a significant sex ratio imbalance, high frequency of fission and low recruitment as previously reported (Alves *et al*., 2002).

The hydrological influence of upwelling and the predominance of asexual reproduction are relevant factors that can influence the genetic structure of populations. Furthermore, considering that sexual reproduction may favor dispersal (by producing planktonic larvae) and that asexual reproduction enhances survivorship of the offspring (by producing clones), the trade-off between dispersal (which can enhances gene flow) and clonal colonization (which enhances survivorship) may model pattern of genetic variation of populations at a mesogeographical scale.

Since existing data published on population genetics of *C. tenuispina* on the Brazilian coast was limited to three close populations (Ventura *et al*., 2004), this study aims to investigate the population genetic structure and gene flow among populations of this species, including new populations, not previously investigated.

MATERIALS AND METHODS

Sample collections

Samples of *Coscinasterias tenuispina* were collected from seven localities along the coast of Rio de Janeiro State (Fig. 1): Paraty (23º13'S, 42º01'W), Itaipu (22º59'S, 43º04'W), Prainha (22º57'S, 42º00'W), Cabo Frio Island (23º00'S, 42º00'W), Forno (22º57'S, 42º01'W), Peró (22º52'S, 44º42'W) and Ferradura (22º45'S, 41º53'W). The minimum linear distance between two localities was 5 km (Cabo Frio Island and Prainha) and the maximum was approximately 320 km (between Ferradura and Paraty sites). Populations of *C. tenuispina* are usually associated with rocky shores, however, abundance and vertical distributions of the species differed among the sampled sites. Regarding vertical distribution, Ferradura, Itaipu, Peró, and Prainha presented individuals living in the intertidal and subtidal zones (Alves *et al*., 2002; Ventura *et al*., 2004); individuals from Cabo Frio Island, Forno, and Paraty inhabited only the subtidal zone. Concerning the abundance, the species was very abundant at Ferradura, Itaipu, and Prainha (Alves *et al*., 2002; Ventura *et al*., 2004), however, showed very low densities at Cabo Frio Island, Peró, Forno and Paraty. Therefore, despite the efforts, samples sizes were smaller for populations from Cabo Frio Island, Forno, Paraty, and Peró (Table 2). The sea stars were transported alive to the laboratory and were kept at -20ºC until dissection. Samples of pyloric caeca were homogenized for electrophoresis.

Allozyme electrophoresis

Horizontal gel electrophoresis was performed by standard methods using 12.5% starch gels (Harris & Hopinkson, 1978). The gels were stained for 18 enzyme systems, of which seven gave useful results interpreted as the expression of nine gene loci: catalase (Cat, E.C.1.11.1.6), alpha-esterase (α-Est, E.C. 3.1.1.1), leucine aminopeptidase (Lap, E.C. 3.4.1.1), malate dehydrogenase (Mdh, E.C. 1.1.1.37), phosphogluconate dehydrogenase (Pgd, E.C. 1.1.4.4), superoxide dismutase (Sod, E.C. 1.15.1.1) and xanthine oxidase (Xod, E.C. 1.2.3.2). The buffer systems used were discontinuous lithium hydroxide pH 8.0 (Selander *et al*., 1971) for the enzyme loci α-Est, Lap, Mdh and Sod, and Tris-Citrate pH 8.0 (Ward & Beardmore, 1977) to Cat, Pgd, and Xod. Alleles were labeled alphabetically, in the decreasing electrophoretic mobility order of their corresponding allozymes.

Statistical analyses

Data analyses were carried out using the software packages GENEPOP 3.3 (Raymond & Rousset, 2001), FSTAT 2.9.3 (Goudet, 2001), TFPGA 1.3 (Miller, 1997) and Structure 2.3.3 (Pritchard *et al*., 2000). Genetic variation was estimated at the population level through the number of polymorphic loci, a number of alleles per locus and the mean number of observed and expected heterozygotes (H_{OBS} and H_{EXP}, respectively) per locus (Nei, 1987). Genotypic frequencies observed

