Online journal ISSN - 1676-5680
GMR Genetics and Molecular Research
EVOLUTION AND TECHNOLOGY

Lack of interpopulation genetic structure in the genus *Stegastes* (Perciformes) with indication of local introgression

E.A. Dias Jr., A.S. Souza and W.F. Molina

Departamento de Biologia Celular e Genética,
Universidade Federal do Rio Grande do Norte,
Centro de Biociências, Natal, RN, Brasil
Corresponding author: W.F. Molina
E-mail: molinawf@yahoo.com.br

Genet. Mol. Res. 6 (4): 1097-1106 (2007)
Received August 24, 2007
Accepted November 12, 2007
Published December 4, 2007

ABSTRACT. The family Pomacentridae comprises about 326 species belonging to 28 genera. The genus *Stegastes* is composed of nearly 33 species, and 8 are endemic to the Brazilian Province, inhabiting the Brazilian coast (*Stegastes fuscus*, *S. variabilis*, *S. leucosticus*, *S. uenfi*, and *S. pictus*) or Western Atlantic oceanic islands (*S. trindadensis*, *S. rocasensis* and *S. sanctipauli*). *Stegastes* species play a major role in the reef ecosystem since they interfere significantly with the composition of benthonic organisms. Studies about population genetics and speciation of Neotropical ichthyofauna are scarce, particularly at insular areas from the Western Atlantic. Random amplified polymorphic DNA markers were used to analyze the population genetic structure of the continental species *S. fuscus* and *S. variabilis* (Northeastern Brazil) as well as the insular species *S. sanctipauli* (Saint Paul's Rocks). Analysis of population parameters revealed a high index of intrapopulation genetic variability in the species, except for *S. sanctipauli*, which showed low values. The ϕ_{ST} values in samples of *S. fuscus* and *S. variabilis* obtained at distinct collection sites 35 km apart from each other indicated a lack of population genetic structure. An intermediary profile of species-specific markers was detected in some individuals of *S. fuscus* and *S. variabilis* from Santa Rita, Rio Grande do Norte, suggesting a putative introgression event between the two species. The genetic profiles observed in *Stegastes* populations indicate a higher genetic variability along the shoreline than at oceanic sites, related to a reduced effective population size on

islands. The lack of genetic differentiation among coastal populations suggests that, despite some biological features such as non-migratory behavior and territoriality, the pelagic larval phase of these species is able to promote an interpopulation homogeneity among sampled areas.

Key words: Genetic variability, Pomacentridae, Pomacentrinae, Random amplified polymorphic DNA, *Stegastes*

INTRODUCTION

Coral reefs represent a hotspot of microhabitats, favorable to the high fish diversity found in such environments (Ebeling and Hixon, 1991). The pelagic larval phase of most reef fish plays an important role in the dispersal of species (Mora and Sale, 2002), as well as in the population structure.

The family Pomacentridae (Perciformes) is composed of 28 genera and about 326 species, popularly known as damselfishes. This is one of the most diverse families of marine teleosteans, distributed over tropical, subtropical and temperate regions (Nelson, 1994). Most pomacentrids are found in coastal waters, associated with rocky bottoms at low depths (Allen, 1975; Menezes and Figueiredo, 1985). Often, they represent the dominant group in both number and diversity in reef environments (Menezes and Figueiredo, 1985).

Four genera of Pomacentridae are reported in Brazil, *Stegastes* (eight species), *Chromis* (four species), *Microspathodon* (one species), and *Abudefduf* (one species) (Emery, 1972; Menezes and Figueiredo, 1985; Rosa and Moura, 1997; Rosa et al., 1997; Rocha et al., 1998).

Some species can live up to 15 years (Schwamborn and Ferreira, 2002). Highly territorial, *Stegastes* species show a remarkable habitat micropartition, restricted to zones of relatively defined depths and characteristic bottoms (Menezes and Figueiredo, 1985). This genus comprises about 33 morphologically conserved species (Emery, 1972; Allen, 1991), and unique features for a single species are rarely reported. Eight *Stegastes* species are endemic to the Brazilian coast (Allen, 1991; Gasparini et al., 1999; Novelli et al., 2000), *S. variabilis*, *S. fuscus*, *S. leucosticus*, *S. uenfi*, and *S. pictus* inhabiting the shoreline, and *S. rocasensis*, *S. sanctipauli* and *S. trindadensis* at the oceanic islands of Rocas Atoll/Fernando de Noronha archipelago, Saint Paul's Rocks and Trinidad and Martin Vaz, respectively (Menegatti et al., 2003).

