

# Genetic diversity of three ornamental reef fishes (Families Pomacanthidae and Chaetodontidae) from the Brazilian coast

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(With 1 figure)

## Abstract

Reef fishes of the families Pomacanthidae (angelfish) and Chaetodontidae (butterflyfish) are popular ornamental species, intensively harvested for the aquarium trade. The impacts of such activity on intra-specific diversity and reef ecosystems are still poorly understood in the south Atlantic. In the present work, a fine-scale genetic analysis using RAPD markers was performed in distinct samples of the queen angelfish (*Holacanthus ciliaris*), French angelfish (*Pomacanthus paru*), and banded butterflyfish (*Chaetodon striatus*) along the Brazilian coast. Most of the genetic variation in the three species was related to intra-population diversity. However, AMOVA results demonstrated that *H. ciliaris* presents a subtle population structure ( $\phi_{st} = 0.132$ ,  $P = 0.003$ ), while *P. paru* and *C. striatus* present low genetic differentiation, especially remarkable in the latter ( $\phi_{st} = 0.090$ ,  $P = 0.001$  and  $\phi_{st} = 0.041$ ,  $P = 0.028$ , respectively). Gene flow ( $Nm$ ) was also higher in *C. striatus* than in the angelfish species. The reported patterns of genetic differentiation contrast with the similar pelagic stage of the selected species, suggesting that larval dispersal per se is a poor predictor of population structure in these reef fishes. Ecological features coupled with biogeographic history and distinct local selective pressures might play a major role on the genetic composition of each species. Although preliminary, the present results provide a baseline for monitoring the genetic variability in these reef species. These differences in the genetic structure among co-occurring species should be taken into consideration for the conservation of eventual evolutionary units along the Brazilian Province.

**Keywords:** angelfish, butterflyfish, dispersal, RAPD, reef fish.

## Diversidade genética em três espécies de peixes ornamentais de recifes (famílias Pomacanthidae e Chaetodontidae) da costa brasileira

### Resumo

Os peixes recifais das famílias Pomacanthidae (peixes-anjo) e Chaetodontidae (peixes-borboleta) são espécies ornamentais populares, intensivamente coletadas para o comércio aquarífilo. Os impactos dessa atividade na diversidade intra-específica e no ecossistema recifal ainda são pouco conhecidos no Atlântico Sul. No presente trabalho, uma análise genética em fina escala usando marcadores RAPD foi realizada em diferentes amostras de *Holacanthus ciliaris* (anjo ciliaris), *Pomacanthus paru* (peixe frade) e *Chaetodon striatus* (borboleta striatus) ao longo da costa brasileira. A maior parte da variação genética nas três espécies relacionou-se à diversidade intrapopulacional. Contudo, resultados da AMOVA demonstraram que *H. ciliaris* apresenta uma estruturação populacional sutil ( $\phi_{st} = 0,132$ ,  $P = 0,003$ ), enquanto *P. paru* e *C. striatus* apresentam uma baixa diferenciação genética, particularmente reduzida na última ( $\phi_{st} = 0,090$ ,  $P = 0,001$  e  $\phi_{st} = 0,041$ ,  $P = 0,028$ , respectivamente). O fluxo gênico ( $Nm$ ) também foi mais alto em *C. striatus* do que nas espécies de Pomacanthidae. Os padrões de diferenciação genética registrados contrastam com o estágio pelágico inicial similar nas espécies selecionadas, sugerindo que a dispersão larval per se não é um bom indicador de estrutura populacional nesses peixes recifais. Fatores ecológicos associados à história biogeográfica e distintas pressões seletivas locais podem desempenhar um papel fundamental na composição genética de cada espécie. Embora preliminares, os resultados apresentados fornecem subsídios para o monitoramento da variabilidade genética dessas espécies recifais. Tais diferenças na estrutura genética entre espécies co-existentz devem ser consideradas para a conservação de eventuais unidades evolutivas dentro da Província Brasileira.

