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Biogeographic patterns in the chromosomal distribution of a satellite DNA in the banded tetra *Astyanax fasciatus* (Teleostei: Characiformes)

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Abstract The As-51 satellite DNA is a transposon-like sequence formerly described for arthropods and physically identifiable by fluorescent in situ hybridization. In the present work, we describe the occurrence of this sequence, as well the C-banding and karyotype composition, in populations of the group *Astyanax fasciatus* from Mogi-Guaçu (Araras-SP), Paranapanema (Angatuba and Pilar do Sul-SP), Ribeira de Iguape (Sete Barras-SP) and Tietê (Indaiatuba and Salesópolis-SP) river basins. The specimens from Sete Barras (10 M + 20SM + 12ST + 6A) and Araras (8 M + 22SM + 12ST + 6A) have 2n=48 chromosomes. The samples from Angatuba, Pilar do Sul and Indaiatuba presented 2n=46 chromosomes (12 M + 20SM + 10ST + 4A). The individuals collected in Salesópolis showed three cytotypes, bearing 2n=46 (12 M + 20SM + 10ST + 4A), 2n=48 (8 M + 22SM + 12ST + 6A) and 2n=50 (8 M + 16SM + 14ST + 12A). C-banding revealed large heterochromatic blocks at terminal chromosomal regions in all populations and/or cytotypes. All analyzed populations have conspicuous blocks carrying the As-51 satellite DNA, although the number of chromosomes bearing this repetitive sequence was variable among them. Such differences were not related to the diploid number of individuals, but rather to a biogeographic pattern. Aspects of the karyotype evolution and distribution of this sequence in distinct populations are discussed.

Keywords *Astyanax fasciatus* · Molecular cytogenetics · Karyotypic evolution · satDNA As-51

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Introduction

Interesting biogeographic features are observed in the freshwater fish fauna along the Brazilian crystalline shield and coastal drainages. The diversity patterns reported suggest that cladogenic events between both structures are ongoing over long stretches of time, and are, in a sense, repetitive, giving rise to similar sister-group phylogenetic patterns, which occurred at different stages in time (Ribeiro 2006).

The rivers encompassing the coastal drainage flow exclusively through the Brazilian territory, ranging from the mouth of the São Francisco River up to Itajaí River (Paiva 1982). Three main hydrographic systems are identified along the coastal region: Paraíba do Sul River, Ribeira de Iguape River and a group of independent Atlantic drainages, also called coastal rivers. This region is known by its high level of endemism, estimated at 95 % (Bizerril 1994). The natural history of the fish fauna in Brazilian coastal drainages is related to the regional geomorphology, mainly influenced by the presence of Serra do Mar Hills—a cliffy mountain range that parallels about 1,000 km of the coast of southern and southeastern Brazil, between the State of Rio de Janeiro and northern Santa Catarina (Almeida and Carneiro 1998). In the State of Paraná, it forms a high mountain chain with ridges of up to 1,800 m in height and, in São Paulo, it comprises a typical plateau border, frequently leveled by heights between 800 and 1,200 m (Almeida and Carneiro 1998). Such a

geological formation is an important divisor between the coastal drainage and inland rivers, such as those from the Tietê, Grande and Paranapanema basins, which, in turn, will give rise to the Paraná River system. Some portions of the Serra do Mar Hills can also separate inland sub-basins, such as the Paranapiacaba Hills, that split the drainages of Ribeira de Iguape and Paranapanema rivers (Almeida and Carneiro 1998).

The frequency of the shared fish fauna between coastal rivers and Paraná and São Francisco rivers is thought to be equal to 17 % and 11 % respectively (Bizerril 1994). However, several species actually comprise 'species complexes' composed of a group of synonymous or cryptic representatives, such as *Astyanax scabripinnis* (Moreira-Filho and Bertollo 1991), *A. altiparanae* (Fernandes and Martins-Santos 2004) and *A. fasciatus* (Pazza et al. 2006), which likely reflects such a biogeographic pattern. Moreover, at least one well-documented case of faunal mixing has been characterized along the region caused by the transposition of a river that used to flow to Grande River (Upper Paraná) and which now flows towards the São Francisco River basin (Moreira-Filho and Buckup 2005).

Astyanax fasciatus has been reported to be an inhabitant of Brazilian rivers. Cytogenetic analyses have indicated that the specimens from Upper Paraná River basin might comprise a more diversified fish group, but which are classified in the same taxon (Pazza et al. 2006). The goal of the present work was to evaluate the chromosomal data regarding the heterochromatin distribution and the As-51 satellite DNA location in species of the *A. fasciatus* complex, and to compare them with the karyo-evolutionary trends observed previously within the genus in order to reveal possible biogeographic relationships.

Materials and methods

Specimens of *A. fasciatus* from four distinct basins in southeastern Brazil were analyzed: 8 from the Mogi-Guaçu River (nearby Araras-SP: S 22 °22.994'/W 47 °25.825'); 14 from the Paranapanema River—12 from Angatuba (S 23 °32.984'/W 48 °30.743') and 2 from Pilar do Sul-SP (S 23 °48.755'/W 47 °42,527'); 9 from the Ribeira de Iguape River (Sete Barras-SP: S 24 °18.678'/W 47 °53.528'); 49 from the Tietê River—47 individuals from Salesópolis-SP (S 23 °31.404'/W 45 ° 51.716') and 2 from Indaiatuba-SP (S 23 °5.652'/W 47 ° 15.636'). The specimens were deposited in the fish collection of the Museum of Federal University of Rio Grande do Sul/Brazil.

