A multi-approach analysis of the genetic diversity in populations of *Astyanax aff. bimaculatus* Linnaeus, 1758 (Teleostei: Characidae) from Northeastern Brazil

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Few reports are available about the ichthyofauna of typical semi-arid rivers, although the regional diversity has been constantly threatened by human activities, mainly related to impoundment and construction of dams. The goal of the present work was to evaluate using different methods, the population genetic structure of a characin fish, *Astyanax aff. bimaculatus*, widespread throughout hydrographic basins of Bahia, Northeastern Brazil. Morphological (meristic and morphometric data), cytogenetic (karyotype and Ag-NOR), and molecular (RAPD and SPAR) analyses were carried out in specimens collected upstream and downstream of Pedra Dam, in the main channel of Contas River (Contas River Basin), and in the Mineiro stream, which belongs to the adjacent Recôncavo Sul basin. Few external differences were detected among populations, where the individuals collected upstream of Pedra Dam were slightly larger than the others. Cytogenetic data also showed a similar karyotypic pattern (2n=50; 6m+28sm+12st+4a; FN= 96) and NORs located on the short arms of up to two chromosome pairs, with numerical inter- and intra-populational variation. Nonetheless, RAPD and SPAR analyses differentiated reliably the three populations, revealing striking differences in the allele frequencies among the localities studied and a significant difference in population structure index (F<sub>st</sub>=0.1868, P<0.0001). The differences between populations within a same river were as significant as those between distinct hydrographic basins, indicating that the dam/reservoir represents an effective barrier to gene flow. Additionally, environmental peculiarities from each locality are also believed to influence the genetic patterns detected herein. On the other hand, the similarity between samples from Contas River and Recôncavo Sul basins could be related to a common evolutionary history, since both basins are geographically close to each other. Finally, the present study shows that a multi-approach analysis is particularly useful in identifying the population structure of widely distributed species and to evaluate the impacts of human activities on natural fish populations.

Key words: Morphometry, Cytogenetics, RAPD, Population structure, Contas River.

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Introduction

The Contas River hydrographic basin is entirely located in the state of Bahia, Northeastern Brazil (12°55’ to 15°30’S, 39°00’ to 42°35’W), comprising a drainage area of 64,933 km², and limited by the Recôncavo Sul, Paraguaçu, São Francisco, Pardo and Eastern hydrographic basins. Its headwaters originate in the Diamantina Plateau (about 1,500 m high) and flow over 620 km before reaching the Atlantic Ocean, encompassing several small to large riverine systems, waterfalls and a high urban concentration (SRHSH, 1993; CRA, 2001; MMA, 2006a). The Contas River Basin is influenced by an array of climate types, ranging from humid to dry. A tropical rain climate is found along the coast and it becomes progressively drier inland, where it shows semi-arid characteristics. Therefore, three physiogeographic regions can be distinguished along this basin: upper, middle and lower Contas River, characterized by semi-arid (Caatinga biome), semi-arid/tropical transition and humid climates (Atlantic rain forest), respectively (SRHSH, 1993; MMA, 2006b).

Contas River is the main river of this hydrographic basin and represents one of the most important water systems in the state of Bahia (CRA, 2002; MMA, 2006b). However, little is known about the ichthyofauna of this river and its tributaries, even though several human activities are likely to affect the local fish assemblages.

In the sixties, a large reservoir (Pedra Dam) was built in the main channel of the Contas River, near the municipality of Jequití (semi-arid belt, middle Contas region) in order to control floods and impound water for dry periods and power supply. This reservoir has a water surface of nearly 70 km and a storage capacity of 1.7 billion cubic meters (SRHSH, 1993; MMA, 2006b).

