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Assessing genetic diversity of Brazilian reef fishes by chromosomal and DNA markers

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Abstract

Little is known on genetics of Brazilian coral reef fish and most of this information is limited to chromosome characterization of major representative species. The diploid chromosome number in marine fish varies from $2n = 22$–26 to $2n = 240$–260. Despite of this apparent diversity, most studied marine species have a diploid complement with 48 acrocentric chromosomes. This latter trend is mostly observed among Perciformes, an important major taxon of coral reef fishes. Studies in the families Pomacentridae, Pomacanthidae and Chaetodontidae, for example, have shown a common karyotype pattern entirely formed by 48 uniarmed chromosomes. However, rare numerical and structural chromosome polymorphisms and cryptic chromosome rearrangements involving heterochromatin segments and/or nucleolar organizing sites have been reported among such fishes. Although new chromosome forms can contribute to the establishment of genetically isolated populations, their role in reef fish speciation at marine realm still is an open question. More recently, genomic DNA analyses using RAPD and microsatellites, and sequencing and RFLP of mitochondrial DNA have increasingly been used in Atlantic reef fish species. Genetic homogeneity over wide geographical ranges has been reported for different fish groups, in contrast to several cases of population substructuring related to environmental constraints or evolutionary history. Amazonas outflow and upwelling on the Southeastern coast of Brazil are believed to be strong barriers to dispersal of some reef species. Moreover, it is suggested that the pattern of speciation and population structure at South Atlantic is quite distinctive from Pacific Ocean, even when comparing closely related taxa. Further genetic studies are strongly encouraged in Brazilian reef fishes in order to provide a reliable scenario of the genetic structure in this important and diverse fish group.

Introduction

The Brazilian coast extends over more than 8000 km and it is under the influence of a diverse set of oceanographic and ecological conditions. A considerable part of the tropical fish fauna of the Western Atlantic is found in Brazilian waters.

The coral bottoms from Brazil constitute a formation distributed over 3000 km along Northeastern and Southeastern coasts of Brazil. The Brazilian Province ranges from Orinoco delta at Venezuela, all the Northeastern shore of South America, including oceanic islands (Fernando de Noronha, Rocas Atoll, Saint Paul's Rocks & Trinidad), to South until the coast of Santa Catarina, aggregating a highly diverse ichthyofauna of both ecological and economical relevance (Briggs, 1974; Floeter et al., 2001). Approximately 20% of reef fish species are restricted to the Southwestern Atlantic, and up to 90% of seafood production come from coastal areas, since they sustain richer fish assemblages than oceanic sites.

Alterations in the coastal zones since Cretaceous, modifying the continental shelf, gave rise to diverse microhabitats during the evolution of South Atlantic Ocean (Kegel, 1957). Several bottlenecks might have been present during depression periods, due to habitat losses. Niche diversification and coevolutionary processes among species competition for space and other resources could explain such level of diversification of these reef-associated fishes (Molina, 2000).

Up to date 59 families, comprising 331 species of tropical teleosteans, have been found associated with hard substrates in the continental shelf and oceanic islands from Brazil. The most specious families found in the Brazilian reef complexes are the Serranidae ($N = 31$ species), Gobiidae ($N = 23$), Pomacentridae ($N = 15$), Labridae ($N = 15$), Labrisomidae ($N = 15$), Lutjanidae ($N = 14$), and Muraenidae ($N = 14$). Several endemic species have been identified, revealing that Brazilian reef fish fauna is not a mere extension of Caribbean Province (Floeter & Gasparini, 2001).

Recent ichthyofauna studies about the geographic variation in fish assemblages of reef species from Brazilian shore have discriminated five environmental groups: (I) South coast, with low-water temperature and decreased coral diversity, (II) Northeastern coast, with warm waters and great coral diversity, and oceanic sites, comprising (III) Trinidad island, (IV) Fernando de Noronha and Rocas Atoll, and (V) Saint Paul's Rocks, all isolated areas of low-primary production. In general, the geographical variation in species composition among Brazilian reef sites is strongly correlated to these environmental features (Floeter et al., 2001; Joyeux et al., 2001).

Such patched pattern in the distribution of coastal and oceanic fishes, comprising intricate current and environmental systems, provides a good model for the understanding of structuring and speciation processes in the marine realm.

The dispersal of marine species is often associated to larval developmental stage. Most of reef fishes present life cycles that alternate sedentary adults with pelagic larvae, which allow the species to colonize new regions, distant from spawning sites. This biological feature brings some difficulties in determining the origin of speciation event in some groups, if they occurred by vicariance or by dispersion. It has been assumed that gene flow in the marine realm is multidimensional, highly complex and associated to several variables, synergic or not to genetic exchange between populations (Grosberg & Cunningham, 2001).

By these reasons, the knowledge of the genetic structure of marine fish populations is required, in order to identify their degree of dispersal and recruitment and to contribute with the sustainable management of coral reef species, which are commonly over exploited by fishery activities.

In the last decade, information on species distribution and assemblage composition of reef fishes from both Brazilian coast and oceanic islands have substantially increased due to the efforts of Brazilian ichthyologists. Most of our current genetic data are focused on chromosome studies, as presented in the following issue, where we discuss about the role of chromosome rearrangements in the karyotype diversification and speciation process. In the subsequent sections, different aspects of genetic variability, dispersal, migration, speciation and evolutionary pattern of reef-associated fish from Atlantic Ocean inferred by molecular markers are commented.

Cytogenetical studies

Usually, cytogenetical studies are carried out to characterize the chromosomal set of species, in order to understand the processes of chromosome changes along the evolutionary course of their karyotypical pattern, and how they can be related to diversification of these taxa.

The chromosomal analyses on fish become especially interesting because they compose a particular group amongst vertebrates, occupying a central position in animal evolution, with a large number of quite diverse species with distinct reproductive and behavioral strategies, living in a diversity of habitats (Ohno, 1974). The diploid number in marine fish varies from $2n = 22$–26 in some Nototheniidae species, an Antarctic fish group, to $2n = 240$–260 in some anadromous Acipenseridae species, which show several microchromosomes (Galetti, Aguilar & Molina, 2000).

In contrast, several species are characterized by karyotypical homogeneity.

Traditionally, most of chromosomal information about Neotropical ichthyofauna is focused on freshwater species (Oliveira et al., 1988). From the total of cytogenetical studies performed in those species by far, it was possible to add a remarkable knowledge about Brazilian Neotropical fishes, with detection of groups with heterogeneous or conservative chromosome evolution pattern. On the other hand, cytogenetics of marine fishes from Brazilian shore was only initiated at 1990s and, compared to species diversity, habitat richness, and coastline extension, they can be considered underrepresented. Next, we present a compilation of cytogenetical data concerning to major reef fish groups from Brazilian Province.

Low-chromosome diversity among Perciformes

The Perciformes constitute the most representative and diverse fish order in the marine realm, with 76% of 9023 described species inhabiting sea water (Nelson, 1994). Perch-like species are particularly dominant over coral reefs, as a result of a dramatic radiation during Early Tertiary, apparently simultaneous to coral radiation (Paxton, 1995). It should be pointed out that data about ancient reef sites, from Monte Bolca, Italy, indicate that the most abundant fish assemblage from Eocene belonged to Beryciformes and other non-Perciformes species, an opposite situation to what is found nowadays (Bellwood, 1996). This reveals the Perciformes ability in being adapted and dispersed, allowing that several species preponderated within a short time period.

Moreover, it is well known that Perciformes compose a polyphyletic group, whose phylogenetic relationships of many species remain controversial (Lauder & Liem, 1983). Consequently, species from this order have deserved more attention and concentrate a great part of available karyotypical information. Different researchers have cytogenetically approached some of the most important Perciformes families from Brazilian reef areas (and from Western Atlantic). These include: Acanthuridae, Blenniidae, Chaetodontidae, Haemulidae, Labridae, Mullidae, Pomacanthidae, Pomacentridae, Serranidae, Scaridae among others. To obtain a reliable picture of cytogenetical diversity in reef fishes, some families of coastal species that are not typical from coral reefs but often visit such areas, as Carangidae and Centropomidae, were excluded from the present work. In total, the number of karyotypical descriptions in Brazilian reef species is not over 60 reports (Table 1).

Following a general trend within Perciformes and other Clupeocephala (Brum and Galetti, 1997), a remarkable karyotype stability in species from Brazilian coast was detected. Like the majority of Perciformes species cytogenetically analyzed (about 640 species) (Klinkhardt, Tesche & Greven, 1995), a diploid value equal to 48 chromosomes is highly frequent (Table 1). These data, distributed in several suborders and families, indicate the conservative condition of such karyotype, proposed as primitive for the group.