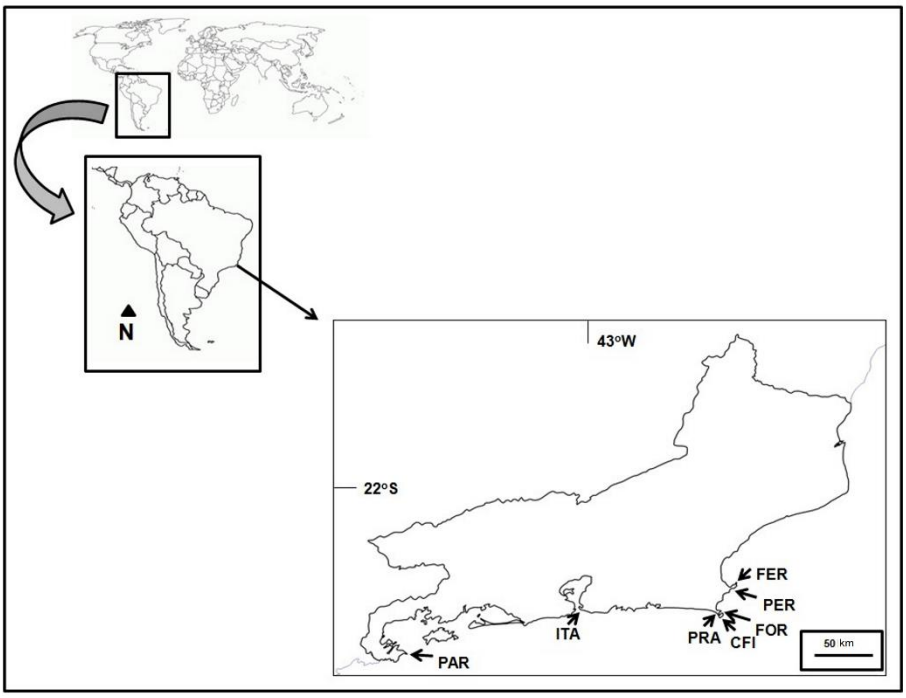

Figure 1. Sample sites on Rio de Janeiro State coast. PAR: Paraty, ITA: Itaipu, PRA: Prainha, CFI: Cabo Frio Island, FOR: Forno, PER: Peró, FER: Ferradura.

in each population at all loci analyzed were tested to conform to Hardy-Weinberg equilibrium using an exact test (Rousset & Raymond, 1995). The null hypothesis tested was a random union of the gametes and the alternative hypothesis was heterozygote deficit or excess. The *P*-values obtained by the exact Markov chain method (Guo & Thompson, 1992), were corrected for multiple testing with Bonferroni technique (Rice, 1989). Genotypic disequilibrium was analyzed by performing exact tests using a Markov chain method and correcting *P*-values obtained with Bonferroni technique (Rice, 1989). The null hypothesis tested was that genotypes at one locus are independent of genotypes at the other locus within each population. The *F*-statistical analysis was used to partition genetic variation within-population (*f*) and between population (θ) components using Weir and Cockerham´s method (Weir & Cockerham, 1984), which takes into account the differences in size among samples. Standard errors and an unbiased θ were obtained by jackknifing over loci and confidence intervals by bootstrapping over loci. Significance tests of *F*-estimates were carried out as described by Krebs (1989). The mean and the $2\times2\theta$ value were used to calculate the number of migrants being exchanged between populations per generation (Nm) using the equation $Nm = ((1/\theta)-1)/4$. Unbiased pairwise genetic distances were calculated according to Nei (1978) and used to cluster populations by the unweighted pair-group method with arithmetic averaging (UPGMA). Confidences for each node were assessed with 1000 bootstrap replicate.

The hypothesis of isolation by distance was tested by the regression of pairwise estimates of $\theta/(1-\theta)$ against geographic distance calculated using the ISOLDE routine of GENEPOP 3.3 under one-dimensional model (Rousset, 1997). The significance of the positive or negative correlation between $\theta/(1-\theta)$ and geographic distance was tested using a rank correlation coefficient computed with two one-sided Mantel´s test (Mantel, 1967) with 10000 random permutations. The slope of the regression line was tested for significant difference from zero, using the Pearson´s correlation coefficient. An analysis of molecular variance (Amova) was performed using differential hierarchical levels of genetic structure (within individuals, within populations, within groups of populations and among groups). Structure 2.3.3 (Pritchard *et al*., 2000) was used for Bayesian analysis of population partitioning. These analyses were performed with 5×10^{10} Monte Carlo Markov Chain (MCMC) steps, discarding the first 1×10^{5} iterations as burn-in. Each analysis was repeated 10 times for each simulated value of K, which ranged from 1 to 7 groups. LnP(D) and ΔK *ad-hoc* statistics (Evanno *et al*., 2005) were used to choose the best estimate of the number of partitions (K).

