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Cytogenetic characterization of *Rhamdia quelen* (Siluriformes, Heptapteridae) from the Bodoquena Plateau, Mato Grosso do Sul, Brazil

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ABSTRACT. We made a cytogenetic study of the fish *Rhamdia quelen* collected from the Bodoquena Plateau, an isolated national park region in Mato Grosso do Sul State, Brazil. The diploid number was 2n = 58, with 36 metacentric + 16 submetacentric + 6 subtelocentric chromosomes. We found one to three B chromosomes, which were metacentric and submetacentric and of medium size, showing both intra- and interindividual variation. The nucleolus organizer region (NOR) was located in the terminal region of the short arm of submetacentric pair 20. Staining with CMA_3 fluorochrome revealed the NOR location, while there was no evidence of fluorescent staining with DAPI. C banding revealed heterochromatin mainly in the terminal regions of the chromosome arms, including the NOR pair. In addition, metacentric pair 2 showed three heterochromatic blocks in the terminal portions and in the pericentromeric region. The B chromosomes appeared euchromatic. The CB + CMA_3 staining combination demonstrated only one chromosome pair with fluorescence, probably the NOR-bearing one, while CB + DAPI gave various fluorescent signals, including metacentric pair 2, indicating that these heterochromatic regions are AT-rich in this population of

R. quelen. The *R. quelen* population in this isolated region of Brazil is chromosomally distinct from that of other populations that have been studied.

Key words: B chromosomes, C banding, Ag-NOR, Chromomycin A_3, DAPI

INTRODUCTION

The Bodoquena Plateau is located in the south-central State of Mato Grosso do Sul (MS) in Brazil, in the proximity of the municipalities of Bonito, Bodoquena, Jardim, and Porto Murtinho (Boggiani, 1999). This region has an area of 76,400 hectares and a broad diversity of species, both in flora and fauna; it was designated a national park on September 20, 2001. This region has various rivers, the main ones being Formoso, Prata, Salobra, and Perdido Rivers, all of them with their headwaters in the Bodoquena national park (Boggiani, 1999). Willink et al., in 2000 (*apud* Cereali, 2006), estimated at least 325 species of fish in this park, reporting that the ichthyofauna of Bodoquena Plateau appeared to be distinctive, with many endemic species. Despite its rich ichthyofauna, cytogenetic studies of the fishes of this region are extremely rare, being limited to only three species of the genus *Hypostomus*: *H. cochliodon*, *Hypostomus* sp 2 - Rio Perdido NUP 4249 and *Hypostomus* sp 3 - Córrego Salobrinha NUP 4247, collected in the Salobra and Perdido Rivers and studied by Cereali (2006). We made a chromosome analysis of specimens of *Rhamdia quelen* from the Bodoquena Plateau, MS.

MATERIAL AND METHODS

We analyzed four specimens of *R. quelen* (two males and two females) collected from Harmonia farm well water, in Porto Murtinho, MS (21° 12' 55" S, 56° 45' 50" W), in Bodoquena Plateau region. A voucher specimen was placed in the fish collection of the Coleção Zoológica de Referência da Universidade Federal do Mato Grosso do Sul, Brazil, under the number ZUFMS 2039. Mitotic chromosome preparations were obtained from kidney cells (Bertollo et al., 1978). Karyotypic analysis was made by conventional staining with Giemsa, and the chromosomes were classified as metacentric (m), submetacentric (sm) or subtelocentric (st), based on Levan et al. (1964), all being considered to have two arms to calculate the fundamental number (FN). Silver nitrate, nucleolus organizer regions (Ag-NORs) and the heterocromatin distribution pattern were evidenced by the techniques of Howell and Black (1980) and Sumner (1972), respectively. Marking with the fluorochromes chromomycin A_3 (CMA_3) and DAPI was done according to Schmid (1980) and Schweizer (1976), respectively.

RESULTS

The specimens had a diploid number of 2n = 58, with a karyotypic formula of 36 m + 16 sm + 6 st and an FN of 116 (Figure 1A); one to three supernumerary or B chromosomes were observed, with both intra- and interindividual variation (Table 1). The B chromosomes were of medium size and were metacentric and submetacentric (Figure 1A).