By damming the rivers, their typical lotic features are affected, with consequent losses and formation of new habitats (Paiva, 1982; Vono et al., 2002). The ecotone mosaic within the impounded waters of a reservoir modify the spatial and temporal patterns of fish communities, such as trophic structure, ecological guilds and species diversity (Pianka, 1974; Winemiller & Leslie, 1992; Welcomme et al., 2005). Moreover, the construction of dams creates a new obstacle to gene flow among populations of aquatic organisms located upstream and downstream of dams, leading to alterations in gene frequencies (Avise & Felley, 1979). The constraints in dispersal and gene flow can affect intra- and inter-population diversity levels, mainly of migratory species (Agostinho et al., 1992; Godinho & Godinho, 1994; Vrijenhoek, 1998; Hatanaka & Galetti, 2003).

Although the small characin fish Astyanax bimaculatus (Linnaeus, 1758) actually refers to specimens from Suriname, the so-called “bimaculatus-group” comprises at least 15 species of generalist and migratory fish, well adapted to both running and stagnant waters and widely distributed throughout Brazilian rivers (Esteves & Galetti, 1995; Agostinho et al., 1997; Garutti & Britski, 2000). Recent morphological studies have separated this group into distinct species such as Astyanax altiparanae from upper Paraná River, Brazil, but most still lack a proper nomination (Garutti, 1998; Garutti & Britski, 2000). Genetic studies carried out in this widespread and closely related group of species have been helpful in understanding the population structure and patterns of geographic isolation, thus providing a baseline for management and conservation programs (Paiva et al., 2006; Domingues et al., 2007; Kantek et al., 2007; Pazza et al., 2007 among others). Such studies, comprising an array of methods from morphology to cytotogenetic and molecular markers, are particularly important to estimate the impact of human activities and environmental effects on Neotropical fish assemblages. However, these features remain unknown along hydrographic basins in the semi-arid region.

In order to evaluate the genetic structure of Astyanax aff. bimaculatus along Contas River Basin, morphometric, cytotogenetic, and molecular studies were performed in populations located upstream and downstream of Pedra Dam (middle Contas River). Another population, from an adjacent hydrographic basin (Recôncavo Sul) was also included in the present work for comparative analyses. These data are discussed based on environmental peculiarities and geographic isolation of each collection site.

Material and Methods

Sampling sites. Specimens of A. aff. bimaculatus were collected using gillnets at three localities: two collection sites in the main channel of the middle Contas River (Contas River Basin), and one in Mineiro stream (Recôncavo Sul Basin). Samples from the Contas River were obtained in the reservoir, 70 km upstream of Pedra Dam, in the city of Maracás, Porto Alegre County (13°52’5”S, 40°14’9”W, 230 m above sea level, site A) and nearly 25 km downstream of Pedra Dam, in the city of Jequití (13°54’84”S, 40°02’54”W, 216 m above sea level, site B). The sampling in Mineiro stream was carried out in the city of Itamari, Mineiro County, at 63 km from Jequití (13°60’54”S, 39°41’54”W, 285 m above sea level, site C) (Figs. 1-2).

Fish samples were transported to the laboratory and kept in separated tanks prior to morphometric, cytotogenetic and molecular analyses. Voucher specimens were identified by Dr. Luiz R. Malabarba (UFRGS, Porto Alegre, RS) and deposited in the fish collection at Universidade Estadual do Sudoeste da Bahia (identification numbers: MN198-202, MN204-208, PA236-241, RC311-317).

