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Research Article

Genetic variation in color morphs of the endangered species, *Paracentrotus gaimardi* (Echinoidea: Echinidae)

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ABSTRACT. *Paracentrotus gaimardi* is a sea urchin reaching a maximum of 42 mm of diameter. It presents five morphotypes in sympatry characterized by their distinctive spine colors (*e.g.*, green, black, gray, brown and rose). *P. gaimardi* is considered as a vulnerable species and it is included on the list of endangered species of the Brazilian coastline. In this work, allozyme electrophoresis was used to study the genetic variation among the five color morphotypes, from two different geographic locations along the coast of Rio de Janeiro State: Itaipu and Prainha, both in sympatry and allopatry. The underlying null hypothesis is that the different spine colors that define the morphotypes, represent only intraspecific variation of *P. gaimardi*. Eight polymorphic *loci* (*Cat*, *α-Est-1*, *α-Est-2*, *Mdh*, *Pep*, *Pgi*, *Pgm* and *Xod*) were assayed. Departures from Hardy-Weinberg equilibrium were tested at each *locus* in each morphotype from each locality. *P. gaimardi* species showed high levels of genetic variation (0.171-0.343) in accordance with the patterns observed for marine invertebrates. Diagnostic *loci* were not found among any of the five morphotypes and neither for the different geographic locations. A U-Mann Whitney test showed values significantly lower in sympatry than in allopatry for both Nei's Genetic Identities and 2 x 2 θ F-statistics. Furthermore, performance of AMOVA analysis considering hierarchical levels of individuals indicates that the differences in genetic variation are sorted among collection sites rather than color morphotypes.

Keywords: echinoderms, morphotypes, allozymes, genetic variation, biochemical systematic, endangered species.

Variación genética en morfotipos de color de la especie en peligro de extinción, *Paracentrotus gaimardi* (Echinoidea: Echinidae)

RESUMEN. *Paracentrotus gaimardi* es un pequeño erizo de mar que presenta un diámetro máximo de 42 mm. Este erizo presenta cinco morfotipos en simpatría caracterizados por sus distintivas espinas coloreadas (*e.g.*, verde, negra, gris, café y rosa). *P. gaimardi* es considerada una especie vulnerable y está incluida en el listado de especies en peligro de la costa brasilera. En este trabajo se usaron caracteres aloenzimáticos para estudiar la variación genética entre cinco morfotipos de color, en dos localidades geográficas diferentes de la costa del Estado de Río de Janeiro: Itaipu y Prainha, ambas en simpatría y alopatría. La hipótesis nula analizada fue: si las diferentes espinas de color definen los morfotipos, representan solamente una variación intraespecífica de *P. gaimardi*. Ocho *loci* polimórficos (*Cat*, *α-Est-1*, *α-Est-2*, *Mdh*, *Pep*, *Pgi*, *Pgm* and *Xod*) fueron examinados. Las salidas del equilibrio de Hardy-Weinberg fueron examinadas en cada *locus* para cada morfotipo en cada localidad. *P. gaimardi* presenta grandes niveles de variación genética (0,171-0,343), lo cual concuerda con los parámetros observados para invertebrados marinos. No se encontró ningún *loci* diagnóstico en ninguno de los cinco morfotipos para ninguna de las áreas geográficas. La prueba del U-Mann Whitney mostró valores con baja significancia en simpatría y alopatría para las identidades genéticas de Nei y la prueba de 2x2 θ F. Adicionalmente, un análisis de AMOVA sugirió niveles jerárquicos para los individuos, indicando que las diferencias genéticas están organizadas alrededor de los sitios de colecta, más que en los morfotipos de color.

Palabras clave: equinodermos, morfotipos, aloenzimas, variación genética, síntesis bioquímica, especies en peligro.

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INTRODUCTION

The genus *Paracentrotus* belongs to the family Echinidae which includes only two species: *Paracentrotus lividus* (Lamarck, 1816) and *Paracentrotus gaimardi* (Blainville, 1825) (Mortensen, 1943). *P. gaimardi* is a small sea urchin reaching a maximum of 42 mm in diameter and is characterized by its delicate and fragile spines (Tommasi, 1966). This organism is distributed along the southeastern Brazilian coast, Angola and the Guinea Gulf (Africa) (Mortensen, 1943). Knowledge on this species is limited (Boudouresque & Yoneshigue, 1987; Villaça & Yoneshigue, 1987; Ventura & Barcellos, 2004; Calderón *et al*., 2009, 2010).