RESULTS

From the total of nine enzyme loci assayed, seven were polymorphic (Cat, α-Est-1, α-Est-2, Lap-2, Mdh, Pdg, and Xod) and only two (Lap-1 and Sod) were monomorphic (Table 1). Observed heterozygosities were high, ranging from 0.310 to 0.535 (mean value overall all loci and populations = 0.386 and variance = 0.051). The proportion of polymorphic loci was also high (77.8%) while the number of alleles per locus varied from 1.9-2.4 (mean value overall loci and populations = 2.2 and variance = 0.028) (Table 2). Departures from Hardy-Weinberg equilibrium were tested for each locus in each population. After Bonferroni corrections ($\alpha = 0.00102$), only one enzyme showed significant deviations from the expected genotypic distribution for three populations (Pgd in Ferradura, Itaipu, and Prainha), due to heterozygote deficits for all of them (Table 3). There was no significant linkage disequilibrium among any loci in any population. The overall Θ-value was 0.017 and F-value 0.081 (Table 4). This value was not significant but indicates that most of the variability exists within samples and is indicative of a generalized deficit of heterozygotes as can be seen in Table 2. Gene flow estimated by overall θ reveals a migration rate of 14.45 individuals per generation and the 2×2θ showed values that ranged from 3 to 28 migrants, but among the far apart populations (Paraty, the western population, and Ferradura, the eastern one) which showed a number of migrations lower than one (0.82 migrants). Genetic identities ranged from 0.882 (between Itaipu and Paraty) to 0.988 (between Ferradura and Prainha).

UPGMA cluster analysis (Fig. 2, Table 5) shows Itaipu as the outside population. However, the bootstrap analysis showed that the Ferradura-Prainha node is the only one well supported (76%). There was no significant correlation between estimates of θ/(1-θ) and geographic distance ($R^2 = 0.0037$). Therefore, genetic distances were not explained by a model of isolation by distance. Results obtained from the analysis of molecular variance (Amova) showed that most of the variance was among individuals within populations rather than among any of the groups defined (Table 6). For Structure analysis, the highest LnP(D) value was achieved for K = 1 (mean LnP(D) over 10 independent runs equals to -1756,85).

DISCUSSION

The high levels of heterozygosity found in this study were in accordance with those found for echinoderms and other marine invertebrates using allozyme electrophoresis technique, for example: the sea anemone *Anthopleura orientalis* Averincev 1967 $H_{ExP} = 0.25$ (Manchenko *et al*., 2000); the coral *Sinularia flexibilis* (Quoy & Gaimard, 1833) $H_{ExP} = 0.32$ (Bastidas *et al*., 2001); the mussel *Perna canaliculus* (Gmelin, 1791) $H_{ExP} = 0.21$ (Apte & Gardner, 2001); and the crustacean *Litopennaeus setiferus* (Linnaeus, 1767) $H_{ExP} = 0.59$ (Ball & Chapman, 2003).

Regarding population genetic structure, the results showed low and not statistically significant genetic divergence. Generally it was expected that species with extended planktonic development would show little genetic divergence (θ ranging from 0.002-0.02, over

Table 1. Allele frequencies at nine gene loci in *Coscinasterias tenuispina* from sites on the Rio de Janeiro coast, Brazil. Abbreviations for sampling sites as in Figure 1. n: sample size.