The presence of 48 acrocentric chromosomes ($2n$ and FN = 48) is considered a sinapomorphy for modern teleosts, especially common in marine species (Brum & Galetti, 1997). This basal karyotype is found in many reef Perciformes species, whether generalist, as Haemulidae (Brum, 1996), or highly specialized, as Pomacanthidae, Chaetodontidae (Affonso et al., 2001) and Pomacentridae (Molina & Galetti, 2004a).

It is often assumed that the reasons for the conservative pattern of chromosome evolution of marine fish are the lacking of well-defined geographical barriers, an intense gene flow (dispersal ability), and the occurrence of large populations, all restraining the fixation of chromosomal rearrangements and karyotypical diversification. This assumption can be corroborated by some examples of species with derived karyotypes from this environment that not fit one or more of these features (Corrêa & Galetti, 1997; Molina & Galetti, 2004b).

However, it should be reminded that the models of marine population distribution are not that clear. While several works indicate a genetic homogenization of populations due to an intense gene exchange between a high number of individuals, favored by a widespread production of pelagic planktonic eggs and larvae, many and significant data do not reveal such correlation (see Grosberg & Cunningham, 2001).

Actually, the dispersal ability of reef fishes during pelagic development stage, interacting with hidrographical, geological and biological factors still remain as a black box, from where more questions than answers are raised (Sale, 1991).

Table 1. List of reef fish species from Brazil cytogenetically analyzed, with their diploid (2*n*) and chromosomal arms (FN) numbers, collection sites (Brazilian States and/or oceanic islands)

Order Family	Species	2*n*	FN[1]	Origin[2]	References
Perciformes					
Acanthuridae	*A. bahianus*	36	52	RN, BA	Affonso et al. (2001)
	A. chirurgus	34	50	RN	Affonso et al. (2001)
	A. coereleus	48	52	RN, BA	Affonso et al. (2001)
Blenniidae	*Parablennius pilicornis*	48	48	RJ	Brum (1996)
	Scartella cristata	48	50	RJ	Brum (1996)
Chaetodontidae	*C. striatus*	48	48	RN, BA	Affonso et al. (2001)
	C. sedentarius	48	48	ES	Unpublished data
Gobiidae	*Bathigobius soporator*	46	48	RN	Unpublished data
Haemulidae	*Anisotremus virginicus*	48	48	RN, RJ	Accioly (2004)
	A. surinamensis	48	48	RN	Accioly (2004)
	A. moricondi	48	48	RN	Accioly (2004)
	Conodon nobilis	48	48	RN	Unpublished data
	H. aurolineatum	48	48	RN, RJ	Unpublished data
	H. striatus	48	–	RN	Unpublished data
	H. flavolineatum	48	–	RN	Unpublished data
	H. parra	48	48	RN	Unpublished data
	H. plumieri	48	48	RN	Unpublished data
	Orthopristes rubber	48	50	RJ	Brum (1996)
	Pomadasys corvinaeformis	48	48	RN	Unpublished data
Labridae	*B. rufus*	48	80	RN	Sena (2003)
	B. pulchellus			ES	Sena (2003
	H. poeyi	48	58	RN, BA, RJ	Sena, 2003)
	H. brasiliensis			RN, RJ	Sena (2003)
	H. radiatus	48	48	SPR	Sena (2003)
Labrisomidae	*Labrisomus nuchipinnis*	48	50	RN, RJ	Unpublished data
Lutjanidae	*Ocyurus chrysurus*	48	48	RN	Unpublished data
Mullidae	*U. parvus*	44	56	RJ	Pauls et al. (1996)
Pomacanthidae	*C. aurantonotus*	48	92	ES, RJ	Affonso and Galetti (2005)
	Holacanthus ciliaris	48	48	CE, PE, BA, ES	Affonso et al. (2001)
	H. tricolor	48	48	BA, ES, RJ	Affonso et al. (2001)
	P. arcuatus	48	50	CE, AL, BA, RJ	Affonso et al. (2002)
	P. paru	48	50	CE, RN, PE, AL, BA, RJ, SP	Affonso et al. (2002)
Pomacentridae	*A. saxatilis*	48	52	RN, BA, RJ, SPR	Aguilar et al. (1998)
	C. flavicauda	39	54	ES	Molina and Galetti (2002)
	C. insolata	46/47	56	ES	Molina and Galetti (2002)
	C. multilineata	48	48	BA, SPR	Molina and Galetti (2002), unpublished data
	M. chrysurus	48	64	BA	Molina and Galetti (2004a)
	S. fuscus	48	90	RN	Molina and Galetti (2004b)
	S. leucostictus	48	88	BA	Molina and Galetti (2004b)
	S. pictus	48	92	CE	Molina and Galetti, (2004b)
	S, sanctipauli	48	92	SPR	Unpublished data
	S. variabilis	48	88	RN	Molina and Galetti (2004b)
Priacanthidae	*Priacanthus arenatus*	52	56	RJ	Bacurau (2003)
Scaridae	*Scarus coelestinus*	48	88	BA	Sena (2003)

Table 1. Continued.

Order Family	Species	2*n*	FN[1]	Origin[2]	References
Perciformes					
	Sparisoma rubripinne	46	70	RN	Sena (2003)
Serranidae	*Alphestes afer*	48	48	RN, BA	Molina et al. (2002)
	Diplectrum formosum	48	50	RJ	Aguilar and Galetti (1997)
	D. radiale	48	48	RJ	Aguilar and Galetti (1997)
	E. adscensionis	48	48	RN	Molina et al. (2002)
	E. guaza	48	48	RJ	Unpublished data
	E. marginatus	48	48	RJ	Unpublished data
	M. acutirostris	48	48	RJ	Aguilar and Galetti (1997)
	M. rubra	48	48	RJ	Aguilar and Galetti (1997)
	Serranus flaviventris	48	48	BA	Molina et al. (2002)
Sparidae	*Diplodus argenteus*	48	48	RJ	Unpublished data
	Pagrus pagrus	48	50	RJ	Unpublished data
Beryciformes					
Holocentridae	*H. adscensionis*	50	78	CE, RN	Bacurau (2003)
	Myripristis jacobus	48	48	RN, SPR	Bacurau (2003)
Scorpaeniformes	*Dactylopterus volitans*	48	78	RJ	Corrêa and Galetti (1997)
	Scorpaena brasiliensis	46	60	RJ	Corrêa and Galetti (1997)
	S. isthmensis	40	54	RJ	Corrêa and Galetti (1997)
Tetraodontiformes					
Tetraodontidae	*Sphoeroides testudineus*	46	74	RN	Sá-Gabriel (2004)
	Sphoeroides greeleyi	46	70	RJ	Brum (1996)
	S. tyleri	46	58	RJ	Brum (1996)
	S. spengleri	46	66	RJ	Brum (1996)
Balistidae	*Balistes vetula*	44	44	BA	Sá-Gabriel (2004)
	Melichthys niger	40	40	SPR	Sá-Gabriel (2004)
Diodontidae	*Chilomycterus spinosus*	52	68	RJ	Sá-Gabriel (2004)
	Diodon holocanthus	46	66	RN	Sá-Gabriel (2004)
Monacanthidae	*Stephanolepis hispidus*	33 (male)	36	BA, RJ	Sá-Gabriel (2004)
		34 (female)	36		
Batrachoidiformes					
Batrachoididae	*Porichthys porosissimus*	44		RJ	Brum et al. (2002)

[1] Metacentric, submetacentric and subtelocentric chromosomes were considered as bi-armed and acrocentric as one-armed elements.
[2] Northeastern Coast: CE = Ceará State, RN = Rio Grande do Norte State, PE = Pernambuco State, AL = Alagoas State, BA = Bahia State. Southeastern Coast: ES = Espírito Santo State, RJ = Rio de Janeiro State, SP = São Paulo State. Island: SPR = St. Paul's Rocks.

Considering the Brazilian Atlantic shore, this kind of information is weakened by the scarcity of studies about distribution of fish species, particularly at pelagic stages.

In addition, the conservative pattern of chromosomal evolution observed in Perciformes can be related to pre-zygotic mechanisms of reproductive isolation. The diverse behavioral strategies of reef fishes (territoriality, ontogenetic and reproductive polychromic signs, sound emission, among others) (Sale, 1991) are able to assure a genetic cohesion of incipient species and further speciation processes without karyotypic changes (Molina, Maia-Lima & Affonso, 2002).