Morphological studies. Eighty-four specimens were collected for meristic and morphometric analyses (Table 1). The meristic characteristics considered were the number of scales in the lateral line (LLS), number of rays in the anal fin (AFR) and number of rays in the dorsal fin (DFR), according to Garutti (1998). Variance analysis (α = 5%) was performed to compare the average meristic values, using the GLM procedure in the software SAS (2004). The morphometric characters were: total length, head length, body height, caudal peduncle height,
Tissue samples were obtained from (A) Levan (M), submetacentric (SM), subtelocentric (ST) and acrocentric arranged in decreasing size order and classified as metacentric karyotyping and NOR analysis. The chromosome pairs were Imagemlink Kodak and Black, 1980). The best metaphases were photographed in were detected by silver nitrate staining (Ag-NOR) (Howell et al., 1977). The morphometric data were converted to body proportions and expressed in percentage in order to indicate the relationship between total length, head length, interorbital width, ocular diameter, preanal length, predorsal length, head height, and distances between dorsal and pectoral fins, pectoral and pelvic fins, pelvic and anal fins, dorsal and anal fins, anal and adipose fins, and dorsal and adipose fins (Lagler et al., 1977). The morphometric data were entered into a binary matrix, assuming that each band represented a Mendelian locus of dominant behavior with a non-detectable recessive allele (Lynch & Milligan, 1994). The software ARLEQUIN (Schneider et al., 1997) was used to perform the analysis of molecular variance (AMOVA) and to estimate the inter-population variation by providing F values, considered the best parameters of population structure for RAPD studies (Excoffier et al., 1992; Hartl & Clark, 1997). According to Wright (1978), values of 0 to 0.05 indicate little genetic differentiation, 0.05 to 0.15 indicates moderate differentiation; 0.15 to 0.25 suggests a high differentiation, and values over 0.25 represent a very high genetic differentiation. The proportion of polymorphic loci and degree of population differentiation using Fisher’s Exact test were carried out using the software TFPGA – Tools For Population Genetic Analysis (Miller, 1997).

**Molecular studies.** Tissue samples were obtained from epaxial muscle or gill filaments of about 25 specimens per collection site (Table 1) and fixed in 95% ethanol prior to molecular analyses. DNA extraction followed the CTAB protocol reported by Boyce et al. (1989), with slight modifications. The amplification reactions were performed according to Williams et al. (1990), comprising a final volume of 13 ml (3.42 ml H2O; 1.3 ml 10x buffer with 1 M MgCl2, 1.04 ml 2.5 mM dNTP; 1.05 ml bovine serum albumin, 3 ml primer at 2.5 mM, 0.2 ml 5 U/ml Taq polymerase and 3 ml of 2.5 ng template DNA). PCR reactions were carried out in a Mastercycler Gradient Eppendorf thermocycler with one initial heating step at 92°C for 2 min, 40 cycles at 92°C for 1 min, 35°C for 1 min and 72°C for 2 min, followed by a final extension step at 72°C for 5 min. The amplified products were run for 3 h at 110V in 1.5% agarose gel and stained with ethidium bromide. The fragments were visualized under ultraviolet light and photographed for further analyses. The fragment size was estimated using a 1-kb molecular weight ladder (Fermentas Life Technologies).

Fifty RAPD and 17 SPAR primers (both provided by Operon Technologies) were screened. The rate of missing values per marker was estimated and the primers showing more than 25% of unrecorded data were excluded. Therefore, six RAPD primers (OPA-18, OPA-20, OPA-02, OPA-09, OPA-11, OPD-02) and five SPARs (SPAR1, SPAR2, SPAR17 SPAR16, SPAR15) were selected, since they produced an adequate number of scorable, polymorphic, and well-defined bands. Each individual was codified as a string of 1 and 0 indicating the presence or absence of amplification products and data entered into a binary matrix, assuming that each band represented a Mendelian locus of dominant behavior with a non-detectable recessive allele (Lynch & Milligan, 1994). The software ARLEQUIN (Schneider et al., 1997) was used to perform the analysis of molecular variance (AMOVA) and to estimate the inter-population variation by providing F values, considered the best parameters of population structure for RAPD studies (Excoffier et al., 1992; Hartl & Clark, 1997). According to Wright (1978), values of 0 to 0.05 indicate little genetic differentiation, 0.05 to 0.15 indicates moderate differentiation; 0.15 to 0.25 suggests a high differentiation, and values over 0.25 represent a very high genetic differentiation. The proportion of polymorphic loci and degree of population differentiation using Fisher’s Exact test were carried out using the software TFPGA – Tools For Population Genetic Analysis (Miller, 1997).