**Palavras-chave:** peixe-anjo, peixe-borboleta, dispersão, RAPD, peixes recifais.

## 1. Introduction

The ecological diversity and the wide taxonomical array in coral reefs are as rich as those found in tropical forests, representing a hot spot of fish biodiversity. Reef fishes compose a complex group of numerous lineages with distinctive life-history features, responsible for a major portion of fishery resources in developing countries (Galetti et al., 2006; Choat, 2006).

Angelfishes (Pomacanthidae) and the closely related butterflyfishes (Chaetodontidae) comprise typical species of tropical reef bottoms, widespread in the Atlantic, Indian, and Pacific Oceans. Like many other reef inhabitants, they present an attractive coloration and are easy to maintain in captivity, and are commercially exploited as ornamental fishes (Stratton, 1994; Fenner, 1996; McMillan et al., 1999; Littlewood et al., 2004). Juveniles and young adults of as many as 24 millions reef fishes belonging to 1,470 species are annually harvested from the wild to supply the fast-developing aquarium trade, especially in the USA, Japan, and European Union (Shuman et al., 2004; Muldoon et al., 2005, among others). Although Indonesia and Philippines represent the major suppliers, Brazil also exports high (and probably underestimated) numbers of live reef fishes (Sadovy and Vincent, 2002; Gasparini et al., 2005). The increasing number of exported individuals, coupled with high mortality rates (30-40%), indicate that natural populations from several species may be overexploited, but the impacts of such activity on reef ecosystems are still poorly understood (Job, 2005).

Along the Brazilian Province (the largest tropical province of the South Atlantic), five species of Pomacanthidae are reported and patchily distributed over reef and rocky bottoms from the northeastern to the southern coast, including oceanic islands (Floeter and Gasparini, 2000). All of them are intensively harvested for aquarium trade. For instance, Monteiro-Neto et al. (2003) reported that the State of Ceará exported 22,969 specimens of French angelfish (*Pomacanthus paru* (Bloch, 1787)) from 1995 to 2000.

Some populations have already been affected by this activity, especially at coastal areas. Due to the harvesting pressure on juveniles, the number of small individuals of *P. paru* at three island sites near Guarapari (State of Espírito Santo) was inversely related to the distance from the coast, while the number of adult specimens remained nearly unchanged among sites. Similarly, the abundance of *Pomacanthus* spp. was significantly higher at protected areas (Gasparini et al., 2005), a trend also observed in other reef species (Floeter et al., 2006). The high fishing pressure on the specialist spongivore queen angelfish (*Holacanthus ciliaris* (Linnaeus, 1758)), combined with environmental degradation and chaotic urban development, has decreased southeastern populations over the last years. Nowadays, this species is considered threatened in the State of São Paulo (Wasko et al., 2004a; Floeter et al., 2006). Similar findings were reported after a heavy collection period of the endemic *Holacanthus clarionensis*

(Gilbert, 1891) from the Pacific coast of Mexico (Wood, 2001) and *H. passer* (Valenciennes, 1846) from Gulf of California (Sanchez-Alcantara et al., 2006).

Furthermore, even remote populations can suffer the impacts of harvesting. Specimens of an endemic and isolated population of *H. ciliaris* at Saint Paul's Rocks, located 1,100 km from the Brazilian coast (1° 04' S and 29° 24' W), present unique color morphs (e.g. blue, yellow, green, or white) and have been harvested to supply specialized markets, where a single individual can reach very high prices (Luiz Jr., 2003; Gasparini et al., 2005).

In Chaetodontidae, six species are collected as ornamental fishes in Brazil, although the pressure is usually not as high as in Pomacanthidae. The banded butterflyfish, *Chaetodon striatus* (Linnaeus, 1758) is the most widely distributed chaetodontid species at Brazilian coastal sites (Ferreira et al., 2004; Gasparini et al., 2005).