Locus	Sample site						
	PAR	ITA	PRA	CFI	FOR	PER	FER
Cat							
A	0.429	0.414	0.442	0.269	0.333	0.455	0.553
B	0.571	0.386	0.519	0.673	0.611	0.227	0.342
C	0.000	0.200	0.039	0.058	0.056	0.318	0.105
n	7	35	26	26	9	11	38
α-Est-1							
A	0.333	0.174	0.412	0.250	0.429	0.500	0.447
B	0.500	0.326	0.471	0.643	0.357	0.500	0.386
C	0.167	0.217	0.088	0.107	0.214	0.000	0.185
D	0.000	0.283	0.029	0.000	0.000	0.000	0.000
n	6	23	17	14	7	2	19
α-Est-2							
A	0.500	0.310	0.276	0.477	0.500	0.300	0.355
B	0.500	0.550	0.645	0.386	0.500	0.500	0.508
C	0.000	0.140	0.079	0.137	0.000	0.200	0.137
n	7	50	38	22	8	10	62
Lap-1							
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
n	2	15	17	20	10	7	21
Lap-2							
A	0.500	0.477	0.463	0.560	0.437	0.541	0.429
B	0.500	0.430	0.389	0.400	0.313	0.417	0.459
C	0.000	0.093	0.148	0.040	0.250	0.042	0.122
n	4	43	27	25	8	12	49
Mdh							
A	0.667	0.687	0.717	0.696	0.500	0.714	0.727
B	0.333	0.313	0.283	0.304	0.500	0.286	0.273
n	6	24	23	23	5	14	33
Pgd							
A	0.917	0.200	0.446	0.583	0.600	0.562	0.423
B	0.083	0.800	0.500	0.417	0.400	0.438	0.500
C	0.000	0.000	0.054	0.000	0.000	0.000	0.077
n	6	10	28	24	5	8	26
Sod							
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
n	8	53	48	13	7	17	65
Xod							
A	0.583	0.625	0.500	0.571	0.500	0.423	0.593
B	0.417	0.375	0.500	0.429	0.500	0.577	0.407
n	6	28	35	21	7	13	43

Table 2. Number of individuals collected and levels of gene variation (standard deviation in parenthesis) for the seven populations of *Coscinasterias tenuispina* analyzed. Abbreviations for sampling sites as in Figure 1. *A locus was considered polymorphic when more than one allele was detected.

Population	n	Mean sample size	Nº alleles per locus	Polymorphism* (%)	Mean heterozygosity	
					Observed	Expected
CFI	29	20.9 (±1.5)	2.2 (±0.3)	77.8	0.430 (±0.091)	0.400 (±0.085)
FER	79	39.6 (±5.6)	2.3 (±0.3)	77.8	0.392 (±0.082)	0.432 (±0.085)
FOR	10	7.3 (±0.3)	2.1 (±0.3)	77.8	0.535 (±0.120)	0.454 (±0.088)
ITA	66	31.2 (±5.0)	2.3 (±0.3)	77.8	0.362 (±0.093)	0.425 (±0.090)
PAR	8	5.8 (±0.6)	1.9 (±0.2)	77.8	0.310 (±0.106)	0.387 (±0.086)
PER	17	10.4 (±1.5)	2.1 (±0.3)	77.8	0.318 (±0.115)	0.445 (±0.088)
PRA	68	28.8 (±3.4)	2.4 (±0.3)	77.8	0.357 (±0.070)	0.419 (±0.082)
Average	39.57	20.6	2.2	77.8	0.386	0.423

Table 3. Probabilities (P) of conformance to Hardy-Weinberg equilibrium (H_o: random union of gametes) for the seven populations of *Coscinasterias tenuispina* analyzed. Abbreviations for sampling sites as in Figure 1. *Significant values after Bonferroni correction ($\alpha = 0.00102$).

Pop/loci		Cat	α-Est-1	α-Est-2	Lap-2	Mdh	Pgd	Xod
FER	*P*	0.273	0.115	0.528	0.002	0.037	0.000*	0.206
	D	+0.146	-0.268	-0.009	-0.080	+0.354	-0.466	-0.190
FOR	*P*	0.383	0.582	0.008	0.277	0.841	0.527	0.560
	D	+0.434	+0.238	+0.875	+0.084	+0.080	-0.250	-0.204
ILH	*P*	0.345	0.340	0.795	0.478	0.045	0.100	0.258
	D	+0.281	+0.075	+0.103	+0.270	+0.406	-0.329	-0.241
ITA	*P*	0.115	0.088	0.031	0.052	0.032	0.000*	0.883
	D	+0.013	-0.365	-0.149	-0.087	+0.424	-1	-0.027
PAR	*P*	0.073	0.678	0.560	0.021	0.301	1.000	0.064
	D	+0.625	-0.250	-0.204	-1	+0.375	+0.000	-0.686
PER	*P*	0.068	0.046	0.008	0.277	0.166	0.021	0.040
	D	+0.490	-1	-0.081	-0.399	+0.350	-0.762	-0.545
PRA	*P*	0.438	0.020	0.064	0.023	0.071	0.000*	0.799
	D	-0.080	-0.429	-0.224	-0.228	+0.364	-0.232	-0.042

Table 4. F-statistics values are not significant for seven enzyme loci in *Coscinasterias tenuispina*.