Furthermore, a great number of marine species are karyotypically unknown, most of them Perciformes (only 7% of all described species were analyzed). This strengthens the relevance of continuing studies aiming to define a scenario of chromosomal evolution in some marine groups. In fact, as long as cytogenetical studies on marine fish are enhanced, new trends can be established.

Chromosome rearrangements and speciation

Comparative analyses based on cytogenetical data of Atlantic Perciformes species (Table 2) provided a good measure about the frequency of chromosomal rearrangements in karyotypes from this important fish group. To accomplish these results, each chromosome of each species per family was individually counted, where acrocentric chromosomes were considered free from rearrangements (maintenance), and bi-armed elements were interpreted as inverted (if $2n = 48$) or fusioned (if $2n < 48$) chromosomes. Extra chromosomes, determining $2n > 48$ were considered to have a fission origin. Such approach indicated that pericentric inversion is the main mechanism of chromosomal evolution of Perciformes (18.7% against 1.9% of fusions, and 0.2% of fissions).

Even within conservative families, exceptions of derived karyotypes can be found, if the number of analyzed representatives is increased. This is the case, for example, of the family Pomacanthidae. Species from the genus *Holacanthus* present a highly conserved karyotype ($2n = 48$, FN $= 48$), but pericentric inversions are indicated in *Pomacanthus* species ($2n = 48$, FN $= 50$) and more evident in *Centropyge aurantonotus* ($2n = 48$, NF $= 92$) (Affonso et al., 2001, 2002; Affonso and Galetti, 2005). Similarly, the Atlantic wrasses, *Bodianus* spp. and *Halichoeres* spp. (family Labridae) were distinguished by the number of metacentric, submetacentric and subtelocentric chromosomes, maintaining a diploid value of $2n = 48$ (Sena, 2003). The same situation was encountered in some Pomacentridae species (genera *Abudefduf*, *Stegastes*, *Microspathodon*). In the latter, a positive correlation between FN increasing and short pelagic stage was found, suggesting an important role of dispersion potential on the karyotype evolution of these fishes (Molina and Galetti, 2004a, b).

The inversions were also found among other phylogenetic marine groups, such as Tetraodontiformes and Batrachoidiformes (Brum, 1996; Brum et al., 2002), what indicates that they are not restricted to Perciformes but widespread throughout

Table 2. Analysis of chromosomal types in karyotypes of Brazilian reef fish species

Family	Chromosomal rearrangements									
	Maintenance		Inversion		Fusion		Fission		Total	
	n	%	*N*	%	*N*	%	*N*	%	*n*	%
Chaetodontidae	96	100.0	–	–	–	–			96	4.1
Haemulidae	380	99.5	2	0.5	–	–	–	–	382	16.4
Serranidae	350	99.5	2	0.5	–	–	–	–	382	16.4
Blenniidae	94	97.9	2	2.1	–	–	–	–	96	4.1
Sparidae	94	97.9	2	2.1	–	–	–	–	96	4.1
Labrisomidae	46	95.8	2	4.2	–	–	–	–	48	2.0
Gobiidae	44	95.7	–	–	2	4.3	–	–	46	1.9
Scaridae	30	31.9	62	66.0	2	2.1	–	–	94	4.0
Pomacentridae	256	49.6	248	48.0	12	2.3	–	–	516	22.2
Labridae	102	70.8	42	29.2	–	–	–	–	144	6.2
Mullidae	28	63.6	12	27.3	4	9.1	–	–	44	1.8
Pomacanthidae	192	80.0	48	20.0	–	–	–	–	240	10.3
Acanthuridae	82	69.5	10	8.5	26	22.0	–	–	118	5.1
Priacanthidae	46	88.5	2	3.8	–	–	4	7.7	52	2.2
Total	1840	79.2	434	18.7	46	1.9	4	0.2	2324	100.0

Individual chromosomes were counted and interpreted for the presence or absence of each chromosomal rearrangement. The absolute values (*N*) and respective frequencies (%) of each event per family are shown.

the chromosomal diversification of marine teleosteans.

Additionally, cases of chromosomal polymorphisms were described in some species, as *Chromis flavicauda* and *C. insolata* (family Pomacentridae). Most of polymorphic situations found in Atlantic marine fishes involve changes in the chromosome number, associated to the occurrence of Robertsonian fusions. In the case of Chrominae species, a succession of chromosomal fusions between formerly acrocentric types was detected, giving rise to large metacentric pairs, evident in both species (Molina & Galetti, 2002). Similar Robertsonian polymorphisms are reported in other representatives of Chrominae from Pacific Ocean (genus *Dascyllus*) (Takai & Ojima, 1995) indicating that numerical rearrangements play an important role in the chromosomal evolution of species from this subfamily. It is yet to be known if such polymorphism reflects a novelty within the populations or if they are already fixed.

Another evident example is found in *Acanthurus* species. The few cytogenetical reports in Acanthuridae are restricted to some Pacific species, all characterized by a basal karyotype (48 acrocentric chromosomes) (Arai & Inoue, 1976). In Brazil, three species are described, *A. coereleus*, *A. bahianus* and *A. chirurgus*. While the former presents $2n = 48$, the remaining species have suffered a significant $2n$ reduction ($2n = 36$ and 34, respectively), with several large metacentric chromosomes, and little deviation from the original FN value. It seems that Robertsonian fusions determined the karyotypical differentiation in Acanthurids from Atlantic, but differently from Chrominae species, they are established, being useful as species-specific markers (Affonso, Maia-Lima & Molina, 2001).

Preliminary studies in other Perciformes families, as Scaridae (Sena, 2003), and different orders such as Scorpaeniformes (Corrêa & Galetti, 1997) and Batrachoidiformes (Brum et al., 2002), also revealed the occurrence of numerical rearrangements, suggesting that they can be more common than previously thought among marine species, particularly at Western Atlantic.

Another chromosomal peculiarity already described in Brazilian reef fish includes the presence of supernumerary chromosomes in the dwarf goatfish *Upeneus parvus* (Mullidae) (Pauls et al., 1996). These chromosomes were characterized by the small size and a remarkable inter- and intra-individual variation, following what is commonly described about B-chromosomes in Neotropical fish, but unusual in typically marine species.

Chromosomal banding procedures in some Perciformes groups, apparently cytogenetically conserved, have revealed a surprising microstructural variability.

The characterization of the number and position of nucleolar organizer regions (NOR) has widely been used in fish and can constitute excellent cytotaxonomical markers in some groups (Foresti, Almeida-Toledo & Toledo-Filho, 1981; Galetti, 1998).

Within Perciformes, the presence of a single NOR pair at interstitial position seems to be the most frequent situation, especially in species with conserved karyotypes. It is suggested that this NOR phenotype represents the basal pattern for some families as Serranidae and Pomacanthidae (Aguilar and Galetti, 1997; Affonso et al., 2001). At a lower frequency, it should be mentioned a subterminal or terminal location of NORs on the short arms of bi-armed chromosomes, and at telomeric position on the long arms, with rare examples of multiple NORs (family Pomacentridae) (Molina et al., 2004a, b). In some groups, the location of NORs in different chromosome pairs can characterize significant differences between similar karyotypes (e.g., Serranidae species) (Martinez et al., 1989).

The heterochromatin distribution patterns are also particularly useful for identification of species-specific markers. The presence of conspicuous heterochromatin in the centromeric region of acrocentric chromosomes is a common feature in teleosteans, and it is widely distributed among Neotropical fish groups. Apparently, this heterochromatin is responsible for the frequent centromere-association between distinct chromosomes and can favor to the establishment of karyotypical rearrangements, mostly centric fusions (Aguilar, Côrrea & Galetti, 1998; Molina & Galetti, 2002).

Although the majority of reef species have followed the typical pattern of heterochromatin distribution in fish (centromeres and NOR-associated), some exceptions were reported. For example, individuals of *C. aurantonotus* (Pomacanthidae) presented conspicuous GC-rich heterochromatic blocks on short arms of submetacentric and subtelocentric chromosomes.

Such findings were related to the occurrence of heterochromatin addition, which seems to be a rare phenomenon in Perciformes (Affonso & Galetti, 2005). Similar examples of variation in amount and/or distribution of heterochromatin segments are also observed in Scaridae (Sena, 2003) and Batrachoididae (Brum et al., 2002).

The molecular cytogenetics for *in situ* localization of DNA fragments at metaphase chromosomes (FISH) also begins to play an important and innovating role in the characterization of marine species. Multiple gene sequences can easily be observed in fish species. Among them, ribosomal cistrons are the most commonly used.