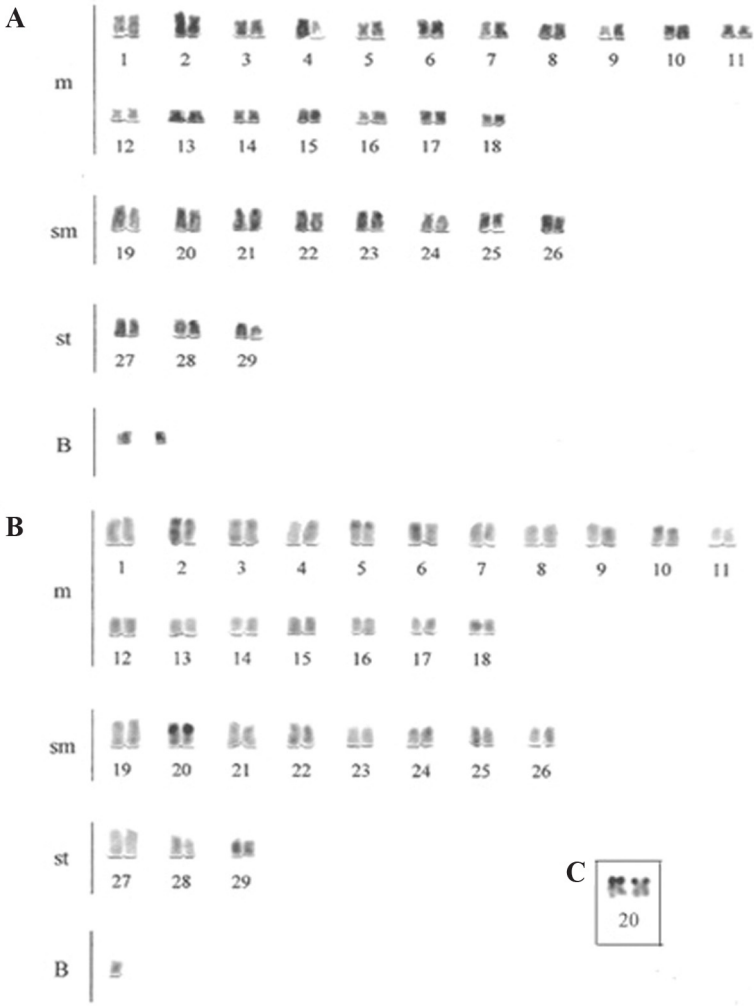

Figure 1. Karyotype of *Rhamdia quelen*. **A.** Giemsa staining. **B.** C banding. **C.** NOR-bearing pair. m = metacentric; sm = submetacentric; st = subtelocentric; B = B chromosomes.

Table 1. B chromosome frequency in somatic cells of *Rhamdia quelen* (Siluriformes, Heptapteridae) from Bodoquena Plateau.

Specimens	Sex	Number of B chromosomes				Total number of cells
		0	1	2	3	
43	♂	10	7	6	2	25
44	♀	3	7	5	4	19
45	♀	11	4	0	0	15
46	♂	6	13	0	0	19
Total		30	31	11	6	78
(%)		38.46%	39.74%	14.1%	7.7%	100%

Heterochromatin was distributed in the terminal regions of almost all the chromosomes, with some chromosomes showing terminal staining in both arms (Figure 1B). Metacentric pair 2 showed, besides terminal heterochromatic blocks on both arms, a block in the pericentromeric region; a difference was observed between the homologs, as one of them had much more evident heterochromatin (Figure 1B). Pair 20 had strong heterochromatic staining in the terminal region of the short arm and the B chromosomes appeared euchromatic (Figure 1B).

Ag impregnation revealed the NOR in the terminal region of the short arm of chromosome pair 20 (Figure 1C). CMA_3 fluorochrome gave positive staining in pair 20, corresponding to the Ag-NOR (Figure 2a), and DAPI fluorochrome gave no staining (Figure 2b). Only pair 20 stained with the combination of C-banding stain (CB) + CMA_3 (Figure 2c), while various chromosomes displayed fluorescent terminal regions with CB + DAPI, especially a large metacentric pair, probably pair 2 (Figure 2d).

