

B chromosomes in *Astyanax scabripinnis* (Pisces, Characidae)

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A karyotypic analysis was carried out using conventional staining and C-banding in 32 specimens of *Astyanax scabripinnis* (Pisces, Characidae) from Campos do Jordão (São Paulo State, Brazil). Twenty-eight individuals (87.5 per cent of the sample studied) showed 1–2 extra B-chromosomes, similar in size and shape (metacentrics) to pair 1 of the karyotype, with a high intra-individual constancy. Two types of B could be identified on the basis of their constitutive heterochromatin patterns. The hypothesis of the origin of the B-chromosome from non-disjunction of chromosome 1, followed by a heterochromatinization process, is postulated.

Keywords: *Astyanax scabripinnis*, B-chromosomes, C-banding, karyotype.

Introduction

The growing number of papers on fish cytogenetics published over the last 20 years have significantly contributed to our knowledge of the karyotype structure of many fish species. Among the many discoveries reported are cases of accessory or B chromosomes. Accessory chromosomes were probably mentioned by Taylor (1967) in a study of *Eptatretus stoutii*. Pauls & Bertollo (1983) reported the first detection of a B chromosome among Ostariophysi, in *Prochilodus scrofa*. Since this report, other cases have been detected in 18 species belonging to seven different families of bony fish (Table 1). Some of these cases can be characterized as B chromosomes, whereas others are reports of more sporadic occurrences of extra chromosomes. Karyotype studies conducted on *Astyanax scabripinnis* have demonstrated chromosome diversity in this species, with the detection of diploid numbers equal to 46, 48 and 50 in different populations (Moreira-Filho *et al.*, 1978; Morelli *et al.*, 1983; Martins *et al.*, 1984; Moreira-Filho & Bertollo, 1986; Rocon-Stange & Passamani, 1988; Maistro *et al.*, 1990; Salvador & Moreira-Filho, 1990).

Moreira-Filho & Bertollo (1991) conducted karyotypic and morphological studies on seven populations of *A. scabripinnis*. Some populations were distinguishable by morphological traits, others by karyotypic traits, and still others by both types of characteristics. Six of the seven populations studied presented unique or exclusive traits at the morphological and/or chromo-

some level which permitted their distinction and were therefore proposed to be distinct species.

This species complex is restricted to the headwaters of small tributaries. Thus, its diversity should be initially interpreted as a function of each microbasin in particular and later analysed in a comparative manner between larger draining systems (Moreira-Filho & Bertollo, 1991).

Materials and methods

Thirty-two specimens (22 females and 10 males) of *Astyanax scabripinnis* (Fig. 1) were collected from the 'Córrego das Pedras' stream, municipality of Campos do Jordão, State of São Paulo, Brazil. This region presents a considerably irregular relief with altitudes ranging from 1800 to 730 m, the collection site being located at 1720 m.

Mitotic cells were obtained by the technique of Egozcue (1971), adapted by Bertollo (1978), and C banding was obtained by the method of Sumner (1972).

The chromosomes were measured with the aid of a dry-tip compass and pachymeter. The length of their short and long arms and the total length were obtained. The mean values were then calculated for each chromosome pair. The relative length (per cent) of the different pairs was calculated from these values in relation to the total length of the haploid lot. B chromosomes were included in the calculation.

