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

Vanessa de Carvalho Cayres Pamponet[1], Paulo Luiz Souza Carneiro[2], Paulo Roberto Antunes de Mello Affonso[2], Viviam Souto Miranda[2], Juvenal Cordeiro Silva Júnior[2], Claudine Gonçalves de Oliveira[1] and Fernanda Amato Gaiotto[1]

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_{st}=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.

Poucos estudos ictiofaunísticos estão disponíveis em rios típicos do semi-árido, apesar da constante ameaça à diversidade local devido a influências antrópicas, com destaque para o represamento e construção de barragens. O presente trabalho teve como objetivo avaliar, por meio de diferentes metodologias, a estrutura genética de populações de uma espécie de caracídeo, *Astyanax* aff. *bimaculatus*, amplamente distribuída em bacias hidrográficas da Bahia, Nordeste do Brasil. Análises morfológicas (dados merísticos e morfométricos), citogenéticas (cariótipo e Ag-RONs) e moleculares (RAPD e SPAR) foram realizadas em espécimes coletados à montante e à jusante da Barragem da Pedra, na calha principal do médio rio de Contas (bacia do Rio de Contas) e no ribeirão Mineiro, pertencente à bacia adjacente do Recôncavo Sul. Poucas diferenças externas foram detectadas entre as populações, sendo os indivíduos originários do reservatório, à montante da barragem, ligeiramente maiores. Os dados citogenéticos também mostraram padrões cariotípicos semelhantes (2n=50; 6m+28sm+12st+4a; FN= 96) e RONs situadas nos braços curtos de até dois pares cromossômicos, com variação numérica inter- e intra-populacional. Contudo, as análises por RAPD e SPAR diferenciaram as três populações de forma eficiente, revelando freqüências alélicas significativamente diferentes entre as localidades amostradas e índices significativos de estruturação populacional (F_{st}=0.1868, P<0.0001). As diferenças entre populações do mesmo rio foram tão significativas quanto entre bacias hidrográficas distintas, indicando que a represa constitui uma barreira eficiente ao fluxo gênico. Além disso, acredita-se que peculiaridades ambientais de cada localidade possam também influenciar os padrões genéticos encontrados. Por outro lado, a similaridade entre amostras das bacias do Rio de Contas e Recôncavo Sul pode estar relacionada a uma história evolutiva comum, já que ambas estão geograficamente próximas. Por fim, o presente estudo demonstra que a realização de estudos envolvendo diferentes marcadores é extremamente útil para a identificação de estruturas de populações em espécies amplamente distribuídas e para avaliação dos impactos das atividades humanas sobre as populações naturais de peixes.

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

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[1]Universidade Estadual de Santa Cruz, Dep. Ciências Biológicas. Rodovia Ilhéus-Itabuna, km 16, 45662-000, Ilhéus, BA, Brazil.
[2]Universidade Estadual do Sudoeste da Bahia, Dep. Ciências Biológicas. Av. José Moreira Sobrinho, s/n., 45206-510, Jequié, BA, Brazil. paulomelloaffonso@yahoo.com.br

Introduction

The Contas River hydrographic basin is entirely located in the state of Bahia, Northeastern Brazil (12º55’ to 15º30’S, 39º00’ to 42º35’W), comprising a drainage area of 64,933 km^2, 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 Jequié (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-populational 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 cytogenetic 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, cytogenetic, 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 (13º52’5”S, 40º14’9”W, 230 m above sea level, site A) and nearly 25 km downstream of Pedra Dam, in the city of Jequié (13º54’84”S, 40º02’54”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 Jequié (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, cytogenetic 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 (á = 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,

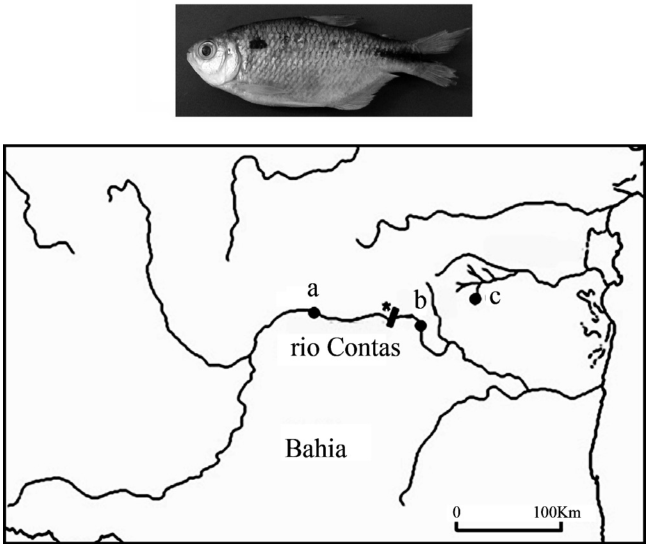

Fig. 1. Map of the studied area in the State of Bahia, Brazil, showing the hydrographic system and collection sites of *Astyanax* aff. *bimaculatus*: (a) site A - Contas River, upstream of Pedra Dam, Porto Alegre County (b) site B - Contas River, downstream of Pedra Dam, city of Jequié (Contas River Basin), (c) site C - Mineiro stream, city of Itamari (Recôncavo Sul Basin) and (*) location of Pedra Dam in Contas River. A specimen of *Astyanax* aff. *bimaculatus* is illustrated in detail (total length = 6.65 cm).