Along the southeast Brazilian coast *P. gaimardi* exists in five morphotypes in sympatry that are characterized by the colors of their spines (green, black, gray, brown and rose) (Fig. 1) (Tommasi, 1966). Color variation is a feature that has been regarded as important at the time of defining taxonomic status for different species groups (Endler *et al*., 2005), including different groups of marine invertebrates such as the starfish genus, *Echinaster* (Tuttle & Lindahl, 1980), the anemone, *Actinia equine* (Linnaeus, 1767) (Quicke *et al*., 1983), the sponge, *Oscarella lobularis* (Schmidt, 1862) (Boury-Esnault *et al*., 1992); the soft coral, *Alcyonium coralloides* (Pallas, 1766) (McFadden, 1999), and the nemertean genus, *Quasitetrastemma* (Zaslavskaya *et al*., 2010). In all of these cases, allozyme electrophoresis was the method used for species identification.

P. gaimardi is considered a vulnerable species and is included on the list of endangered species of the Brazilian coastline (Machado *et al*., 2008), especially because its over-exploitation and threats to its native habitat due to pollution (habitat destruction). In recent years, there has been a dramatic increase in the number of endangered species and hence extinction can no longer be regarded as a natural phenomenon. The endangerment of species usually occurs due to the destructiveness of human activities of natural resources, leading to a loss of biodiversity. Adequate information on the nature and the extent of genetic diversity in such species is a useful requirement for developing a suitable strategy for its conservation and management.

In the present work, allozyme electrophoresis was used to study genetic variation among the five color morphotypes of *P. gaimardi* that occur on the southeast Brazilian coast, both in sympatry and allopatry. The underlying null hypothesis is that the different colors of spines that define the morphotypes represent only intraspecific variation of *P. gaimardi*.

MATERIALS AND METHODS

Study sites

Samples of all *P. gaimardi* morphotypes were collected from two localities along the coast of Rio de Janeiro State (Fig. 2): Itaipu (22º58'26"S, 43º02'49"W) and Prainha (22º57'37"S, 42º01'10"W); the linear distance between the two localities is approximately 110 km.

The sea urchins were transported alive to the laboratory and were sorted by morphotypes, dissected, the tissues (gonad, guts and lantern muscle) washed with sea water, and kept at -20ºC until electrophoresis. Specimens examined in this study comprise a total of 172 individuals (109 from Itaipu and 63 from Prainha).

Allozyme electrophoresis

Horizontal gel electrophoresis was performed by standard methods using 12.5% starch gels (Harris & Hopinkson, 1978). The gels were stained for 30 enzyme systems using three tissues and four buffers. Eight enzymes gave useful results, interpreted as the expression of nine gene *loci* with two tissues and three buffers. The buffer systems used were discontinuous Tris-citrate/borate (pH 8.1/8.7) (Poulik, 1957) for the enzyme *loci* peptidase (Pep, E.C. 3.4.11.1), superoxide dismutase (Sod, E.C. 1.15.1.1) and xanthine oxidase (Xod, E.C. 1.2.3.2) with gut tissue; discontinuous lithium hydroxide pH 8.0 (Selander *et al*., 1971) for the enzyme *loci* alpha-esterase (α-Est, E.C. 3.1.1.1), malate dehydrogenase (Mdh, E.C. 1.1.1.37) and phosphoglucomutase (Pgm, E.C. 2.7.5.1) with gut tissue, and Tris-EDTA maleate (pH 7.4) (Brewer, 1970) for the enzyme *loci* catalase (Cat, E.C. 1.11.1.6) and glucose phosphate isomerase (Pgi, E.C. 5.3.1.9) with lantern muscle tissue. Alleles were labeled alphabetically, in order of decreasing electrophoretic mobility of their corresponding allozymes.

Statistical analyses

Data analyses were carried out using the programs BYOSIS-2 (Swofford & Selander, 1997), GENEPOP 3.3 (Raymond & Rousset, 2001) FSTAT 2.9.3 (Goudet, 2001), Arlequin 3.5.1.3 (Excoffier *et al*., 2005) and SPSS (SPSS Inc, 2001).

Genetic variation was estimated at the morphotype-locality level through the number of polymorphic *loci*, number of alleles per *locus* and the mean number of observed and expected heterozygotes (H_{OBS} and H_{EXP}, respectively) per *locus* (Nei, 1978).

