

First Cytogenetic Description of the Species *Rhamdella microcephala* (Pisces, Hepapteridae)

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Summary Cytogenetic studies were carried out on *Rhamdella microcephala* collected from the headwaters of the Machado river, a tributary of the Sapucaí river, Minas Gerais, Brazil. Sixteen specimens exhibited $2n=56$ chromosomes, with 18 metacentrics, 30 submetacentrics and 8 subtelocentric-acrocentrics (FN=84). The nucleolar organizing regions (NORs) were identified in the interstitial position on the long arm of a submetacentric pair (pair 12). Chromomycin A₃ staining evidenced only the NOR-bearing segments. Positive C-band segments were identified in a pericentromeric position in most of the chromosomes and the NOR-bearing segments were also C-band positive. Some aspects related to the chromosomal characteristics of *Rhamdella microcephala* are discussed.

Key words *Rhamdella microcephala*, Cytogenetics, C-band, NOR, Hepapteridae.

According to Nelson (1994), the order Siluriformes is a group of fish with about 2400 species distributed among 34 families and 412 genera. Although several taxonomic revisions of Siluriformes families have appeared, the systematics of the groups that constitute this order is still unresolved (de Pinna 1998).

The cytogenetic data available to the Hepapteridae family are scarce but members of this groups exhibit an extensive karyotypic variability (Oliveira and Gosztonyi 2000). For the genus *Rhamdella*, only 1 species, *Rhamdella* sp. from Bahia state, Brazil, was cytogenetically studied (Souza *et al.* 1994) and the results obtained showed that it had $2n=56$ chromosomes (26M-SM+30ST-A). The objective of the present paper is to describe for the first time the karyotype, the number and location of the nucleolus organizer regions, the pattern of distribution of C-band positive segments, and the pattern of chromomycin A₃ (CMA₃) staining of *Rhamdella microcephala*. The data were compared with others available for the family Hepapteridae in order to achieve a better understanding of the chromosomal evolution of this family.

Materials and methods

A cytogenetic survey was performed on 16 specimens (not sexed) of *Rhamdella microcephala* collected from the Machado river, a tributary of the Sapucaí river which passes through the town of São João da Mata, state of Minas Gerais, Brazil (S 22°04.471'–W 46°02.810'). The fishes were identified and are deposited in the fish collection of the Laboratório de Pesquisas em Genética, UNIFENAS, Brazil.

Mitotic cells were obtained from gill and kidney tissues by the technique described by Foresti *et al.* (1993). Chromosome morphology was determined on the basis of arm ratios as proposed by Levan *et al.* (1964) and the chromosomes were classified as metacentrics (M), submetacentrics

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(SM), submetacentrics (ST) and acrocentrics (A). The fundamental number (FN) was determined considering M and SM chromosomes with 2 arms and ST and A chromosomes with 1 arm. C-banding was performed by the method of Sumner (1972) and silver staining of nucleolus organizer regions (NORs) by the method of Howell and Black (1980). Chromomycin A₃ staining was performed by the method of Schweizer (1980).

Results and discussion

The diploid number found in *R. microcephala* was $2n=56$ (18M+30SM+8ST-A) with FN=84 (Fig. 1). Only 1 species of this genus, *Rhamdella* sp. from Itaetê town, Bahia, Brazil, has been cytogenetically studied and presented $2n=56$ chromosomes (26M-SM+30ST-A) and FN=82 (Souza *et al.* 1994). A diploid number of $2n=56$ is the most frequent among Hepapteridae species and may be the ancestor diploid number for the order Siluriformes (Oliveira and Gosztanyi 2000).

In *Rhamdella microcephala* the NORs were found in an interstitial position on the long arm of a large submetacentric pair (pair 12) and exhibited a large size heteromorphism in some individuals (Fig. 3a). This heteromorphism has been commonly described among Neotropical fishes (Almeida-Toledo and Foresti 1985). Considering that the presence of only 1 NOR-bearing chromosome pair with terminal NORs is the most common feature found among teleost fishes (Almeida-Toledo and Foresti 1985, Klinkhardt 1998), the occurrence of the NORs located in an interstitial position on the chromosomes of *R. microcephala* and of some other species of Siluriformes could be a derived trait for the species that have them (Oliveira and Gosztanyi 2000).



