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Research Article

Multiple pericentric inversions and chromosomal divergence in the reef fishes *Stegastes* (Perciformes, Pomacentridae)

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Abstract

Damselfishes (Pomacentridae, Perciformes) occur in all major oceans of the world and, with approximately 320 species, represent one of the most diverse families of marine Teleostei. The taxonomy of these reef fishes is problematic because of the large number of complex species and the range of color patterns they display, which vary among individuals and populations of the same species. In this study, we examined the cytogenetic composition of four species of *Stegastes* (*S. pictus, S. fuscus, S. variabilis* and *S. leucostictus*) found along the coast of Brazil. *Stegastes pictus* had a chromosomal number of 2n = 48 (14m+28sm+2st+4a, fundamental number (FN) = 92), *S. fuscus* had 2n = 48 (20m+22sm+6a, FN = 90), *S. variabilis* had 2n = 48 (18m+22sm+8a, FN = 88), and *S. leucostictus* had 2n = 48, (18m+22sm+8a, FN = 88). The nucleolar organizing regions were single and homologous in all of the species, and were located in the interstitial region on the short arm of the first submetacentric pair. The heterochromatin segments were reduced in size and were distributed conservatively over the centromeric and pericentromeric regions of most of the chromosomes. The marked divergence in the number of chromosomal arms, compared to other Perciformes (2n = 48, FN = 48), indicated that varying degrees of multiple pericentric inversions had occurred during the karyotypic evolution of the Pomacentridae. Subtle karyotypic differences between *S. variabilis* and *S. leucostictus* suggested a recent divergence or that their karyotypes were less susceptible to changes. These results indicate that cytogenetic analyses could provide important complementary data for the characterization of populations and species of *Stegastes* and damselfishes in general.

Key words: pericentric inversions, pomacentridae, *Stegastes*, 5S genes.

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Introduction

The family Pomacentridae (Perciformes) contains 28 genera and approximately 320 species known as damselfishes. This family is one of the most diverse among marine teleosts and occurs in tropical, sub-tropical, and temperate regions of all the major oceans (Nelson, 1994). The taxonomy of damselfishes is complicated by the large number of complex species and the color patterns that vary among individuals and populations of the same species. Several species are of growing economic interest because of their diverse color patterns, and this has led to their exploitation.

Several reports have provided karyotypic descriptions for the Pomacentridae, especially from the Pacific region, which has the greatest variety of damselfishes (Ojima, 1983; Klinkhardt *et al*., 1995). However, very little is known about structural aspects of the karyotype, such as the heterochromatic patterns and the number, position and frequency of the nucleolar organizing regions (NORs).

In this work, the karyotypes of the damselfishes *Stegastes fuscus*, *S. pictus*, *S. variabilis*, and *S. leucostictus* found along the coast of Brazil have been described. FISH with probes of the ribosomal 5S subunit were used on the species *S. fuscus* and *S. variabilis*, and rDNA 18S probes on *S. fuscus*.

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Material and Methods

Cytogenetic analyses were done using 24 *Stegastes fuscus* (11 males and 13 females) and seven *S. variabilis* (4 males and 3 females) from the coast of the State of Rio Grande do Norte in northeastern Brazil, and four *S. leucostictus* (2 juveniles, 1 female and 1 male) and five *S. pictus* (3 males and 2 females) collected off the coast of the State of Bahia in northeastern Brazil. Voucher specimens were deposited in the Museum of Zoology of the Univer-