The coastal species *S. fuscus* and *S. variabilis* are sympatric and very common in Northeastern Brazil (Moura et al., 1999). Although easily differentiated by the juvenile color pattern, both species are hardly distinguished when adults, displaying a uniform dark brown coloration with blue stripes in *S. fuscus* that may disappear along the head in *S. variabilis*. *S. sanctipauli* has a bright yellow body with small brown marks on the back.

Several population genetic studies using molecular markers have been performed in pomacentrids in the Pacific Ocean, showing some genetic differentiation on a small geographic scale (Bell et al., 1982; Lacson, 1994; Lacson and Clark, 1995; Planes and Doherty, 1997; Planes et al., 1998) as well as a tendency of genetic homogeneity on a macrogeographic scale (Shaklee, 1984; Lacson and Morizot, 1991; Lacson, 1992; among others). However, the patterns of variability and genetic differentiation remain unknown in most species from the Western Atlantic. Cytogenetic studies in *Stegastes* species from the Western Atlantic - *S. fuscus*, *S. variabilis*, *S. pictus*, and *S.*

sanctipauli (Molina and Galetti Jr., 2004b; Molina, 2006) - revealed cytotaxonomical markers, mainly related to multiple pericentric inversions, helpful in distinguishing each species.

A large number of studies using random amplified polymorphic DNA (RAPD) markers in fishes have shown their potential in providing species-specific profiles (Foo et al., 1995; Liu et al., 1999) besides estimates of phylogenetic relationships between species and subspecies (Callejas and Ochando, 1998) and evaluation of genetic similarities among geographically isolated populations (Bielawski and Pumo, 1997; Hatanaka and Galetti Jr., 2003). RAPD markers were successfully used by Bardakci and Skibinski (1994) to identify tilapia subspecies, revealing an intrapopulation variation undetected by mtDNA polymorphisms.

In the present study, RAPD markers were used to determine the degree of interpopulation differentiation and the levels of genetic variability in the species *S. fuscus* and *S. variabilis*, composing large coastal populations, and *S. sanctipauli*, endemic to the highly isolated Saint Paul's Rocks.

MATERIAL AND METHODS

Species and collection sites

Specimens of *S. fuscus* and *S. variabilis* were collected in sympatry at two coastal regions, Santa Rita (05° 41' 57.1" S; 035° 11' 36.3" W) (*S. fuscus*, N = 12; *S. variabilis*, N = 12) and Búzios (06° 00' 20.9" S; 035° 06' 30.4" W) (*S. fuscus*, N = 20; *S. variabilis*, N = 20), 35 km apart from each other, on the coast of Rio Grande do Norte, Northeastern Brazil. Twenty-nine individuals of *S. sanctipauli* (N = 29) were collected at Saint Paul's Rocks (0° 55' 02" N; 29° 20' 42" W) (Figure 1), situated on the mid-Atlantic ridge, 960 km from Cape Saint Roque (Brazil) and 1890 km from Senegal, Africa. Ascension and Saint Helena, the only other tropical islands on the mid-Atlantic ridge are 1940 and 3150 km from the two coasts, respectively. The closest area to Saint Paul's Rocks is the archipelago of Fernando de Noronha (630 km). The samples were collected from January 2003 up to December 2004.

DNA extraction and amplification

Tissue samples (liver and/or fins) were obtained just after the specimen collection, preserved in ethanol/methanol (1:1) and then stored at -20°C. The DNA extraction was performed according to Sambrook et al. (1989). The samples were macerated in liquid nitrogen and digested in a solution containing 0.1 M NaCl, 0.01 M Tris-HCl, 0.025 M EDTA, 0.5% SDS, 0.1 mg/mL RNAse, and 0.1 mg/mL proteinase-K; the DNA was then precipitated with ethanol and stored at -20°C.