Mitotic chromosomes were obtained according to Gold et al. (1990) and C-banding performed according to the procedure of Sumner (1972). Fluorescent in situ hybridization (FISH) (Pazza et al. 2006; Pinkel et al. 1986) was carried out in at least two specimens from each cytotype using a satellite DNA probe, As-51, formerly isolated from a population of *A. scabripinnis* (Mestriner et al. 2000). The FISH experiments were performed in all slides simultaneously in order to avoid artifacts.

The preparations were analyzed under an optical microscope and images captured (5 megapixel resolution) with CoolSnap Pro and Image Pro Plus (Media Cybernetics, Bethesda, MD, USA) image-analysis software. The chromosomes were classified into four types according to arm ratio (AR), as proposed by Levan et al. (1964): M—metacentric (AR=1.00–1.70); SM—submetacentric (AR=1.71–3.00); ST—subtelocentric (AR=3.01–7.00); A–acrocentric (AR = >7.00).

Results

Different diploid numbers and distinguishable karyotype formulae were identified in individuals throughout the distinct hydrographic basins sampled (Fig. 1). The results and available reports are summarized in Table 1. No karyotypical differences related to sex were observed in the specimens analyzed.

The specimens from Sete Barras presented 2n=48 chromosomes (10 M + 20SM + 12ST + 6A) (Fig. 2a). The population from Salesópolis was characterized by three cytotypes bearing 2n=46 (12 M + 20SM + 10ST + 4A) (Fig. 2c), 2n=48 (8 M + 22SM + 12ST + 6A) (Fig. 2e) or 2n=50 chromosomes (8 M + 16SM + 14ST + 12A) (Fig. 2g). On one hand, the specimens from Indaiatuba, Pilar do Sul and Angatuba presented 2n=46 chromosomes and a similar karyotype formula (12 M + 20SM + 10ST + 4A) (Fig. 3a, c, e respectively). On the other hand, the individuals from Araras showed 2n=48 chromosomes (8 M + 23SM + 12ST + 6A) (Fig. 3g). In all populations, the karyotypes presented conspicuous heterochromatin blocks, with the exception of the samples from Sete Barras/SP, whose heterochromatin segments were located preferentially at pericentromeric regions of chromosomes (Fig. 4). An evaluation of the C-banding location in both the Angatuba and Araras populations was performed previously and discussed by Pazza et al. (2008b).

The satDNA As-51 was present in all populations studied (Figs. 2 and 3), but differences in the number of chromosomes bearing this repetitive DNA sequence were observed. Such differences were not related to the diploid number of the individuals; instead, they were closely related to the basins where the specimens were collected. The population from Sete Barras presented homologies with the As-51 probe on a single chromosomal pair, showing positive signals at the interstitial region in a pair of acrocentric chromosomes (Fig. 2b). The cytotypes from Salesópolis bearing 2n=46 and 48 presented 2 pairs of acrocentric chromosomes carrying such satellite DNA (Fig. 2d, f). On one hand, the