Representatives from both families play an important role on reef community structure, supposed to be greater than their relative abundance might suggest, since they are usually seen in pairs or small aggregations (Ferreira et al., 2004; Bonaldo et al., 2005). For example, juveniles of *P. paru* and *P. arcuatus* (Linnaeus, 1758) act as regular cleaners on a variety of client assemblages, ranging from small herbivores to large carnivores (Sazima et al., 1999), and intensive harvesting may disrupt such inter-specific associations and affect the entire ecosystem (Gasparini et al., 2005).

As long as the natural environment is damaged and populations of several species are either overexploited or depleted, the necessity of useful genetic information for conservation management increases in order to provide an accurate measure of biodiversity. Moreover, because of the pelagic dispersal mode at the initial development stage of most reef fishes (including angelfish and butterflyfish), the characterization of populations and species as discrete entities is dependent on indirect evidences, such as molecular markers.

Among the several available molecular markers, RAPD (randomly amplified polymorphic DNA) stands for a simple and cost-efficient assay to evaluate genetic variability, regardless any previous genomic information about the selected organism (Dinesh et al., 1993; Wasko et al., 2004b). Nonetheless, since many factors can affect the changes in RAPD profiles, a proper optimization is required in order to generate reliable results (Atienzar and Jha, 2006). This approach has been providing useful information for species management and conservation of several freshwater neotropical fishes (Wasko and Galetti, 2002; Hatanaka and Galetti, 2003; Wasko et al., 2004b).

Studies involving genetic markers in Pomacanthidae and Chaetodontidae at South Atlantic are limited to cytogenetic (see Galetti et al., 2006), enzymatic (Chung and Woo, 1998), and mitochondrial (Bellwood et al., 2004; Littlewood et al., 2004; Bowen et al., 2006a) analyses, mainly regarding taxonomic and evolutionary issues.

Therefore, the genetic diversity in distinct samples of three reef fish species (*Holacanthus ciliaris*,

*Pomacanthus paru*, and *Chaetodon striatus*), commonly harvested for aquarium trade along the Brazilian coast, was investigated through RAPD markers in the present work. The obtained data represent the first information about the population genetic structure of these important and exploited reef fishes.

## 2. Material and Methods

### 2.1. Study area and sampling collection

Forty-one specimens of *H. ciliaris*, 68 of *P. paru*, and 68 of *C. striatus* were collected while snorkeling or SCUBA diving, using hand nets at different shallow reef sites along the Brazilian coast. Some individuals of *H. ciliaris* were also collected at the oceanic island of Saint Paul's Rocks (Table 1).

In order to make the sampling more representative, some biogeographic, ecological, and environmental features of each collection site were taken into consideration. This procedure was based on recent reports about biogeography of Brazilian reef species (Floeter and Gasparini, 2000; Floeter et al., 2001; Rocha, 2004; Ferreira et al., 2004). According to these authors, the Brazilian coastal zones can be reliably separated into northeastern, southeastern, and oceanic islands. The northeastern shore presents warm waters, high coral diversity, and narrow shelf, while the southeastern coast is characterized by cold waters, high primary productivity, and a wide shelf. Oceanic islands are distinguishable because of their high degree of isolation in open sea.

Additionally, between the States of Rio Grande do Norte (4° 59' S) and Pernambuco (7° 51' S), the South Equatorial Current splits into two branches to become the northward flowing North Brazil Current and the southward flowing Brazil Current (Feitoza et al., 2005). Thus, animals of planktonic dispersal located above Rio Grande do Norte State are expected to have few chances of reaching southern areas. Therefore, samples from Ceará State were treated as a distinct group in the northeastern region. Specimens from Bahia State were also clustered apart to constrain the geographical range of collection sites, providing a fine-scale analysis (otherwise, the northeastern sample would comprise an area of more than 2,000 km, in contrast with the other sample groups, limited to nearly 1,000 km of extension).