In general, the visualization of major ribosomal genes (45S) by FISH in Atlantic reef species have corroborated the results obtained by conventional silver nitrate (Ag-NOR) staining (e.g., families Pomacanthidae, Chaetodontidae, Pomacentridae and Serranidae) (Affonso, 2000; Affonso & Galetti, 2005; Sola et al., 2000; Molina & Galetti, 2004a).

On the other hand, FISH analyses using 5S rRNA gene probes in some few Perciformes species from Brazilian reef sites have demonstrated a great karyotypical diversification.

In the family Pomacentridae, the chromosomes bearing 5S rRNA genes were involved in a numerical polymorphism in the genus *Chromis* and constituted an evidence to confirm the occurrence of centric fusions (Molina & Galetti, 2002). In angelfishes from the family Pomacanthidae, 5S genes presented different topology and frequency (from two sites in *Pomacanthus* spp. up to 18 in *C. aurantonotus*), sometimes reflecting heterochromatin dispersion, as detected in *C. aurantonotus*. Based on these results, it seems that the role of present population structure on fixation of chromosomal rearrangements is not significant, as all angelfishes are characterized by an initial pelagic development stage. Thus, it is possible that cytogenetical differences would be a direct effect of one or more population bottlenecks during evolutionary process, favoring to a faster establishment of a new karyotypical pattern by drift effects (Affonso, 2000; Affonso & Galetti, 2005).

Briefly, the present cytogenetical data of Brazilian reef fishes indicates that chromosomal changes in marine species may be closely related to evolutionary history of coral reefs, characterized by drastic habitat reduction followed by sea level elevation, and not only to populational structure and dispersal abilities at present. Furthermore, the available cytogenetical information about reef fishes from the Brazilian Province suggests distinct chromosomal mutation rates between Atlantic and Pacific species, due to environment peculiarities and particular biogeographic constraints of each Ocean.

DNA studies

Molecular genetic analyses are able to provide important insights into the evolutionary biology of marine fishes, helpful in the understanding of the origin and maintenance of biodiversity in marine ecosystems. The application of molecular tools permits to explore the origin of high specific diversity levels in face of the apparently intense gene flow observed in most coral reef fish species, including populations separated by thousands of kilometers at open sea. The high mutation rates of DNA markers allow tracking of demographically relevant levels of larval dispersal and uncovered biogeographic and phylogenetic history of reef fishes (Shulman, 1998). Moreover, genetic considerations on population structure are also useful to captive breeding and marine stock-enhancement programs in order to avoid inbreeding depression and impacts of artificial selection on fitness (Vrijenhoek, 1998; Shaklee & Bentzen, 1998).

Here, we present some results of molecular analyses carried out in Atlantic reef fishes with distinct molecular markers. When convenient, data from other oceanic regions, such as reef sites at Pacific, were included.

Larval dispersal, biogeography and population structure

In marine organisms, including tropical reef fishes, a pelagic larval stage seems to increase the opportunities for long-distance dispersal and it is often associated to little genetic differentiation over large geographical distances, although the extent of gene flow due to dispersal of the planktonic life stage is generally unknown (Palumbi, 1996).

Several cases of intense gene flow between isolated regions favored by dispersal abilities were reported in reef fish species. For example, the sequencing of a portion of the mitochondrial control region demonstrated that early life-history characteristics, including larval spatial distributions, affect the rates of dispersal and, therefore, the levels of genetic partitioning among reef fishes from Gulf of California (Riginos & Victor, 2001). Three blennioid fishes with markedly different early life histories were investigated. *Axoclinus nigricaudus* has a short larval duration (18 days) and develops inshore, *Malacoctenus hubbsi* has an intermediate larval duration (24 days) and most individuals develop inshore and *Ophioblennius steindachneri* has a long larval life (50 days) and disperses offshore. Estimates of genetic partitioning from mtDNA control region sequences differed greatly between these species and were in the same rank order as predicted by their early life-history characteristics. *A. nigricaudus*, the species that shows the shortest larval stage, presented the highest variance estimates while *O. steindachneri*, with higher dispersal ability, showed no genetic differences between populations, suggesting that larval strategies may be good predictors of population genetic substructuring in marine fishes (Riginos & Victor, 2001).

Likewise, the genetic population structure of the red grouper, *Epinephelus morio* and the scamp, *Mycteroperca phenax*, from the Southeastern US Atlantic coast and the Gulf of Mexico was recently examined using nuclear microsatellites (Zatcoff, Ball and Sedberry, 2004). Genetic variation was assessed at four microsatellite loci in *E. morio* and six loci in *M. phenax*. The microsatellite loci were highly polymorphic, with an average expected heterozygosity of 0.75 in *E. morio* and 0.68 in *M. phenax*. Minimal genetic differences distinguished southeastern US Atlantic or Mexican *E. morio* from other localities and no indication of genetic differentiation was observed in *M. phenax* samples, although physical and biological data indicate that relatively isolated populations of these fish may exist. This large-scale genetic homogeneity may be attributed to ongoing gene flow and/or historical contact between present-day populations (Zatcoff, Ball & Sedberry, 2004). The same suggestions are argued to explain the absence of genetic divergence over great distances in *Coris julis* (Labridae), a species of remarkable dispersal ability (Aurelle et al., 2003).

However, different situations can be found. Dispersal, gene flow and endemism were formerly investigated in eight species of Caribbean reef fishes that varied in egg type (pelagic and non-pelagic) and length of planktonic (usually larval) life, two life-history traits that are supposed to affect dispersal ability (Shulman & Bermingham, 1995). The degree of genetic differentiation among six populations of *S. leucostictus, O. atlanticus*, *A. saxatilis*, *Gnatholepis thompsoni, Haemulon flavolineatum*, *H. bivittatus*, *Holocentrus ascencionis* and *Thalassoma bifasciatum* was estimated by mtDNA restriction fragment length polymorphism (RFLP) markers. The analyzed populations of each species came from widely separated localities, occupying Northern and Southern current tracks within the Caribbean. Mean sequence divergence observed among conspecific Caribbean mtDNA haplotypes in each of the eight fish species was small, less than 0.7% for most species. The predominant mtDNA haplotype of each species was widespread in the Caribbean. Also, populations located under different major surface currents were no more differentiated from those in the same current track. This result suggests that there is considerable gene flow throughout the Caribbean, which has not been constrained by present-day ocean currents. Nevertheless, though between-population variance accounted for only 8–17% of the total, statistically significant population substructuring could be observed for *S. leucostictus* (nonpelagic eggs; short planktonic life); *G. thompsoni* (nonpelagic eggs; long planktonic life); and *H. bivittatus* (pelagic eggs; short planktonic life). These findings imply that neither egg type nor length of larval life may be a simple predictor of geographic structure in reef fish populations. Probably, the genetic patterns of these species would be related to evolutionary history involving bottleneck events (Shulman & Bermingham, 1995).

Similar suggestions were inferred from molecular studies in several reef species from quite distinct localities. Mitochondrial analysis of the anemonefish *Amphiprion ocellaris* at 11 sites throughout Southeast Asia indicated a considerable intrapopulation genetic variation along the board off the Sunda Shelf, in contrast to low levels of variation within shelf populations (Nelson et al., 2000). The Sunda shelf was exposed during sea level reductions associated to periods of glaciation. Thus, much of the phylogeographic substructuring observed today for *A. ocellaris* could be explained by a geographic isolation during the last ice-age and re-colonization onto the shelf with the subsequent rising of the sea level (Nelson et al., 2000).

The analysis of 354bp of the mitochondrial control region from individuals of nine populations of *Chlorurus sordidus* (Scaridae) scattered throughout most of the species Indo-central Pacific range showed high levels of both nucleotide and haplotype diversity and strong genetic subdivision. Nested clade analysis and isolation-by-distance tests pointed out that the genetic substructuring of *C. sordidus* is probably the result of multiple contemporary and historical processes, including long-distance colonization and range expansion. These findings therefore suggest that the pattern of population substructuring recorded in *C. sordidus* is caused by large local population sizes, high gene flow, and a recent history of repeated fragmentation and remixing of populations resulting from fluctuating sea levels (Bay et al., 2004). It should be reminded that previous studies were unable to detect genetic substructuring among the mid- and outer-shelf populations of this species (Dudgeon, Gust & Blair, 2000).