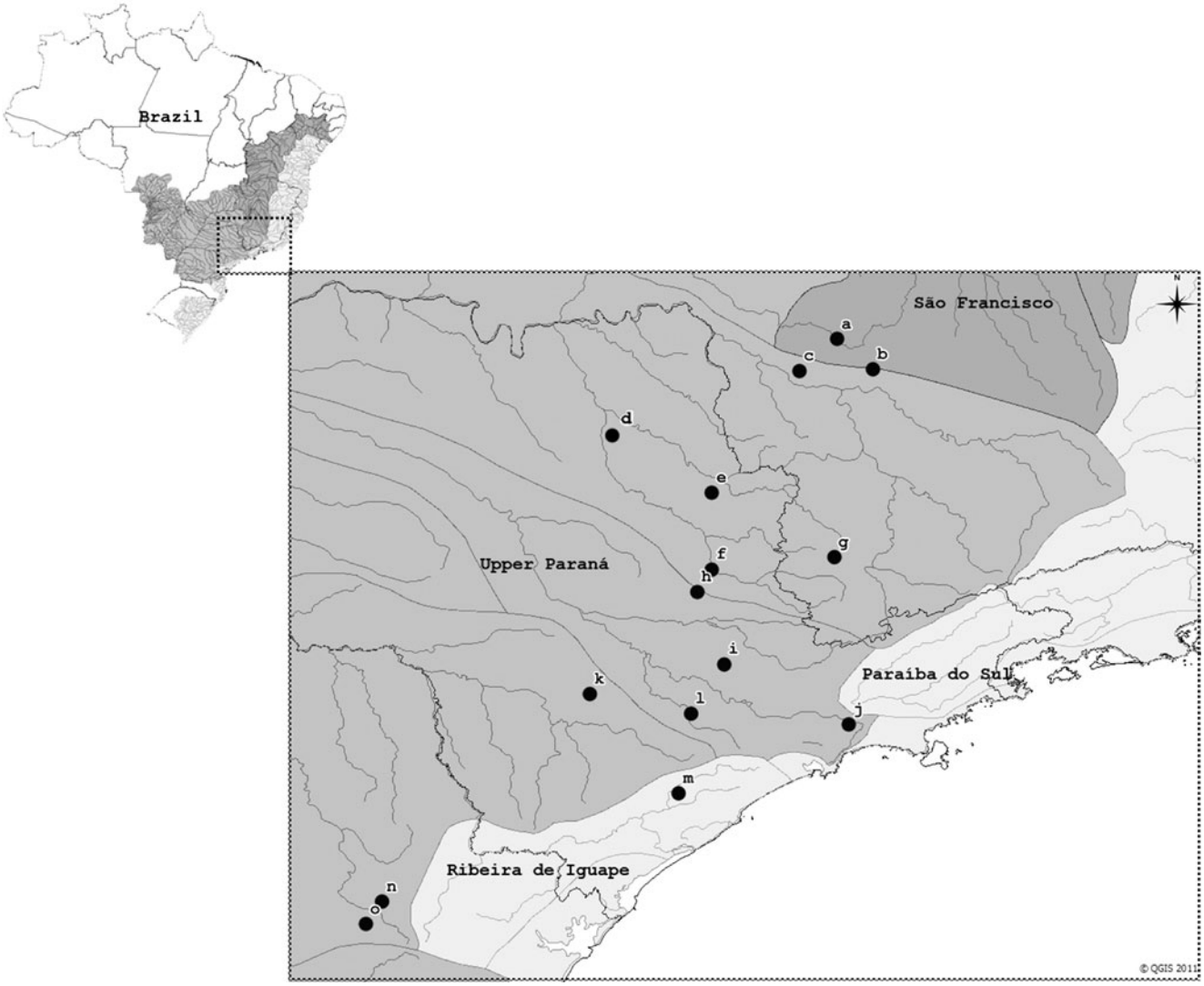

Fig. 1 Map showing southeastern Brazil with the major hydrographic systems. The letters indicate the samples' location from the literature **a**, **b**, **c**, **d**, **e**, **g**, **h**, **n**, **o**) and studied in the present work **f**, **i**, **j**, **k**, **l**, **m** (see Table 1)

cytoype with 2n=50 chromosomes presented an additional positively-marked ST pair, besides a size heteromorphism in the site located on the twentieth (acrocentric) pair (Fig. 2h). On the other hand, the specimens from Indaiatuba presented ten chromosomes bearing positive signals for the As-51 satellite DNA probe, including a ST chromosome harboring two syntenic sites (Fig. 3b). The populations from Angatuba and Pilar do Sul presented 14 and 17 chromosomes bearing As-51 satellite DNA respectively (Fig. 3f, d). The highest number of As-51 sites was detected in the sample from Araras, comprising 18 marks (Fig. 3h).

Discussion

Several examples of chromosomal variation in natural populations have been reported in neotropical fishes, where centric fusions/fissions, inversions and occurrence of supernumerary chromosomes seem to be the most frequent events, followed by some cases of polyploidy.

The first cytogenetic analyses in *A. fasciatus* were carried out in the 1970s (Jin and Toledo 1975), and this group has been intensively studied ever since. The *A. fasciatus* species complex is considered one of the most diversified groups within the genus *Astyanax*, and remarkable variation in the diploid number, ranging from 2n=45 to 2n=50 chromosomes, is well known (Gross et al. 2004; Jin and Toledo 1975; Morelli et al. 1983; Pazza et al. 2006). Even amongst cytotypes bearing the same chromosome number, there may be differences in the karyotype formulae and/or occurrence of odd chromosomes lacking similar-shaped homologues (Pazza et al. 2006). Cases of natural triploidy have been reported (Gross et al. 2004).

In order to increase the information about polymorphism in *A. fasciatus*, the present study analyzed the standard