Table 1 Supernumerary chromosomes in Osteichthyes fish

Family species	Individual analysis	Individuals with extra chromosomes	$2n$	Number of extra chromosomes	Size	Type	Reference
Prochilodontidae							
<i>Prochilodus scrofa</i>	62	61	54	0-5	Micro	—	1
<i>P. scrofa</i>			54	0-7	Small	M	2
<i>P. cearensis</i>	7	5	54	0-2	Micro	—	1
Curimatidae							
<i>Cyphocharax modesta</i> cit. <i>Curimata modesta</i>	10	1	$3x = 81$	1	Small	—	3
<i>Cyphocharax modesta</i>	17	1	54	1	Micro	—	4
Parodontidae							
<i>Apareiodon piracicabae</i>	20	1	54	0-1	Large	M	5
Characidae							
<i>Oligosarcus pinto</i> cit.	19	2	50	0-1	Large	M	5
<i>Paroligosarcus pinto</i>							
<i>Moenkhausia intermedia</i>	14	8	50	0-1	Small	—	6
<i>M. sanctafilomenae</i>	30	30	50	1-8	Micro	—	7
<i>Astyanax eigenmanniorum</i>	3	1	50	0-1	Large	M	8
<i>A. scabripinnis</i>	29	3	50	0-2	Small	—	9
<i>A. scabripinnis</i>	15	1	50	0-1	Large	M	10
<i>A. scabripinnis</i>	32	28	50	0-2	Large	M	11
<i>Characidium cf. zebra</i>	28	1	50	0-1	Small	A	12
<i>Piabina argentea</i>	12	1	52	0-1	Small	—	13
Pimelodidae							
<i>Pimelodella kronei</i>	5	1	50	0-1	Micro	—	14
<i>Bergiaria westermanni</i>	7	7	56	0-5	Small	—	15
<i>Rhadia quelen</i>	30	13	58	0-2	Small	—	16
<i>R. hilarii</i>	51	50	58	0-5	Small	—	17
Callichthyidae							
<i>Corydoras aeneus</i>	33	9	60	0-3	Small	—	18
<i>Callichthys callichthys</i>	27	18	58	0-16	Middle	M/SM	19
Loricariidae							
<i>Microlepidogaster</i>							
<i>M. leucofrenatus</i>	34	9	54	0-2	Large	M	20
Cichlidae							
<i>Gymnogeophagus balzanii</i>	4	3	48	0-4	Micro	—	21

References:

- | | | |
|------------------------------------|---|--------------------------------------|
| 1. Pauls & Bertollo (1990) | 11. Present paper | M = metacentric |
| 2. Cavallaro & Bertollo (1990) | 12. Miyazawa (1991) | A = acrocentric |
| 3. Venere & Galetti Jr (1985) | 13. Wasko, (1991) | M/SM = metacentric or submetacentric |
| 4. Venere (1991) | 14. Almeida Toledo <i>et al.</i> (1985) | |
| 5. Falcão <i>et al.</i> (1984) | 15. Dias (1987) | |
| 6. Portela <i>et al.</i> (1988) | 16. Hochberg & Erdtmann (1988) | |
| 7. Foresti <i>et al.</i> (1989) | 17. Fenocchio & Bertollo (1990) | |
| 8. Stripeck <i>et al.</i> (1985) | 18. Oliveira <i>et al.</i> (1986) | |
| 9. Rocon-Stange & Passamani (1988) | 19. Erdtmann <i>et al.</i> (1990) | |
| 10. Maistro <i>et al.</i> (1990) | 20. Andreato (1991) | |
| | 21. Feldberg & Bertollo (1984) | |

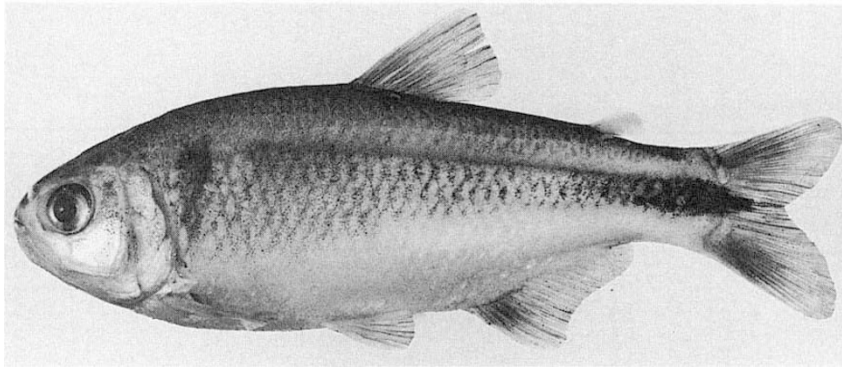


Fig. 1 *Astyanax scabripinnis* specimen from the 'Córrego das Pedras' stream, Campos do Jordão, SP, Brazil. Standard length = 8.0 cm.

The chromosomes were identified using the arm ratio (AR) criteria proposed by Levan *et al.* (1964) and classified as metacentrics (M: AR = 1.00–1.70), submetacentrics (SM: AR = 1.71–3.00), subtelocentrics (ST: AR = 3.01–7.00), and acrocentrics (A: AR > 7.01).

Results

A total of 502 metaphase preparations from males and females were analysed, and 367 cells were found to have 50 chromosomes, corresponding to 73.1 per cent of the total number examined. Thus, the diploid number of *A. scabripinnis* from the Campos do Jordão site is $2n = 50$. Chromosome measurements and classification showed that the basic karyotype consists of three pairs of metacentric chromosomes, 11 pairs of submetacentrics, five pairs of subtelocentrics, and six pairs of acrocentrics, with a fundamental number (FN) equal to 88 (Table 2 and Fig. 2).