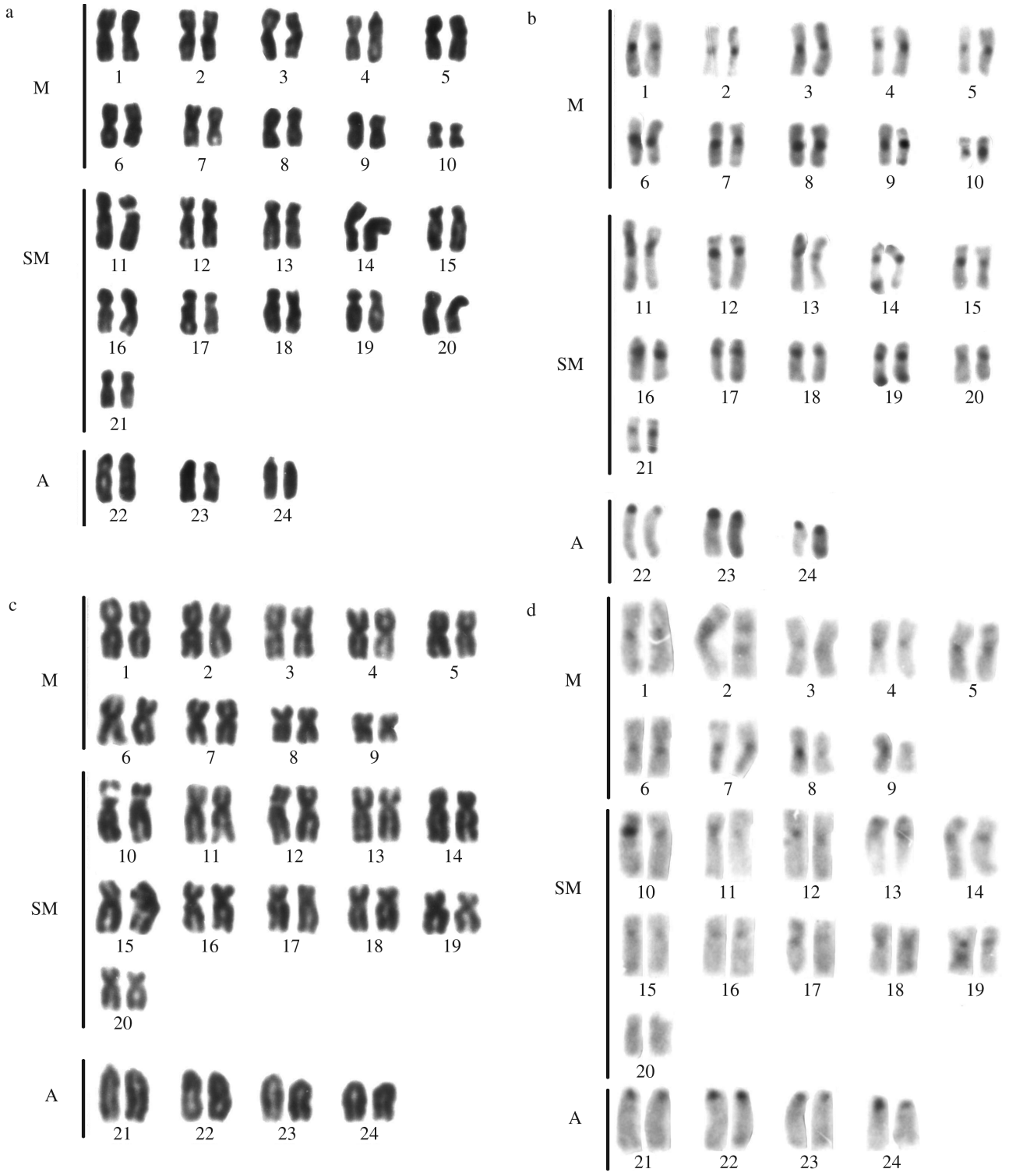

Figure 1 - Karyotypes stained by Giemsa and C banding patterns in (a, b) *S. fuscus* (2n = 48, FN = 90) and (c, d) *S. variabilis* (2n = 48, FN = 88). Secondary constrictions are present on pairs 11 and 10, respectively. A common heterochromatin pattern can be seen in the centromeric and pericentromeric regions.

sidade Federal da Paraíba. Chromosomal preparations were obtained from kidney tissue dissociated in 9.5 mL of RPMI 1640 culture medium with five drops of colchicine for 30 min followed by hypotonic treatment for 25 min at room temperature. The material was then fixed in methanol: acetic acid (3:1, v/v). The NORs and the heterochromatic regions were identified by the methods of Howell and Black (1980) and Sumner (1972), respectively. Fluorescence *in situ* hybridization (FISH) was done in *S. fuscus* and *S. variabilis* using a probe from the 5S rRNA gene of the fish

Figure 2 - Karyotypes stained by Giemsa and C banding patterns in (a, b) *S. leucostictus* (2n = 48, FN = 88) and (c, d) *S. pictus* (2n = 48, FN = 92). The NOR-bearing chromosomes are located in the tenth and eighth pairs, respectively.