Table 1 Satellite DNA As-51 in the *Astyanax* genus from Brazil

Species	Map	Location	2n	Markers	Hydrographic system	Remarks	Ref
A. altiparanae	–	Salesópolis (SP)	50	2 ST	Upper Tietê	(8 M + 20SM + 10ST + 12A)	Kavalco et al. (2011)
	–	Pilar do Sul (SP)	50	5 M/SM/A	Paranapanema	(8 M + 22SM + 14ST + 6A)	
A. aff. bimaculatus	–	Sete Barras (SP)	50	0	Ribeira de Iguape	(8 M + 24SM + 12ST + 6A)	
	–	Cachoeiras de Macacu (RJ)	50	2 ST	Guapimirim	(8 M + 24SM + 12ST + 6A)	
A. bockmanni	–	São Miguel Arcanjo (SP)	50	0	Paranapanema	(10 M + 12SM + 12ST + 16A)	Kavalco et al. (2009b)
	–	Pilar do Sul (SP)	50	0	Paranapanema	(10 M + 12SM + 12ST + 16A)	
A. aff. fasciatus	h	Piracicaba river—Piracicaba (SP)	46	14 ST/A	Upper Paraná	(12 M + 20SM + 10ST + 4A)	Abel et al. (2006)
	e	Mogi-Guaçu river—Cachoeira de Emas (SP)	45 46 47 48	±16 ST/A	Upper Paraná	2n=48 (8 M + 22SM + 12ST + 6A), 2n=46 (12 M + 20SM + 10ST + 4A), 2n=45, 2n=46 variant and four cytotypes with 2n=47	Pazza et al. (2008a)
	g	Mogi-Guaçu river—Ouro Fino (MG)	46 48	±16 ST/A	Upper Paraná	2n=48 (8 M + 22SM + 12ST + 6A), 2n=46 (12 M + 20SM + 10ST + 4A)	
	d	Pardo river (SP)	46 48	±16 ST/A	Upper Paraná	2n=48 (8 M + 22SM + 12ST + 6A), 2n=46 (12 M + 20SM + 10ST + 4A)	
	c	Grande river—Furnas Dam (MG)	48	8 to 14 ST/A	Upper Paraná	(8 M + 20SM + 16ST + 4A)	Peres et al. (2009)
	a	Minhoca Stream—Piumhi river (MG)	48	8 to 14 ST/A	São Francisco	(8 M + 20SM + 16ST + 4A)	
	a	São Francisco river—Piumhi downstream (MGS)	48	8 to 14 ST/A	São Francisco	(8 M + 20SM + 16ST + 4A)	
	b	Araras Stream (MG)	48	2 ST/A	São Francisco	(8 M + 20SM + 16ST + 4A)	
	b	Pimenta Stream (MG)	48	2 ST/A	São Francisco	(8 M + 20SM + 16ST + 4A)	
	f	Mogi-Guaçu river—Araras (SP)	46	18 ST/A	Upper Paraná	(8 M + 22SM + 12ST + 6A)	P
	k	Angatuba (SP)	46	16 ST/A	Upper Paraná	(12 M + 20SM + 10ST + 4A)	
	l	Pilar do Sul (SP)	46	17 ST/A	Upper Paraná	(12 M + 20SM + 10ST + 4A)	
	m	Sete Barras (SP)	48	2 ST/A	Ribeira de Iguape	(10 M + 20SM + 12ST + 6A)	
	j	Salesópolis (SP)	46 48 50	4 to 5 ST/A	Upper Paraná	2n=46 (12 M + 20SM + 10ST + 4A), 2n=48 (8 M + 22SM + 12ST + 6A), 2n=50 (8 M + 16SM + 14ST + 12A) with an heremorphic pair	
	i	Indaiatuba (SP)	46	10 ST/A	Upper Paraná	(12 M + 20SM + 10ST + 4A) One chromosome bearing two syntenic sites	
	b	das Araras river—Piumhi (MG)	48	1 A	São Francisco	(8 M + 20SM + 16ST + 4A)	Kantek et al. (2009)
	a	Piumhi river—Piumhi (MG)	48	3 ST	São Francisco	(8 M + 20SM + 16ST + 4A)	
	c	Furnas reservoir—Capitólio (MG)	48	13 SM/ST/A	Upper Paraná	(8 M + 20SM + 16ST + 4A)	
	n	Cara-Cará river—Ponta Grossa	48	48 14 ST/A	Upper Paraná	(8 M + 18SM + 14ST + 10A)	
		(PR)	50	10 SM/ST/A			
	o	Tibagi river—Ponta Grossa (PR)	50	9ST/A	Upper Paraná	(8 M + 18SM + 14ST + 10A)	
A. giton	–	Jacuí Stream and Paraitinga river—Cunha (SP)	50	0	Paraíba do Sul	(6 M + 8SM + 8ST + 28A)	
A. hastatus	–	Distrito Ypiranga (RJ)	50	0	Coastal rivers	(4 M + 8SM + 10ST + 28A)	Kavalco et al. (2007)
A. hastatus A. intermedius	–	Santana do Japuíba (RJ)	50	0	Coastal rivers	(8 M + 10SM + 14ST + 18A)	Kavalco et al. (2009a)
	–	Cachoeiras de Macacu (RJ)	50	0	Coastal rivers	(6 M + 8SM + 4ST + 32A)	
	–	Paraitinga river—Cunha (SP)	50	0	Paraíba do Sul	(6 M + 8SM + 4ST + 32A)	
A. janeiroensis	–	Castro and São Luiz do Purunã (PR)	50	13 to 14 ST/A	Ribeira de Iguape	(6 M + 14SM + 14ST + 16A)	Kavalco et al. (2007)
A. mexicanus	–	Aquaculture—MX[a]	50	±4 ST/A	Central America	(8 M + 18SM + 12ST + 12A) Subtle marks	Vicari et al. (2008) and Kantek et al. (2009)

Table 1 (continued)