The amplification reaction comprised 1 µL template DNA (25 ng/µL), 1 µL primer (100 ng/µL), 10.5 µL nuclease-free water, and 12.5 µL polymerase chain reaction Master Mix - kit Promega (50 U/mL *Taq* DNA polymerase, 400 µL dNTPs, 3 mM $MgCl_2$) to a final volume of 25 µL. The RAPD reactions were performed in a thermocycler PTC-100 (MJ Research, Inc.). The polymerase chain reaction cycles comprised a first step at 94°C for 4 min, followed by 45 cycles at 94°C for 1 min, 36°C for 1 min and 72°C for 2 min, plus a final step at 72°C for 7 min (Barman et al., 2003).

Figure 1. Map showing the collection sites on Northeastern Brazilian shore (Santa Rita and Búzios) for *Stegastes fuscus*, *S. variabilis* and Saint Paul's Rocks for *S. sanctipauli*.

The samples were amplified using 25 arbitrary primers obtained from the University of British Columbia Biotechnology Laboratory (UBC) and Operon Technologies (OPP), and eight of them (OPP-7, GTCCATGCCA; OPP-8, ACATCGCCCA; OPP-9, GTGGTCCGCA; UBC-101, GCGCCTGGAG; UBC-174, AACGGGCAGG; UBC-428, GCGGAGGTCC; UBC-457, CGACGCCCTG; UBC-459, GCGTCGAGGG) were selected based on the intensity and reproducibility of bands. Replicates were performed at different intervals in 5% of the samples and their products were run on the same gel in order to determine the authenticity of amplification products.

Analyses of amplification products

Matrices based on population genetic data were analyzed using the software Popgene version 1.31 (Yeh et al., 1999) and Arlequin 3.1 (Schneider et al., 2000; available at http://cmpg.unibe.ch/software/arlequin3/). The Shannon index (I) (Zar, 1974), Nei's genetic diversity (*h*), Nei's

genetic identity and distance, number of migrants (N*m*) between populations based on Nei's genetic variation (G_{st}) (N*m* = 0.5 (1 - G_{st}) / G_{st}) and the number of polymorphic loci were estimated for each species (Nei, 1973). Analysis of molecular variance (AMOVA) was used to estimate the variation among populations, providing ϕ_{ST} values (Excoffier, 1992), analogous to F_{ST} (Wright, 1978), which represents the degree of genetic differentiation or population subdivision.

In order to confirm the ϕ_{ST} values, AMOVA data were submitted to 1023 independent permutations and P values lower than 0.05 were considered significant. Genetic analyses were performed per species and each collection site was discriminated for shore species.

RESULTS

Forty polymorphic bands (85.11%) were identified for *S. fuscus*, and 35 polymorphic bands (81.40%) were found in the two localities analyzed for *S. variabilis*. *S. sanctipauli* samples showed 26 polymorphic bands (39.39%). The fragments ranged from 440 to 2500 bp (Figure 2). Species-specific bands were identified for each species, allowing their differentiation. G_{st} values of 0.2474 and 0.2717 and N*m* equal to 1.58 and 1.34 were reported for the species *S. fuscus* and *S. variabilis*, respectively, at Búzios and Santa Rita, Rio Grande do Norte, Northeastern Brazil. The genetic diversity in the insular population of *S. sanctipauli* (G_{st} = 0.1703) was much lower than that of coastal species (Table 1).