Genotypic frequencies observed in each morphotype from each locality at all *loci* analyzed were tested for conformation to Hardy-Weinberg equilibrium using an exact test (Rousset & Raymond, 1995). The null hypo-

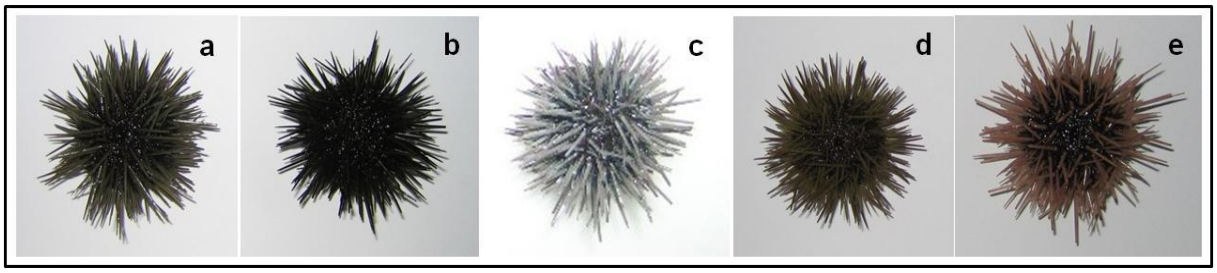

Figure 1. Morphotypes of *Paracentrotus gaimardi* characterized by their distinctive spine colors: a) green, b) black, c) gray, d) brown and e) rose.

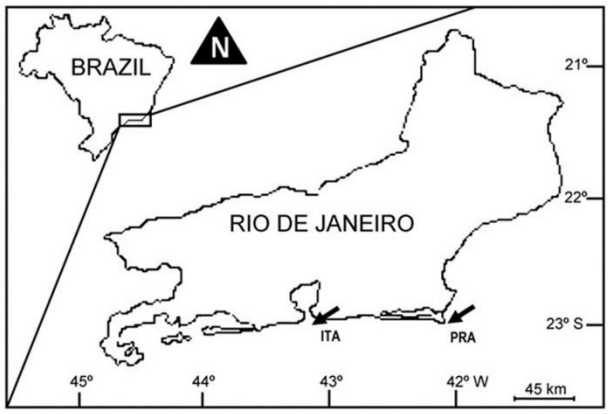

Figure 2. Sample sites on the coast of the Rio de Janeiro State: ITA: Itaipu and PRA: Prainha.

thesis 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 the Bonferroni technique (Rice, 1989).

Linkage disequilibrium was analyzed by performing exact tests using a Markov chain method and correcting *P*-values obtained with the Bonferroni technique (Rice, 1989). The null hypothesis tested was that genotypes at one *locus* are independent from genotypes at the other *locus* within each morphotype-locality.

F-statistics analysis was used to partition genetic variation within morphotype-locality (f) and between morphotypes-locality (θ) components using Weir & Cockerham´s (1984) method, 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).

An analysis of molecular variance (AMOVA) was performed using differential hierarchical levels of genetic structure (within individuals, within populations and within groups of populations, among groups). Significance was tested using 10.000 permutations. Unbiased pairwise genetic identities were calculated according to Nei (1972), and used to cluster morphotypes-locality by the unweighted pair-group method with arithmetic averaging (UPGMA). The results for Genetic Identities and 2 x 2 θ were used to test if differences in genetic variation are sorted among collection sites or color morphotypes with an U-Mann Whitney test.

RESULTS

From the nine enzyme *loci* assayed, eight were polymorphic (Cat, α-Est-1, α-Est-2, Mdh, Pep, Pgi, Pgm and Xod) and only one (Sod) was monomorphic (Table 1). The average percentage of polymorphic *loci* was 73.4% (varied from 55.6% for Prainha rose morphotype to 88.9% for Prainha brown and black morphotypes) and the number of alleles per *locus* varied from 1.7 to 2.1 (mean value overall *loci* and populations = 1.9). The sampled morphotype-locality did not show exclusive alleles for any *loci* in sympatric or allopatric morphotypes.

Observed heterozygosities ranged from 0.171 to 0.343 (Table 1) and the mean value overall of all *loci* and morphotype-locality was 0.232, indicating high levels of heterozygosity. When observed and expected heterozygosities were compared, observed heterozygosities were generally smaller than expected heterozygosities (Table 1), although this heterozygote deficit was not statistically significant.