Based on these predictions, five sampling groups were established for the present analyses, named as: 1) Ceará, 2) Northeastern (from Rio Grande do Norte to Alagoas), 3) Bahia, 4) Southeastern (southern Espírito Santo to northern Santa Catarina) and 5) SPR (Saint Paul's Rocks) (Figure 1). Besides increasing the number of specimens per collection site, these groupings are likely to reflect more precisely the effective populations of each studied species.

### 2.2 DNA isolation, PCR, and electrophoresis

Fish DNA samples were obtained from fin and/or liver tissues, previously fixed in a solution of methanol: ethanol (1:1). Genomic DNA extraction was accomplished using the phenol: chlorophorm protocol (Sambrook et al., 1989).

Twenty-six decamer oligonucleotides of arbitrary sequence were tested as single primers, based on previous works with distinct aquatic organisms (Tassanakajon et al., 1997; Mamuris et al., 1998; Koh et al., 1999; Hatanaka and Galetti, 2003). Five of them were polymorphic and useful for genetic analyses in the three species, named B-07 (5' GTGAGGCGTC 3'), C-05 (5' GTGGGCTGAC 3'), C-10 (5' GCGCCTGGAG 3'), D-02 (5' CGACGCCCTG 3'), and D-03 (5' CGACGCCCTG 3').

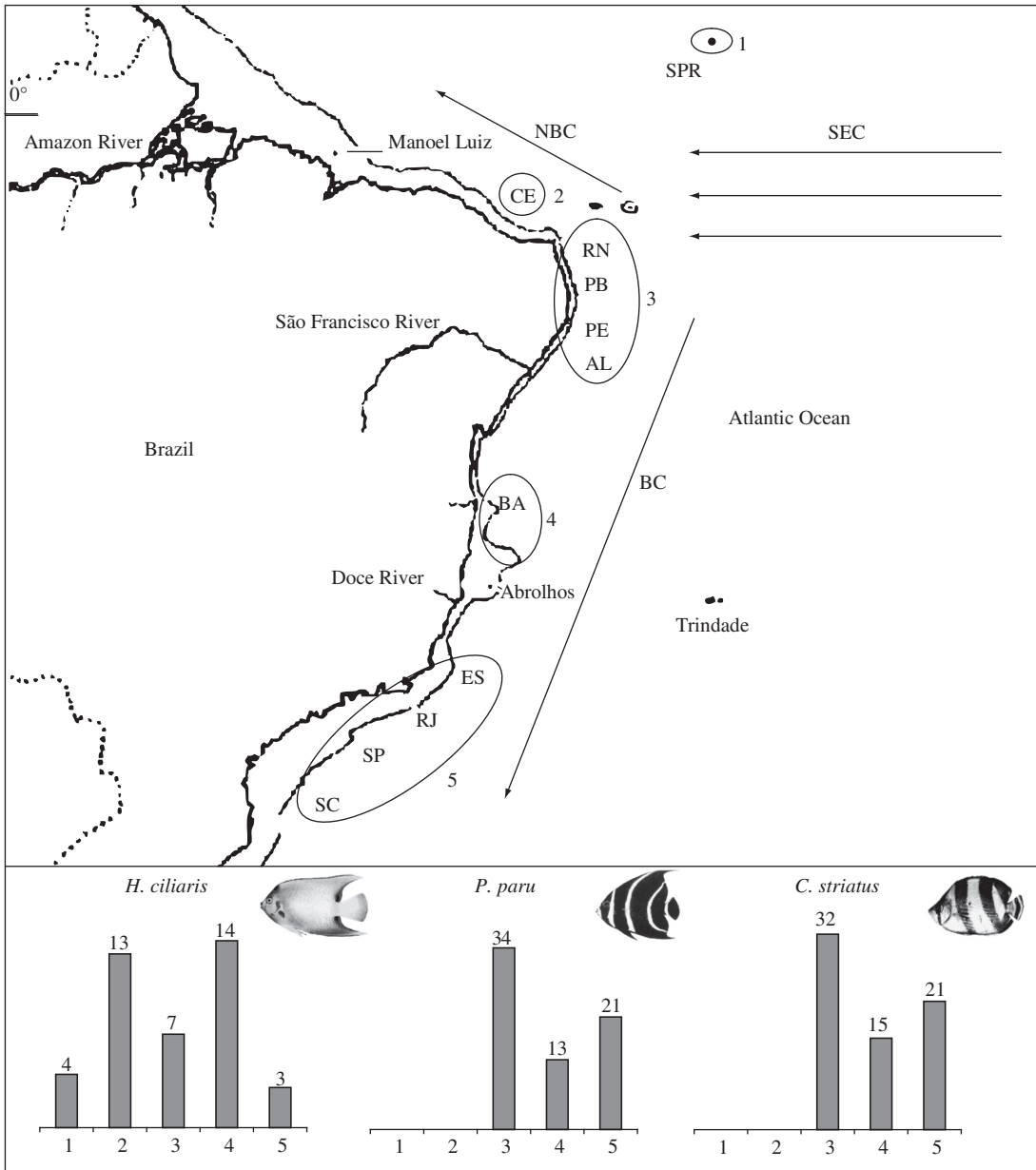
The PCR Master Mix kit (Promega) was used in RAPD reactions after testing different concentrations of template DNA, primer, and magnesium chloride, to obtain a reliable amplification pattern. Therefore, the standard PCR was carried out in 25 µL reactions containing 2.5 U.mL<sup>-1</sup> Taq polymerase, 1.5 mM MgCl<sub>2</sub>, 50-100 ng of template DNA, 100 ng of primer, and 200 µM dNTP. Amplification was performed in a PTC-100 MJ Research thermocycler programmed for one cycle at 94 °C for 4 minutes, 40 cycles at 92 °C for 1 minute, 37 °C for 1 minute and 30 seconds, 72 °C for 3 minutes, and one final cycle at 72 °C for 3 minutes (Hatanaka and Galetti, 2003).