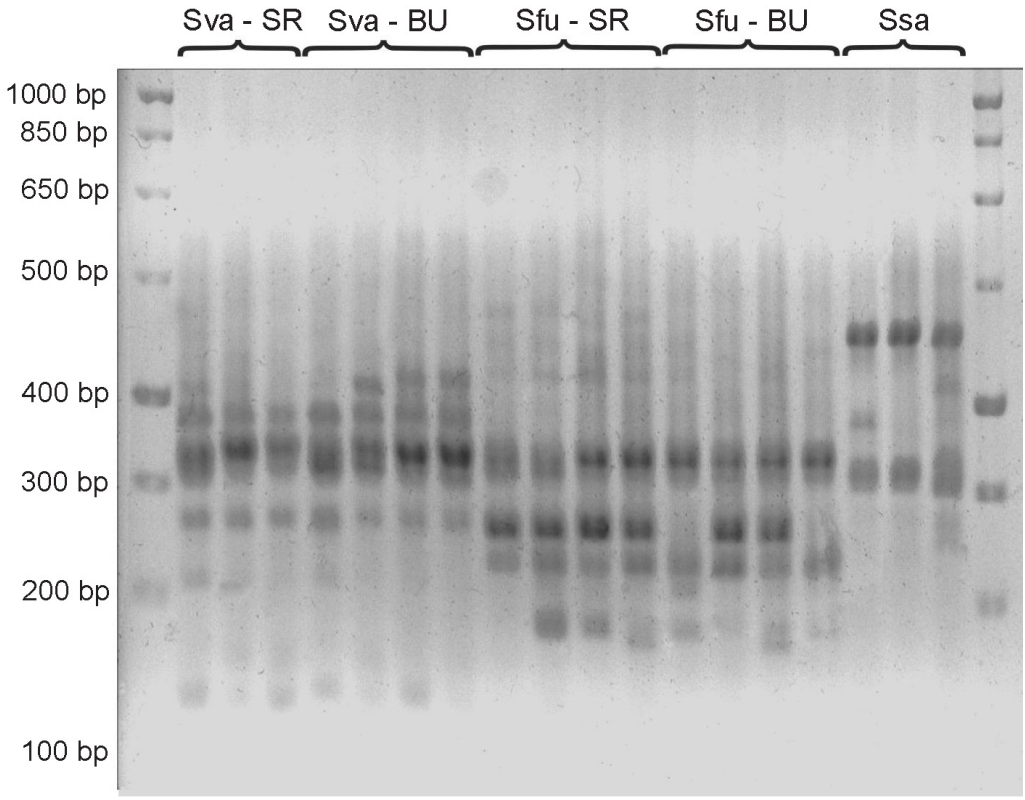

Figure 2. DNA amplification profiles using the primer UBC-459 in the species *Stegastes variabilis* (Sva), *S. fuscus* (Sfu) and *S. sanctipauli* (Ssa) from Santa Rita (SR) and Búzios (BU) beach (Rio Grande do Norte coastline, Brazil).

Table 1. Genetic parameters observed using eight random amplified polymorphic DNA primers in the geographic samples of the *Stegastes* species.

Species/site	Polymorphic bands	Shannon index	Nei's genetic diversity
S. fuscus			
Búzios, RN	29 (61.70%)	0.3604	0.2474
Santa Rita, RN	26 (55.32%)	0.3499	0.2452
Búzios + Santa Rita	40 (85.11%)	0.4766	0.3224
S. variabilis			
Búzios, RN	28 (65.12%)	0.3812	0.2603
Santa Rita, RN	22 (51.16%)	0.3085	0.2129
Búzios + Santa Rita	35 (81.40%)	0.4768	0.3259
S. sanctipauli			
Saint Paul's Rocks	26 (39.39%)	0.2449	0.1703

Analyses based on a three-group matrix (one comprising *S. sanctipauli* samples and the others representing the two geographic samples of *S. fuscus* and *S. variabilis* from Búzios and Santa Rita) were performed using AMOVA data. The results indicated a level of genetic variation of 27.46% between species. Little variation was detected between geographic samples of each species, and most of the variation was observed within populations (Table 2).

Table 2. Comparative analyses of molecular variance among populations and *Stegastes* species.

Source of variation	d.f.	χ^2	% Variation
Among species	2	17,586	27.46
Among populations within a species	2	1,704	1.40
Within populations	88	57,871	71.14
Total	92	77,161	100.00

d.f. = degrees of freedom; χ^2 = chi-square.

The ϕ_{ST} values among *Stegastes* populations indicated a lack of population genetic structure between Santa Rita and Búzios in each coastal species (*S. fuscus* and *S. variabilis*). The highest values of genetic differentiation were found between the species *S. sanctipauli* and *S. variabilis* ($\phi_{ST} \geq 0.35765$). Despite a significant genetic differentiation among species, the ϕ_{ST} values between samples of *S. fuscus* and *S. variabilis* from Santa Rita were very low ($\phi_{ST} = 0.08914$), differing remarkably from the remaining inter-specific analyses (Table 3).

Table 3. Values of ϕ_{ST} among species and populations of *Stegastes*.

