



Short Communication

## Comparative cytogenetics among populations of *Astyanax altiparanae* (Characiformes, Characidae, *Incertae sedis*)

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### Abstract

Cytogenetic data are presented for *Astyanax altiparanae* populations from three Brazilian hydrographic systems. The chromosomal data obtained in *A. altiparanae* support the hypothesis of diploid number conservation. However, small differences in the karyotype formula and number of nucleolar organizer regions were observed in these populations. The apparent karyotypical similarity among the studied populations strongly suggests a close relationship among them with some chromosomal divergences due to gene flow restriction.

**Key words:** cytotaxonomy, karyotype diversification, fish cytogenetics.

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*Astyanax* is a genus from the family Characidae widely distributed throughout South and Central America and previously assigned to the subfamily Tetragonopterinae (Géry, 1977). This group has been recently considered *Incertae sedis* for not exhibiting consistent evidence of monophyly (Lima *et al.*, 2003). *Astyanax altiparanae* is a typical species of the upper Paraná River Basin above Sete Quedas, Brazil (Garutti and Britski, 2000), with the exception of one population from a location in the Iguazu River situated below this region (Graça and Pavanelli, 2002).

The cytogenetics of Neotropical fishes has greatly contributed to their systematics and taxonomy. There are numerous examples among *Astyanax*, mainly from the “*scabripinnis*” and “*fasciatus*” groups, in which the intraspecific cytogenetic variation among populations was shown to be much larger than that detected through morphological systematics, thereby forming true species complexes (Moreira-Filho and Bertollo, 1991; Artoni *et al.*, 2006; Vicari *et al.*, 2008a).

The aim of this work was to characterize the karyotypes of three *A. altiparanae* populations and to compare them with those described in the literature. This comparative analysis could reveal population markers and help to

establish cytosystematic, evolutionary and/or biogeographical relationships among the populations.

Forty-nine specimens of *A. altiparanae* were analyzed: nine specimens (five males and four females) from the Pântano Stream in the Tietê River Basin of the state of São Paulo; eight specimens (five males and three females) from the Feijão Stream in the Mogi-Guaçu River Basin in the state of São Paulo; and 32 specimens collected at the Salto Segredo hydroelectric power plant on the Jordão River in the Iguazu River Basin in the state of Paraná. The procedures were approved by the Ethics Committee on Animal Experimentation of the Universidade Estadual de Ponta Grossa (Process no. 04741/08).

Chromosome preparations were obtained from anterior kidney cells using the *in vivo* colchicine treatment (Bertollo *et al.*, 1978). Nucleolar organizer regions (NORs) were detected after silver nitrate staining (Ag-NOR) according to Howell and Black (1980) and C-banding was obtained as described in Sumner (1972).

Fluorescent *in situ* hybridization (FISH) was performed with a biotinylated 18S rDNA probe from the fish *Prochilodus argenteus* (Hatanaka and Galetti Jr, 2004) and with a biotinylated 5S rDNA probe from the fish *Leporinus elongatus* (Martins and Galetti Jr, 1999). An Olympus BX50 epifluorescence microscope was used for the analysis.