Geographical differences of mtDNA RFLP markers in three reef-associated species, *Lutjanus campechanus*, the red snapper; *E. morio*, the red grouper and *Seriola dumerili*, the greater amberjack, were investigated at Gulf of Mexico and along the Southeastern Atlantic coast of the United States (Gold & Richardson, 1998). These species represent an important commercial resource and so, information on stock structure would be useful to identify genetically meaningful management units. Although the nucleotide sequence diversity was substantially higher in *S. dumerili* (0.55%) than in *L. campechanus* (0.22%) and in *E. morio* (0.06%), no evidence of population substructuring was observed in the three species. In contrast to what might be expected on the basis of their adult life history, the patterns of mtDNA haplotype variation observed in these three reef species indicate that each one is composed by a single breeding population in the Gulf. However, the low levels of mtDNA variation observed in *L. campechanus* and in *E. morio* are consistent with the occurrence of past or present-day bottleneck events (Gold & Richardson, 1998). Lacking of population substructuring in *L. campechanus* was latter confirmed by microsatellite analysis (Gold, Pak & Richardson, 2001).

Habitat peculiarities and ecological interactions might also represent another source of population substructuring in some reef fish species in both Pacific and Atlantic Oceans. Genetic variation estimated through sequencing of the mitochondrial control region DNA in a small subtidal rock-reef fish, *A. nigricaudus* from the Gulf of California was significantly partitioned between relatively short geographical regions (< 200 km, in some cases) (Riginos & Nachman, 2001). Phylogenetic relationships among mtDNA haplotypes revealed a deep division between Northern and central Gulf populations. This genetic discontinuity coincides with an abrupt change in ecological characteristics, including temperature and salinity, but does not coincide with known oceanographic circulation patterns or to any known historic barriers. An overall relationship of increasing genetic distance with increasing geographical distance between population pairs was also evidenced. A significant habitat-by-geographical-distance interaction indicated that populations separated by discontinuous habitat (sand) or deep open water were more genetically distinct than populations separated by continuous habitat (rock). These results indicate that levels of genetic differentiation among populations of *A. nigricaudus* cannot be explained by a single factor, but are due to the combined influences of biogeography, geographical distance and availability of suitable habitat (Riginos & Nachman, 2001).

As previously commented, the major Atlantic biogeographic provinces are discontinuous and most of reef fishes from tropical Atlantic are patchily distributed over wide geographical ranges. Coral reefs in the Gulf of Guinea (West Africa) are separated from Brazilian reefs by an oceanic gap of approximately 4000 km. Caribbean and Brazilian reefs are separated by 2300 km of coastline dominated by soft bottoms and riverine outflows from the Amazon and Orinoco rivers. The freshwater outflow of the Amazon basin is recognized as a major barrier between Brazilian and Caribbean faunas. These barriers are invoked to explain the high endemism of each Province, and this level of isolation may be higher than is currently recognized (Floeter & Gasparini, 2000; Floeter et al., 2001). The vast oceanic distances between Brazil and the mid-Atlantic ridge islands also represent another substantial barrier. Besides, reef fish assemblages along the Brazilian shore seems to be constrained by distinct environmental conditions and/or trophic interactions, which would be able to affect their phylogeographical patterns at different levels (Joyeux et al., 2001; Ferreira, Gonçalves & Coutinho, 2001).

In order to evaluate the possible influences of some of these aspects over the phylogeography of Atlantic reef species, sequencing of mitochondrial cytochrome *b* gene (Cyt*b*) was performed in three Atlantic surgeonfishes (Acanthuridae), *A. bahianus*, *A. chirurgus* and *A. coeruleus* (Rocha et al., 2002). These species have similar life histories but different adult habitat preferences. Samples were obtained from seven locations at Western Atlantic and oceanic islands in the South and Central Atlantic. The Cyt*b* data showed no population substructuring between Brazil and the mid-Atlantic islands, indicating that this oceanic barrier is readily traversed by the pelagic larval stage of all three surgeonfishes, which spend approximately 45–70 days in the pelagic environment. Nevertheless, the genetic separation between Brazilian and Caribbean populations showed a strong relationship with the habitat specificity of adults of the three species. The Amazon River showed to be a strong barrier to dispersal of the reef-associated *A. bahianus*, a moderate barrier for the reef-and-sponge associated *A. coeruleus*, and had no discernible effect in *A. chirurgus*, frequently collected under the Amazon plume. Thus, adult habitat preferences seem to have an additional effect in the differentiating phylogeographical patterns of such species (Rocha et al., 2002).

Similarly, analyses by random amplified polymorphic DNA (RAPD) markers in angelfishes (Pomacanthidae) and butterflyfishes (Chaetodontidae) from Brazilian coast revealed that species composing large assemblages and able to explore different bottoms tend to present a higher genetic similarity between populations, along high levels of genetic diversity (Affonso, 2004). Individuals of the banded butterflyfish, *Chaetodon striatus*, from three localities along the Northeastern and Southeastern reef sites from Brazil were no statistically different from each other and a high intra-populational diversity was responsible for nearly 96% of overall genetic variation. In contrast, the queen angelfish, *H. ciliaris*, presented a moderate but significant population substructuring. The duration of larval period in representatives from both closely related families is similar (45–50 days), but *C. striatus* is characterized by wide geographical range, special swimming abilities at the end of the pelagic phase and higher adaptability to distinct substratum when compared to angelfishes (Stobutzki, 1998; Ferreira, Gonçalves & Coutinho, 2001; Floeter et al., 2001). Thus, it is likely that these specific abilities related to dispersal and habitat colonization could favor an intense gene flow among samples of the Chaetodontid representative (Affonso, 2004).

In *A. saxatilis* (Pomacentridae) from different Brazilian coastal and island sampling sites, RAPD analyses showed population geographical differences, indicating a certain reduction of gene flow. *A. saxatilis* populations from the Southern Atlantic coast are each other more genetically related than they are with the Northern coast populations. However, fishes from the isolated populations of Rocas Atoll and St Paul's Rocks are not distinguishable, although a remarkable morphological differentiation was observed. It was suggested that such genetic similarity could be due to a recent and common origin of these two fish populations (Molina, 2000). Lacking of genetic divergence was already reported among populations of *A. luridus* from the islands of Azores and Madeira, using nuclear microsatellites (Carvalho et al., 2000).

A fine-scale genetic differentiation was observed in the French angelfish *P. paru* from Brazil. Slight differences were identified in samples from distinct areas along the Brazilian shore by RAPD markers and they were further supported by preliminary microsatellite analyses. Coupled with data from other reef-associated angelfish species, it was verified that populations on the Southeastern coast can be so differentiated from Northeastern samples as individuals from oceanic islands, although a Northern–Southern dispersal is expected due to influence of the Brazilian Current. Thus, the genetic divergence between both inshore populations might be determined by local selective pressure, mainly related to upwelling at Cabo Frio region (23° S). This phenomenon would be responsible for a drastic modification of environmental conditions from Northeastern to Southern of the Brazilian coast (Affonso, 2004).

Also attempting to clarify general biogeographic patterns in Atlantic reef species, analyses of mtDNA Cyt*b* gene sequences were performed in two amphi-Atlantic reef fishes from major biogeographic zones within their ranges (Carlin, Robertson & Bowen, 2003). Samples of the rock hind *E. adscencionis* and the greater soapfish *Rypticus saponaceous* were collected from multiple locations within the Caribbean, Brazilian, mid-Atlantic biogeographic provinces, and Eastern Atlantic region. The results indicated relatively ancient separations as well as recent surmounting of biogeographic barriers by dispersal or colonization. *E. adscencionis* showed slight but significant population genetic differentiation across much of the tropical Atlantic Ocean, but deep divergence between the Southeastern United States and seven other localities from the Bahamas to the South, Central and East Atlantic. In contrast, highly significant population genetic substructuring was observed among *R. saponaceous* from the SW Caribbean, Brazil, and mid-Atlantic ridge, with a deep evolutionary separation distinguishing putative *R. saponaceous* from West Africa. The widespread haplotype sharing by *E. adscencionis* indicates that larvae of this species cross-oceanic expanses of as much as 2000 km. However, such a situation is difficult to reconcile with the isolation of populations in Florida and Bahamas separated by only 250 km. These findings suggest that populations of some species in disjunct biogeographic zones may be isolated for long periods, perhaps sufficient for allopatric speciation, but rare gene flow between zones may prevent such evolutionary divergence in other species, thus indicating how complex interactions may be involved in simple predictors of population structure, such as dispersive larvae, restricted habitat type, surface current patterns and straight-line distances between habitats (Carlin, Robertson & Bowen, 2003).

Speciation and sibling species identification

There are many definitions of species, but almost all of them include some level of reproductive isolation (Avise, 2000). Molecular and biochemical methods have already proven to be useful for illuminating aspects of marine speciation. One of the first generalizations that emerge from these studies is that cosmopolitan 'species' are often a group of sibling species that upon a closer examination can also be distinguished by morphological or ecological characters. The genetic analysis of cryptic species may provide important insights into the evolutionary history of the world's oceans and how to preserve their biodiversity (Knowlton, 1993, 2000).

