

B Chromosomes in Four Different Populations of *Rhamdia quelen* (Siluriformes, Heptapteridae): A Comparative Study of Frequency and Distribution

Vivian Patrícia Oliveira DE MORAES, Juliana DE SOUZA CARNEIRO and Ana Lúcia DIAS

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In the present study 25 specimens of *Rhamdia quelen* from four different localities were analyzed cytogenetically. All showed a diploid number of 58 chromosomes, with a karyotypic formula of $36m+16sm+6st$ and $FN=116$. Metacentric B chromosomes showing inter- and intraindividual variation were observed in all populations. C-banding revealed differences in the heterochromatin distribution pattern, with evidence of completely heterochromatic B chromosomes in three populations: Água das Pedras, Água dos Patos and Taquari rivers, and partially heterochromatic B chromosomes in the population at the fish farm station in Timbó/SC. The occurrence of B chromosomes in such distinct populations suggests that they could have arisen from the same ancestral state, before the geographic dispersal of this species.

Key words: B chromosomes, populations, *Rhamdia quelen*.

Vivian Patrícia Oliveira DE MORAES, Juliana DE SOUZA CARNEIRO and Ana Lúcia DIAS, Universidade Estadual de Londrina, Centro de Ciências Biológicas, Departamento de Biologia Geral. 86051-970, Londrina, Paraná, Brasil.
E-mail: anadias@uel.br

B chromosomes, also known as supernumerary or accessory chromosomes, are additional chromosomes to the normal "A" complement, which can be found in some populations of some species; they probably arose from a chromosome of the standard complement but followed their own evolutionary path (CAMACHO *et al.* 2000).

In the family Heptapteridae, this type of chromosome was found in *Pimelodella kronei* studied by ALMEIDA-TOLEDO *et al.* (1992), but it occurs much more frequently in the genus *Rhamdia* (VISOTTO *et al.* 1999; FENOCCHIO *et al.* 2000; ABUCARMA & MARTINS-SANTOS 2001; among others).

B chromosomes in *Rhamdia* present intra- and interindividual variation, occurring as chromosomes of different sizes (micro, small and medium); medium sized metacentric B chromosomes are the most frequent (FENOCCHIO *et al.* 2000; STIVARI & MARTINS-SANTOS 2004; SWARÇA *et al.* 2003). When submitted to C-banding, these chromosomes appear completely or partially heterochromatic, although there are reports of euchromatic B chromosomes for *Rhamdia voulezi* and *Rhamdia* sp. described by ABUCARMA & MAR-

TINS-SANTOS (2001) and for a population of *Rhamdia quelen* examined by MORAES *et al.* (2007).

Since B chromosomes are very common in *Rhamdia*, the aim of this study was to present a comparative analysis of the occurrence, frequency, type and heterochromatin distribution pattern of B chromosomes in four distinct populations of *Rhamdia quelen* and discuss their probable origin in relation to other populations and species of the genus.

Material and Methods

Twenty five specimens of *Rhamdia quelen* from four distinct localities in Brazil were analyzed: 9 specimens (3 males, 5 females and 1 unknown sex) collected in Água dos Patos river (Iepê, São Paulo State) 5 specimens (1 male and 4 females) from Água das Pedras (Londrina, Paraná State), 7 individuals (3 males, 3 females and 1 sex unknown) from Taquari river (Jataizinho, Paraná State) and 4 specimens from the fish farm station named FUNPIVI (Fundação de Piscicultura do Vale do Itajaí), located at Timbó (Santa Catarina State).

Mitotic chromosomes were obtained from kidney cells from the populations of Água das Pedras, Água dos Patos and Taquari rivers according to BERTOLLO *et al.* (1978) and from lymphocyte culture following the method of FENOCCHIO and BERTOLLO (1988) from the FUNPIVI and the Água das Pedras River populations. Chromosomal morphology was determined according to LEVAN *et al.* (1964): metacentric (m), submetacentric (sm) and subtelocentric (st) chromosomes were considered as biarmed. C-banding was obtained using the method described by SUMNER (1972).

Results

The 25 specimens studied had a diploid number of $2n = 58$ chromosomes with a karyotypic formula of $36m + 16sm + 6st$ and $FN = 116$ (Fig. 1A). B chromosomes of the medium metacentric type were found in all the populations and C-banding

showed that they were entirely heterochromatic for the populations from the Água das Pedras, Água dos Patos and Taquari rivers (Fig. 1B), while those in specimens from the fish farm station were partially heterochromatic (Fig. 1C).