**Table 1.** Localities and sample sizes of *Astyanax aff. bimaculatus* for morphologic (N1), cytogenetic (N2), and molecular (N3) analyses (*Contas River Basin, ** Recôncavo Sul Basin).

<table>
<thead>
<tr>
<th>Samples (sites)</th>
<th>Locality</th>
<th>N1</th>
<th>N2</th>
<th>N3</th>
</tr>
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<tbody>
<tr>
<td>Contas River (A)*</td>
<td>Upstream Pedra Dam, Porto Alegre County</td>
<td>30</td>
<td>5</td>
<td>24</td>
</tr>
<tr>
<td>Contas River (B)*</td>
<td>Downstream Pedra Dam, Jequié</td>
<td>24</td>
<td>19</td>
<td>25</td>
</tr>
<tr>
<td>Mineiro stream (C)**</td>
<td>Mineiro County, Itamari</td>
<td>30</td>
<td>17</td>
<td>24</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>84</td>
<td>41</td>
<td>73</td>
</tr>
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</table>
Results

Morphological studies. The mean and standard deviation values for the number of lateral line scales (LLS), and number of rays in the anal (AFR) and dorsal fins (DFR) of each A. aff. bimaculatus population are shown in Table 2. Such meristic features demonstrated little or no variation (e.g., DFR) among distinct collection sites, with a modal number of 33, 26 and 11 for LLS, AFR and DFR, respectively. Variance analysis also supported this finding, revealing no population effects in relation to the characteristics examined (P > 0.05).

Analyzing the mean absolute values for total length, head length and body height, we observed that specimens from the reservoir (site A) displayed higher values than individuals from other localities, thus supporting empirical findings that fish upstream of Pedra Dam were usually larger. However, inter-population differences were remarkably small when comparing percentage values in relation to total length, head length and body height. The only exceptions include the proportion between interorbital width, head height and head length, and between the caudal peduncle height and body height (Table 3).

Cytogenetic studies. The diploid number found in the three populations of A. aff. bimaculatus was equal to 2n = 50. A similar karyotype, composed of 6 metacentric, 28 submetacentric, 12 subtelocentric and 4 acrocentric chromosomes (FN = 96) was observed in both males and females from all collection sites (Figs. 3a-c).

Molecular studies. The percentage of polymorphic loci in the three populations of A. aff. bimaculatus ranged from 85.71% in both Contas River samples (sites A and B) to 100% in Mineiro stream (site C). Based on Fisher’s exact test, 42.8% (12) of the 28 loci analyzed showed significant differences (P < 0.05) among collection sites. The populations were also significantly different when information from all loci were considered (P < 0.0001).

The amount of genetic variability among and within the three populations of A. aff. bimaculatus inferred by analysis of molecular variance (AMOVA) was equal to 18.69 and 81.31%, respectively, with significant values. Although intra-population variation was responsible for most of the genetic diversity in the studied samples, a high genetic differentiation was detected (Fst = 0.1868, P < 0.0001), indicating a population structure in A. aff. bimaculatus from the Northeastern river basins (Table 4).

Pairwise comparisons based on AMOVA also revealed highly significant divergences (P < 0.0001). A divergence level of 22.02% was detected between populations upstream and downstream of Pedra Dam (sites A and B, respectively). A lower divergence (about 13%) was found between populations from site A (Pedra Dam reservoir, Contas River Basin) and site C (Mineiro stream, Recôncavo Sul Basin) (Table 5).
Discussion

Studies on the ichthyofauna of Northeastern Brazil still lack a detailed characterization and semi-arid regions, such as Caatinga (dry shrubland), have been formerly regarded as low diversity ecosystems. Nevertheless, a high level of endemism has been suggested for some fish groups inhabiting this biome, as a response to specific evolutionary processes provided by peculiar climate and hydrological features (Rosa, 2004; Rapini et al., 2006). For instance, recent studies reported nearly 240 fish species in semi-arid riverine systems, 57% of them being endemic (Rosa et al., 2003).