Departures from Hardy-Weinberg equilibrium were tested at each *locus* in each morphotype from each locality. After the Bonferroni correction (α = 0.00098) (Rice, 1989), only one enzyme for one morphotype

Table 1. Allele frequencies at nine gene *loci** in *P. gaimardi* from sites on the Rio de Janeiro coast, Brazil. I: Itaipu, P: Prainha, N: sample size, A,B,C: alleles, H_{OBS}: Observed heterozygosities, H_{EXP}: expected heterozygosities.

Locus		Sample									
		I-Brown	I-Gray	I-Black	I–Rose	I-Green	P-Brown	P-Gray	P-Black	P-Rose	P-Green
α-Est-1*	A	0.063	0.750	0.400	0.250	0.091	0.042	0.333	0.200	0.000	0.167
	B	0.874	0.250	0.400	0.750	0.773	0.583	0.500	0.500	0.500	0.833
	C	0.063	0.000	0.200	0.000	0.136	0.375	0.167	0.300	0.500	0.000
	N	8	2	5	4	11	12	6	5	3	3
	H_{OBS}	0.250	0.500	0.400	0.500	0.455	0.250	0.333	0.200	0.333	0.333
	H_{EXP}	0.242	0.500	0.711	0.429	0.394	0.540	0.667	0.689	0.600	0.333
α-Est-2*	A	0.286	0.900	0.750	0.750	0.714	0.658	0.750	0.444	0.000	0.375
	B	0.714	0.100	0.250	0.250	0.286	0.342	0.250	0.556	1.000	0.625
	N	14	5	4	4	14	19	6	9	3	8
	H_{OBS}	0.286	0.200	0.500	0.500	0.286	0.368	0.167	0.444	0.000	0.250
	H_{EXP}	0.423	0.200	0.429	0.429	0.423	0.462	0.409	0.523	0.000	0.500
Cat*	A	0.583	0.500	0.800	0.389	0.500	0.812	0.125	0.500	0.250	0.400
	B	0.417	0.500	0.200	0.611	0.500	0.188	0.875	0.500	0.750	0.600
	N	12	4	5	9	8	8	4	2	2	10
	H_{OBS}	0.333	0.500	0.400	0.333	0.500	0.375	0.250	0.000	0.500	0.200
	H_{EXP}	0.507	0.571	0.356	0.503	0.533	0.325	0.250	0.667	0.500	0.505
Mdh*	A	0.325	0.125	0.227	0.200	0.227	0.344	0.083	0.333	0.200	0.389
	B	0.650	0.875	0.728	0.700	0.750	0.593	0.917	0.445	0.700	0.611
	C	0.025	0.000	0.045	0.100	0.023	0.063	0.000	0.222	0.100	0.000
	N	20	4	11	5	22	16	6	9	5	9
	H_{OBS}	0.300	0.250	0.182	0.200	0.091	0.188	0.167	0.556	0.600	0.111
	H_{EXP}	0.483	0.250	0.437	0.511	0.394	0.542	0.167	0.680	0.511	0.503
Pep*	A	1.000	1.000	1.000	1.000	1.000	0.944	1.000	0.909	1.000	0.937
	B	0.000	0.000	0.000	0.000	0.000	0.056	0.000	0.091	0.000	0.063
	N	11	10	7	3	19	18	9	11	5	8
	H_{OBS}	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.182	0.000	0.125
	H_{EXP}	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.173	0.000	0.125
Pgi*	A	0.133	0.286	0.091	0.111	0.083	0.000	0.000	0.625	0.125	0.300
	B	0.700	0.714	0.773	0.667	0.834	0.786	0.750	0.375	0.750	0.600
	C	0.167	0.000	0.136	0.222	0.083	0.214	0.250	0.000	0.125	0.100
	N	15	7	11	9	12	7	4	4	4	5
	HOBS	0.467	0.286	0.091	0.222	0.333	0.143	0.500	0.750	0.500	0.400
	HEXP	0.480	0.440	0.394	0.523	0.304	0.363	0.429	0.336	0.464	0.600
Pgm*	A	0.000	0.000	0.000	0.500	0.333	0.667	1.000	0.833	1.000	1.000
	B	1.000	1.000	1.000	0.500	0.667	0.333	0.000	0.167	0.000	0.000
	N	3	1	1	3	6	3	2	3	2	2
	HOBS	0.000	0.000	0.000	1.000	0.333	0.000	0.000	0.333	0.000	0.000
	HEXP	0.000	0.000	0.000	0.600	0.485	0.533	0.000	0.333	0.000	0.000
Sod*	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	N	35	13	18	9	34	22	9	10	5	16
Xod*	A	0.885	0.643	0.833	0.833	0.750	0.944	0.937	0.875	0.900	0.750
	B	0.115	0.357	0.167	0.167	0.250	0.056	0.063	0.125	0.100	0.250
	N	13	7	3	3	6	9	8	4	5	8
	HOBS	0.077	0.429	0.333	0.333	0.500	0.111	0.125	0.250	0.200	0.250
	HEXP	0.212	0.495	0.333	0.333	0.409	0.111	0.125	0.250	0.200	0.400
Mean	HOBS	0.190	0.240	0.212	0.343	0.278	0.159	0.171	0.302	0.237	0.185
	HEXP	0.261	0.273	0.296	0.370	0.327	0.332	0.227	0.428	0.253	0.330