In the population from the Água dos Patos river, all 9 individuals had 1 B chromosome at a frequency of 18.1% and 2 Bs in 3.8% of the cells examined, with both inter- and intra-individual variation (Table 1). In specimens from the Água das Pedras river, all the individuals also had Bs, with a frequency of 21% with 1 B and 5.9% with 2 Bs (Table 1). In the Taquari river, only one individual was found without a B chromosome, while the frequency of 1 B was 25.73% and 13.45% for 2 Bs (Table 1). In the population of *R. quelen* from the fish farm station, 2 of the 4 individuals studied showed Bs, and the frequency of cells with 1 B was 23.8% and with 2 Bs, 11.11%, which was similar to the frequency found in the wild populations (Table 1).

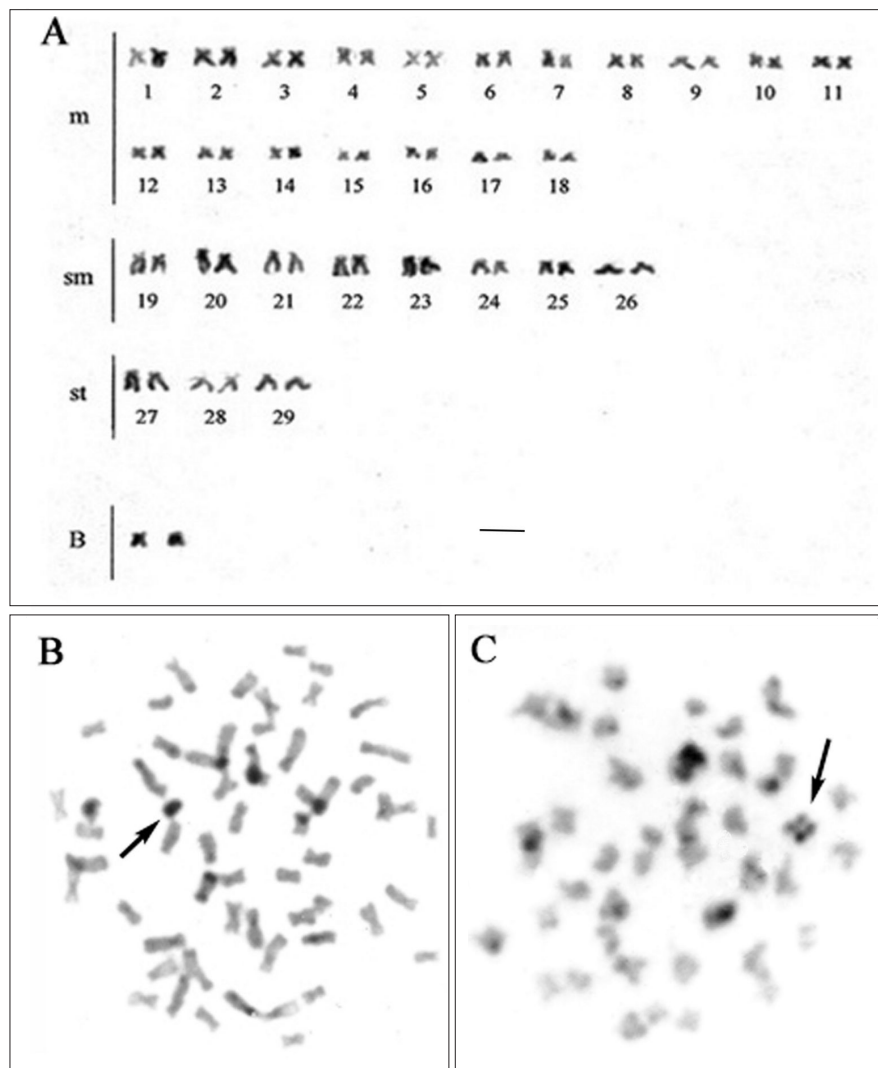


Fig. 1. (A) Karyotype of *Rhamdia quelen*; (B) and (C) Somatic metaphases of *Rhamdia quelen* showing B chromosomes completely and partially heterochromatic (arrows), respectively. Bar = 10 μ m.