Besides the scarce information about regional fish fauna, many hydrographic basins in the semi-arid region are also threatened by environmental disturbances. The construction of dams and reservoirs are likely to decrease the local biodiversity before we even get to know it. Dams are able to disrupt the gene flow between upstream and downstream aquatic populations and affect the dispersal rate of several migratory fish (Avise & Felley, 1979; Agostinho et al., 1992; MMA, 2006a). They are responsible for changes in the water flow within a hydrographic system, with consequent losses of original habitats (Vono et al., 2002). Under specific circumstances, the reservoirs may lead to local extinction of populations unable to adapt to the drastic environmental modifications imposed (Godinho & Godinho, 1994). As a result, the constraints on dispersal, gene flow and fitness usually affect both inter- and intra-populational diversity (Vrijenhoek, 1998).

Actually, the localities selected in the present study are among several other hydrographic systems throughout the eastern Atlantic basin that have been deeply disturbed by human activities (CRA, 2002; MMA, 2006b). These practices have certainly altered natural ecosystems to an unknown extent. Such may be the case of the Pedra Dam in the middle portion of Contas River (Jequié-BA) (Paiva, 1982; SRHSH, 1993; MMA, 2006a).

The morphological analyses in populations of *A. aff. bimaculatus* from the Contas and Recôncavo Sul basins revealed a remarkable homogeneity of both meristic and morphometric characters among individuals, regardless of the collection site. Few exceptions to this pattern were observed, such as the differential proportion of the caudal peduncle height in the Recôncavo Sul sample. Moreover, specimens collected upstream of Pedra Dam (Porto Alegre County, site A) were, on average, larger than those from other samples (Table 3).

Similarly, morphological analyses in allopatric populations of *Astyanax altiparanae* from upper Paraná River Basin revealed several overlaps among measurements in individuals at distinct sites but significant differences were observed regarding the caudal peduncle height (Domingues et al., 2007). These data suggest that such morphometric character could be a valuable parameter in distinguishing, morphologically, populations in these fish groups.

The apparent lack of a remarkable morphological differentiation as observed in the samples of *A. aff. bimaculatus* studied could indicate that populations, mainly within the same basin, are connected. Nonetheless, it should be recalled that morphological variations within several fish species become detectable when considered over large geographical distances (e.g., Molina et al., 2006) or in the presence of highly effective geographic barriers. For instance, Paiva et al. (2006) analyzed meristic characters in populations of *A. bimaculatus* from the Doce River Basin and found significant differences based on F statistics (P < 0.05) between samples from the Santana and Casca rivers, currently separated by Grande Falls. This wa-

<table>
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<th>Character</th>
<th>Site A</th>
<th>Site B</th>
<th>Site C</th>
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<tr>
<td>Total length (mm)</td>
<td>74.90</td>
<td>69.47</td>
<td>69.46</td>
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<tr>
<td>Head length (mm)</td>
<td>18.20</td>
<td>17.44</td>
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<td>29.89</td>
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<td>27.94</td>
</tr>
<tr>
<td>Percentage of total length (%)</td>
<td>24.36</td>
<td>25.15</td>
<td>25.78</td>
</tr>
<tr>
<td>Body height</td>
<td>39.81</td>
<td>40.39</td>
<td>40.25</td>
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<tr>
<td>Caudal peduncle height</td>
<td>12.38</td>
<td>12.58</td>
<td>12.44</td>
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<tr>
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<td>53.02</td>
<td>52.67</td>
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<td>Preanal length</td>
<td>67.95</td>
<td>66.31</td>
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<td>Dorsal-anal fin distance</td>
<td>43.80</td>
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<tr>
<td>Percentage of head length (%)</td>
<td>31.51</td>
<td>31.71</td>
<td>31.15</td>
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<tr>
<td>Interorbital width</td>
<td>41.94</td>
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<tr>
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<td>97.96</td>
<td>103.23</td>
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<tr>
<td>Percentage of body height (%)</td>
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<td>31.18</td>
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<th>Variance components</th>
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<tr>
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<td>2</td>
<td>30.365</td>
<td>1.03302</td>
<td>18.69</td>
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<tr>
<td>Within populations</td>
<td>72</td>
<td>4.495</td>
<td>4.49464</td>
<td>81.31</td>
</tr>
<tr>
<td>Total</td>
<td>74</td>
<td>5.192</td>
<td>5.52766</td>
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|$F_{ST} = 0.1868$
A multi-approach analysis of the genetic diversity of *Astyanax aff. bimaculatus*