In order to assess information on how evolutionary processes are influenced by differences in oceanography and geography, DNA sequences of the mitochondrial cytochrome *b* gene of two species of blennies were analyzed (Muss et al., 2001). *O. atlanticus* is found in the Western Atlantic, throughout the Greater Caribbean and along the coast of Brazil from the Northeastern to approximately São Paulo, including islands of Fernando de Noronha, St Paul's Rocks and Trinidad, Mid-Atlantic ridge islands of Ascension and Santa Helena and the Eastern Atlantic islands of São Tomé, Cape Verde, Canary Islands, Madeira and Azores. *O. steindachneri* is distributed along the Eastern Pacific Coast from the Sea of Cortez to Peru and inhabits all off-shore islands in this region. Samples were collected in 10 Atlantic locations and four Eastern Pacific locations. A deep genetic substructuring was observed in *O. atlanticus*, corresponding to the five biogeographic provinces (Caribbean, Brazilian, St. Helena/Ascension Island, Gulf of Guinea, and Azores/Cape Verde regions). The Atlantic phylogeny is consistent with a Pliocene dispersal from the Western to Eastern Atlantic. The degree of these separations, along with prior morphological studies (Springer, 1962), indicates that at least three of these lineages are likely at or beyond the transition between population differentiation and speciation. Differently, the Eastern Pacific species *O. steindachneri* was characterized by a markedly weaker substructuring than *O. atlanticus* and no concordance between genetic structure and biogeographic provinces was found. This contrast between genetic signatures of Atlantic and Eastern Pacific blennies demonstrates how differences in geology and oceanography have influenced evolutionary radiation within each region. The phylogeographic pattern in *O. atlanticus* may be attributed to dispersal during the reorganization of Atlantic circulation patterns that followed the shoaling of the Isthmus of Panama. The low degree of substructuring of *O steindachneri* in the Eastern Pacific is probably due to coastal habitat continuity and unstable circulation, which increase the potential for high gene flow (Muss et al., 2001).

Conversely, the circumtropical trumpetfishes that comprises the genus *Aulostomus* constitute a relevant model to study the consequences of a renewed sympatry. Three species are recognized: *A. chinensis*, an Indian-Pacific species that occurs from the Gulf of Panama to South Africa; *A. strigosus*, distributed from Madeira to the Gulf of Guinea at East Atlantic, and *A. maculatus* found in the West Atlantic, from Bermuda to Brazil. Mitochondrial Cyt*b* gene sequencing was performed in samples from 16 worldwide locations, including Brazilian reef zones (Bowen et al., 2001). Low levels of mtDNA diversity were observed in the three species. Based on molecular clock, the separation of West Atlantic and Indian-Pacific species fits the shoaling of the Isthmus of Panama, 3–4 million years ago. Trumpetfishes in the East Atlantic seemed to have been isolated from the Indian Ocean about 2.5 million years ago, coinciding with the advent of glacial cycles and cold-water upwelling around South Africa. Interestingly, 100% of the samples from coastal Brazil and St Paul's Rocks, locations that were previously described as *A. maculatus* habitats, contained the most common haplotype from *A. strigosus*, contradicting the accepted distribution of trumpetfish species. This may indicates a recent colonization of Brazilian waters by East Atlantic species. Alternatively, it suggests that, after 3–4 million years and a globe-spanning series of vicariant and dispersal events, trumpetfish lineages have come back into contact in the Southwest Atlantic and appear to be merging in Brazil, following a ring species pattern. It is possible that such phenomenon may occur in a broad array of marine organisms as well, with clear effects on the production and maintenance of biodiversity in marine ecosystems (Bowen et al., 2001).

Another good example of sibling species is reported in *Acanthochromis polyacanthus*, a Pacific reef fish that lacks a pelagic phase. Three-color morphs are reported along the modern Great Barrier Reef (GBR) and the Coral Sea, and they seem to hybridize at two zones of secondary contact. Allozyme electrophoreses have revealed strong differences between morphs from the Southern GBR zone but few differences between morphs from the Northern counterpart, suggesting different contact histories (Doherty, Mather & Planes, 1994; Planes and Doherty, 1997). The mitochondrial cytochrome *b* region sequences analysis revealed three major clades: (1) black fish collected from the Southern GBR; (2) bicolored fish collected from the GBR and one reef (Osprey) from the Northern Coral Sea; and (3) black and white monomorphs collected from six reefs in the Coral Sea. Sequence divergences were very high between the major clades (mean = 7.6%) as well as within them (2.0–3.6%). Within clades, most reefs segregated as monophyletic assemblages. The extreme divergence between color morphs and the large differentiation among reefs suggest long periods of isolation, whereas the limited diversity within most reefs indicates some recent modifications in the population size, as recent founder events or recent bottlenecks in population size leading to lineage extinction. These data suggest that the monospecific status of *Acanthochromis* should be revisited as the sequence divergences found among these samples from the modern GBR and the Coral Sea are substantially greater than those recorded among well-recognized species in other reef fishes (Planes, Doherty & Bernardi, 2001).

Peculiar situations are also reported at Atlantic Ocean and may provide a quite distinct scenario. Hamlets of the genus *Hypoplectrus* (Serranidae) are described in the Caribbean region. There is a long-running debate whether they are different species or merely a single species, *H. unicolor*, with distinctive color and marking phases. Based on these external patterns, up to nine species are currently recognized. Analyses of mtDNA in these 'species' throughout their geographical range revealed a mosaic, indicating low divergence among them. Nevertheless, two major clades were identified. These results, besides evidence of natural hybridization, initially suggest that *Hypoplectrus* complex represents a result of a recent speciation. Nevertheless, the data fit as well with a model of formerly allopatric species (isolated populations during past sea level reductions) that, nowadays, due to a new contact, suffer a certain degree of introgression, partially restrained by mating preferences among similar colormorphs (Ramon, Lobel & Sorenson, 2003).

On the other hand, analyses of 12S and 16S mtDNA sequences in angelfishes (Pomacanthidae) and their putative sister taxon, the butterflyfishes (Chaetodontidae), supported the monophyly of both families. The relationships of 24 representative Pomacanthidae species were consistent with traditional genetic boundaries and fossil calibrated estimates, excepting in the genus *Centropyge*. Terminal Tethyan Event and the closure of Panama Isthmus have most likely impacted the family Pomacanthidae and species-level diversification within the family is restricted to a single pygmy angelfish clade with an origin near the Oligocene–Miocene boundary (Bellwood, van Herwerden & Konow, 2004)

All these results obtained from DNA studies, commonly paradoxical, shows that little genetic differentiation over large geographical distances seems to be related to larval dispersal ability, even though in several cases such direct relationship is equivocal. Thus, coupled to larval strategies and life-cycle history, many other factors such as biogeography, geographical distance, ecological conditions (local selective pressure) and habitat preference should be considered to explain the genetic pattern of reef fishes. Furthermore, most of genetic divergences in cryptic species groups and world widespread species have suggested that the monospecific status should be revisited. It is also demonstrated that speciation process in the marine realm is equally complex.

Final conclusions

Despite of its physical continuity, the marine environment is ecologically heterogeneous by both biotic and abiotic features (Briggs, 1961). Opposite to freshwater systems, the distribution of marine species is related to several factors not readily distinguishable. Therefore, the characterization of populations is mostly dependent on indirect evidences generated by genetic markers.

Undoubtedly, genetic analyses in reef species can be very useful for delimitation of species distribution, populational structure and identification of sibling species, providing intricate and dynamic evolutionary models. Molecular and cytogenetical studies corroborate that, although life-cycle patterns may be helpful in predicting the genetic structure and speciation mode, oceanographic, historical, behavioral and geographic factors have also to be taken into account when trying to understand the evolutionary processes of reef fish.

Unfortunately, these aspects are poorly known in marine fishes from the Brazilian Province and they remain as an open question in most of reef-associated groups. However, the available data, involving both DNA and chromosomal markers, indicate that most species from South Atlantic Ocean present peculiar and distinct evolutionary rates from those at Pacific Ocean. Therefore, inter-oceanic comparisons and generalizations should be carefully interpreted with basis on particular biogeographic pattern of each environment. Moreover, mostly of genetic studies in tropical Atlantic reef fishes are focused on Perciformes species, which compose a polyphyletic group. Differences in the genetic structure, related to specific evolutionary pathways, are commonly reported in species from a same family or even within the same genus, once again limiting general assumptions.