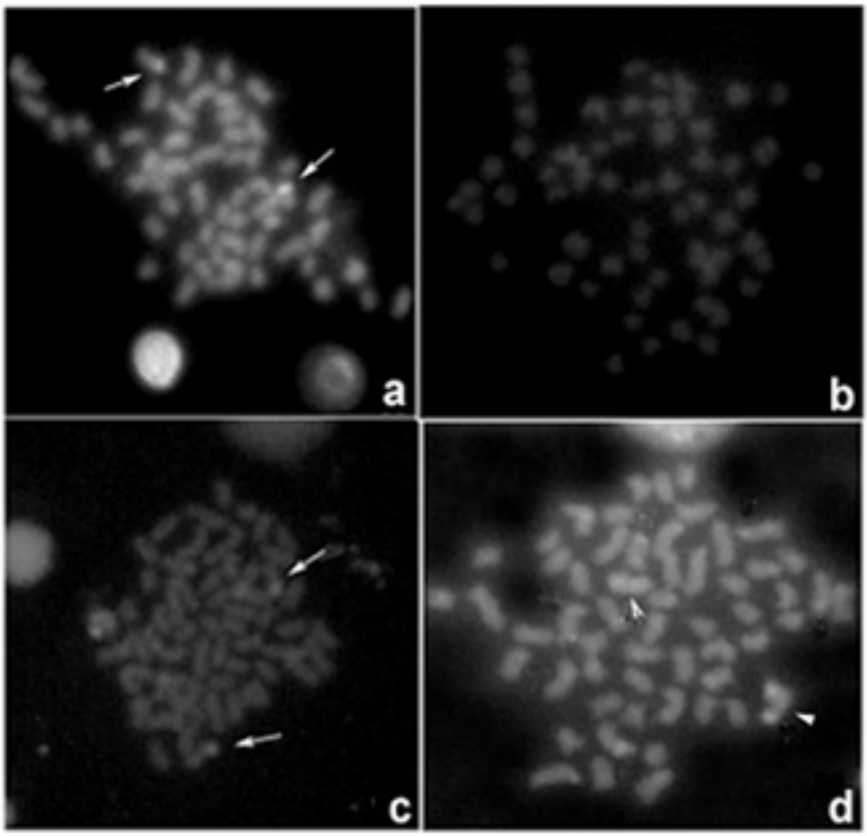

Figure 2. Somatic chromosome metaphases of *Rhamdia quelen* with: **a.** CMA_3. **b.** DAPI. **c.** CB + CMA_3. **d.** CB + DAPI staining. Arrows indicate the NOR-bearing pair. Arrowheads indicate the pair 2.

DISCUSSION

The specimens of *R. quelen* from the region of the Bodoquena Plateau, MS, had the 2n chromosome number characteristic for this genus (Fenocchio et al., 2000; Garcia et al., 2003;

Stivari and Martins-Santos, 2004). This number has been observed in most of the species of this genus, along with a high FN, greater than 100, which is also characteristic of *Rhamdia* spp, due to the 2-arm chromosomes. However, the karyotypic formula varies among the populations studied; the 36 m + 16 sm + 6 st that we observed differs from that of all other *R. quelen* populations. However, there are differences in classification among authors, since the chromosomes are very small in this genus.

The four specimens that we examined had, besides a normal chromosome complement, one to three B chromosomes with both intra- and interindividual variation (Table 1). Two of these individuals (45 ♀ and 46 ♂) had only one medium metacentric type B chromosome, while the other two (43 ♂ and 44 ♀) had one to three, these being metacentric and submetacentric and medium size. The frequency with which these chromosomes occurred among these individuals was very high. In individuals 43, 44 and 46, the proportion of cells with B chromosomes was greater than that of cells without this type of chromosome; 61.54% of the cells examined contained B chromosomes. The variation in the number of B chromosomes observed within and among individuals demonstrates mitotic instability, which must be a consequence of non-Mendelian segregation; this is a characteristic of these chromosomes.

The type of B chromosome most frequently found in *R. quelen* is the medium metacentric type (Hochberg and Erdtmann, 1988; Fenocchio et al., 2000; Swarça et al., 2003), but there are reports of submetacentric and acrocentric B chromosomes of small and medium size (Guilherme, 2005). The largest number and the types of B chromosomes found in *R. quelen* were described by Guilherme (2005) for a population from the Uberabinha River in Minas Gerais State, which had up to seven B chromosomes of the metacentric, submetacentric and acrocentric types that were small- to medium-sized.

According to Fenocchio et al. (2000), various species and populations of *Rhamdia* have B chromosomes, despite their wide geographic distribution, indicating an ancestral origin for this chromosome. The finding of B chromosomes in *R. quelen* from the Bodoquena Plateau, MS, in an isolated region, again indicates that this type of chromosome is characteristic of the genus *Rhamdia*, also supporting its origin from a common ancestor.