Table 1
Frequency of B chromosomes in the somatic cells of *R. quelen* from Água dos Patos, Água das Pedras, Taquari rivers and from the fish farm station

Locality	Specimens	Sex	Number of B chromosomes			Total number of cells
			0	1	2	
Água dos Patos	2936/435	♀	24	2	0	26
	2937/436	♀	22	2	0	24
	2938/437	♀	12	1	0	13
	2939/438	♀	17	6	2	25
	3346	♂	24	4	1	29
	3347	♀	19	11	5	35
	3372	?	16	4	0	20
	3374	♂	16	4	0	20
	3375	♂	14	4	0	18
	Total		164	38	8	210
%		78.1	21	3.8		
Água das Pedras	2831	♀	25	3	1	29
	2845	♀	17	1	1	19
	2846	♀	17	16	5	38
	2847	♀	11	2	0	13
	431	♂	17	3	0	20
	Total		87	25	7	119
%		73.1	21	5.9		
Taquari	1959	♀	16	9	2	27
	1961	?	22	1	2	25
	2474	♀	19	0	0	19
	2804	♂	17	16	9	42
	2805	♂	11	7	1	19
	2911	♂	11	5	7	23
	3305	♀	8	6	2	16
	Total		104	44	23	171
%		60.82	25.73	13.45		
Fish farm station	211	–	17	0	2	19
	212	–	8	2	3	13
	213	–	4	13	2	19
	214	–	12	0	0	12
	Total		41	15	7	63
%		65.08	23.81	11.11		

Discussion

The results for *Rhamdia quelen* obtained in the present study reveal the same diploid number observed in other populations of *Rhamdia* studied by FENOCCHIO *et al.* (2003a,b), SWARÇA *et al.* (2003), STIVARI & MARTINS-SANTOS (2004), among others, as well as a fundamental number greater than 100.

B chromosomes of the medium metacentric type were found in all the populations studied here, but their number and frequency in each varied greatly. The presence of Bs in the genus *Rhamdia* is characteristic of the group, found in 72.98% of the dif-

ferent populations and/or species analyzed and in different types and sizes with intra- and inter-individual variation, thereby conferring a high rate of occurrence for these chromosomes in this genus (Table 2).

The inter- and intra-individual variation of B chromosomes in the populations examined here demonstrates the mitotic instability of this chromosome, probably due to its non-Mendelian behavior during cell division. All populations had at least one specimen in which the frequency of Bs was greater in relation to other individuals, i.e. individual number 3347 from the Água dos Patos river with a frequency of 45.71%; 2846 from the

Table 2

B chromosomes in the genus *Rhamdia*. 2n – diploid number; Bs – B chromosomes; m – metacentric; sm – submetacentric; a – acrocentric Rf. – references

Genus/Species	Locality	2n	Bs	Type/Size	Heterochromatin	Rf.
<i>R. branneri</i>	Iguaçu river/PR	58	0-4	m and sm, medium	partially	1
<i>R. hilarii</i>	Onça river / SP	58	–	–	–	2
<i>R. hilarii</i>	Monjolinho reservoir/ SP	58	0-5	m, small to medium	partially	3
<i>R.cf. hilarii</i>	Jurumirim reservoir/SP	58	0-3	m, small	totally	4
<i>R.cf. hilarii</i>	Quinta stream/SP	58	0-3	m, small	totally	4
<i>R.cf. hilarii</i>	Jacutinga stream/ SP	58	0-3	m, small	totally	4
<i>R.cf. hilarii</i>	Hortelã stream/ SP	58	0-3	m, small	totally	4
<i>R.cf. hilarii</i>	Araquá river/SP	58	0-3	m, small	totally	4
<i>R.cf. hilarii</i>	Pardo river/SP	58	0-3	m, small	totally	4
<i>R. hilarii</i>	Lobo reservoir /SP	58	0-3	m, medium	partially	5
<i>R. hilarii</i>	“29” reservoir / SP	58	0-5	m, medium	partially	5
<i>R. hilarii</i>	Mogi-Guaçu river/SP	58	–	–	–	5
<i>R. hilarii</i>	Aguapey river/Ar	58	–	–	–	5, 7
<i>R. hilarii</i>	São Francisco river/MG	58	2	m, medium	partially	5
<i>R. hilarii</i>	Mogi-guaçu river/SP	58	0-2	m, medium	partially	6
<i>R. quelen</i>	Guaíba river/RS	58	0-2	m, medium	totally	8
<i>R. quelen</i>	Mogi-Guaçu river/SP	58	4	m, medium	partially	5
<i>R. quelen</i>	Iguaçu river/SC	58	0-1	m, medium	partially	5
<i>R. quelen</i>	Paraná river/Ar	58	–	–	–	5, 7
<i>R. quelen</i>	Iguaçu river/PR	58	0-1	m	totally	9
<i>R. quelen</i>	Taquarussu river/PR	58	1-4	m, medium to large	partially	10
<i>R. quelen</i>	Maringá stream/PR	58	–	–	–	10
<i>R. quelen</i>	Bodoquena Plateau/ MS	58	1-2	m and sm, small	euchromatic	13
<i>R. quelen</i>	Água dos Patos river/SP	58	1-2	m, small	totally	14
<i>R. quelen</i>	Água das Pedras river/PR	58	1-2	m, small	totally	14
<i>R. quelen</i>	Taquari river/PR	58	1-2	m, small	totally	14
<i>R. quelen</i>	Fish farm station FUNPIVI/SC	58	1-2	m, small	partially	14
<i>R.sapo</i>	Buenos Aires/Ar	58	0-1	m and sm	–	11
<i>R.sp.</i>	Iguaçu river/PR	58	0-2	m, small	euchromatic	1
<i>R.sp.</i>	Grande stream/SP	58	0-4	m, small	totally	12
<i>R. voulezi</i>	Iguaçu river/PR	58	0-2	m	euchromatic	1