Populations	ϕ_{ST}	Significance
S. sanctipauli - *S. fuscus* (Búzios)	0.23144	$P < 0.05$
S. sanctipauli - *S. fuscus* (Santa Rita)	0.19801	$P < 0.05$
S. sanctipauli - *S. variabilis* (Búzios)	0.35765	$P < 0.05$
S. sanctipauli - *S. variabilis* (Santa Rita)	0.36264	$P < 0.05$
S. fuscus (Búzios) - *S. fuscus* (Santa Rita)	0.07755	$P > 0.05$
S. fuscus (Búzios) - *S. variabilis* (Búzios)	0.31322	$P < 0.05$
S. fuscus (Búzios) - *S. variabilis* (Santa Rita)	0.35734	$P < 0.05$
S. fuscus (Santa Rita) - *S. variabilis* (Búzios)	0.12249	$P < 0.05$
S. fuscus (Santa Rita) - *S. variabilis* (Santa Rita)	0.08914	$P > 0.05$
S. variabilis (Búzios) - *S. variabilis* (Santa Rita)	-0.02914	$P > 0.05$

Another AMOVA was performed grouping distinct populations of the same species, thus generating a single population for each species. The results demonstrated a higher genetic variation within species than among them (Table 4).

Table 4. Comparative analysis of molecular variance among species and populations of *Stegastes* (*S. fuscus*, *S. variabilis* and *S. sanctipauli*).

Source of variation	d.f.	χ^2	% Variation
Among species	2	19,738	32.03
Within species	90	56,950	67.97
Total	92	76,688	100.00

d.f. = degrees of freedom; χ^2 = chi-square.

DISCUSSION

Population genetic analysis in *Stegastes variabilis*, *S. fuscus* and *S. sanctipauli*

A planktonic phase is particularly widespread in marine fish, with more than 90% of species showing a first pelagic larval period. In most freshwater species, this strategy was evolutionarily lost (Bonhomme and Planes, 2000). An association between dispersal and pelagic larval period has been commonly reported, playing a major role in population recruitment, population structure, speciation, and extinction (Strathmann, 1993). In pomacentrids, an inverse correlation between karyotypic differentiation and duration of the pelagic larval period was observed - i.e., species of restricted dispersal abilities displayed a higher number of pericentric inversions than those of high dispersal (Molina and Galetti Jr., 2004a).

Several studies have emphasized a broad scale genetic homogeneity in marine fish (Ward et al., 1994). In the present study, the values of genetic diversity in the species *S. fuscus* (h = 0.2474 and 0.2452), as well as the I of 0.3604 and 0.3499 for the populations from Búzios and Santa Rita, respectively, indicate a high degree of intrapopulation variability, remarkably higher than that observed in *S. sanctipauli* (h = 0.1703; I = 0.2449). These data suggest a reduced number of ancestor founders in the genetic composition of the species *S. sanctipauli*. Nowadays, this species is restricted to a geographical range of 500 m^2, comprising an extremely low population effective size when compared to coastal representatives.

The level of introgression between both populations of *S. fuscus* based on the number of migrants per generation revealed that, although reduced (Nm = 1.58), it is enough to keep a cohesive and unstructured population effective size (panmitic population).

A high number of reports have revealed some barriers to dispersal, often unclear, such as river outflows and marine currents, that could determine a historical genetic differentiation among marine populations a few kilometers apart (Floeter and Gasparini, 2000; Rocha, 2003). Nevertheless, despite the geographical distance between *S. fuscus* and *S. variabilis* populations (Búzios and Santa Rita, comprising about 35 km) and the presence of a large river mouth (Potengi River, S 05° 45' 23.0"/W 035° 12' 02.9") and a small one (Pium River, S 05° 58' 57.8"/W 035° 07' 22.6") within the area, they did not represent a barrier to gene flow along the coastline populations. This feature was corroborated by the high level of genetic similarity among geographic samples of both *S. variabilis* and *S. fuscus*.

The index of genetic diversity in populations of *S. variabilis* from Búzios and Santa Rita was very similar to that observed in the sympatric species *S. fuscus* at the same area, suggesting that they share some biological features favorable to an effective gene flow among regions.

The coastal species *S. fuscus* and *S. variabilis* demonstrated a number of polymorphic bands two-fold higher than that of the insular species *S. sanctipauli*, indicating a significantly reduced genetic variation in the latter, a feature likely related to the population effective size of each population studied (high in the coastal samples and low for the islands).