Locus	*f*	F	θ
Cat	-0.164	-0.103	0.052
α-Est-1	0.264	0.283	0.026
α-Est-2	0.046	0.053	0.007
Lap-2	0.095	0.084	-0.012
Mdh	-0.382	-0.391	-0.007
Pgd	0.415	0.446	0.054
Xod	0.190	0.181	-0.011
Average	0.081	0.096	0.017

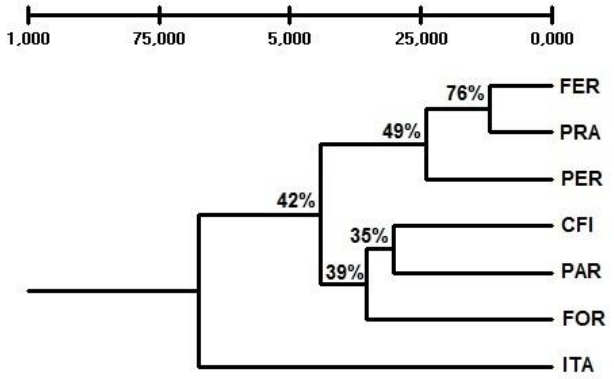

Figure 2. UPGMA cluster analysis for the seven populations of *Coscinasterias tenuispina* based on Nei's genetic distance. Abbreviations for sampling sites as in Figure 1.

distances greater than 1,000 km, Johnson & Black, 1984; Watts *et al*., 1990; William & Benzie, 1998; Apte & Gardner, 2001; Sköld *et al*., 2003). Nonetheless, *C. tenuispina* is also able to reproduce asexually, producing large individuals with low dispersal capability. Although some studies observed genetic divergence between populations of species that can reproduce both sexually and asexually (Stickle *et al*., 1992; Russo *et al*., 1994; Miller & Ayre, 2004), this is

Table 5. Nei's (1978) unbiased measures of genetic identity (above diagonal) and pairwise θ values (below diagonal) for the six populations of *Coscinasterias tenuispina*. Abbreviations for sampling sites as in Figure 1.

Population	CFI	FER	FOR	ITA	PAR	PER	PRA
CFI	*****	0.962	0.968	0.936	0.970	0.955	0.974
FER	0.0320	*****	0.964	0.967	0.947	0.978	0.988
FOR	-0.0009	0.0035	*****	0.928	0.962	0.945	0.973
ITA	0.0481	0.0097	0.0307	*****	0.882	0.937	0.961
PAR	-0.0030	0.233	-0.0270	0.0804	*****	0.940	0.952
PER	0.0263	-0.0088	0.0088	0.0093	0.0054	*****	0.975
PRA	0.0169	-0.0005	-0.0145	0.0184	0.0155	-0.0053	*****

Table 6. Analyses of molecular variance (AMOVA) among seven samples of *Coscinasterias tenuispina* separated into three groups: West of Upwelling (Paraty, Itaipu, and Prainha), Upwelling (Cabo Frio Island), and East of Upwelling (Forno, Peró, and Ferradura). df: degrees of freedom.

	Source of variation	df	Variance components	% variation	Fixation Indices	*P*
Panmixia	Among populations	6	-0.03553	-6.64	ST = -0.06638	1.00000
	Within populations	545	0.57081	106.64		
	Total	551	0.53528			
Three groups	Among groups	2	-0.00033	-0.06	ΦCT = -0.00061	0.65982
	Among populations within groups	4	-0.03529	-6.59		
	Within populations	545	0.57081	106.66		
	Total	551	299.120			

not the case for populations of *C. tenuispina* investigated in this work.

Another interesting possibility for limiting gene flow among *C. tenuispina* populations was the upwelling phenomenon that affects the region studied. The occurrence of the upwelling events along the coastal region of Cabo Frio is well described elsewhere (Knoppers *et al*., 2002) and is considered an unusual system in the western boundary of the Atlantic Ocean (Carbonel, 2003). Alterations of physical and chemical conditions of the sea water mass associated with this phenomenon could act as a physical barrier to larval dispersal. Some studies have shown the effect of upwelling phenomena limiting gene flow of a variety of marine invertebrates.