The B chromosomes of this population, examined by C banding, were found to be euchromatic, which is the first report of such a finding for *R. quelen*. This finding differs from that of other populations of this species, which had totally or partially heterochromatic B chromosomes (Fenocchio et al., 2000; Swarça et al., 2003; Stivari and Martins-Santos, 2004). The only other finding of euchromatic B chromosomes in the genus *Rhamdia* was reported by Abucarma and Martins-Santos (2001) in two other species, *Rhamdia* sp and *R. voulezi*, from the Iguaçu River, collected from the power plant reservoir of Usina Hidrelétrica de Salto Segredo in Guarapuava, Paraná State. It appears that the difference between the *R. quelen* population in our study compared to other populations is the process of heterochromatinization of the B chromosomes, which has been occurring more slowly in the population from Bodoquena Plateau, since it is located in a hydrographic system that is isolated from other groups.

The heterochromatin distribution pattern in the normal chromosome complement for this population of *R. quelen* differed from that found by others, since the pattern most commonly seen in this species is limited to weak staining in some terminal regions of one of the chromosome arms and in some centromeres (Fenocchio et al., 2003b; Swarça et al., 2003), while heterochromatin

in the population from Bodoquena Plateau was found in the terminal region of both chromosome arms. Pair 2 (m) showed three heterochromatic blocks, two in the terminal portion of the chromosome arms and one block in the pericentromeric region, where one of the homologs of this pair had more evident bands. This type of heterochromatin distribution in one chromosome pair has not been found in any of the previously studied populations, suggesting thus that besides the B chromosomes, the specimens from the Bodoquena Plateau have a marker pair (metacentric pair 2), which in addition to the euchromatic B chromosomes, would differentiate this population from others.

The C banding also showed more evident heterochromatic staining in the terminal portion of the short arm of chromosome pair 20 (sm) than in the rest of the chromosomes. Ag impregnation did not show this chromosome to be NOR-bearing, which agrees with CMA_3 fluorochrome-staining evidence. Therefore, besides being heterochromatic, this region is rich in GC base pairs. This coincidence of NOR with heterochromatin was observed earlier by Fenocchio et al. (2000) and by Swarça et al. (2003), as well as the fact that NORs are GC-rich (Swarça et al., 2003; Stivari and Martins-Santos, 2004; Tsuda, 2005). After treatment with DAPI, the chromosomes were uniformly stained. None of the chromosomes showed regions rich in AT base pairs. Swarça et al. (2003) examined specimens of *R. quelen* from the Iguaçu River and obtained the same result as we obtained with DAPI, observing that the NOR was slightly paler.

The localization of the NOR in submetacentric chromosomes that we found in our specimens has previously been reported; NORs were found to be more frequent in submetacentric and in subtelocentric chromosomes (Fenocchio et al., 2000; Garcia et al., 2003; Stivari and Martins-Santos, 2004). NORs in acrocentric chromosomes have been observed infrequently (Hochberg and Erdtmann, 1988; Fenocchio et al., 2003a); this is also the case for metacentric chromosomes, recently described by Guilherme (2005) for *R. quelen* from the Uberabinha River, Minas Gerais State. This variation in the NOR-bearing pair is again an indication of chromosome rearrangements.

Based on CB + CMA_3 staining, only the heterochromatin associated with NOR is GC-rich; CB + DAPI revealed a large metacentric chromosome, which was probably pair 2, with a very strongly fluorescent signal, showing that the heterochromatin in one of the homologs of this pair is rich in AT bases. This finding reinforces the notion that this type of chromosome can be a marker for this population. In addition to this pair, other fluorescent regions were found. It is believed that pre-treatment with C banding relaxes DNA and increases accessibility of fluorochromes (Swarça et al., 2003), allowing a more detailed analysis of heterochromatin composition. This would explain why CB + DAPI gave different results compared to simple treatment with this fluorochrome.

Swarça et al. (2003) observed various CB + CMA_3 staining patterns in the terminal regions, besides the NOR in *R. quelen* from the Iguaçu River, Paraná State. Using CB + DAPI, they also found evidence of various terminal staining patterns; the heterochromatin patterns in their population were different from what we found.