Amplification products (10 µL) were analyzed by electrophoresis (3 hours at 85 V) in 1.4% agarose gel, stained with ethidium bromide, and documented using Electrophoresis Documentation and Analysis System (Edas 290 - Kodak). To avoid some limitations caused by RAPD-PCR method, such as sensitivity to reaction conditions, contamination, and occasional non-reproducible fragments, PCR reactions were repeated twice after

**Table 1.** Number of analyzed individuals in the present work per sampling site.

Species	Sampling site*											Total
	SC	SP	RJ	ES	BA	AL	PE	PB	RN	CE	SPR	
<i>H. ciliaris</i>	1	-	-	2	14	-	5	-	2	13	4	41
<i>P. paru</i>	1	2	18	-	13	3	4	-	27	-	-	68
<i>C. striatus</i>	-	-	21	-	15	-	-	1	31	-	-	68

\* The sites are represented by the states where the animals were collected; SC = Santa Catarina, SP = São Paulo, RJ = Rio de Janeiro, ES = Espírito Santo, BA = Bahia, AL = Alagoas, PE = Pernambuco, PB = Paraíba, RN = Rio Grande do Norte, CE = Ceará, and SPR = Saint Paul's Rocks.



**Figure 1.** Map of the Brazilian coast showing the sampled groups, named as 1) Saint Paul's Rocks – SPR, 2) Ceará – CE, 3) Northeastern – NE, 4) Bahia – BA, and 5) Southeastern (SE). The arrows indicate the flow of the main currents at south-western Atlantic (SEC = South Equatorial Current, NBC = North Brazil Current, BC = Brazil Current). On the bottom, the number of individuals analyzed per sampling group (1 to 5) in each selected species.

standardization, and a negative control (free of template DNA) was used in all experiments. Furthermore, only those bands clearly distinguishable and routinely repeatable were selected for genetic analyses.

### 2.3. Data analysis

Each individual was scored as 1 or 0 for the presence or absence of every amplification product and data entered into a binary matrix, assuming that each band

represents a Mendelian locus of dominant behavior with a non-detectable recessive allele (Lynch and Milligan, 1994).