showed significant deviations from the expected genotypic distribution (Mdh in Itaipu green morphotype), due to heterozygote deficiency. Significant linkage disequilibrium was not detected for any combination of *loci* among any morphotype-locality.

The genetic structure of morphotypes from the different localities was analyzed by means of Weir & Cockerham´s (1984) method, the overall θ-value and *f*-value were 0.062 and 0.329, respectively, which were not significantly different from zero, showing lower genetic differentiation among morphotypes and localities (Table 2). The 2x2 θ shows values which were not significant in sympatry (U-Mann Whitney test, $P = 0.353$), but showed a difference for Itaipu (-U Mann Whitney test, $P = 0.008$) and near signi-ficance for Prainha (U-Mann Whitney test, $P = 0.083$). In the same way, Nei's Genetic Identities (GI) between morphotypes showed high values for sympatric morphotypes (ranging from a maximum of 1 and a minimum of 0.887) and low values for allopatric morphotypes (ranging from a maximum of 0.986 and a minimum of 0.686) (Table 3). U-Mann Whitney test for Genetic Identities also showed values which were not significant for sympatry ($P = 0.473$) but different in allopatry for both cases (Itaipu, $P = 0.003$ and Prainha, $P = 0.007$). These results indicate that the differences in genetic variation are sorted among collection sites rather than color morphotypes (Table 3). Results obtained from the analysis of molecular variance (AMOVA) also indicated the greatest genetic variance within regional populations (10.07%, $P = 0.00684$) rather than morphotypes (-7.34%, $P = 0.86510$) (Table 4). The UPGMA dendrogram illustrates samples grouped by sites and not by morphotypes (Fig. 3), the only exception is Prainha brown morphotype which is clustered within Itaipu group.

Table 2. F-statistics (Weir & Cockerham, 1984) for eight polymorphic enzyme *loci* in *P. gaimardi*. *($P < 0.05$).

Locus	F	Θ	F
α-Est-1	0.3359	0.0525	0.3708
α-Est-2	0.2716	0.1466	0.3783
Cat	0.2820	0.0575	0.3233
Mdh	0.5092*	-0.0195	0.4996
Pep	0.3988	-0.0185	0.3877
Pgi	0.2518	0.0070	0.2570
Pgm	0.2871	0.3909	0.5658
Xod	0.1684	-0.0140	0.1568
Average	0.3297	0.0620	0.3713

DISCUSSION

The present study could not identify any diagnostic *loci* among the nine *loci* sampled for the five morphotypes of *P. gaimardi* from two different geographic locations. Furthermore, the results clearly indicate that in this species, spine color variation represents an intraspecific polymorphism, since genetic identities (GI) were significantly high for morphotypes living in sympatry and low for morphotypes living in allopatry. Analysis of molecular variance (AMOVA) is clear cut concerning this conclusion which is well represented by the dendrogram, which showed morphotypes clustered by sampling locations instead of color of spines. The only exception is Prainha brown morphotype, which is not an unexpected result since cluster analysis done by the UPGMA algorithm is sensitive to high values of genetic identities as is the case for morphotypes and localities studied here. Calderón *et al*. (2010) and Lopes & Ventura (2012) showed some asymmetry in relation to crossings between morphotypes of *P. gaimardi*. Despite any such preferential crossings, allozyme data clearly indicate that this restriction to gene flow has not been enough to determine a relevant isolation between these morphotypes. Asymmetric gametic isolation cannot stop gene flow between morphotypes. If genes from one morphotype freely enter the genome of the other in sympatry, even in one direction, recombination would be sufficient to prevent the formation of a new species (Lessios, 2011).