Fig. 3. Giemsa-stained karyotypes of *Astyanax aff. bimaculatus* (2n = 50, FN = 96) from sites A (a), B (b) and C (c). In (d), a somatic metaphase after silver nitrate staining in a specimen from Contas River, showing four positive signals (arrows). The bar equals 5µm.

The waterfall is the largest (15m) within the sample range and could represent an effective barrier between populations by affecting the survival of eggs and larvae flowing downstream. In some cases, even genetically distinguishable populations can keep their morphological resemblance, as corroborated by the absence of visible morphological differences in individuals of *Astyanax fasciatus* from the Mogi-Guaçu River bearing distinct cytotypes (Pazza et al., 2007).

On the other hand, some morphometric studies in other *Astyanax* representatives can reveal a high degree of differentiation, such as the one observed in samples of *A. fasciatus* collected at different sites along Recôncavo Sul and Contas River basins, suggesting that this species could have a higher phenotypic plasticity than *A. aff. bimaculatus*. Furthermore, in the same report, the morphological data were supported by chromosomal differences (Medrado et al., 2008).

In the present work, the karyotypes of *A. aff. bimaculatus* populations were identical and the diploid number found (2n=50) is the same as described elsewhere for this species group. Actually, cytogenetic studies in several populations of *A. bimaculatus* and closely related species (e.g. *Astyanax altiparanae*) have also shown a chromosomal homogeneity, regarding both diploid number and karyotypic formula, when compared to other *Astyanax* species (Morelli et al., 1983; Fernandes & Martins-Santos, 2004; Fernandes & Martins-Santos, 2006; Domingues et al., 2007).

According to Oliveira et al. (2007), migratory rate and population density can influence the karyotypic macrostructure of a species. That is, the greater the mobility and number of individuals of a group, the more stable the karyotypic macrostructure will be, since gene flow would be higher and the fixation of chromosomal rearrangements hindered. Following
this trend, representatives of the “bimaculatus-group” have been referred to as migratory and generalist species (Esteves & Galetti, 1995) and most of the studied populations usually maintain conserved macrokaryotypic features within the same hydrographic basin (Morelli et al., 1983; Paganelli, 1990 among others).

Nonetheless, the present data reveal that populations from the state of Bahia have a differentiated karyotypic formula in relation to other Brazilian hydrographic basins, indicating a high structural chromosomal diversity in the Astyanax group comprising the yellow-tailed characins. These results coupled with the available data in the literature (e.g., high chromosomal diversity, presence of distinct cytotypes and few cases of natural hybrid forms) can putatively indicate inter-specific differences, since Astyanax commonly comprises species complexes (see Pazza & Kavalco, 2007 for a review).