Analysis of molecular variance

AMOVA data indicate a higher genetic variability within populations of *S. fuscus*, *S. variabilis* and *S. sanctipauli* (71.14%) and slight differences among groups (27.46%). These results are in agreement with studies in other marine species, showing high variability and little genetic structure (Ward et al., 1994; Waples, 1998). The reduced population structure among samples of *S. fuscus* and *S. variabilis* from Santa Rita and Búzios, as detected through ϕ_{ST} values, revealed the occurrence of an effective gene flow able to maintain the genetic cohesion in these species.

Molina (2006), using a cytogenetic approach, pointed out that the insular species *S. sanctipauli* would represent a derived form of the coastal *S. fuscus*, based on the karyotype formulae found in both species. Chromosomal differentiation commonly acts as an efficient post-zygotic barrier between species. In this case, a lack of gene flow between the two species is detected by ϕ_{ST} values, which can be associated with the evolutionary process of dispersal and differentiation in *S. sanctipauli*.

On the other hand, the ϕ_{ST} values among *S. variabilis* and *S. fuscus* from Santa Rita, RN, suggest the occurrence of gene flow between species from this region. Although morphological studies have not been performed to differentiate the two species or reveal hybridization events, the ϕ_{ST} values indicate they could hybridize since some individuals showed intermediate profiles between the two species.

Hybridization has been reported in 56 fish families, mainly in freshwater groups (Pyle and Randall, 1994), and they are related to weak pre- and post-zygotic barriers. It can occur when closely related species spawn aggregately (i.e., at the same time and place), favoring the contact between inter-specific gametes in the water column (Frisch and Van Herwerden, 2006) or when a transitory or permanent form is present in an unbalanced sex ratio between species phylogenetically related living in sympatry. Thus, the abundance or an extreme reduction of males in a species could lead to inter-specific mating (Allen, 1975; Pyle and Randall, 1994).

Despite of the absence of information about population dynamics, a prevalence of *S. variabilis* specimens was observed during collection expeditions in Santa Rita, contrary to what has been found in other surrounding areas where *S. fuscus* is more frequent. Considering the high complexity of ethological and ecological patterns in reef fishes (Sale, 1991), hybridization in this region could be associated with a breakdown of weak mechanisms of reproductive isolation (Campton, 1987). Unfortunately, there are no reports about ecological and behavioral aspects of hybridization in the genus *Stegastes* from the Atlantic Ocean.

Molina and Galetti Jr. (2004b) demonstrated that *S. fuscus* and *S. variabilis* share a karyotype of 2n = 48, mainly composed of meta-submetacentric chromosomes. However, they differ in the number of acrocentric chromosomes (three pairs in *S. fuscus* and four pairs in *S. variabilis*). The

differences in the karyotype formulae of these species constitute an effective cytotaxonomic marker, helpful in identifying, cytologically, an eventual occurrence of natural hybridization between them. If hybrids between the two species can be formed, introgression could lead to a reduction in genetic diversity, losses of local adaptation or even to species substitution (Grant et al., 2002).

According to the present data, the level of genetic variability within populations is quite higher in the coastal species than in the insular representative. This feature is putatively linked to a high degree of inbreeding in *S. sanctipauli*, coupled with a reduced population effective size. Albeit some cases of genetic divergence have been reported in other pomacentrids within a small geographic scale (Lacson and Clark, 1995; Planes et al., 1998), the geographic samples of *S. fuscus* and *S. variabilis* from Búzios and Santa Rita displayed an unstructured genetic pattern, and each species could be regarded as a single population.

ACKNOWLEDGMENTS

Research supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazil.

REFERENCES

Allen GR (1975). Damselfishes of the south seas. T.F.H. Publications Inc., Hong Kong.

Allen GR (1991). Damselfishes of the world. Mergus Publishers, Melle.

Bardakci F and Skibinski DO (1994). Application of the RAPD technique in tilapia fish: species and subspecies identification. *Heredity* 73: 117-123.

Barman HK, Garat A, Yadav BM, Banerjee S, et al. (2003). Genetic variation between four species of Indian major carps as revealed by random amplified polymorphic DNA assay. *Aquaculture* 217: 115-123.

Bell LJ, Moyer JT and Numachi K (1982). Morphological and genetic variation in Japanese populations of the anemonefish *Amphiprion clarkii*. *Mar*. *Biol*. 72: 99-108.

Bielawski JP and Pumo DE (1997). Randomly amplified polymorphic DNA (RAPD) analysis of Atlantic Coast striped bass. *Heredity* 78: 32-40.