For example, Smith (1988) and Apte & Gardner (2002) working on *Perna canaliculus* (Gmelin, 1791) (bivalve), Laudien *et al*. (2003) on *Donax serra* Röding, 1798 (bivalve) and Waters & Roy (2004) and Ayers & Waters (2005) with *Patriella regularis* (Verrill, 1867) (asteroid), related the genetic differentiation found among populations of such marine invertebrates with the upwelling phenomena occurring in their studied regions. This study failed to reveal any interference from the upwelling phenomenon to the genetic structure of *C. tenuispina*.

The hypothesis that the data were distributed in a pattern of isolation by distance (Wright, 1943) was tested but showed no correlation whatsoever between genetic distances (or θ) and geographical distances. Furthermore, Structure analysis showed only one population. It should be bear in mind that allozymes are relatively conserved molecular markers, thus, it cannot be discarded the possibility that they were unable to detect population divergence which is due to more recent and intermittent physical boundaries such as upwelling. Therefore, further investigation using different molecular markers are desirable.

The population of Itaipu exhibited a strong sex ratio imbalance (only males were found) and there has been no record of small individuals (≤5 mm) (Alves *et al*., 2002). These facts suggested that recruitment for this population occurs mainly through clonal rather than sexual reproduction. It also suggested that this site was not receiving recruits from nearby populations or arriving recruits were not surviving. When analyzing genetic identities and θ values, the Itaipu population was consistently farther from any other population. However, a Mann-Whitney test over genetic identities

and pairwise θ values failed to review any statistically significant ($P > 0.05$) results for this apparent higher genetic difference between Itaipu and the other populations. Data on levels of heterozygosity ($H_{OBS} = 0.362$) for Itaipu population were very similar to those found for the other studied populations and most marine invertebrates that reproduce sexually. There is no evidence of significant linkage disequilibrium between any loci. Furthermore, we expect that clonal species should display either deficits or excess of heterozygotes because genotypic frequencies will be a simple reflection of the genotypes of the most successful clones (Ayre, 1984), but that was not the case for the population from Itaipu. Therefore, the genetic data obtained in this study did not support the hypothesis that clonal reproduction is the most important mechanism maintaining the Itaipu population. Nevertheless, evidence for clone reproduction requires greater sample sizes in order to infer for each individual its multi-loci genotype, what was not achieved in this study. Concerning the other six populations it was not found any evidence of fission among them, what is expected since, among echinoderms, asexual reproduction by fission occurs only as a temporary strategy in response to stressful conditions and even so alternating with sexual reproduction events (García-Cisneros *et al*., 2017).

In conclusion, the major results of our allozyme survey of *C. tenuispina* populations can be summarized as follow: 1) all seven populations exhibited high levels of intra-populational variation, 2) there was no evidence of genetic structure, neither from upwelling nor from geographical distance between populations, 3) the hypothesis that the Itaipu population is mainly maintained by asexual reproduction was not supported by the genetic data, although this needs further confirmation from multi-locus genetic in the future.

ACKNOWLEDGMENTS

We are thankful to the staff of the Instituto de Estudos do Mar Alte. Paulo Moreira (IEAPM–Brazilian Navy), for providing facilities and support during fieldwork. Also, we are grateful to Carlos Eduardo F. Leite, Joel Creed, and Fábio B. Pitombo for helping to sample specimens. We would like to acknowledge heartfelt the contribution of Tim O'Hara (Melbourne, Australia) in correcting the English of this paper. C.R.R.Ventura thanks the Brazilian Scientific Council (CNPq) for the Research Productivity fellowship and financial support (Proc. 474485/2004-8).

REFERENCES

Alves, S.L.S., A.D. Pereira & C.R.R. Ventura. 2002. Sexual and asexual reproduction of *Coscinasterias tenuispina* (Echinodermata: Asteroidea) from Rio de Janeiro, Brazil. Mar. Biol., 140: 95-101.

Apte, S. & J.P.A. Gardner. 2001. Absence of population genetic differentiation in the New Zealand green shell mussel *Perna canaliculus* (Gmelin, 1971) as assessed by allozyme variation. J. Exp. Mar. Biol. Ecol., 258: 173-194.

Apte, S. & J.P.A. Gardner. 2002. Population genetic subdivision in the New Zealand green shell mussel (*Perna canaliculus*) inferred from single-strand conformation polymorphism analyses of mitochondrial DNA. Mol. Ecol., 11: 1617-1628.

Ayers, K.L. & J.M. Waters. 2005. Marine biogeographic disjunction in central New Zealand. Mar. Biol., 147: 1045-1052.