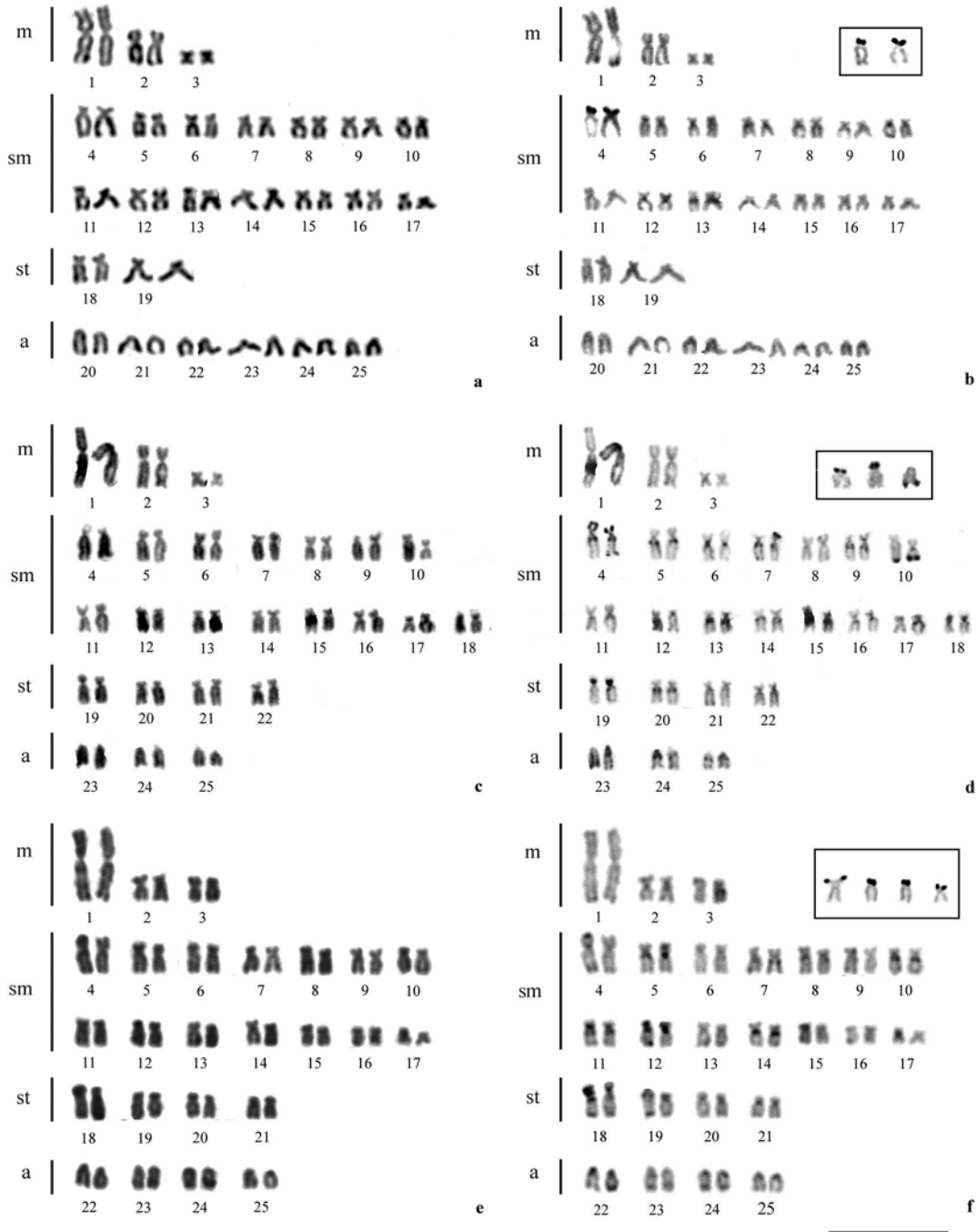
Nearly 30 metaphases were analyzed per specimen to determine the diploid number, karyotype composition and Ag-NOR distribution. Chromosomes were classified as

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metacentric (m), submetacentric (sm), subtelocentric (st) and acrocentric (a) following Levan *et al.* (1964).

Fishes of the genus *Astyanax* have shown considerable karyotype diversity. The diploid and fundamental numbers (2n and FN) allied to some other chromosome markers, allowed cytotaxonomic and evolutionary inferences regarding these fishes. The *A. altiparanae* popula-

tions studied (Pântano Stream, Feijão Stream and Jordão River) have a diploid number of  $2n = 50$  chromosomes (Figure 1). However, differences in the fundamental numbers were found among the three populations analyzed. The specimens from the Pântano Stream displayed a FN = 88, with  $6m+28sm+4st+12a$ . The Feijão Stream population had a FN = 94, with  $6m+30sm+8st+6a$ , whereas the speci-