Based on that, further genetic studies, particularly using refined markers such as microsatellites are strongly encouraged in Brazilian reef fishes in order to provide a reliable scenario of the genetic structure in this important and diverse fish group. Such approach becomes more relevant as long as many reef species and populations from Brazil are threatened either by habitat degradation or overfishing for food or ornamental aquarium trade. Genetic information is able to reveal the ongoing intra-specific diversity and how it is distributed over populations along their distribution range. Thus, management units might be well defined and conservation and/or sustainable exploitation programmes can be properly conducted, avoiding the potential risk of biodiversity losses in reef-associated species from the Brazilian Province.

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References

Accioly, I.V., 2004. Contribuição à citogenética dos gêneros *Pomadasys* e *Anisotremus* (Haemulidae, Perciformes). Graduation monograph. Universidade Federal do Rio Grande do Norte. Natal, Brazil, 43.

Affonso, P.R.A.M., 2000. Caracterização citogenética de peixes de recifes de corais da família Pomacanthidae (Perciformes). Master thesis. Universidade Federal de São Carlos,. São Carlos, Brazil, 143.

Affonso, P.R.A.M., 2004. Marcadores moleculares na análise de espécies e composição populacional de peixes marinhos de recifes de corais da família Pomacanthidae (Perciformes). Doctoral thesis. Universidade Federal de São Carlos. São Carlos, Brazil, 183.

Affonso, P.R.A.M., W. Guedes, E. Pauls & P.M. Galetti Jr., 2001. Cytogenetic analysis of coral reef fishes from Brazil (families Pomacanthidae and Chaetodontidae). Cytologia 66: 379–384.

Affonso, P.R.A.M., F.A. Maia-Lima, Molina W.F., 2001. Rearranjos robertsonianos na diversificação cariotípica de peixes recifais da família Acanthuridae (Perciformes). In Proceedings of the 47th Congresso Nacional de Genética (abstract). Available at http://www.sbg.org.br.

Affonso, P.R.A.M., W. Guedes, E. Pauls & P.M. Galetti Jr., 2002. Close karyotypical relationship between two species of marine angelfishes from South Atlantic: *Pomacanthus arcuatus* and *P. paru* (Perciformes, Pomacanthidae). Caryologia 55: 323–329.

Affonso, P.R.A.M. & P.M. Galetti Jr., 2005. Chromosomal diversification of reef fishes from genus *centropyge* (Perciformes, Pomacanthidae). Genetica 123: 227–233.

Aguilar, C.T. & P.M. Galetti Jr., 1997. Chromosomal studies in South Atlantic serranids (Pisces, Perciformes). Cytobios 89: 105–114.

Aguilar, C.T., M.M.O. Corrêa & P.M. Galetti Jr., 1998. Chromosome associations by centromeric heterochromatin in marine fishes. Chromosome Sci. 2: 73–76.

Arai, R. & M. Inoue, 1976. Chromosomes of seven species of Pomacentridae and two species of Acanthuridae from Japan. Bull. Natl Sci. Mus. Tokyo, Ser. A (Zool) 2: 73–82.

Aurelle, D., T. Guillemaud, P. Afonso, T. Morato, P. Wirtz, R.S. Santos & M.L. Cancela, 2003. Genetic studies of *Coris julis* (Osteichtyes, Perciformes, Labridae) evolutionary history and dispersal abilities. C. R. Biol. 326: 771–785.

Avise, J.C., 2000. Phylogeography: the history and formation of species. Harvard University Press, Cambridge.

Bacurau, T.O.F. 2003. Análise genética de populações e espécies das famílias Holocentridae e Priacanthidae (Pisces) no litoral Nordeste do Brasil e Arquipélago de São Pedro e São Paulo. Master thesis. Universidade Federal do Rio Grande do Norte, Natal, Brazil, 85 pp.

Bay, L.K., J.H. Choat, L. van Herwerden & D.R. Robertson, 2004. High genetic diversities and complex genetic structure in an Indo-Pacific tropical reef fish (*Chlorurus sordidus*): evidence of an unstable evolutionary past? Mar. Biol. 144: 757–767.

Bellwood, D.R., 1996. The Eocene fishes of Monte Bolca: the earliest coral reef fish assemblage. Coral Reefs 15: 11–19.

Bellwood, D.R., L. van Herwerden & N. Konow, 2004. Evolution and biogeography of marine angelfishes (Pisces, Pomacanthidae). Mol. Phylogenet. Evol. 33: 140–155.

Bowen, B.W., A.L. Bass, L.A. Rocha, W.S. Grant & D.R. Robertson, 2001. Phylogeography of the trumpetfishes (*Aulostomus*): ring species complex on a global scale. Evolution 55: 1029–1039.

Briggs, J.C., 1961. The East Pacific barrier and the distribution of marine shore fishes. Evolution 15: 545–554.

Briggs, J.C., 1974. Marine Zoogeography. McGraw-Hill Book Co, New York.

Brum, M.J.I., 1996. Cytogenetic studies of Brazilian marine fish. Braz. J. Genet. 19: 421–427.

Brum, M.J.I. & P.M. Galetti Jr., 1997. Teleostei ground plan karyotype. J. Comp. Biol. 2: 91–102.

Brum, M.J.I., P.R.A.M. Affonso, L.C.G. Mota, E. Pauls & M.R.C.B. Netto, 2002. Cytogenetic characterization of *Porichthys porosissimus* (Valenciennes, 1857) (Batrachoididae, Batrachoidiformes) from the Rio de Janeiro coast, Brazil. Chromosome Sci. 5: 15–18.

Carlin, J.L., D.R. Robertson & B.W. Bowen, 2003. Ancient divergences and recent connections in two tropical Atlantic reef fishes *Epinephelus adscensionis* and *Rypticus saponaceous* (Percoidei: Serranidae). Mar. Biol. 143: 1057–1069.

Carvalho, M.C., R. Streiff, T. Guillemaud, P. Afonso, R.S. Santos & M.L. Cancela, 2000. Isolation and characterization of polymorphic microsatellite markers in *Abudefduf luridus* (Pisces: Pomacentridae). Mol. Ecol. 9: 993–994.

Corrêa, M.M.O. & P.M. Galetti Jr., 1997. Chromosomal diversity in Scorpaenidae (Teleostei, Scorpaeniformes): Cytogenetic studies in *Scorpaena brasiliensis* and *Scorpaena isthmensis* from the coast of Rio de Janeiro, Brazil. Cytologia 62: 397–404.

Doherty, P.J., P. Mather & S. Planes, 1994. *Acanthochromis polyacanthus*, a fish lacking larval dispersal has genetically differentiated populations at local and regional scales on the Great Barrier Reef. Mar. Biol. 121: 11–21.

Dudgeon, C.L., N. Gust & D. Blair, 2000. No apparent genetic basis to demographic differences in scarid fishes across continental shelf of the Great Barrier Reef. Mar. Biol. 137: 1059–1066.

Ferreira, C.E.L., J.E.A. Gonçalves & R. Coutinho, 2001. Community structure of fishes and the habitat complexity on a tropical rocky shore. Env. Biol. Fish. 61: 353–369.

Floeter, S.R. & J.L. Gasparini, 2000. The southwestern Atlantic reef fish fauna: composition and zoogeographic patterns. J. Fish Biol. 56: 1099–1114.

Floeter, S.R. & J.L. Gasparini, 2001. Brazilian endemic reef fishes. Coral Reefs 19: 292.

Floeter, S.R., J.L. Gasparini, L.A. Rocha, R.Z.P. Guimarâes, C.E.L. Ferreira, C.A. Rangel, B.M. Feitoza & G.W. Nunan, 2001. Brazilian reef fish fauna: checklist and remarks. BioBase Project: http://www.biobase.org/BCF/Index.html.

Foresti, F., L.F. Almeida-Toledo & S.A. Toledo-Filho, 1981. Polymorphic nature of nucleolar organizer regions in fishes. Cytogenet. Cell Genet. 31: 137–144.

Galetti, P.M. Jr., 1998. Chromosome diversity in neotropical fishes: NOR studies. Ital. J. Zool. 65(Suppl.): 53–56.

Galetti, P.M. Jr., C.T. Aguilar & W.F. Molina, 2000. An overview of marine fish cytogenetics. Hydrobiologia 420: 55–62.

Gold, J.R. & L.R. Richardson, 1998. Mitochondrial DNA diversification and population structure in fishes from the Gulf of Mexico and western Atlantic. J. Hered. 89: 404–414.