Species	Map	Location	2n	Markers	Hydrographic system	Remarks	Ref
A. parahybae	–	Paraitinga river—Cunha (SP)	48	4 ST/A	Paraíba do Sul	(8 M + 18SM + 12ST + 10A) Sporadic extra pair with interstitial marking	Kavalco and Almeida-Toledo (2007)
A. paranae	–	Quebra Perna Stream, Ponta Grossa (PR)	50	13	Upper Paraná	(8 M + 18SM + 10ST + 4A)	Kavalco et al. (2007)
A. ribeirae	–	Iporanga, Registro e Sete Barras (SP)	50	0	Ribeira de Iguape	4 M + 10SM + 6ST + 30A	Kantek et al. (2009)
A. scabripinnis	–	Centenário Stream—Maringá (PR)	50	+16 ST/A	Paranapanema	(6 M + 20SM + 8ST + 16A) Intra-individual variation	Kavalco et al. (2010)
A. scabripinnis							
A. serratus[b]	–	Marrecas Stream—Londrina (PR)	48	+16 ST/A	Paranapanema	(8 M + 20SM + 12ST + 10A) Intra-individual variation	Mantovani et al. (2004) and Abel et al. (2006)
	–	Viveiro de Mudas Stream—Três Marias (MG)	50	+2 ST/A	São Francisco	(6 M + 30SM + 8ST + 6A) Extra subtle marks on chromosomes	
	–	Curral das Éguas Stream—São Gonçalo do Abaeté (MG)	46	0	São Francisco	(6 M + 22SM + 8ST + 14A)	Abel et al. (2006)
	–	Macacos Stream—Cunha (SP)	50	6 ST/A	Paraíba do Sul	(8 M + 20SM + 8ST + 14A)	
	–	Santo Antonio Stream—Jaguariúva (PR)	48	10 ST/A	Paranapamena	(10 M + 16SM + 10ST + 12A)	Kavalco et al. (2007)
	–	Bicurdo river—Balsa Nova (PR)	50	16 SM/ST/A	Iguaçu	(4 M + 24SM + 6ST + 16A)	Kantek et al. (2009)

[a] Individuals from pet shop, imported from Mexico

[b] Cited originally as *Astyanax* sp. D

SP São Paulo State, *RJ* Rio de Janeiro State, *MG* Minas Gerais State, *PR* Paraná State, *MX* Mexico, *ST* subtelocentric, *M* metacentric, *SM* submetacentric, *A* acrocentric chromosomes, *P* present data

katyotypes with 2n=46 and 2n=48 chromosomes along Tietê (2n=46), Paranapanema (2n=46) and Mogi-Guaçu (2n=48) river basins. A new cytotype was identified in specimens from Ribeira de Iguape River (2n=48), as well as a numerical polymorphism within the population from Upper Tietê River. In the latter, aside from the standard forms, specimens with 2n=50 chromosomes were also reported (Fig. 2g, h)—an unusual diploid number for this group.

The C-banding pattern in *Astyanax* is quite variable, including cases of intra-population polymorphism (Mantovani et al. 2000). In *A. fasciatus*, two main patterns of heterochromatin distribution were detected. The first type comprises C-bands at distal chromosomal regions found in populations from Upper Paraná River (Centofante et al. 2003; Pazza et al. 2008a, b), in the populations studied herein (Fig. 4) and in two samples from Piumhi River—MG (Peres et al. 2009). The population from Piumhi River probably derives from an introduced population originally from Grande River after the transposition of this river to the São Francisco River basin in the 1960s (Moreira-Filho and Buckup 2005). The second pattern, verified only in individuals from São Francisco and Ribeira de Iguape rivers, is characterized by C-bands located preferentially at pericentromeric regions of several chromosomes (Justi 1993; Fig. 4), or at the distal region of a single chromosomal pair (Peres et al. 2009). Therefore, the heterochromatin distribution pattern in this group might represent an important chromosomal marker for inter-population studies may potentially indicate that populations from the São Francisco and Paraná rivers should not correspond to a same taxon (Pazza et al. 2008a).

The remarkable variation within the group *A. fasciatus* might be related to intrinsic genomic features, such as the presence of the As-51 satellite DNA, once a variation in this satDNA is observed amongst different hydrographic basins. The repetitive DNA closely resembles a transposable sequence already described for arthropods, and its accumulation on *Astyanax* could constitute an evolutionary trend in these fishes (Mestriner et al. 2000). More specific studies must be carried out to test such a hypothesis.

The distinct populations analyzed in the present work show an interesting panorama regarding the chromosomal location of the As-51 satellite DNA in the cytotypes of *A. fasciatus* and the basins in which they occur. The population from Ribeira de Iguape River basin (Sete Barras), which composes a part of the coastal drainages, carries a single chromosomal pair bearing such repetitive DNA (Fig. 2b). The cytotypes from the headwaters of the Tietê River (Salesópolis), also situated on the eastern portion of the continent, also present few As-51 DNA-bearing chromosomal pairs (Fig. 2d, f, h). These cytotypes are remarkably distinct from those found in inland basins, such as Paranapanema River (Pilar do Sul and Angatuba—Fig. 3d, f), Middle Tietê River (Indaiatuba—Fig. 3b) and Mogi-Guaçu River (Araras—Fig. 3h), in which several sites bearing the satellite DNA were identified by FISH. It is worth mentioning