Extra chromosomes were detected in 28 of the 32 specimens investigated, with 19 females and eight males presenting only one of this kind of chromosome in 100 per cent of their metaphases (Table 3). This chromosome is of the metacentric type, large in size and slightly smaller than the first pair in the basic karyotype complement (Fig. 3). Two accessory chromosomes of similar morphology and size (Fig. 4) were detected in 100 per cent of complete metaphases obtained from one female (Table 3). In the remaining individuals, two females and two males, no extra chromosome was detected in any of the cells examined.

C-banding permitted the characterization of at least two types of supernumerary chromosomes in terms of the heterochromatin pattern. One of them was fully heterochromatic and the other presented a median euchromatin segment on each side of the centromere, with distal heterochromatin blocks (Fig. 5). One or the other of these chromosome types was observed in the specimens with a single supernumerary chromosome. Both chromosome types were present in the individual with two extra chromosomes (Fig. 5).

Table 2 Chromosome measurements and types in *Astyanax scabripinnis* from Campos do Jordão, SP, Brazil

Pair	Pair means					Type
	<i>p</i>	<i>q</i>	<i>TL</i>	<i>RL</i> (%)	<i>AR</i>	
1	11.67	15.67	27.34	8.17	1.34	M
2	5.92	7.42	13.34	3.99	1.25	M
3	5.15	7.32	12.47	3.73	1.42	M
4	6.02	10.95	16.97	5.07	1.82	SM
5	3.65	8.15	11.80	3.53	2.23	SM
6	3.70	8.07	11.77	3.52	2.18	SM
7	3.25	7.95	11.20	3.35	2.44	SM
8	3.20	7.95	11.15	3.33	2.45	SM
9	3.30	7.50	10.80	3.23	2.27	SM
10	3.40	7.22	10.62	3.17	2.12	SM
11	2.70	7.07	9.77	2.92	2.62	SM
12	2.69	7.07	9.76	2.92	2.62	SM
13	3.00	5.30	8.30	2.48	1.76	SM
14	2.57	5.41	7.98	2.39	2.10	SM
15	1.97	8.40	10.37	3.10	4.26	ST
16	2.57	7.75	10.32	3.08	3.01	ST
17	1.60	8.50	10.10	3.02	5.31	ST
18	2.10	7.45	9.55	2.85	3.54	ST
19	2.37	7.15	9.52	2.85	3.01	ST
20	1.25	12.22	13.47	4.02	9.77	A
21	0.75	12.45	13.20	3.94	16.60	A
22	1.10	10.35	11.45	3.42	9.41	A
23	0.80	10.52	11.32	3.38	13.15	A
24	1.20	9.05	10.25	3.06	7.54	A
25	0.50	7.45	7.95	2.38	14.90	A
B ¹	10.45	11.85	22.30	6.67	1.13	M
B ²	9.65	11.85	21.50	6.43	1.22	M

p = short arm.

q = long arm.

TL = total length.

RL (%) = relative length.

AR = arm ratio.

M = metacentric

SM = Submetacentric

ST = Subtelocentric

A = acrocentric

B¹ = B chromosome type 1

B² = B chromosome type 2

The first supernumerary chromosome type had 6.67 per cent participation in the relative length of the haploid lot, and the second, 6.43 per cent. Thus, the two chromosome types taken together represented 13

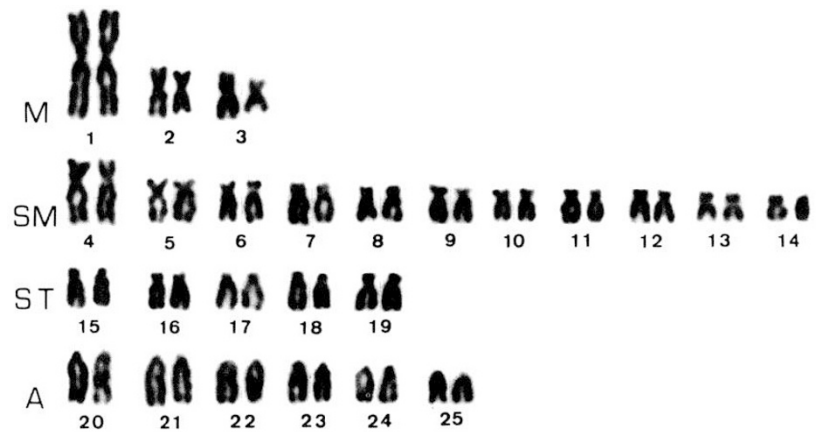


Fig. 2 Basic karyotype of an *Astyanax scabripinnis* male from Campos do Jordão, SP, Brazil.