Leporinus elongatus (Anostomidae, Characiformes) (Martins and Galetti, 1999). An 18S rDNA probe was obtained from total DNA of *Prochilodus affinis* (Characiformes) (kindly provided by Dr. T. Hatanaka - Universidade Federal de São Carlos) by PCR amplification using the probes NS1 (5'-GTAGTCATATGCTTGTC TC-3') and NS8 (5'-TCCGCAGGTTCACCTACGGA-3') (White *et al*., 1990; Hizume, 1994). This rDNA probe was used to investigate the major ribosomal sites in *S. fuscus*.

Results

Stegastes fuscus (20m+22sm+6a), *S. variabilis* (18m+22sm+8a) (Figure 1), *S. leucostictus* (18m+22sm+8a), and *S. pictus* (14m+28sm+2st+4a) (Figure 2) had

2n = 48 and an elevated number of chromosomal arms (FN = 90, 88, 88 and 92, respectively). The heterochromatic regions were distributed in the centromeric and pericentromeric portions in most of the chromosomal pairs, with blocks that were more evident in the secondary constriction (Figures 1 and 2). NORs occurred at an interstitial position on the short arm of the largest submetacentric pair (pair 11 in *S. fuscus*, 10 in *S. variabilis* and *S. leucostictus*, and 12 in *S. pictus*) (Figure 3). In *S. fuscus*, hybridization with the 5S rDNA probe identified sequences located interstitially on the short arms of two unidentified chromosomal pairs, with one of these pairs having marks that were more evident than in the other. In contrast, signals were detected on only a single pair of chromosomes in *S. variabilis* (Figure 4). In *S. fuscus*, FISH using the 18S rDNA probe confirmed the sites identified by silver staining (Figure 4).

Discussion

The cytogenetic data currently available for marine Perciformes indicates a high degree of chromosomal conservation in which a large number of species show only minor deviations in the chromosomal organization and fundamental number. A karyotype with 48 chromosomes is considered ancestral for the Teleosts (Ohno, 1974), and occurs in 211 of the 660 Perciformes species analyzed so far (Klinkhardt *et al*., 1995).

Almost all of the species of Pomacentridae that have been analyzed cytogenetically have 2n = 48, with fundamental numbers that vary between 48 and 92 (Table 1). A karyotype with 2n = 48 and FN = 48, considered ancestral in the group, has been observed in only a few species, such as *Pomacentrus coelestis* (Arai and Inoue, 1976), *Chromis chromis* (Alvarez *et al*., 1980) and *C. multilineata* (Molina and Galetti, 2002). Some representatives of the subfamily Chrominae show marked numerical polymorphism in their chromosomes while maintaining the number of chromosomal arms. This pattern suggests Robertsonian fusion in the karyotypic evolution of this group. Nevertheless, only 10% of the family has diploid values below the basal number. Despite the importance of polymorphism, this does not appear to be the main evolutionary tendency in the karyotype of this family, but may be characteristic of specific groups.

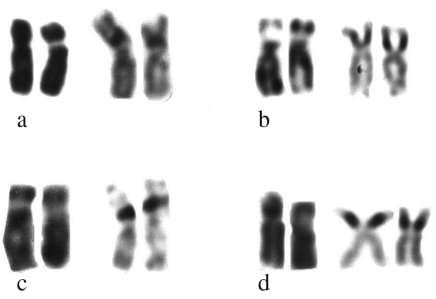

Figure 3 - NOR-bearing chromosomes. Giemsa and Ag-NOR staining. Note the NORs in an interstitial position on the short arm in pair 11 in *S. fuscus* (a), in pair 10 in *S. variabilis* (b) and *S. leucostictus* (c), and in pair 8 in *S. pictus* (d).