This is the first cytogenetic description of *R. quelen* from the Bodoquena Plateau. Some of the findings, the occurrence of euchromatic B chromosomes, distributions and composition of heterochromatin on chromosomes of the normal complement, mainly in the metacentric pair 2, are characteristic of the fish in this isolated region, since they have not been reported for other populations. These factors indicate population differentiation, suggesting an intrinsic type of *R. quelen* in Bodoquena Plateau.

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REFERENCES

Abucarma M and Martins-Santos IC (2001). Karyotype and B chromosome of *Rhamdia* species (Pisces, Pimelodidae) endemic in the river Iguaçu basin. *Cytologia* 66: 299-306.

Bertollo LAC, Takahashi CS and Moreira-Filho O (1978). Cytotaxonomic considerations on *Hoplias lacerdae* (Pisces, Erythrinidae). *Braz. J. Genet*. 1: 103-120.

Boggiani PC (1999). Por que Bonito é bonito? In: Nos jardins submersos da Bodoquena (Scremin-Dias E, Pott VJ, Hora RC and Souza PR, eds.). Universidade Federal de Mato Grosso do Sul, Campo Grande, 10-23.

Cereali SS (2006). Estudos citogenéticos de Loricariidae (Siluriformes) do Planalto da Bodoquena - Mato Grosso do Sul. M.Sc. thesis, Universidade Estadual de Londrina, Londrina.

Fenocchio AS, Bertollo LA, Takahashi CS and Camacho JP (2000). B chromosomes in two fish species, genus *Rhamdia* (Siluriformes, Pimelodidae). *Folia Biol*. 48: 105-109.

Fenocchio AS, Bertollo LAC, Takahashi CS, Dias AL, et al. (2003a). Cytogenetic studies and correlate considerations on Rhamdiinae relationships (Pisces, Siluroidei, Pimelodidae). *Cytologia* 68: 363-368.

Fenocchio AS, Swarca AC, Cestari MM and Dias AL (2003b). Karyotypic characterization and NOR analysis by different banding techniques of *Rhamdia quelen* (Pisces, Pimelodidae) from the first plateau of the Iguacu River (Brazil). *Folia Biol*. 51: 219-222.

Garcia C, Moreira-Filho O, Bertollo LAC and Centofante L (2003). B chromosomes and natural triploidy in *Rhamdia* sp. (Pisces, Siluriformes, Heptapteridae). *Cytologia* 68: 403-411.

Guilherme LC (2005). Estudos reprodutivos e citogenéticos na população de *Rhamdia quelen* (Pisces, Rhamdiidae) do rio Uberabinha no município de Uberlândia - MG e desenvolvimento de sistema artesanal de recirculação d'água para criação de peixes. Ph.D. thesis, Universidade Federal de Uberlândia, Uberlândia.

Hochberg VBM and Erdtmann B (1988). Cytogenetical and morphological considerations on *Rhamdia quelen* (Pisces, Pimelodidae) - The occurrence of B chromosomes and polymorphic NOR regions. *Rev. Bras. Genet*. 11: 563-576.

Howell WM and Black DA (1980). Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. *Experientia* 36: 1014-1015.

Levan A, Fredga K and Sandberg AA (1964). Nomenclature for centromeric position on chromosomes. *Hereditas* 52: 201-220.

Schmid M (1980). Chromosome banding in amphibia. IV. Differentiation of GC- and AT-rich chromosome regions in Anura. *Chromosoma* 77: 83-103.

Schweizer D (1976). Reverse fluorescent chromosome banding with chromomycin and DAPI. *Chromosoma* 58: 307-324.

Stivari MK and Martins-Santos IC (2004). Karyotype diversity in two populations of *Rhamdia quelen* (Pisces, Heptapteridade). *Cytologia* 69: 25-34.

Sumner AT (1972). A simple technique for demonstrating centromeric heterochromatin. *Exp. Cell Res*. 75: 304-306.

Swarça AC, Fenocchio AS, Cestari MM and Dias AL (2003). Analysis of heterochromatin by combination of C-banding and CMA3 and DAPI staining in two fish species (Pimelodidae, Siluriformes). *Genetica* 119: 87-92.

Tsuda JR (2005). Análise citogenética em *Rhamdia quelen* (Pisces, Heptapteridae) do ribeirão Lindóia: ocorrência de triploidia natural. Bachelor's Monography, Universidade Estadual de Londrina, Londrina.