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 converted to body proportions and expressed in percentage in order to indicate the relationship between total length, head length, body height and other morphometric measurements.

Cytogenetic studies. Two males and 3 females of *A.* aff. *bimaculatus* from site A (Contas River, upstream Pedra Dam), 11 males and 8 females from site B (Contas River, downstream of Pedra Dam), and 17 individuals, including 5 males, 11 females and one unidentified, from site C (Mineiro stream) were cytogenetically analyzed (Table 1). Mitotic stimulation was performed on collected specimens 72h prior to cytogenetic procedures, as described by Molina (2001). Metaphasic chromosomes were obtained from kidney cells according to Bertollo *et al.* (1978). The slides were stained with 5% Giemsa for conventional analyses. The nucleolar organizer regions were detected by silver nitrate staining (Ag-NOR) (Howell and Black, 1980). The best metaphases were photographed in Imagelink Kodak^{TM} ISO 25 for chromosomal measurements, karyotyping and NOR analysis. The chromosome pairs were arranged in decreasing size order and classified as metacentric (M), submetacentric (SM), subtelocentric (ST) and acrocentric (A) (Levan *et al.*, 1964).

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 H_2O; 1.3 ml 10x buffer with 1 M $MgCl_2$, 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.*, 2000) was used to perform the analysis of molecular variance (AMOVA) and to estimate the inter-population variation by providing F_{st} 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 (N_1), cytogenetic (N_2), and molecular (N_3) analyses (*Contas River Basin, ** Recôncavo Sul Basin).

Samples (sites)	Locality	N_1	N_2	N_3
Contas River (A)*	Upstream Pedra Dam, Porto Alegre County	30	5	24
Contas River (B)*	Downstream Pedra Dam, Jequié	24	19	25
Mineiro stream (C)**	Mineiro County, Itamari	30	17	24
Total		84	41	73

Fig. 2. Partial view of collection sites of *Astyanax* aff. *bimaculatus* in the State of Bahia, Brazil: (a) Contas River, upstream Pedra Dam, Porto Alegre County – site A, (b) Contas River, downstream Pedra Dam, city of Jequié – site B, and (c) Mineiro stream, Recôncavo Sul Basin, city of Itamari – site C. In (d), view of Pedra Dam reservoir in Middle Contas River, city of Jequié.

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).

Silver nitrate staining revealed an intra- and inter-individual polymorphism in the number of NOR-bearing chromosomes within populations of *A.* aff. *bimaculatus*. Positive signals, ranging from one to four Ag-NOR sites, were located in the telomeric region on short arms of SM/ST chromosome pairs (Fig. 3d) in all populations studied.

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 ($F_{st} = 0.1868$, $P < 0.0001$), 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.

Table 2. Mean and standard deviation values for the number of lateral line scales (LLS), rays in the anal fin (AFR) and rays in the dorsal fin (DFR) of each population of *Astyanax* aff. *bimaculatus* (*Contas River Basin, ** Recôncavo Sul Basin).

Collection sites	LLS	AFR	DFR
Contas River, upstream Pedra Dam (A)*	33.13 ± 0.43	26.63 ± 0.85	11.00 ± 0.00
Contas River, downstream Pedra Dam (B)*	32.61 ± 0.62	26.09 ± 1.10	11.00 ± 0.00
Mineiro stream (C)**	32.49 ± 0.60	25.85 ± 0.83	11.00 ± 0.00

Table 3. Mean morphometric data and body proportions of *Astyanax* aff. *bimaculatus*.

Character	Site A	Site B	Site C
Total length (mm)	74.90	69.47	69.46
Head length (mm)	18.20	17.44	17.89
Body height (mm)	29.89	28.02	27.94
Percentage of total length (%)			
Head length	24.36	25.15	25.78
Body height	39.81	40.39	40.25
Caudal peduncle height	12.38	12.58	12.44
Predorsal length	52.42	53.02	52.67
Preanal length	67.95	66.31	66.40
Dorsal-pectoral fin distance	43.80	43.65	42.89
Dorsal-anal fin distance	41.92	42.14	41.83
Anal-adipose fin distance	37.49	37.69	37.38
Dorsal-adipose fin distance	38.29	38.30	39.54
Percentage of head length (%)			
Ocular diameter	31.51	31.71	31.15
Interorbital width	41.94	39.48	38.80
Head height	97.96	103.23	104.5
Percentage of body height (%)			
Caudal peduncle height	31.20	31.18	44.48

Table 4. Analysis of molecular variance for the three populations of *Astyanax* aff. *bimaculatus* studied (all values showed statistically significant differences, P<0.0001).