Table 3 Frequency of B chromosome per cell in females and males of *A. scabripinnis* from Campos do Jordão, SP, Brazil

Fish	Number of B chromosomes/cell			Number of B	Number of cells analysed	Cells with B (%)
	0	1	2			
6164 ♀	23	—	—	0	23	0
6166 ♀	—	31	—	1	31	100
6167 ♀	—	16	—	1	16	100
6168 ♀	17	—	—	0	17	0
6176 ♀	—	02	—	1	02	100
6911 ♀	—	08	—	1	08	100
6912 ♀	—	05	—	1	05	100
6916 ♀	—	12	—	1	12	100
6917 ♀	—	05	—	1	05	100
6944 ♀	—	17	—	1	17	100
6956 ♀	—	14	—	1	14	100
6977 ♀	—	06	—	1	06	100
6979 ♀	—	20	—	1	20	100
6985 ♀	—	06	—	1	06	100
6986 ♀	—	02	—	1	02	100
6987 ♀	—	04	—	1	04	100
6988 ♀	—	07	—	1	07	100
6989 ♀	—	09	—	1	09	100
6990 ♀	—	—	20	2	20	100
6991 ♀	—	24	—	1	24	100
7550 ♀	—	09	—	1	09	100
7581 ♀	—	07	—	1	07	100
6169 ♂	—	15	—	1	15	100
6175 ♂	—	15	—	1	15	100
6919 ♂	—	06	—	1	06	100
6943 ♂	22	—	—	0	22	0
6945 ♂	—	08	—	1	08	100
6972 ♂	—	06	—	1	06	100
6973 ♂	—	04	—	1	04	100
6980 ♂	—	05	—	1	05	100
6981 ♂	—	02	—	1	12	100
7107 ♂	20	—	—	0	20	0
Total number of cells analysed					367	

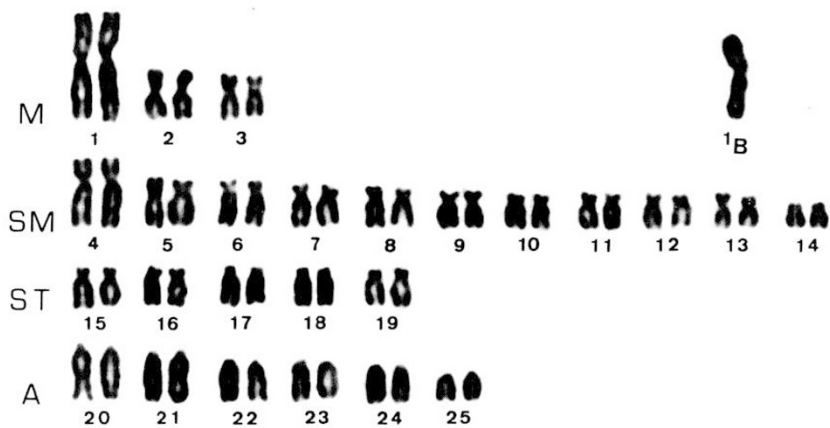


Fig. 3 Karyotype of an *Astyanax scabripinnis* male from Campos do Jordão, SP, Brazil, showing a B chromosome.



Fig. 4 Karyotype of an *Astyanax scabripinnis* female from Campos do Jordão, SP, Brazil, showing two B chromosomes.

per cent of the haploid complement of the species (Table 2).

Discussion

A. scabripinnis from Campos do Jordão (SP) presents a diploid number equal to 50 chromosomes and its karyotype complement is similar to those detected by Moreira-Filho and Bertollo (1992) in two populations from different sites (Salesópolis and São Carlos: State of São Paulo, Brazil) but differs in the number of ST and A chromosomes. This difference may be due in part to the fact that several of these chromosomes present an AR close to the classifying limit of these two chromosome classes. However, *A. scabripinnis* from Campos do Jordão shows the peculiarity of extra chromosome in its karyotype. The occurrence of these chromosomes at the population level, as well as their characteristics permit us to consider them to be B chromosomes.