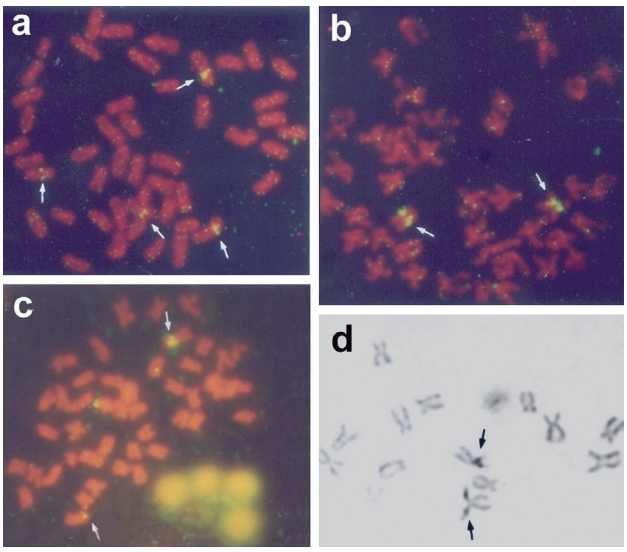

Figure 4 - Chromosomes bearing ribosomal sites. Fish with the 5S rDNA probe in (a) *S. fuscus* (four signals) and (b) *S. variabilis* (two signals). (c) Fluorescence *in situ* hybridization with the 18S rDNA probe showing the rDNA sites located in the secondary constrictions in *S. fuscus*. (d) Ag-NOR signals in *S. fuscus*.

Even with a conserved chromosomal number there is considerable variation in the FN values in this family. This finding is a strong indication that pericentric inversions probably play an important role in the karyotypic diversification of this group of fish. Very little is known about structural aspects of the karyotype in the Pomacentridae. The first indications of C-banding in the Pomacentridae showed a pattern with reduced heterochromatic regions that was basically restricted to centromeric and pericentric areas. This pattern is common to most Perciformes analyzed so far (Galetti *et al*., 2000; Molina *et al*., 2002), and seems to define an array of characteristics that is common to karyotypes with little heterochromatin. A small content of heterochromatin appears to reduce or eliminate the dynamics provided by the heterochromatic segments in the karyotype of a given species. One of the most evident consequences of this small content of heterochromatin is the presence of single NORs in Perciformes and their limited distribution in the karyotype.

The occurrence of two chromosomal pairs with sites for the 5S rRNA gene in *S. fuscus* represents a derived condition in fish (Martins and Galetti, 1999). The presence of only one site in *S. variabilis* may reflect the fact that some sites are so small that they are incapable of emitting a detectable signal. Most probably, however, this single site represents an ancestral condition. Other species of this family have a single 5S rDNA locus, *e.g. Abudefduf saxatilis*, or two loci for the 5S gene, as in *Chromis insolata* and *Chromis flavicauda* (Molina and Galetti, 2002). In the latter two species, the 5S sequences are located in the

Table 1 - Chromosome numbers in the family Pomacentridae.