A pairwise comparison of RAPD bands was evaluated by Jaccard's similarity coefficient using the software NTSYS-PC (Rohlf, 1993) and the coefficient of variation was obtained as follows: standard deviation/mean Jaccard's similarity x 100. The gene frequency for each locus, percentage of polymorphic loci, and Nei's

genetic distance and identity (Nei, 1978) among sampled areas were determined using the software POPGEN 1.31 (Yeh et al., 1999). The software ARLEQUIN (Schneider et al., 2000) was used to perform the analysis of molecular variance (AMOVA) and estimate the inter-population variation by providing  $\phi_{st}$  values, considered the best parameters of population structure for RAPD studies (Excoffier et al., 1992; Hartl and Clark, 1997). Values from 0 to 0.05 indicate little genetic differentiation, from 0.05 to 0.15 indicate a moderate differentiation, from 0.15 to 0.25 suggest a high differentiation, and values over 0.25 represent a very high genetic differentiation (Wright, 1978). AMOVA data were subjected to a non parametric procedure, comprising 10,000 independent permutations, to test the significance levels of  $\phi_{st}$  values. P values lower than 0.05 were considered significant. The mean number of migrants per generation (Nm) of each species was calculated using  $\phi_{st}$  as a  $F_{st}$  analogous in the equation  $Nm = 0.25(1/F_{st} - 1)$  (Wright, 1951).

### 3. Results

RAPD profiles based on five decamer primers in individuals of *H. ciliaris*, *P. paru*, and *C. striatus* allowed identifying, respectively, 59, 45, and 50 polymorphic bands. The mean Jaccard's similarity and variation coefficients, considering all primers for each species, were: 62.8/38.5 for *H. ciliaris*, 56.0/39.3 for *P. paru*, and 56.8/42.9 for *C. striatus*. Significant differences ( $P < 0.05$ ) in allele frequency between populations were found for 21 loci in *H. ciliaris*, 10 loci in *P. paru*, and 5 loci in *C. striatus*.

Intra-population analyses (Table 2) indicated that *H. ciliaris* presented the lowest percentage of polymorphic loci and genetic diversity, excepting samples from Bahia. Indeed, the apparent higher diversity within this sample can be related to a larger number of individuals collected in Bahia. Specimens from Saint Paul's Rocks (SPR) were the less polymorphic ones. On the other hand, polymorphic loci were more frequent in *P. paru* and *C. striatus*. Both species presented a higher genetic diversity in the so-called northeastern population and a decreased number of polymorphic loci in Bahia, what can be related to the discrepancy between the number of individuals collected per region.

Genetic identity and distance values in *H. ciliaris* (Table 3) showed that SPR and northeastern populations are more similar, while the distinct samples were more

differentiated when compared to the remaining species. The genetic distance was lower among sites in *P. paru* (Table 4) and, remarkably, in *C. striatus* (Table 5).

AMOVA data (Table 6) demonstrated that intra-population variation was responsible for most of the genetic diversity in the three species. However, *H. ciliaris* presented a moderate population structure ( $\phi_{st} = 0.132$ ,  $P = 0.003$ ), while *P. paru* and, particularly, *C. striatus* showed a low genetic differentiation ( $\phi_{st} = 0.090$ ,  $P = 0.001$  and  $\phi_{st} = 0.041$ ,  $P = 0.028$ , respectively). Gene flow (Nm) was also much higher in *C. striatus* than in Pomacanthidae species, indicating a significant lack of genetic structure.

### 4. Discussion

The present results demonstrate that the genetic differences among individuals from the same locality were usually high in the three analyzed species (Tables 2 to 5). Actually, AMOVA results revealed that a major portion of genetic variability was distributed within populations, particularly in *P. paru* and, remarkably, in *C. striatus* (Table 6). Furthermore, unique populational RAPD bands, able to discriminate each sampled area, were detected in neither species, despite significant differences in some allele frequencies among sites.