The constancy of these chromosomes in 100 per cent of the cells from the individuals bearing them (Table 3) indicates that some mechanism must exist to insure the stability of these chromosomes in the karyotype complement, although it has not yet been possible to conduct a study on the behaviour of these chromosomes during meiosis. On this basis, the B chromosomes of *A. scabripinnis* present interesting characteristics related not only to their high incidence in the population (87.5 per cent of the individuals analysed) but also to their intra-individual constancy.

Volobujev (1981) raised two hypotheses to explain the origin of B chromosomes. One proposes that B chromosomes may be relics of structural rearrangements that occurred during the evolution of the ancestral karyotype. The other proposes that these chromosomes may result from autosome or sex chromosome non-disjunction followed by a process of genetic inactivation.

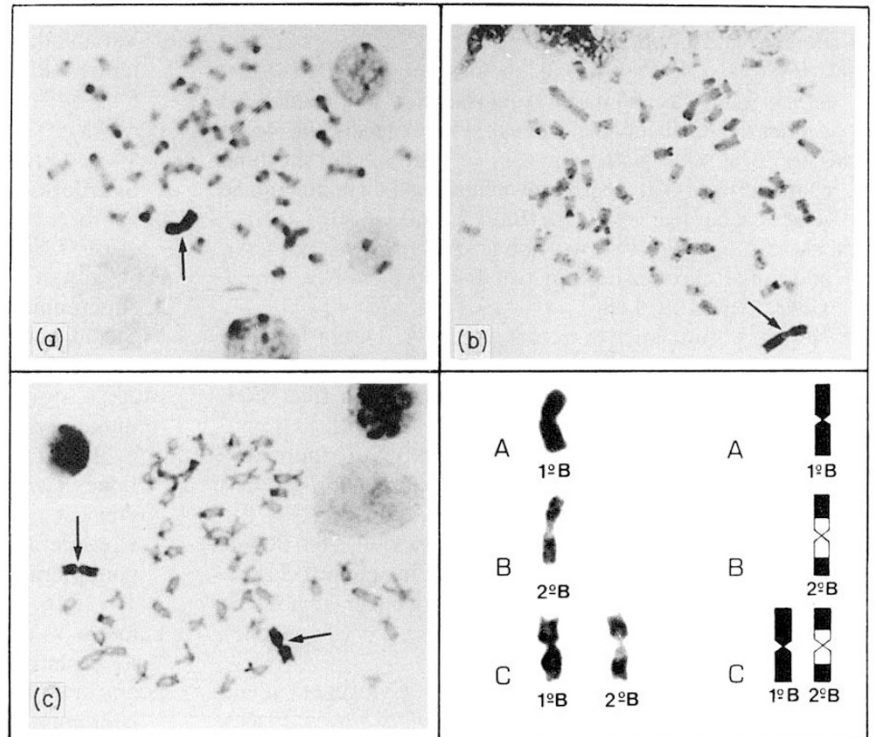


Fig. 5 Types of B chromosomes detected by C-banding in *Astyanax scabripinnis* from Campos do Jordão, SP, Brazil. (a) Metaphase showing a fully heterochromatic B chromosome (arrow). (b) Metaphase with a partially euchromatic B chromosome in the middle region (arrow). (c) Metaphase with two B types present (arrows). The inset at lower right gives details of the two B types observed.

Scheel (1973) and later Morelli *et al.* (1983) and Falcão & Bertollo (1985) have emphasized as a widely diffuse characteristic of Characidae the presence of a first metacentric chromosome pair which is higher than the remaining chromosomes in the karyotype complement. This chromosome pair is also present in the basic karyotype of *A. scabripinnis* from Campos do Jordão. In turn, the B chromosomes detected in this population are similar in both size and shape to the first pair in the complement. On this basis, it may be suggested that they probably originated from non-disjunction of one of these chromosomes followed by heterochromatinization, in agreement with the second hypothesis raised by Volobujev (1981).

According to Rejon *et al.* (1987), the heterochromatinization of the B chromosome may be of a selective evolutionary nature because this chromosome may have harmful effects on the organism which would be minimized by a process of heterochromatinization. The heterochromatin patterns presented by the B chromosomes of *A. scabripinnis* from Campos do Jordão (Fig. 5) may represent distinct stages of heterochromatinization and consequently of genetic inactivation of these chromosomes. The high incidence of B in the population analysed may be justified by the specific characteristics of the *scabripinnis* group, which forms small isolated populations and reproduces at different times of the year, a fact that would favour an easier fixation of these chromosomes in the population.

Acknowledgements

The authors are grateful to Dr Heraldo Britski for taxonomic identification. Funds to support this study were provided by FAPESP and CNPq.

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