1) ABUCARMA & MARTINS-SANTOS (2001); 2) TOLEDO & FERRARI (1976); 3) FENOCCHIO & BERTOLLO (1990); 4) VISSOTTO *et al.* (1999); 5) FENOCCHIO *et al.* (2000); 6) MAISTRO *et al.* (2002); 7) FENOCCHIO *et al.* (2003a); 8) HOCHBERG & ERDTMANN (1988); 9) SWARÇA *et al.* (2003); 10) STIVARI & MARTINS-SANTOS (2004); 11) VALCARCEL *et al.* (1993); 12) GARCIA *et al.* (2003); 13) MORAES *et al.* (2007); 14) present study.

Água das Pedras river with 55.26%; 2804 from the Taquari river with 59.52% and 213 from the fish farm station with a frequency of 78.95%. This reinforces once again the former reports about the unstable behavior of this B chromosome.

Variation in the frequency of Bs among the populations, despite the difference in the number of specimens, may indicate population differentiation for *Rhamdia quelen*. The greatest frequency obtained was in the population from the Taquari

River, where 39.18% of the cells examined had 1 to 2 B chromosomes, followed by populations from the fish farm (34.92%), Água das Pedras River (26.9%) and lastly Água dos Patos River (21.9%).

CAMACHO *et al.* (2000) reported that the differences in the incidence of B chromosomes among populations depend on selection factors (such as the relation between Bs and environmental conditions including temperature and altitude), historical factors (such as the number of generations from

the origin of the Bs in the population or even in the species), transmission factors (related to mechanisms of accumulation) and random factors. These four types of factors likely act simultaneously, making it difficult to evaluate the action of each individually, even when conducting a more detailed study.

In 61.54% of the populations of *R. quelen* studied, including those of the present work, Bs were found to be entirely heterochromatic, and 30.77% were shown to be partially heterochromatic, these being the most common patterns for this species (Table 2). To date, only one population of *Rhamdia quelen* has been found to have euchromatic B chromosomes (MORAES *et al.* 2007).

In other species of the genus *Rhamdia*, the Bs are completely or partially heterochromatic, occurring as euchromatic chromosomes only in a few cases (Table 2), suggesting that these chromosomes are actually in distinct phases of their evolutionary process or, alternatively, that this process follows independent pathways in isolated populations.

Studying the occurrence of Bs in various species of the genus *Rhamdia*, FENOCCHIO *et al.* (2000) observed that many of the populations studied possessed B chromosomes which were similar in size, morphology, C-banding pattern, as well as irregular mitotic behavior, independent of the wide geographic distribution. This led the authors to suggest that the presence of these chromosomes indicates that they could have originated from a common ancestor, justifying their occurrence in distant or even isolated populations, and that this could have occurred before the differentiation and distribution of this species.

The finding of Bs in four different populations of *R. quelen*, in which three were from the same basin (Parapanema river) and one from a fish farm, reinforces the hypothesis proposed by FENOCCHIO *et al.* (2000), indicating that these B chromosomes arose from a common ancestor but are dispersed in different populations and undergo differentiation independently. The occurrence of Bs in the genus *Rhamdia*, as cited previously, is characteristic of this group of fishes, being an integral part of their evolutionary process.

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