Gold, J.R., E. Pak & L.R. Richardson, 2001. Microsatellite variation among red snapper (*Lutjanus campechanus*) from the Gulf of Mexico. Mar. Biotech. 3: 293–304.

Grosberg, R.K. & C.W. Cunningham, 2001. Genetic structure in the sea. From populations to communities, in edited by

M.D. Bertness, S. Gaines & M.E. Hay. Sinauer Associates, Sunderland.

Joyeux, J-C., S.R. Floeter, C.E.L. Ferreira & J.L. Gasparini, 2001. Biogeography of tropical reef fishes: the South Atlantic puzzle. J. Biogeogr. 28: 831–841.

Kegel, W., 1957. Contribuição ao estudo da bacia costeira do Rio Grande do Norte, in Estudos de Paleontologia Potiguar. Escola Superior de Agricultura de Mossoró, Coleção Mossoroense, v. 167, Mossoró.

Klinkhardt, M., M. Tesche & H. Greven, 1995. Database of Fish Chromosomes. 1st ednWestarp-Wissenschaften, Magdeburg.

Knowlton, N., 1993. Sibling species in the sea. Annu. Rev. Ecol. Syst. 24: 189–216.

Knowlton, N., 2000. Molecular genetic analyses of species boundaries in the sea. Hydrobiologia 420: 73–90.

Lauder, C.V. & K.F. Liem, 1983. The evolution and interrelationships of the actinopterygian fishes. Bull. Mus. Comp. Zool. 150: 95–197.

Martinez, G., G. Thode, M.C. Alvarez & J.R. Lopez, 1989. C-banding and Ag-NOR reveal heterogeneity among karyotypes of serranids (Perciformes). Cytobios 58: 53–60.

Molina, W.F., 2000. Análise da diversidade genética na família Pomacentridae (Pisces, Perciformes), utilizando métodos combinados de citogenética, marcadores moleculares e morfometria. Doctoral thesis. Universidade Federal de São Carlos, São Carlos, Brazil, 167.

Molina, W.F. & P.M. Galetti Jr., 2002. Robertsonian rearrangements in the reef fish *Chromis* (Perciformes, Pomacentridae) involving chromosomes bearing 5S rRNA genes. Genet. Mol. Biol. 24: 373–377.

Molina, W.F. & P.M. Galetti Jr., 2004a. Karyotypic changes associated to the dispersive potential on Pomacentrridae (Pisces, Perciformes). J. Exp. Mar. Biol. Ecol. 309: 109–119.

Molina, W.F. & P.M. Galetti Jr., 2004b. Multiple pericentric inversions and chromosomal divergence in the reef fishes *Stegastes* (Perciformes, Pomacentridae). Genet. Mol. Biol. 27: 543–548.

Molina, W.F., F.A. Maia-Lima & P.R.A.M. Affonso, 2002. Divergence between karyotypical pattern and speciation events in Serranidae fish (Perciformes). Caryologia 55: 299–305.

Muss, A., D.R. Robertson, C.A. Stepien, P. Wirtz & B.W. Bowen, 2001. Phylogeography of *Ophioblennius*: the role of ocean currents and geography in reef fish evolution. Evolution 55: 561–572.

Nelson, J.S., 1994. Fishes of the World. 3rd ed John Wiley & Sons Inc, New York.

Nelson, J.S., R.J. Hoddell, L.M. Chou, W.K. Chan & V.P.E. Phang, 2000. Phylogeographic structure of false clownfish, *Amphiprion ocellaris*, explained by sea level changes on the Sunda shelf. Mar. Biol. 137: 727–736.

Ohno, S., 1974. Protochordata, Cyclostomata, and Pisces, in edited by B. John. Gebruder-Borntrager, Berlin.

Oliveira, C., L.F. Almeida-Toledo, F. Foresti, H.A. Britski & S.A. Toledo-Filho, 1988. Chromosome formulae of neotropical freshwater fishes. Brazil. J. Genet. 11: 577–624.

Palumbi, S.R., 1996. Macrospatial genetic structure and speciation in marine taxa with high dispersal abilities, in edited by J.D. Ferraris &S.R. Palumbi. Wiley-Liss Inc., New York.

Pauls, E., P.R.A.M. Affonso, M.R.C.B. Netto & M.L. Pacheco, 1996. Supernumerary chromosomes on marine fish *Upeneus parvus* (Poey 1853, Mullidae) from Atlantic Ocean. Arch. Zootec. 45: 295–299.

Paxton, J.R., 1995. Habitats and adaptations, in edited by J.R. Paxton &W.N. Eschemeyer. Academic Press Inc, San Diego.

Planes, S. & P.J. Doherty, 1997. Genetic relationships of the color morphs of *Acanthochromis polyacanthus* (Pomacentridae) in the northern Great Barrier Reef. Mar. Biol. 130: 109–117.

Planes, S., P.J. Doherty & G. Bernardi, 2001. Strong genetic divergence among populations of a marine fish with limited dispersal, *Acanthochromis polyacanthus*, within the Great Barrier Reef and the Coral Sea. Evolution 55: 2263–2273.

Ramon, M.L., P.S. Lobel & M.D. Sorenson, 2003. Lack of mitochondrial genetic structure in hamlets (*Hypoplectrus* spp.): recent speciation or ongoing hybridization? Mol. Mol. Ecol. 12: 2975–2980.

Riginos, C. & M.W. Nachman, 2001. Population subdivision in marine environments: the contributions of biogeography, geographical distance and discontinuous habitat to genetic differentiation in a blennioid fish, *Axoclinus nigricaudus*. Mol. Ecol. 10: 1439–1453.

Riginos, C. & B.C. Victor, 2001. Larval spatial distributions and other early life-history characteristics predict genetic differentiation in eastern Pacific blennioid fishes. Proc. R. Soc. Lond. 268: 1931–1936.

Rocha, L.A., A.L. Bass, D.R. Robertson & B.W. Bowen, 2002. Adult habitat preferences, larval dispersal, and the comparative phylogeography of three Atlantic surgeonfishes (Teleostei: Acanthuridae). Mol. Ecol. 11: 243–252.

Sá-Gabriel, L.G., 2004. Inferências sobre a evolução cariotípica em Balistidae, Monacanthidae e Tetraodontidae (Pisces, Tetraodontiformes). Exemplo de extensa diversificação numérica. Master thesis, Universidade Federal do Rio Grande do Norte, Natal, Brazil, 79 pp.

Sale, P.F., 1991. The Ecology of Fishes on Coral Reefs. 1Academic Press, California.

Sena, D.C.S., 2003. Diversificação cariotípica em espécies das famílias Labridae e Scaridae (Pisces – Perciformes) do Atlântico Ocidental. Master thesis, Universidade Federal do Rio Grande do Norte, Natal, Brazil, 70 pp.

Shaklee, J.B. & P. Bentzen, 1998. Genetic identification of stocks of marine fish and shellfish. Bull. Mar. Sci. 62: 589–621.

Shulman, M.J., 1998. What can population genetics tell us about dispersal and biogeographic history of coral-reef fishes?. Aust. J. Ecol. 23: 216–225.

Shulman, M.A. & E. Bermingham, 1995. Early life histories, ocean currents, and the population genetics of Caribbean reef fishes. Evolution 49: 897–910.

Sola, L, S. De Innocentis, E. Gornung, S. Papalia, A.R. Rossi, G. Marino, P. De Marco & S. Cataudella, 2000. Cytogenetic analysis of *Epinephelus marginatus* (Pisces: Serranidae), with the chromosome localization of the 18S and 5S rRNA genes and of the (TTAGGG)n telomeric sequence. Mar. Biol. 137: 47–51.

Springer, V.G., 1962. A review of the Blenniid fishes of the genus *Ophioblennius* Gill. Copeia 1962: 426–433.

Stobutzki, I.C., 1998. Interspecific variation in sustained swimming ability of late pelagic stage reef fish from two families (Pomacentridae and Chaetodontidae). Coral Reefs 17: 111–119.

Takai, A. & Y. Ojima, 1995. Chromosome evolution associated with Robertsonian rearrangements in Pomacentrid fish (Perciformes). Cytobios 84: 103–110.

Vrijenhoek, R.C., 1998. Conservation genetics of freshwater fish. J. Fish Biol. 53(Suppl. A): 394–412.

Zatcoff, M.S., A.O. Ball & G.R. Sedberry, 2004. Population genetic analysis of red grouper, *Epinephelus morio*, and scamp, *Mycteroperca phenax*, from the southeastern US Atlantic and Gulf of Mexico. Mar. Biol. 144: 769–777.