Diversity in coloration is a frequent phenomenon in marine invertebrates, but its ecological significance is often not fully understood. Variations in color may be related to age (Medioni *et al*., 2001), to light or wave exposure (Stoletzki & Schierwater, 2005), to diet (Tlusty & Hyland, 2005) or to behavioural patterns (Pryke, 2007). In sea urchins color variation is widespread (Millot, 1964; Gras & Weber, 1977; Growns & Ritz, 1994; Coppard & Campbell, 2004). Some species are capable of changing the intensity of their dermal coloration (Kleinholz, 1938; Millot, 1968; Jensen, 1974), whereas others maintain the same color throughout their lives. As with most marine invertebrates, the ecological implications of color variation specific to echinoids remain poorly understood.

Binks *et al*. (2011a, 2011b) show that for the Western Australian sea urchin *Heliocidaris erythrogramma* (Valenciennes, 1846), in addition to color variation, there are extensive variations in spine morphology. *P. gaimardi* shows no obvious differences in other aspects of morphology, habitat preferences, light or wave exposure or diet between the different color morphotypes, all of which can be found on the same rock (Calderón *et al*., 2010). Therefore, despite the fact that color variation has been used in taxonomic classification for several groups of marine invertebrates (Meroz-Fine *et al*., 2003; Tarjuelo *et al*., 2004; Hizi-

Table 3. Nei's (1978) unbiased measures of genetic identity (above diagonal) and pairwise θ values (below diagonal) for the populations of *P. gaimardi*. I: Itaipu, P: Prainha.

Population	I-Brown	I-Gray	I-Black	I-Rose	I-Green	P-Brown	P-Gray	P-Black	P-Rose	P-Green
I–Brown	****	0.887	0.956	0.951	0.965	0.905	0.783	0.872	0.827	0.853
I–Gray	0.1798	****	0.999	0.955	0.949	0.850	0.822	0.825	0.686	0.770
I–Black	0.0552	-0.0373	****	0.966	0.980	0.942	0.807	0.856	0.740	0.795
I–Rose	0.0525	0.0132	0.0119	****	1.000	0.986	0.983	0.976	0.895	0.973
I–Green	0.0578	0.0188	-0.0103	-0.0566	****	0.974	0.917	0.929	0.861	0.923
P–Brown	0.0876	0.1074	-0.0326	0.0174	0.0202	****	0.917	0.968	0.902	0.939
P–Gray	0.2313	0.1521	0.1363	-0.0104	0.0797	0.0981	****	0.940	0.932	0.959
P–Black	0.1163	0.1346	0.0882	0.0284	0.1122	0.0254	0.0991	****	0.978	1.000
P–Rose	0.1567	0.3272	0.2144	0.1320	0.1827	0.1011	0.1528	0.0111	****	0.979
P–Green	0.0589	0.1378	0.0998	-0.0167	0.0546	0.0539	0.0598	-0.0586	-0.0221	****

Table 4. Analyses of molecular variance (AMOVA) among 10 samples of *P. gaimardi* separated into two regional populations (Itaipu and Prainha) and five morphotypes (green, black, gray, brown and rose). df: degrees of freedom, *Significant ($P < 0.05$).

	Source of variation	df	Variance components	% variation	Fixation indices	*P*
Panmixia	Among populations	9	-0.01436	-7.28	ST = -0.07281	0.99881
	Among individuals within populations	200	-0.13128	-66.57		
	Within individuals	210	0.34286	173.85		
	Total	419	0.19722			
Regional populations	Among groups	1	0.17473	10.07	ΦCT = 0.10080	0.00684*
	Among populations within groups	8	0.06454	3.72		
	Among individuals within populations	200	0.46843	27.02		
	Within individuals	210	102.570	59.17		
	Total	419	173.340			
Morphotypes	Among groups	4	-0.12076	-7.34	ΦCT = -0.07341	0.86510
	Among populations within groups	5	0.27170	16.51		
	Among individuals within populations	200	0.46843	28.47		
	Within individuals	210	102.570	62.35		
	Total	419	164.507			

Degany *et al*., 2007; Pérez-Portela *et al*., 2007; Pleijel *et al*., 2009; Ozgo, 2011; Freckelton *et al*., 2012), this seems not to be the case for *P. gaimardi* color variation. Thus, although color variation can be an important feature in defining taxonomic status for different species groups, this trait does not necessarily indicate evolutionary divergence, especially as coloration can evolve rapidly, outpacing other morphological characters (Endler *et al*., 2005).