Ayre, D.J. 1984. The effects of sexual and asexual reproduction on geographic variation in the sea anemone *Actinia tenebrosa*. Oecology, 62: 222-229.

Baker, M. 1978. Descriptions of the larvae of *Stichaster australis* (Verril) and *Coscinasterias calamaria* (Gray) (Echinodermata: Asteroidea) from New Zealand, obtained from a laboratory culture. Biol. Bull., 177: 32-46.

Ball, A.O. & R.W. Chapman. 2003. Population genetic analysis of white shrimp, *Litopenaeus setiferus*, using microsatellite genetic markers. Mol. Ecol., 12: 2319-2330.

Bastidas, C., J.A.H. Benzie, S. Uthicke & K.E. Fabricius. 2001. Genetic differentiation among populations of a broadcast spawning soft coral, *Sinularia flexibilis*, on the Great Barrier Reef. Mar. Biol., 138: 517-525.

Carbonel, C.A.A.H. 2003. Modeling of upwelling-downwelling cycles caused by variable wind in a very sensitive coastal system. Cont. Shelf Res., 23: 1559-1578.

Clark, A.M. & M.E. Downey. 1992. Starfishes of the Atlantic. Chapman and Hall, London, 794 pp.

Emson, R.H. & I.C. Wilkie. 1980. Fission and autonomy in echinoderms. Oceanogr. Mar. Biol., 18: 155-250.

Evanno, G., S. Regnaut & J. Goudet. 2005. Detecting the number of clusters of individuals using the software Structure: a simulation study. Mol. Ecol., 14: 2611-2620.

Garcia-Cisneros, A., R. Pérez-Portela, O.S. Wangensteen, M. Campos-Canet & C. Palacín. 2017. Hope springs eternal in the starfish gonad: preserved potential for sexual reproduction in a single-clone population of a fissiparous starfish. Hydrobiologia, 787: 291-305.

Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). [http://www.unil.ch/izea/softwares/fstat.html]. Reviewed: 17 October 2016.

Guo, G.W. & E.A. Thompson. 1992. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics, 48: 361-372.

Harris, H. & D.A. Hopkinson. 1978. Handbook of enzyme electrophoresis in human genetics. North-Holland Publishing Company, Amsterdam, 310 pp.

Heyden, S., M. Beger, R.J. Toonen, L. Herwerden, M.A Juinio-Meñez, R. Ravago-Gotanco, C. Fauvelot & G. Bernardi. 2014. The application of genetics to marine management and conservation: examples from the Indo-Pacific. Bull. Mar. Sci., 90(1): 123-158.

Johnson, M.S. & R. Black. 1984. Pattern beneath the chaos: the effect of recruitment on genetic patchiness in an intertidal limpet. Evolution, 38: 1371-1383.

Knoppers, B., W. Ekau, A.G. Figueiredo & A. Soares-Gomes. 2002. Zona costeira e plataforma continental do Brasil. In: A. Soares-Gomes & R.C. Pereira (eds.). Biologia marinha. Interciências, Rio de Janeiro, pp. 353-361.

Krebs, C.J. 1989. Ecological methodology. Harper & Row Publishers, New York, 654 pp.

Laudien, J., N.S. Flint, F.H. Van Der Bank & T. Brey. 2003. Genetic and morphological variation in four populations of the surf clam *Donax serra* (Röding) from southern Africa sandy beaches. Biochem. Syst. Ecol., 31: 751-772.

Manchenko, G.P., T.N. Dautova & Y.Y. Latypov. 2000. High levels of genetic divergence between sympatric color morphs of the littoral sea-anemone *Anthopleura orientalis* (Anthozoa: Actinaria). Biochem. Syst. Ecol., 28: 737-750.

Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Res., 27: 209-220.

Miller, M.P. 1997. TFPGA 1.3: tools for population genetic analyses. Available from: [http://www.marksgenetic software.net/tfpga.htm]. Reviewed: 10 September 2016.

Miller, K.J. & D.J. Ayre. 2004. The role of sexual and asexual reproduction in structuring high latitude populations of the reef coral *Pocillopora damicornis*. Heredity, 92: 557-568.

Mladenov, P.V. & R.H. Emson. 1990. Genetic structure of population of two closely related brittle star with contrasting asexual life histories, with observations on the genetic structure of a second asexual species. Mar. Biol., 104: 265-274.