Furthermore, banding techniques, such as Ag-NOR staining, could eventually indicate a higher degree of differentiation among apparent homogeneous populations (Kantek et al., 2007). In fact, the number and location of active NORs can be a useful tool to discriminate fish species and/or populations with similar karyotypes, as observed in A. altiparancae (Pacheco et al., 2001). The present results reinforce the polymorphic nature of the major ribosomal sites and the structural chromosomal variation usually detected in Astyanax species, since the number of Ag-NORs ranged from one to four signals. However, no cytogenetic populational marker was evident by analyzing the number and/or location of active NORs in this study, since they varied both within and among the populations of A. aff. bimaculatus studied. Moreover, it should be pointed out that the present cytogenetic results were based on conventional analyses. Additional studies using distinct and refined chromosomal markers could eventually reveal inter-population differences within each sample, as observed in several other studies within Astyanax (Fernandes & Martins-Santos, 2006; Kantek et al., 2007 among others).

In spite of the high morphological and cytogenetic resemblance among the A. aff. bimaculatus populations studied, our molecular data revealed a significant populational structure ($F_{ST} = 0.1868$). Curiously, a higher divergence level was observed between populations within Contas River Basin and isolated by the dam than between basins (Contas and Recôncavo Sul) (Table 5).

The levels of genetic differentiation among populations are supposed to reflect either their period or degree of isolation (Hartl & Clark, 1989). Although recently constructed (nearly 40 years ago) (CRA, 2002; MMA, 2006a, b), Pedra Dam has been responsible for profound scenario changes (a former narrow fast-flowing river free from relevant physical barriers turned into an impounded reservoir) and already seems to represent an effective barrier to gene flow.

In the case of Neotropical fish, the few reports addressing the relationship between genetic structure of populations separated by artificial barriers (dams) and consequent differences in environmental features have found similar results. Hatanaka & Galetti (2003), using RAPD markers in populations of Prochilodus marggravii in the São Francisco River Basin, revealed that the fish collected close to Três Marias Dam have a higher similarity coefficient than those from other downstream sites far from the dam. Additionally, significant differences in the band frequencies were observed among localities. According to the authors, both findings suggest the presence of structured populations in distinct natural P. marggravii stocks. The same scenario was observed in populations of another Neotropical fish species, Brycon lundii, where different allele frequencies were fixed upstream and downstream of Três Marias Dam (Wasko & Galetti, 2002). Furthermore, the waters impounded after the construction of dams and reservoirs often provide a secondary contact between populations previously isolated (totally or partially) by natural obstacles. For instance, studies of genetic differentiation based on RAPD markers and coloration pattern in fish of the genus Steindachnerina from the upper Paraná River Basin indicated the occurrence of two different species. Spotted specimens were identified as Steindachnerina brevipinna, formerly found downstream of Sete Quedas Falls, suggesting that this species must have overcome the geographical barrier after the building of Itaipu Dam, which submerged the waterfalls - a former obstacle between upper and middle Paraná River basins (Oliveira et al., 2002). Similar evidence has also been reported in other fish species along this floodplain, such as Hemisorubim platyrynchus (Prioli et al., 2004).

Apart from human-imposed environmental changes, particular biotic and abiotic features can also play a key role in the divergence pattern observed among populations. Environmental factors influence phenotypes both directly and indirectly, via trait correlations and interactions with other environmental variables, as demonstrated in several fish species (Langerhans et al., 2007). Analogously, they are also supposed to determine the genetic pattern of natural populations as well. A significant genetic differentiation among sites and no relationship between genetic differences and geographical distances were detected by RAPD markers in populations of the African cyprinid Barbus neumayeri. These data suggest that population structure is more related to habitat-specific selection pressures (different water flow and hypoxia levels per site) on dispersers, rather than insufficient dispersal (Chapman et al., 1999).

As a matter of fact, recent colonization and adaptation to new habits have been proved to affect the genetic structure of migratory fish and to result in fast reproductive isolation among populations. Genetic evidences for the salmonid fish Oncorhynchus nerka consistently showed that nearby populations introduced into divergent environments evolved to reproductively isolated “ecotypes” after fewer than 13 generations (Hendry et al., 2000). Considering that Astyanax species show remarkable ability to adapt to different habitats and a short life cycle (Garuti, 1989; Orsi et al., 2004), their populations may putatively change even faster.