P. gaimardi showed high levels of genetic variation, however, within the range of heterozygosities found for other marine invertebrates (Nei, 1978; Nevo, 1978; Beaumont & Beveridge, 1984; Schaeffer *et al*., 1985; Benzie & Ballment, 1994; Diehl & Biesiot, 1994; Manchenko *et al*., 2000; Addison & Hart, 2004) and echinoderms (Marcus, 1977; Williams & Benzie, 1998; Uthicke *et al*., 1998, 2001; Benzie, 1999; Moberg & Burton, 2000; Manchenko & Yakolev, 2001; Matsuoka & Asano, 2003). Where the species is still common and maintain preserved its habitat it can occur in relative high densities (Matsuoka *et al*., 1995) and, therefore, in these cases, high levels of genetic variation can be easily explained by a neutral model. However, biochemical and ecological studies, as well as the use of different molecular markers are desirable in order to properly evaluate the role of selection and genetic drift in shaping the patterns of biochemical genetic variation observed for natural populations of *P. gaimardi*.

Results obtained for echinoderms generally are consistent with a model of high dispersion and high genetic homogeneity. Benzie & Wakeford (1997) studying six populations of the Australian sea star, *Acanthaster planci* (Linnaeus, 1758), in the Great Coral Barrier (130 km apart), found for nine allozymic *loci*

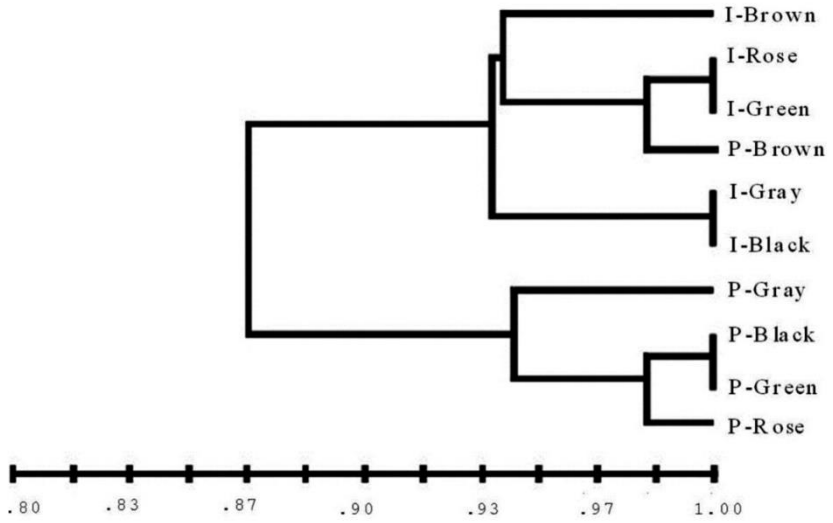

Figure 3. UPGMA cluster analysis of five morphs from two populations of *P. gaimardi* based on Nei's (1972) genetic identity. I: Itaipu, P: Prainha.

no significant values of F_{ST} as low as 0.001. Similarly, Uthicke *et al*. (2001) studying four populations of the sea cucumber, *Holothuria atra* Jaeger, 1833, and five populations of the sea cucumber, *Stichopus chloronotus* Brandt, 1835, also in the Australiam region, found no significant $F_{ST,}$ although these were higher than that those found for *A. planci* in the Great Coral Barrier (0.06 and 0.306, respectively). The results presented here for *P. gaimardi* are similar to those results (Benzie & Wakeford, 1997; Uthicke *et al*., 2001) showing that for this tropical species evidence of high dispersion and high genetic homogeneity is also present.

The *P. gaimardi* species have high levels of genetic variation, in accordance to generally observed patterns for marine invertebrates. Diagnostic *loci* were not found among morphotypes, and genetic variation is clearly distributed following sampling sites rather than color pattern. Allozyme results also clearly indicate evidence of high dispersion and genetic homogeneity within a distance of 110 km. Finally, it is important to remember that for solving taxonomic problems, a multidisciplinary approach should be applied, including allozyme analysis which is still an excellent method for solving problems concerning species systematics and is especially relevant for genetic orientated problems in population genetics.

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