Species	2n	FN	Formulae	NORs	References
Amphiprioninae					
Amphiprion clarkii	48	78	14m+16sm+18a	-	Arai and Inoue (1976)
A. frenatus	48	84 (92)	14m+22sm+12st-a	-	Arai *et al.* (1976)
A. frenatus	48	92	14m+22sm+8st+4a	-	Molina (2000)
A. ocellaris	48	84	14m+22sm+12st-a	-	Arai *et al*. (1976)
Pomacentrinae					
Abudefduf leucozonus	48	48 (52)	4st+44a	-	Arai and Inoue (1976)
A. coelestinus	48	52	2m+46a	-	Takai and Ojima (1987)
A. notatus	48	50 (52)	2m+2st+44a	-	Arai and Inoue (1976)
A. oxyodon	48	-	?		Takai and Ojima (1987)
A. saxatilis (RJ)	48	52	2m+2sm+44a	S, T	Aguilar *et. al*. (1998)
A. saxatilis (RN)	48	52	2m+2sm+44a	S, I	Molina (2000)
A. sordidus	48	52 (54)	2m+2sm+2st+42a	-	Arai and Inoue (1976)
A. vaigiensi	48	52 (54)	2m+2sm+2st+42a	-	Arai and Inoue (1976)
Amblyglyphidodon curacao	48	76	6m+22sm+20st-a	-	Ojima (1983)
Cheilioprion labiatus	48	70	2m+20sm+26st-a	-	Ojima (1983)
Dischistodus prosopotaenia	48	64	6m+10sm+32st-a	-	Ojima (1983)
Glyphidodontops hemicyaneus	48	80	32sm+16st-a	-	Ojima (1983)
G. cyaneus	48	48	48st-a	-	Ojima (1983)
G. rex	48	78	8m+22sm+18st-a	-	Ojima (1983)
Microspathodon chrysurus	48	64	6m+10st+32a	S*,T	Molina (2000)
Paraglyphidodon nigroris	48	80	8m+24sm+16st-a	-	Ojima (1983)
Plectroglyphidodon lacrymatus	48	50	2sm+46st-a	-	Ojima (1983)
P. leucozonus	48	48	48st-a		Ojima (1983)
Pomacentrus coelestis	48	48	48a	-	Takai and Ojima (1995)
P. coelestis	48	48	48a	-	Arai and Inoue (1976)
P. moluccensis	48	84	8m+28sm+12st-a	-	Ojima (1983)
P. amboinensis	48	-	-	-	Ojima (1983)
P. fuscus	48	48	48a	-	Takai and Ojima (1995)
P. rhodonotus	48	82	8m+26sm+14st-a	-	Ojima (1983)
P. sp.	48	84	10m+26sm+12st-a	-	Ojima (1983)
P. philippinus	48	76	6m+22sm+20st-a	-	Ojima (1983)
Stegastes fuscus	48	90	20m+22sm+6a	S, I	Present paper
S. pictus	48	92	14m+28sm+2st+4a	S, I	Present paper
S. leucostictus	48	88	18m+22sm+8a	S, I	Present paper
S. variabilis	48	88	18m+22sm+8a	S, I	Present paper
S. lividus (= *Eupomacentrus*)	48	78	6m+24sm+18st-a	-	Ojima (1983)
S. nigricans (= *Eupomacentrus*)	48	52	2m+2sm+44st-a	-	Ojima (1983)
Chrysiptera cyanea	42	64 (66)	6m+16sm+2st+18a	S, T	Takai and Ojima (1995)
C. leucopoma	48	74 (80)	4m+22sm+6st+16a	S, T	Takai and Ojima (1995)
C. rex	36	58	12m+10sm+14st-a	S, T	Takai and Ojima (1995)
C. starckii	48	60	-	-	Takai and Ojima (1987)
C. hemicyanea	48	78	-	-	Takai and Ojima (1991)
Chrominae					
Chromis chromis	48	48	48a	-	Alvarez *et al*. (1980)
C. insolata	46-47	56	4-3m+6sm+36-38a	S*, T	Molina and Galetti (2002)
C. multilineata	48	48	48a	S, T	Molina and Galetti (2002)
C. flavicauda	39	54	9m+6sm+24a	S*, T	Molina and Galetti (2002)
C. chrysura	48	50	2m+46sta	-	Ojima (1983)
C. caerulea	48	48	48sta	-	Ojima (1983)
Dascyllus trimaculatum	47	48	1m+46a	-	Arai and Inoue (1976)
D. trimaculatus	47-48	48	0-1msm+46-48sta	-	Ojima and Kashiwagi (1981)
D. reticulatus	34-37	48	14-11m-sm+20-26st-a	-	Ojima and Kashiwagi (1981)
D. aruanus	27-33	48	21-15m-sm+6-18st-a	-	Ojima and Kashiwagi (1981)
D. melanurus	48	48	48sta	-	Ojima and Kashiwagi (1981)

S, single NORs; *non-homologous chromosomes; T, telomeric; I, interstitial. The FN values indicated in parentheses are based on the assumption that the ST chromosomes have two arms.

pericentromeric region of two pairs involved in a Robertsonian fusion.

In contrast to other fish groups (Galetti *et al*., 1984), the NORs were not very informative cytotaxonomic markers in the *Stegastes* species examined here. FISH using an 18S rDNA probe confirmed the location of the major ribosomal sites identified by silver staining in *S. fuscus*. In this group, maintenance of the rDNA cluster on the same chromosomal pair among species reflected both the narrow phylogenetic proximity of the species and a lower dynamism of the internalized sites in relation to those located in telomeric positions.

Multiple pericentric inversions are an important mechanism of post-zygotic isolation (King, 1992). Events of this magnitude could favor speciation in extremely territorial coral species such as *Stegastes*. High fundamental numbers indicate that pericentric inversions in concert are widespread in the karyotypic evolution of the Pomacentrinae and Amphiprioninae. In both of these groups, biological characteristics that regulate the gene flow (territorialism, adhesive eggs and a sessile form) contribute to the break/split of the marine environment in defined micro-regions that may favor the fixation of chromosomal rearrangements.

The high FN values observed in *S. variabilis* (88), *S. leucostictus* (88), *S. fuscus* (90), and *S. pictus* (92) suggest the occurrence of rearrangements in chain acting upon the karyotypes of the species. The increase in the fundamental numbers seen here may be indicative of different levels of phylogenetic relationship among the species analyzed.

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