**Figure 1** - Karyotypes of *Astyanax altiparanae*: population from the Pântano Stream after conventional Giemsa staining (a) and sequential C-banding (b); in the inset, the chromosomes with Ag-NORs. Population from the Feijão Stream after conventional Giemsa staining (c) and sequential C-banding (d); in the inset, the chromosomes with Ag-NORs. Population from the Jordão River after conventional Giemsa staining (e) and sequential C-banding (f); in the inset, the chromosomes with Ag-NORs. The bar represents 10 μm.

mens from the Jordão River showed a FN = 92, with 6m+28sm+8st+8a (Figure 1, Table 1). In the Jordão River population, chromosome pair 2 was smaller than the same pair in the Pântano and Feijão Streams populations (Figure 1). Variations in diploid number were observed between populations of species from the *A. scabripinnis* and *A. fasciatus* complexes and resulted from chromosome translocations (Mantovani *et al.*, 2000; Artoni *et al.*, 2006; Vicari *et al.*, 2008a). Nevertheless, *A. altiparanae* exhibited a conserved 2n = 50 chromosomes in all the populations karyotyped (Table 1). Despite presenting the same 2n, different fundamental numbers were detected due to chromosome morphology variations. Non-Robertsonian rear-

rangements, such as pericentric inversions were suggested to explain the karyotype differences among *A. altiparanae* populations (Domingues *et al.* 2007).

After C-banding, heterochromatic blocks were observed in the centromeric or pericentromeric regions of all chromosome pairs and in the telomeric regions of some chromosomes with small differences among the analyzed populations (Figure 1b, d and f). This results are similar to findings previously described for other *A. altiparanae* populations (Fernandes and Martins-Santos, 2004; Domingues *et al.*, 2007). The small variations in constitutive heterochromatin distribution combined with the large variation in FN found among the *A. altiparanae* populations (Table 1)

**Table 1** - Chromosome data from Brazilian populations of *Astyanax altiparanae*. Updated from Fernandes and Martins-Santos (2004).

<i>A. altiparanae</i> (River/Stream, City, State)	River basin	2n	Karyotypic formulae				FN	Ag-NORs	N° of 18S sites	N° of 5S sites	Ref.
			m	sm	st	a					
Iguaçu. Salto Caxias, PR	Iguaçu	50	10	26	4	10	90	-	-	-	1
Iguaçu. Curitiba, PR	Iguaçu	50	6	30	8	6	94	2	2	2	2
Tibagi. Ponta Grossa, PR	Tibagi	50	6	28	8	8	92	2-3	7	2	2
Tibagi. Londrina, PR	Tibagi	50	6	22	8	14	86	-	-	-	3
Tibagi. Londrina, PR	Tibagi	50	10	22	6	12	88	-	-	-	3
Tibagi. Sertãoópolis, PR	Tibagi	50	10	22	8	10	90	-	-	-	3
Couro de Boi. Jataizinho, PR	Parapanema	50	8	20	10	12	88	-	-	-	3
Três Bocas. Londrina, PR	Parapanema	50	10	28	4	8	92	-	-	-	3
*Três Bocas. Londrina, PR	Parapanema	50	12	28	2	8	92	1-6	-	-	4
*Três Bocas. Londrina, PR	Parapanema	50	6	22	16	6	94	1-6	-	-	4
*Três Bocas. Londrina, PR	Parapanema	50	18	16	8	4	88	1-6	-	-	4
Claro. Tamarana, PR	Parapanema	50	10	26	4	10	90	1-4	-	-	5
Claro. Tamarana, PR	Parapanema	50	10	24	4	12	88	1-4	-	-	5
Claro. Tamarana, PR	Parapanema	50	10	22	4	14	86	1-4	-	-	5
Parapanema. Salto Grande, SP	Parapanema	50	10	22	6	12	88	-	-	-	6
Tatuteba. Maringá, PR	Ivaí	50	6	26	6	12	88	3	4	2	7
Keçaba. Maringá, PR	Pirapó	50	6	26	6	12	88	1	7	2	7
Maringá. Maringá, PR	Pirapó	50	6	26	6	12	88	3	4	2	7
*Sarandi. Maringá, PR	Pirapó	50	34	-	16	-	100	-	-	-	8
Maringá. Maringá, PR	Pirapó	50	10	22	6	12	88	-	-	-	3
Índios. Cianorte, PR	Ivaí	50	6	30	4	10	90	10	-	-	9
Paraná. Porto Rico, PR	Paraná	50	6	26	6	12	88	2	4	2	7,9
*Mogi-Guaçu. Pirassununga, SP	Mogi-Guaçu	50	10	24	4	12	88	-	-	-	10
*Mogi-Guaçu. Pirassununga, SP	Mogi-Guaçu	50	6	24	12	8	92	-	-	-	11
Pedras. Uberlândia, MG	Grande	50	16	26	4	4	96	-	-	-	12
Jataí. Uberlândia, MG	Grande	50	16	22	6	6	94	-	-	-	12
*Meia Ponte. Goiânia, GO	Meia Ponte	50	-	26	-	24	76	-	-	-	13
Pântano. São Carlos, SP	Tietê	50	6	28	4	12	88	1-2	2	2	14
Feijão. São Carlos, SP	Mogi-Guaçu	50	6	30	8	6	94	1-3	4	2	14
Jordão. Mangueirinha, PR	Iguaçu	50	6	28	8	8	92	2-4	6	2	14