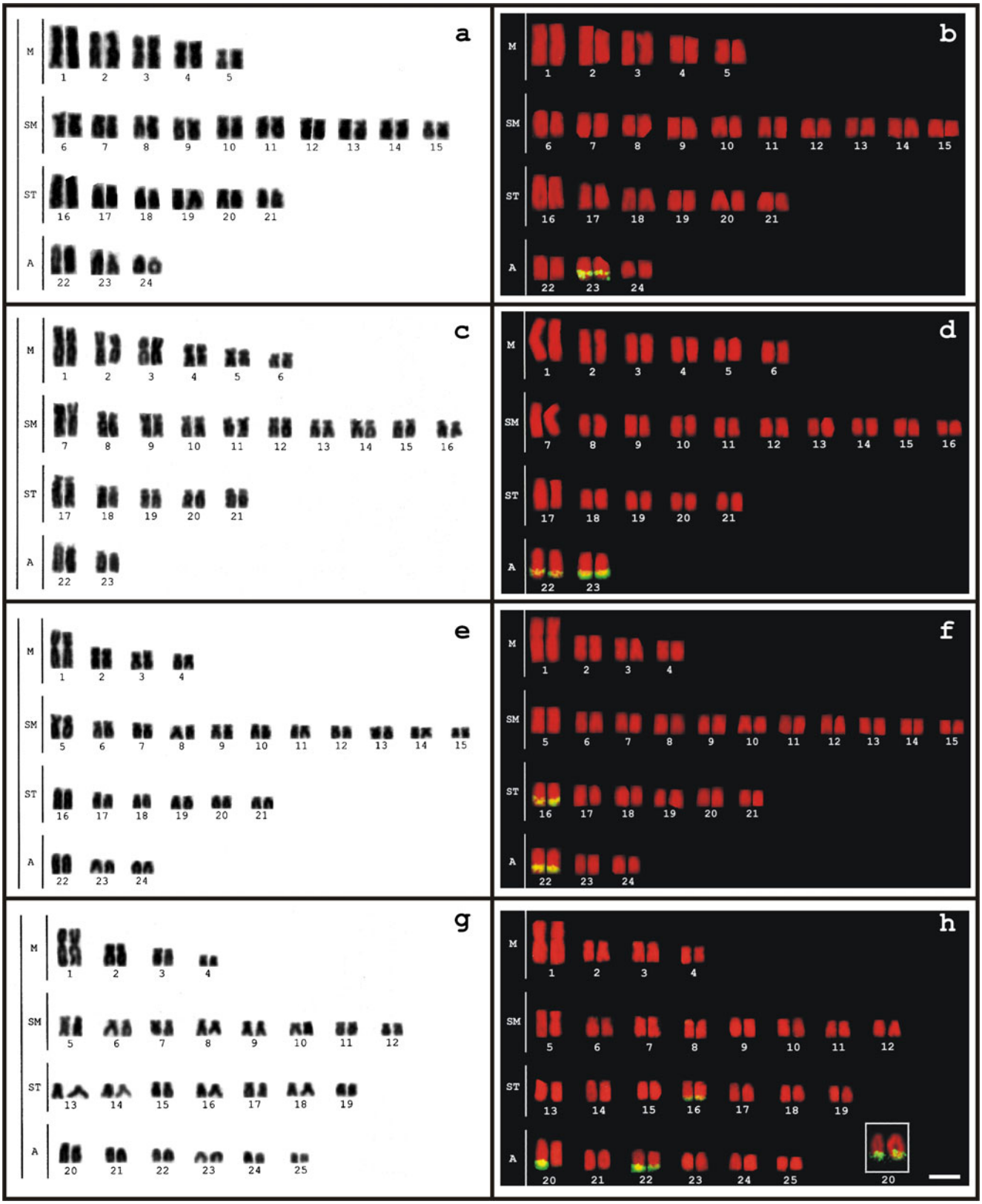

Fig. 2 Karyotypes of *A. fasciatus* with Giemsa staining and after FISH with satDNA As-51 probe. Individuals from Sete Barras/SP—Ribeira de Iguape river basin **a**, **b**; and Salesópolis/SP—Tietê river basin [2n=46 **c**, **d**; 2n=48 **e**, **f**; 2n=50 **g**, **h**]. In the box, the twentieth pair of cytotypes with 2n=50 chromosomes bearing two homomorphic sites of satDNA As-51. Bar = 5 μm