Bonhomme F and Planes S (2000). Some evolutionary arguments about what maintains the pelagic interval in reef fishes. *Environ*. *Biol*. *Fishes* 59: 365-383.

Callejas C and Ochando MD (1998). Identification of Spanish barbel species using the RAPD technique. *J*. *Fish Biol*. 53: 208-215.

Campton DE (1987). Natural hybridization and introgression in fishes. Methods of detection and genetic interpretations. In: Population genetics in fishery management (Ryman N and Utter F, eds.). University of Washington Academic Press, Seattle, 161-192.

Ebeling AW and Hixon MA (1991). Tropical and temperate reef fishes: comparison of community structures. In: The ecology of fishes on coral reefs (Sale PF, ed.). Academic Press, London, 509-563.

Emery AR (1972). A new species of damselfish (Pisces: Pomacentridae) from the Eastern Coast of South America. *Copeia* 2: 330-335.

Excoffier L (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA data. *Genetics* 131: 479-491.

Floeter SR and Gasparini JL (2000). The southwestern Atlantic reef fish fauna: composition and zoogeographic patterns. *J*. *Fish Biol*. 56: 1099-1114.

Foo CL, Dinesh KR, Lim TM, Chan WK, et al. (1995). Inheritance of RAPD markers in the guppy fish, *Poecilia reticulata*. *Zool*. *Sci*. 12: 535-541.

Frisch A and Van Herwerden L (2006). Field and experimental studies of hybridization between coral trouts, *Plectropomus leopardus* and *Plectropomus maculatus* (Serranidae), on the Great Barrier Reef, Australia. *J*. *Fish Biol*. 68: 1013-1025.

Gasparini JL, Moura RL and Sazima I (1999). *Stegastes trindadensis* n. sp. (Pisces: Pomacentridae), a new damselfish from Trindade Island, of Brazil. *Bol*. *Mus*. *Biol*. *Mello Leitão* (Nova série) 10: 3-11.

Grant GC, Vondracek B and Sorensen PW (2002). Spawning interaction between sympatric brown and brook trout may contribute to species replacement. *Trans*. *Am*. *Fish*. *Soc*. 131: 569-576.

Hatanaka T and Galetti PM Jr (2003). RAPD markers indicate the occurrence of structured populations in a migratory freshwater fish species. *Genet*. *Mol*. *Biol*. 26: 19-25.

Lacson JM (1992). Minimal genetic variation among samples of six species of coral reef fishes collected at La Parguera, Puerto Rico, and Discovery Bay, Jamaica. *Mar. Biol.* 112: 327-331.

Lacson JM (1994). Fixed allele frequency differences among Palauan and Okinawan populations of the damselfishes *Chrysiptera cyanea* and *Pomacentrus coelestis*. *Mar. Biol.* 118: 359-365.

Lacson JM and Morizot DC (1991). Temporal genetic variation in subpopulations of bicolor damselfish (*Stegastes partitus*) inhabiting coral reefs in the Florida Keys. *Mar. Biol.* 110: 353-357.

Lacson JM and Clark S (1995). Genetic divergence of Maldivian and Micronesian demes of the damselfishes *Stegastes nigricans*, *Chrysiptera biocellata*, *C. glauca* and *C. leucopoma* (Pomacentridae). *Mar. Biol.* 121: 585-590.

Liu ZJ, Argue BJ and Dunham RA (1999). Random amplified polymorphic DNA markers: usefulness for gene mapping and analysis of genetic variation of catfish. *Aquaculture* 174: 59-69.

Menegatti JV, Vescovi DL and Floeter SR (2003). Interações agonísticas e forrageamento do peixe-donzela, *Stegastes fuscus* (Perciformes: Pomacentridae). *Natureza on line* 1: 45-50.

Menezes NA and Figueiredo JL (1985). Manual de peixes marinhos do sudeste do Brasil. V. Teleostei (4). Museu de Zoologia. Universidade de São Paulo, São Paulo.

Molina WF (2006). Chromosome changes and stasis in marine fish groups. In: Fish cytogenetics (Pisano E, ed.). Science Publisher, Enfield, 69-110.

Molina WF and Galetti PM Jr (2004a). Karyotypic changes associated to the dispersive potential on Pomacentridae (Pisces, Perciformes). *J. Exp. Mar. Biol. Ecol.* 309: 109-119.