\*Cited as *Astyanax bimaculatus*. m = metacentric; sm = submetacentric; st = subtelocentric; a = acrocentric; FN = fundamental number; PR = Paraná State; SP = São Paulo State; MG = Minas Gerais State; GO = Goiás State. Ref. = References : 1. Porto and Martins-Santos (2002 apud Fernandes and Martins-Santos, 2004); 2. Domingues *et al.* (2007); 3. Pacheco (2001 apud Fernandes and Martins-Santos, 2004); 4. Takahashi (1995 apud Fernandes and Martins-Santos, 2004); 5. Pacheco *et al.* (2001); 6. Daniel-Silva and Almeida-Toledo (2001); 7. Fernandes and Martins-Santos (2006); 8. Vale and Martins-Santos (1998 apud Fernandes and Martins-Santos, 2004); 9. Fernandes and Martins-Santos (2004); 10. Morelli *et al.* (1983); 11. Paganelli (1990 apud Fernandes and Martins-Santos, 2004); 12. Barbosa *et al.* (2002 apud Fernandes and Martins-Santos, 2004); 13. Jim and Toledo (1975 apud Fernandes and Martins-Santos, 2004). 14. Present study.

supports the hypothesis that this structural variation is not due to the amplification of heterochromatic sequences. Thus, the different FNs observed in *A. altiparanae* populations, sometimes even within the same hydrographic basin, such as the Tibagi, Paranapanema and Mogi-Guaçu Rivers, must be mainly due to pericentric inversions.

Silver-nitrate staining (Ag-NORs) and FISH with the 18S rDNA probe evidenced a single NOR-bearing pair in the population of the Pântano Stream, while the populations of the Feijão Stream and the Jordão River exhibited multiple terminal signals on different chromosomes (Figure 1, in the insets; and Figure 2a, c and e). Chromosome rearrangements and transference of ribosomal sites could cause these variations, but transposition events have been suggested as the main mechanism to explain the majority of cases of NORs variability in the genome of these animals (Mantovani *et al.*, 2000; Vicari *et al.*, 2008b). Together with variations in the fundamental number, Ag-NORs are important cytogenetic markers to determine differences among populations in this species (Pacheco *et al.*, 2001; Fernandes and Martins-Santos, 2004; 2006). Domingues *et al.* (2007) car-