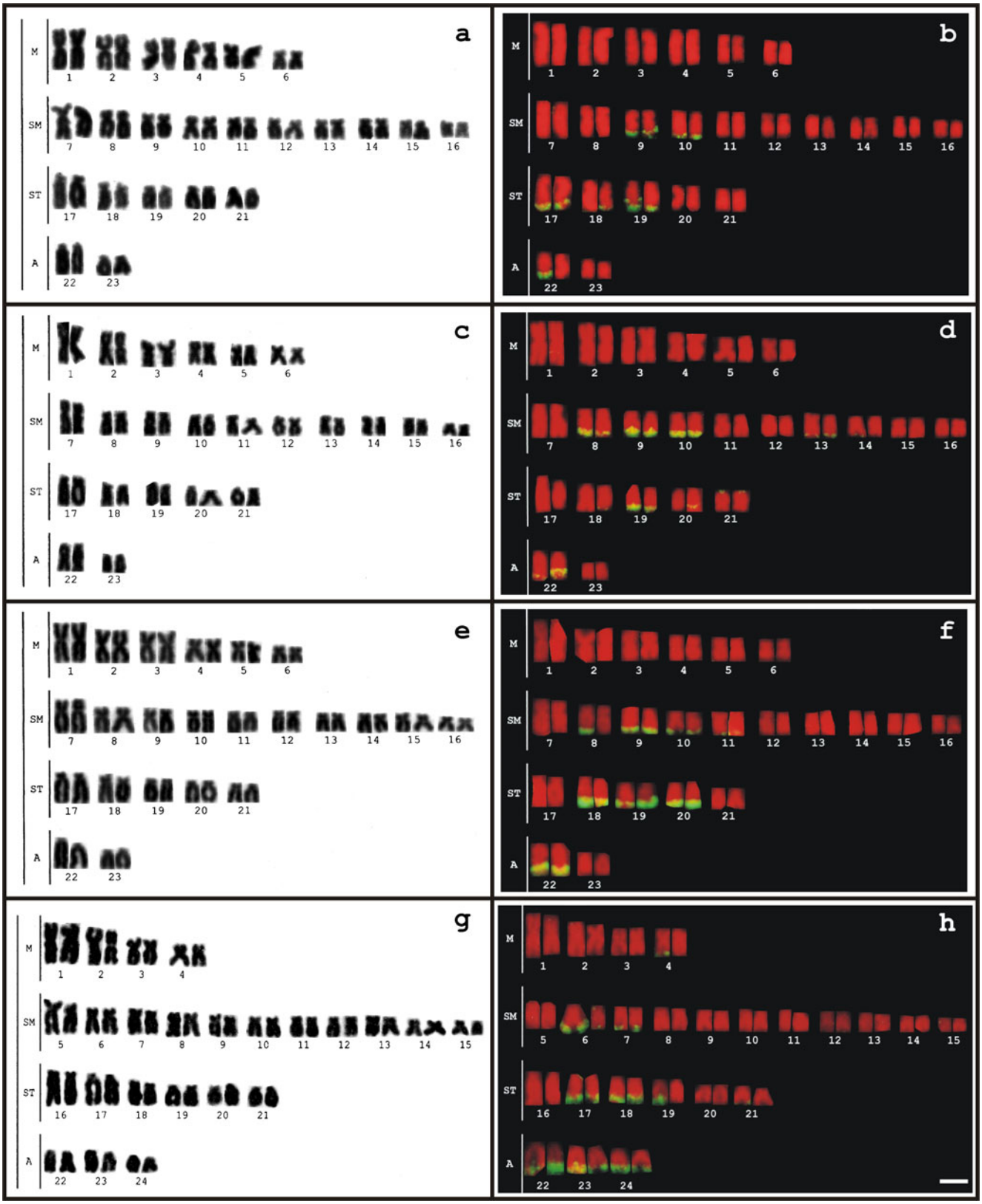

Fig. 3 Karyotypes of *A. fasciatus* with Giemsa staining and after FISH with satDNA As-51 probe. Individuals from Indaiatuba/SP—Tietê river basin **a**, **b**; Pilar do Sul/SP—Paranapanema river basin **c**, **d**; Angatuba/SP—Paranapanema river basin **e**, **f**; and Araras/SP—Mogi-Guaçu river basin **g**, **h**. Bar = 5 μm

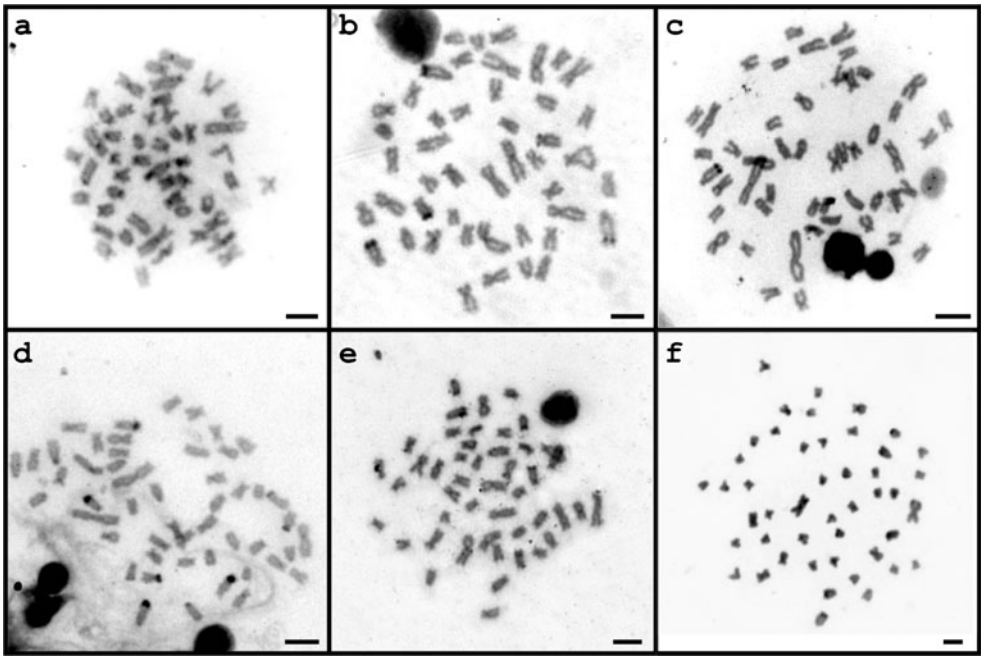

Fig. 4 Metaphases of *A. fasciatus* after C-banding. Individuals from Sete Barras/SP—Ribeira de Iguape river basin **a**; Salesópolis/SP—Tietê river basin [2n=46 **b**; 2n=48 **c**; 2n = 50 **d**]; Indaiatuba/SP—Tietê river basin **e**; and Pilar do Sul/SP—Paranapanema river basin **f**. Bars = 5 μm

that the populations characterized by little amounts of As-51 satDNA display a marginal distribution along the Brazilian coast. In the face of the regional geological history, these populations could have been formed by vicariant events in the Atlantic paleo-river, which, prior to the invasion of the Atlantic Ocean, was responsible for draining the rivers along the Brazilian coast (Ab'Saber 1957).