Molina WF and Galetti PM Jr (2004b). Multiple pericentric inversions and chromosomal divergence in the reef fishes *Stegastes* (Perciformes, Pomacentridae). *Genet. Mol. Biol.* 27: 543-548.

Mora C and Sale PF (2002). Are populations of coral reef fish open or closed. *Trends Ecol. Evol.* 17: 422-428.

Moura RL, Gasparini JL and Sazima I (1999). New records and range extensions of reef fishes in the Western South Atlantic, with comments on reef fish distribution along the Brazilian coast. *Rev. Bras. Zool.* 16: 513-530.

Nei M (1973). Analysis of gene diversity in subdivided populations. *Proc. Nat. Acad. Sci. USA* 70: 3321-3323.

Nelson JS (1994). Fishes of the World. 3rd edn. John Willey and Sons Inc., New York.

Novelli R, Numan GW and Lima NRW (2000). A new species of the damselfish genus *Stegastes* Jenyns, 1842 (Teleostei: Pomacentridae) from the coast of Brazil. *Bol. Mus. Nac. N. S. Zool.* 413: 1-12.

Planes S and Doherty PJ (1997). Genetic and color interactions at a contact zone of *Acanthochromis polyacanthus*: a marine fish lacking pelagic larvae. *Evolution* 51: 1232-1243.

Planes S, Parroni M and Chauvet C (1998). Evidence of limited gene flow in three species of coral reef fishes in the lagoon of New Caledonia. *Mar. Biol.* 130: 361-368.

Pyle RL and Randall JE (1994). A review of hybridization in marine angelfishes (Perciformes: Pomacanthidae). *Environ. Biol. Fishes* 41: 127-145.

Rocha LA (2003). Patterns of distribution and processes of speciation in Brazilian reef fishes. *J. Biogeogr.* 30: 1161-1171.

Rocha LA, Rosa IL and Rosa RS (1998). Peixes recifais da costa da Paraíba, Brasil. *Rev. Bras. Zool.* 15: 553-566.

Rosa RS and Moura RL (1997). Visual assessment of reef fish community structure in the Atol das Rocas Biological Reserve, of Northeastern Brazil. Proceedings of the 8th International Coral Reef Symposium, Panamá, 983-986.

Rosa RS, Rosa IL and Rocha LA (1997). Diversidade da ictiofauna de poças de maré da praia de Cabo Branco, João Pessoa, Paraíba, Brasil. *Rev. Bras. Zool.* 14: 201-212.

Sale PF (Editor) (1991). The Ecology of Coral Reef Fishes. Academic Press, San Diego.

Sambrook J, Fritsch EF and Maniatis T (1989). Molecular cloning: a laboratory manual. Cold Springer Harbor Laboratory Press, Cold Springer Harbor.

Schneider S, Roessli D and Excoffier L (2000). Arlequin version 2000: a software for population data analysis. University of Geneva, Geneva.

Schwamborn SHL and Ferreira BP (2002). Age structure and growth of the dusky damselfish, *Stegastes fuscus*, from Tamandaré reefs, Pernambuco, Brazil. *Environ. Biol. Fishes* 63: 79-88.

Shaklee JB (1984). Genetic variation and population structure in the damselfish, *Stegastes fasciolatus*, throughout the Hawaiian archipelago. *Copeia* 1984: 629-640.

Strathmann RR (1993). Hypothesis on the origins of marine larval. *Annu. Rev. Ecol. Evol. Syst.* 24: 89-117.

Waples RS (1998). Separating the wheat from the chaff; patterns of genetic differentiation in high gene flow species. *J. Hered.* 89: 438-450.

Ward RD, Woodwark M and Skibinski DOF (1994). A comparison of genetic diversity levels in marine, freshwater and anadramous fishes. *J. Fish Biol.* 44: 213-232.

Wright S (1978). Evolution and genetics of populations: the theory of gene frequencies. University of Chicago Press, London.

Yeh FC, Boyle T, Yeh Z and Xiyan JM (1999). POPGENE Version 1.31: Microsoft Window based freeware for population genetic analysis. University of Alberta and Center for International Forestry Research, Edmonton.

Zar JH (1974). Biostatistical analysis. Prentice-Hall, Englewood Cliffs.