Fig. 1. Karyotype of *Rhamdella microcephala* collected from the Machado river headwaters after Giemsa staining.

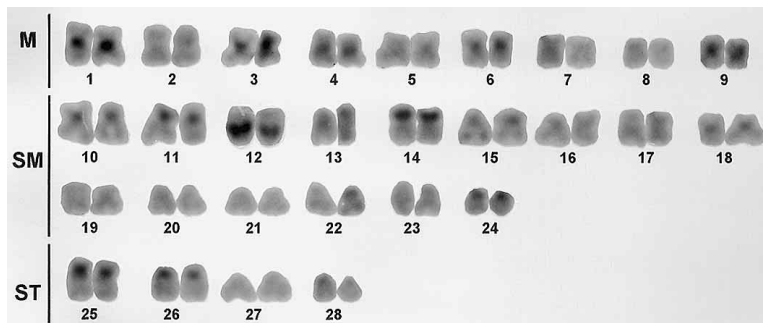


Fig. 2. Karyotype of *Rhamdella microcephala* collected from the Machado river headwaters after C-banding.

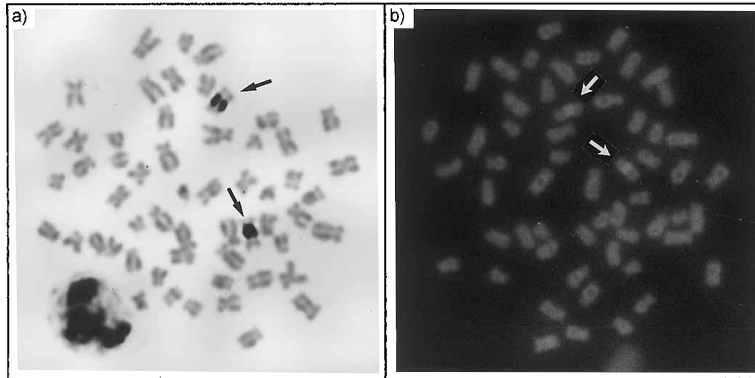


Fig. 3. Metaphase chromosomes of *Rhamdella microcephala*. a) Ag-NOR-bearing chromosomes (arrows) and b) CMA₃ staining (arrows).

Chromomycin A₃ is considered to be a GC-specific fluorochrome (Schweizer 1980) that yields fluorescent bands at NOR sites of the chromosomes of lower vertebrates, including fish (Amemiya and Gold 1986, Galetti-Jr. and Rasch 1993, Maistro *et al.* 2000, 2001, among others). In *R. microcephala* only the NOR-bearing chromosome pair exhibited a bright signal after CMA₃ staining (Fig. 3b), indicating that the NOR region may contain NOR-associated heterochromatin rich in GC base pairs. On the other hand, these data suggest that the other C-band-positive segments found in this species are not rich in GC base pairs. This characteristic has been commonly found in several fish species (Amemiya and Gold 1986, Maistro *et al.* 2000, Swarça *et al.* 2001, among others).

In *R. microcephala*, C-banding showed the occurrence of heterochromatin in the pericentromeric region of most chromosomes, more clearly visible on pairs 1, 3, 4, 6, 9, 11, 24, 25 and 26 (Fig. 2). The NOR segment and the entire short arm of pair 14 was also found to be C-band positive. Although a small amount of heterochromatin has been commonly described among Hepapteridae species and many other teleost species (Gold *et al.* 1990), this was not observed in *R. microcephala*.

The data obtained in the present study indicate that, besides the conserved diploid number ($2n=56$), rearrangements in the karyotypes, such as changes in the number and position of the NORs and in the among of heterochromatin, might be important mechanisms of chromosome diversification in some Hepapteridae species. The occurrence and fixation of these rearrangements might be facilitated by fact that some species of *R. microcephala* live in the headwaters of small streams, rivers in mountain regions or small rivers in caverns isolated from other populations. The association of rapid changes in karyotypic features and population structure has been suggested for several fish species like *Astyanax scabripinnis* (Moreira-Filho and Bertollo 1991, Maistro *et al.* 1998) and the genus *Trichomycterus* (Torres *et al.* 1998).

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