Furthermore, data in the literature support this scenario. A population of *A. parahybae* from the Paraíba do Sul River basin (coastal drainage) also presents just 4-5 As-51 satDNA-bearing sites, with a heteromorphism in one of the bearing pairs (Kavalco et al. 2007). The present results reinforce the close relationship between *A. parahybae* and the *A. fasciatus* cytotypes from Upper Tietê River. *Astyanax parahybae* has been formerly regarded as a subspecies of *A. fasciatus* (Melo 2001)—both share several chromosomal similarities, such as a diploid number of 2n=48 and a karyotype comprising several submetacentric chromosomes (Kavalco and Moreira-Filho 2003). According to Kavalco et al. (2007), *A. scabripinnis* from Paraíba do Sul River also presents a reduced number of As-51 sites in relation to other populations from Paranapanema River analyzed by Mantovani et al. (2004) and Abel et al. (2006). Besides, the drainages from Upper Tietê and Paraíba do Sul rivers present an ancient geological history, and some events, such as headwater capture from the Tietê River by the Paraíba do Sul River, seem to have taken place (Ab'Saber 1957). Consequently, although both drainages flow in opposite directions nowadays, some degree of resemblance is expected among related species occurring next to Upper Tietê and Paraíba do Sul River basins.

Conversely, previously analyzed populations of *A. fasciatus* from inland basins belonging to the current Paraná River drainage are rich in chromosomal sites bearing As-51 satellite DNA (Abel et al. 2006; Pazza et al. 2008a). The occurrence of chromosomes bearing two syntenic As-51 satDNA sites has been already reported in a cytotype with 2n=47 chromosomes from Mogi-Guaçu River (Pazza et al. 2008a); nonetheless, in the present work, positive signals were observed in a single homolog (Fig. 3b). The data obtained in the present study strengthen the hypothesis about the distribution of the cytotypes of *A. fasciatus* in the Paraná River basin suggested by Pazza et al. (2006). Similar to the situation observed in the population from Salesópolis, where cytotypes bearing 2n=46, 48 and 50 chromosomes were detected (Fig. 2), Pazza et al. (2006) reported a chromosomal polymorphism in the region of the Emas waterfalls in the Mogi-Guaçu River. In this location, a very high frequency of individuals bearing 2n=46 chromosomes was observed, which is in contrast to the findings from samples upstream and downstream of Mogi-Guaçu River. This seems to indicate the effects of a typical invasive cytotype (cytotype 2n=46), with cytotype 2n=48 being the resident form in this basin (Pazza et al. 2006, 2007). The presence of a similar paracentric inversion and 46 chromosomes in specimens from the Middle Tietê River (Indaiatuba region) reinforces the idea that fish fauna introduction putatively occurred in the Mogi-Guaçu River from Tietê drainages thus characterizing this cytotype as an invader species.

Analogously, the available data from São Francisco River basin populations also present a gradation in the quantity of As-51 satellite DNA on chromosomes (Table 1). They clearly show that the higher the distance from the boundaries of the Upper Paraná River basin, the lower the number of As-51 DNA sites (Kantek et al. 2009; Peres et al. 2009). Therefore, the drainages of Grande, Tietê and Paranapanema Rivers

comprise an area where the As-51 satellite DNA is present in a higher amount and is more disperse on chromosomes of *A. fasciatus* than the marginal hydrographic systems (Middle São Francisco River and coastal basins).

Although processes such as duplication cannot be disregarded, a hypothesis that could explain this peculiar feature involves a later introgression of such sequences in the populations from marginal basins (São Francisco and coastal rivers) in relation to the Paraná River basin, as the dispersal throughout the host genome is a trend commonly observed in transposable elements. In addition, the populations from the Central Plateau in the Paraná basin, as well as those from Angatuba and Pilar do Sul (Paranapanema River basin), Araras (Mogi-Guaçu River basin) and Indaiatuba (Middle Tietê River basin), might represent more ancient stocks, when the Central Plateau was located over the Brazilian shield and where the most ancient rocks of the region are found. The further dispersal of this species to other basins could be a result of geological movements and changes in the rivers, coupled with vicariance—a common event in fauna from headwaters, as pointed out by Castro (1999).

Ribeiro (2006) reported a recent vicariant pattern between the fish fauna from the crystalline shield and coastal drainages, as evidenced by the species exchange, including the formation of real hybridization zones. However, it is not possible to state precisely when this satellite DNA first arose in the genome of *Astyanax* species. We can only infer that it happened more than 4.5 My ago—the date estimated for the first colonization of *A. mexicanus* from Central America (Strecker et al. 2004), a species that presents signs of partial homology with the As-51 satellite DNA (Kavalco and Almeida-Toledo 2007).

Although all these cytotypes have been clustered in the same species group (*A. fasciatus*), we can conclude that their distinctiveness in relation to diploid numbers, karyotype formulae and intrinsic genome features within populations from each major hydrographic system (Paraná River, São Francisco River and Coastal Rivers basins) may lead to a higher degree of chromosomal differentiation, potentially able to establish a definitive reproductive isolation among these populations, which may already be present among populations from other Brazilian basins. Therefore, these data indicate that *A. fasciatus* is likely to represent a group comprising a large number of cryptic species, each one bearing particular chromosomal features.

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