The species herein studied, like all pomacanthids, chaetodontids and most reef fishes, present an early pelagic planktonic phase. Since adults usually present a benthonic, site-fidelity behavior, the pelagic larval duration (PLD) is usually related to the dispersal ability of species. Such strategy is also commonly referred as a proxy for the apparent connectivity of several marine species (Fisher, 2005). Therefore, considering that *H. ciliaris*, *P. paru*, and *C. striatus* are characterized by a similar life cycle (PLD = 40-50 days) (Thresher and Brothers, 1985; Stobutzki, 1998), an undifferentiated pattern of population structure among the analyzed species would be expected.

In fact, the selected species presented little genetic differentiation among samples, although at different levels according to each representative. Usually, the values of genetic distance among populations of a single species, as inferred by dominant markers, are lower than 0.3, while higher values would indicate inter-specific differentiation (Thorpe, 1982; Govindaraju and Jayasankar, 2004; Mamuris et al., 1998). The present results support such statement, since all genetic distance values were

**Table 2.** Percentage of polymorphic loci and mean genetic diversity (in parentheses) within samples of the studied species (according to Nei, 1973).

Sampling site	<i>H. ciliaris</i>	<i>P. paru</i>	<i>C. striatus</i>
Saint Paul's Rocks	47.46 (0.197)	-	-
Ceará	66.1 (0.257)	-	-
Northeastern	54.24 (0.227)	91.11 (0.348)	90.0 (0.467)
Bahia	72.88 (0.310)	68.89 (0.272)	56.0 (0.334)
Southeastern	57.63 (0.240)	80.0 (0.277)	88.0 (0.302)

below 0.3, although some inter-populational comparisons in *H. ciliaris* were relatively close to the threshold (e.g., Ceará x SE, Ceará x SPR, Table 3). Therefore, a subtle, but significant genetic structure, is indicated in *H. ciliaris*, while the analyses carried out in *P. paru* and *C. striatus* revealed a higher inter-population genetic similarity (Tables 4 and 5). This suggestion is particularly evident in *C. striatus* which also presented the highest gene flow (Nm) (Table 6).

Several studies have also shown that PLD and genetic or phylogeographic patterns of reef fishes are not always related (Bay et al., 2006; Choat, 2006; Craig et al., 2007). Bowen et al. (2006b) point out that larval retention and self-recruitment may be higher than previously expected. These data suggest that other environmental aspects might influence both dispersal and speciation of reef fishes. Distinctive local selective pressures could explain how nearby populations under a same current

**Table 3.** Values of genetic identity and distance (above and below the diagonal, respectively) (Nei, 1978) among the sampling sites of *H. ciliaris*.

Sampling site	CE	NE	BA	SE	SPR
CE	-	0.899	0.903	0.795	0.806
NE	0.107	-	0.809	0.816	0.841
BA	0.102	0.211	-	0.849	0.797
SE	0.230	0.203	0.164	-	0.831
SPR	0.216	0.173	0.227	0.185	-

**Table 4.** Values of genetic identity and distance (above and below the diagonal, respectively) (Nei, 1978) among the sampling sites of *P. paru*.

Sampling site	NE	BA	SE
NE	-	0.923	0.945
BA	0.077	-	0.876
SE	0.056	0.133	-

**Table 5.** Values of genetic identity and distance (above and below the diagonal, respectively) (Nei, 1978) among the sampling sites of *C. striatus*.

Sampling site	NE	BA	SE
NE	-	0.954	0.970
BA	0.047	-	0.932
SE	0.030	0.070	-

**Table 6.** Analysis of molecular variance (AMOVA) in the studied species, showing inter- and intra-population variation and degree of population differentiation ( $\phi_{st}$  index). The P value was obtained after 10,000 replications and gene flow (Nm) was based on  $\phi_{st}$  values (Wright, 1951).