Source of variation	DF	Mean square	Variance components	Variation rate (%)
Among populations	2	30.365	1.03302	18.69
Within populations	72	4.495	4.49464	81.31
Total	74	5.192	5.52766	100.0
F_{ST} = 0.1868				

Table 5. Percentage of genetic variability in pairwise comparisons of *Astyanax* aff. *bimaculatus* populations, as estimated by analysis of molecular variance (all values showed statistically significant differences, P<0.0001).

Collection sites	A	B
A (Contas River, upstream Pedra Dam)	-	-
B (Contas River. Downstream Pedra Dam)	22.02	-
C (Mineiro stream)	13.03	20.28

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-

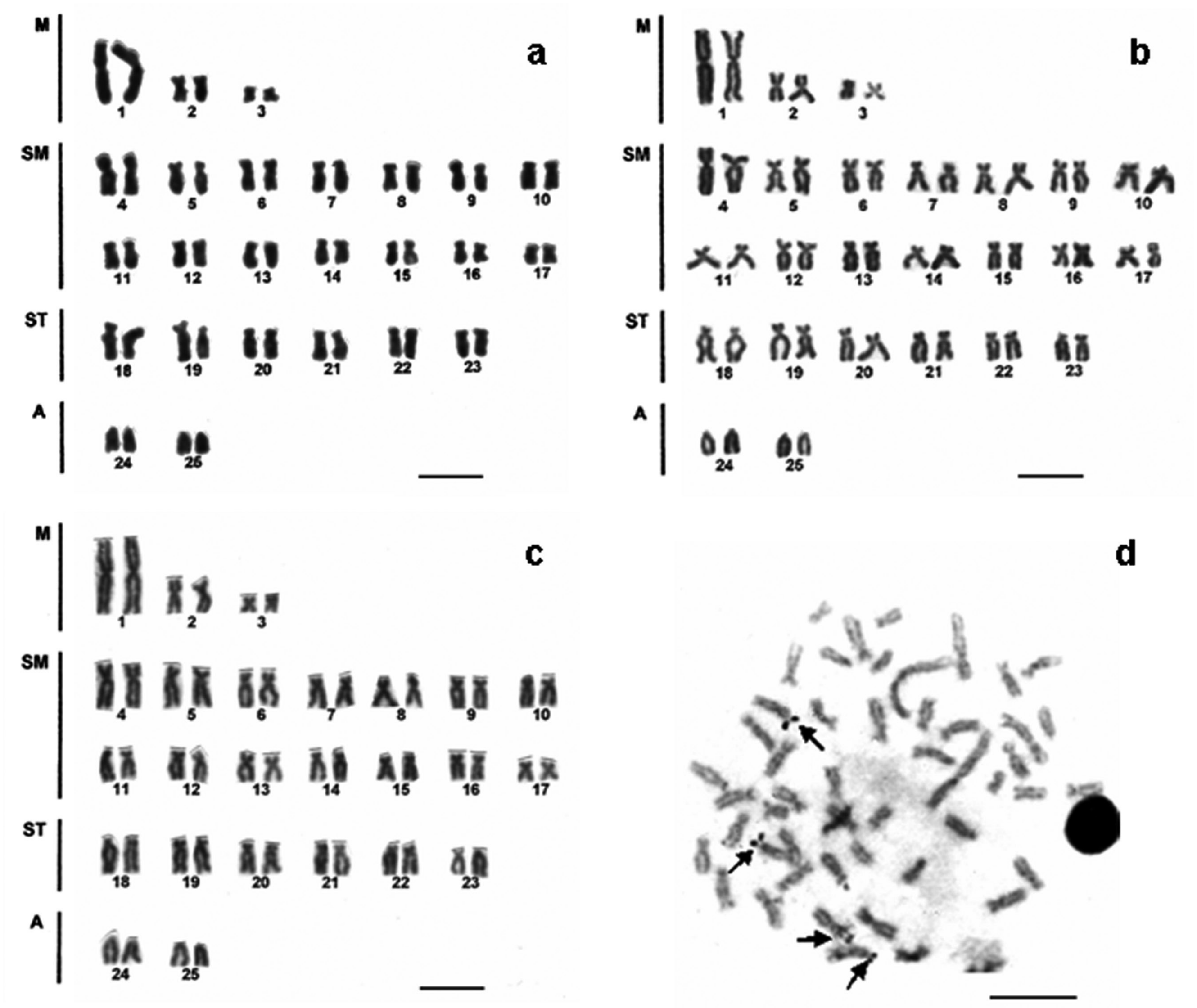

Fig. 3. Giemsa-stained karyotypes of *Astyanax* aff. *bimaculatus* (2n = 50, FN = 96) from sites A (a), B (b) and C (c). In (d), a somatic metaphase after silver nitrate staining in a specimen from Contas River, showing four positive signals (arrows). The bar equals 5μm.

terfall is the largest (15m) within the sample range and could represent a 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. altiparanae* (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 platyrhynchos* (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 (Garutti, 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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