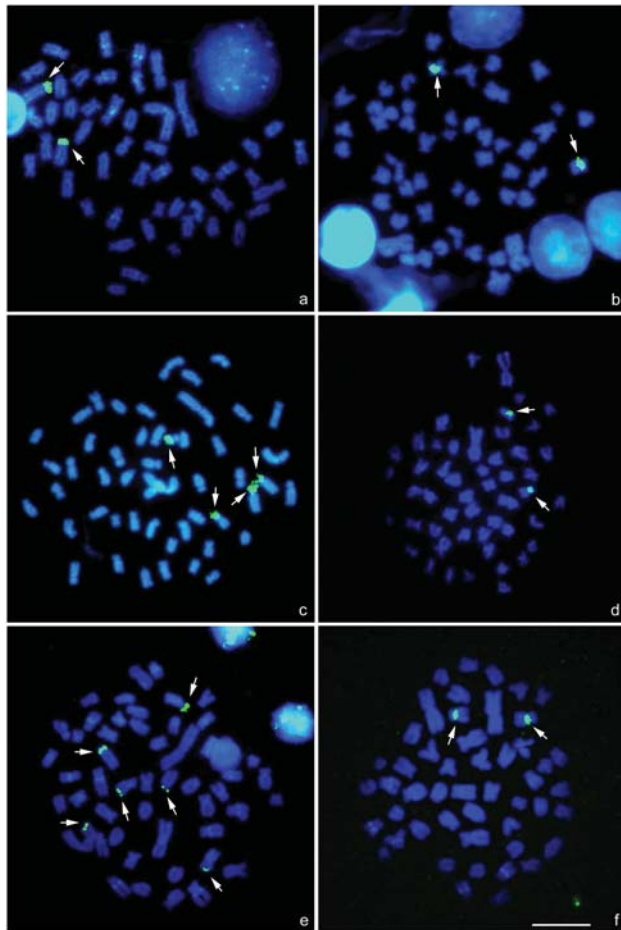
ried out a comparative cytogenetic and morphometric study on *A. altiparanae* populations from the upper Tibagi and Iguauçu Rivers. These authors found a similar karyotype macrostructure in the populations with differences in the number of major rDNA sites between populations. The Ag-NORs and 18S rDNA FISH data (Table 1) also revealed variations between populations of this species both within the same and from different hydrographic basins.

While variations in the number of 18S rDNA sites occurred between populations of *A. altiparanae*, only one signal in the proximal long arm of a sm chromosome was observed after FISH with the 5S rDNA probe (Figure 2b, d and e) (Fernandes and Martins-Santos, 2004; 2006; Domingues *et al.*, 2007; Peres *et al.*, 2008; present study). The 5S rDNA sites seemed to be conserved in the proximal region of the long arm of two chromosome pairs (one m and one a) in populations of the *A. scabripinnis* complex (Ferro *et al.*, 2001; Almeida-Toledo *et al.*, 2002; Mantovani *et al.*, 2005; Vicari *et al.*, 2008a). In *Astyanax* sp. D and in *A. janeiroensis*, the 5S rDNA site was located in the proximal region of the long arm of a single acrocentric pair, similar to what was found in the *A. scabripinnis* complex (Kantek *et al.*, 2007; Vicari *et al.*, 2008b, respectively). Mantovani *et al.* (2000) proposed that 5S rDNA sites in the proximal position of the long arm indicated a conserved pattern for these genes in *Astyanax*. Thus, the chromosomes with 5S rDNA sites would be the same among the *A. altiparanae* populations studied, but would diverge in morphology and number of 5S rDNA sites in other populations of the genus.

The cytogenetic data from the three *A. altiparanae* populations studied herein are similar to those from other populations of this species reported in the literature. This observation strengthens the hypothesis of a conserved  $2n = 50$ . Nevertheless, differences in the karyotypic formula and in the number of nucleolar organizer regions were noticed when comparing the data from these populations to those already described for other populations. The apparent karyotype similarity strongly suggests a close relationship among the studied populations, but the small karyotypic differences detected indicate some evolutionary divergence due to gene flow restrictions.

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**Figure 2** - Chromosome spreads of *Astyanax altiparanae* after FISH: (a) 18S and (b) 5S rDNA sites in the population from the Pântano Stream (c) 18S and (d) 5S rDNA sites in the Feijão Stream population; and (e) 18S and (f) 5S rDNA sites in the Jordão River population. The bar represents 10  $\mu$ m.



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