Species	inter-population (%)	intra-population (%)	$\phi$ index	P value	Nm
<i>H. ciliaris</i>	13.24	86.76	0.132	0.003	1.64
<i>P. paru</i>	9.00	91.00	0.090	0.001	2.53
<i>C. striatus</i>	4.09	95.91	0.041	0.028	8.74

system might be subdivided (Rocha et al., 2002, 2005). In the Brazilian coast, for instance, striking differences in the water temperature would act as the main barrier responsible for the differentiation between northeastern and southeastern regions, despite the potential dispersive pelagic stage (Santos et al., 2006).

A putative role of ecological features on the genetic structure of the analyzed species is also supported in the present work. While *H. ciliaris* represents a specialized spongivore, analyses of *C. striatus* along the Brazilian coast have demonstrated a high plasticity in both substrate preference and feeding behavior (Ferreira et al., 2001; Bonaldo et al., 2005; Floeter et al., 2006). These features may facilitate the colonization of distinct habitats throughout the coast. In addition, larvae of *C. striatus* display a differentiated swimming ability prior to settlement, able to drastically increase its dispersal potential (Stobutzki, 1998; Fisher, 2005). Such ability could favor a wider distribution of individuals at south Atlantic, leading to population connectivity. In fact, the banded butterflyfish is one of the most widespread species along the Brazilian Province, further reported at oceanic islands (Floeter et al., 2001). On the other hand, individuals of *P. paru*, which present an intermediate level of genetic differentiation in relation to the other species, are not as specialized as *H. ciliaris*, including algae in their diet. Underwater observations have also suggested that *Pomacanthus* could explore marginal sites better than *Holacanthus* (Ferreira et al., 2004), thus enhancing its access to different environmental conditions.

The apparent low genetic diversity in individuals of *H. ciliaris* from Saint Paul's Rocks (SPR) might be either influenced by a reduced sample size or dispersal constraints. Morphological studies showed that this population is unique by the presence of several color morphs, probably related to inbreeding effects caused by a restricted gene flow and a high degree of self-recruitment (Luiz Jr., 2003). These features reinforce the vulnerable status of this local population to commercial harvesting for aquarium trade (Gasparini et al., 2005). Actually, this species is considered threatened in the southeastern Brazil coast because of uncontrolled exploitation (Wasko et al., 2004a). However, further studies comprising a larger number of specimens and other molecular markers are required for a deep analysis about the putative endogamy of such *H. ciliaris* population.

The lack of a clear relationship among dispersal mode, current systems, and phylogeographic variation of fish assemblages has also been reported in Pomacanthidae

and Chaetodontidae from the Indo west-Pacific and the Red Sea (Thresher and Brothers, 1985; Roberts et al., 1992; Schultz et al., 2007), and evolutionary events may be equally relevant to processes of genetic differentiation (or connectivity) of reef fishes. Genetic studies based on mitochondrial DNA suggest that past isolation conditions and successive colonization, associated with ecological aspects, are likely to influence both speciation and population structure in several Atlantic fishes (Rocha, 2004; Rocha et al. 2005; Bowen et al., 2006a; Galetti et al., 2006; Craig et al., 2007).

It should be also pointed out that differences in the number of analyzed individuals per locality of each species might bring some noise to the present populational studies, particularly in *H. ciliaris*. Further molecular studies, comprising a higher number of samples and other species, are still required to precisely evaluate the genetic structure of angelfishes and butterflyfishes along the Brazilian coast. For instance, preliminary studies using species-specific microsatellites in *P. paru* from several points at Brazilian coast have indicated a moderate structuring between northeastern and southeastern regions, by the presence of 10 polymorphic alleles (Affonso, 2004).

Thus, the results of the present work could be useful for monitoring and managing the levels of intra- and inter-population diversity of angelfishes and butterflyfishes along the Brazilian coast. Since these ornamental reef fishes are currently threatened by environmental disturbances and/or overexploitation (Gasparini et al., 2005) and less than 1% of Brazilian waters are protected (Floeter et al., 2006), the differences in the genetic structure among co-occurring species should be taken into consideration for the conservation of eventual evolutionary units along the Brazilian Province.

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