If environmental features, whether natural or artificially imposed, are able to affect the genetic structure among populations of a species, the diversity pattern observed by molecular
markers in the populations of *A. aff. bimaculatus* studied here are likely to reflect habitat peculiarities of each collection site. Actually, the first sampled area (site A) is located 70 km upstream of Pedra Dam, at the upper part of the reservoir in the Contas River. It represents a lotic and unpolluted ecosystem, surrounded by typical caatinga vegetation. Large specimens were easily collected at this site which usually indicates the occurrence of more suitable conditions for the development of local populations (Orsi *et al.*, 2004). At collection site B (downstream of the dam, in Contas River), the natural environment is highly damaged by the daily oscillation in the water flow and by both domestic and industrial sewage from the city of Jequié (CRA, 2002). The third collection site (C), located in the Recôncavo Sul Basin, represents a small stream located in the Atlantic rainforest zone (see Fig. 2).

On the other hand, while particular environmental features may explain the differences observed among populations, especially within the same basin (sites A and B), historic facts can provide some insight about the close relationship between populations from distinct basins (Contas and Recôncavo Sul) (Table 5). Mineiro stream is located near Contas River (about 60 km apart), being separated by a small hill system with a putative common evolutionary history. Hypothetically, the occurrence of some past geological events in the area studied, such as headwater capture, could lead to genetic similarity between populations from currently separated (although adjacent) river basins. In fact, recent studies on biogeography in freshwater fishes along Brazilian coastal basins support the idea of an ancient connectivity among rivers from nearby areas (Pazza & Kavalco, 2007). For instance, connectivity and divergence patterns in fish assemblages along southeastern drainages seem to be related to past tectonic movements (Ribeiro, 2006; Ribeiro *et al.*, 2006). Moreover, a database of the quaternary faults and tectonic behavior over the last 1.6 millions of years along the Brazilian territory indicate that several geological events have taken place throughout the northeastern coast (Saadi *et al.*, 2002). Unfortunately, the hydrographic systems in the state of Bahia and most of the northeastern region still lack specific studies focusing on their biogeographic or geological aspects, and thus, the present suggestions remain speculative.

Finally, despite the population structure detected herein, the intra-population genetic variability was higher (81.31%) than among populations, showing that most of the variation refers to differences among individuals within populations. Similar results have been commonly reported by genetic studies with molecular markers in natural fish populations (Leuzzi *et al.*, 2004; Paiva *et al.*, 2006; Affonso & Galetti, 2007). Other studies on small and isolated populations of domestic animals have also demonstrated such pattern, indicating that, even under inbreeding conditions, the levels of genetic variation within populations is usually higher than that observed among populations of a single species (*e.g.*, Albuquerque *et al.*, 2006). The results of the present work are also important for highlighting the applicability of molecular markers in detecting differences within species characterized by a conservative morphological and/or cytogenetic pattern. Unlike from some other freshwater fish populations (*e.g.*, *Astyanax fasciatus*) where distinct morphological and cytogenetic features have corroborated further molecular analyses (Pazza *et al.*, 2007; Medrado *et al.*, 2008), the populations of *A. aff. bimaculatus* studied here showed low levels of both morphometric and meristic differences and a similar karyotypic structure. Such lack of congruence between molecular and morphology/karyotype data is commonly found in species composed of large and highly-connected populations (intense gene flow), such as marine fish (Galetti *et al.*, 2006; Affonso & Galetti, 2007). In these cases, the utilization of highly sensitive DNA markers is essential to help us understand how fish populations respond to natural selection or environmental changes caused by human activities, providing a useful baseline for the conservation and sustainable management of natural populations.

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