Mladenov, P.V. & R.D. Burke. 1994. Echinodermata: asexual propagation. In: K.G. Adiyodi & R.G. Adiyodi (eds.). Reproductive biology of invertebrates, asexual propagation and reproductive strategies. John Wiley & Sons, Chichester, Vol. 6, Part B, pp. 339-382.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics, 89(3): 583-590.

Nei, M. 1987. Molecular evolutionary genetics. University of Columbia Press, New York, 512 pp.

O´Foighil, D. & M.J. Smith. 1995. Evolution of asexuality in the cosmopolitan marine clam *Lasaea*. Evolution, 49: 140-150.

Pritchard, J.K., M. Stephens & P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics, 155: 945-959.

Raymond, M. & F. Rousset. 2001. GENEPOP 3.3: Population genetics software for exact tests and ecumenicism. [ftp://cefe.cnrs-mop.fr]. Reviewed: 20 September 2016.

Rice, W.R. 1989. Analysing tables of statistical tests. Evolution, 43: 223-225.

Rousset, F. 1997. Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. Genetics, 145: 1219-1228.

Rousset, F. & M. Raymond. 1995. Testing heterozygote excess and deficiency. Genetics, 140: 1413-1419.

Russo, C.A.M., A.M. Sole-Cava & J.P. Thorpe. 1994. Population structure and genetic variation in two tropical sea anemones (Cnidaria, Actinidae) with different reproductive strategies. Mar. Biol., 119: 267-276.

Selander, R.K., M.H. Smith, S.Y. Yang, W.E. Johnson & J.R. Gentry. 1971. Biochemical polymorphism and systematics in the genus *Peromyscus*. I: Variation in the old-field mouse (*Peromyscus polionotus*). Studies in Genetics VI, University of Texas, 7103: 49-70.

Sköld, M., S.R. Wing & P.V. Mladenov. 2003. Genetic subdivision of a sea star with high dispersal capability in relation to physical barriers in a fjordic seascape. Mar. Ecol. Prog. Ser., 250: 163-174.

Smith, P.J. 1988. Biochemical-genetic variation in the green-lipped mussel *Perna canaliculus* around New Zealand and possible implications for mussel farming. New Zeal. J. Mar. Freshw., 22: 85-90.

Stickle, W.B., D.W. Foltz, M. Katoh & H.L. Nguyen. 1992. Genetic-structure and mode of reproduction in five species of sea stars (Echinodermata, Asteroidea) from the Alaskan coast. Can. J. Zool., 70: 1723-1728.

Tortonese, E. 1982. Variability and geographic distribution of *Coscinasterias tenuispina* (Lamarck) (Echinodermata: Asteroidea). Quad. Civ. Staz. Idrobiol. Milano, 10: 1-22.

Ventura, C.R.R., S.L.S. Alves, C.E.P. Mauricio & E.P. Silva. 2004. Reproduction and population genetics of *Coscinasteria tenuispina* (Asteroidea: Asteridae) on the Brazilian coast. In: T. Heinzeller & J.N. Nebelsick

(eds.). Echinoderms: München. Taylors & Francis Group, London, pp. 73-77.

Ward, R.D. & J.A. Beardmore. 1977. Protein variation in sympatric freshwater *Lymnaea peregra* and *L. ovata* (Gastropoda: Lymnaeidae). Biol. J. Linn. Soc., 61: 139-149.

Waters, J.M. & M.S. Roy. 2003. Global phylogeography of the fissiparous sea-star genus *Coscinasterias*. Mar. Biol., 142: 185-192.

Waters, J.M. & M.S. Roy. 2004. Phylogeography of a high-dispersal New Zealand sea-star: does upwelling block gene-flow? Mol. Ecol., 13: 2797-2806.

Received: 29 December 2016; Accepted: 6 November 2017

Watts, R.J., M.S. Johnson & R. Black. 1990. Effects of recruitment on genetic patchiness in the urchin *Echinometra mathaei* in Western Australia. Mar. Biol., 105: 145-151.

Weir, B.S. & C.C. Cockerham. 1984. Estimating F-statistics with special regard to systems of mating. Evolution, 19: 395-420.

Williams, S.T. & J.A.H. Benzie. 1998. Evidence of a biogeographic break between populations of a high dispersal starfish: congruent regions within Indo-west Pacific defined by colormorphs, mtDNA, and allozymes data. Evolution, 52: 87-99.

Wright, S. 1943. Isolation by distance. Genetics, 28: 114-138.