Cytogenetic Studies and Correlate Considerations on Rhamdiinae Relationships (Pisces, Siluroidei, Pimelodidae)

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Summary In Pimelodidae fish the available chromosome numbers range from 2n=46 to 2n=63, the value 2n=56 being the most frequent one. In the present paper 6 species belonging to the Rhamdiinae subfamily were analyzed: Rhamdia hilarii, R. quelen, Cetopsorhamdia sp., C. iheringhi, Imparfinis cf. piperatus and Imparfinis aff. schubarti. Chromosome preparations were obtained by direct and short term culture methods from kidney cells and analysed under standard Giemsa staining, C-banding and NOR silver staining. Rhamdia species present a basic karyotype composed by 58 chromosomes and the NORs are located on the short arm of a subtelocentric chromosome pair. Cetopsorhamdia sp., C. iheringhi and Imparfinis aff. schubarti also shows 2n=58, while I. cf. piperatus is the only analyzed species presenting 2n=56. However, these 4 species show an interstitial NOR location. These chromosomal data agree with the occurrence of subgroups in the Rhamdiinae subfamily, proposed on the basis of their morphological traits.

Key words Fish cytogenetics, Cytotaxonomy, Rhamdiinae, Siluriformes.

The Pimelodidae fish family comprises upper 300 neotropical species, showing a wide variability in size and shape. Their systematics and taxonomy need a reanalysis and a better definition in several cases because phylogenetic relationships remains obscure being suggested some internal sub-divisions, i.e. Heptapteridae family, including a number of genera of the classical pimelodids (de Pinna 1998). There are more than fifty Pimelodidae nominal genera and several undescribed species and, at least, 4 major groups can be considered into this family (Lundberg et al. 1988, 1991). Karyotypic data about 40 species, mainly from the Pimelodinae and Sorubiminae subfamilies, are available (Toledo and Ferrari 1976, LeGrande 1981, Hochberg and Erdtmann 1988, Oliveira et al. 1988, Fenocchio and Bertollo 1990, 1992, Dias and Foresti 1993, Martins-Santos et al. 1996, Abucarma and Martins-Santos 2001, Swarça et al. 2000, Vissotto et al. 2001). The diploid number ranges from 2n=46 (Pimelodella sp.) to 2n=63 (Rhamdia hilarii), the value 2n=56 being the most frequent one.

About 26 genera can be found in Rhamdiinae, sharing a number of synapomorphies (Lundberg et al. 1991). However, some subgroups can also be considered into this subfamily on the basis of morphological traits (Lundberg et al. 1991). In this study are presented some karyotypic data of 6 Rhamdiinae species and their probable evolutionary relationships in this fish subfamily.
Materials and methods

Six species, belonging to three genera of Rhamdiinae (*sensu* Lundberg et al. 1991), were studied: *Rhamdia hilarii* (2 females and 2 males from Aguapey river, Corrientes, Argentina), *R. quelen* (3 females from Paraná river, Posadas, Argentina), *Cetopsorhamdia iheringhi* (3 unsexed specimens, 2 from São Francisco river, Três Marias, Minas Gerais, and 1 from Rio das Marrecas, Paraná, Brasil), *Cetopsorhamdia* sp. (2 females and 1 male from Canta Galo stream, Itirapina, São Paulo, Brasil); *Imparfinis aff. schubarti*. (1 female and 4 males from Canta Galo stream, Itirapina, São Paulo, and 2 females and 2 males from Ribeirão Três Bocas, Paraná, Brasil) and *I. cf. piperatus* (1 female and 2 males from Juquiá river, Carlos Botelho, São Paulo, Brasil).

Mitotic preparations were obtained from kidney cells by direct and culture cell methods (Bertollo et al. 1978 and Fenocchio et al. 1991). Nucleolar organizer regions (NORs) were identified by a silver staining method, according to Howell and Black (1980).

The chromosomes were grouped into metacentrics (M), submetacentrics (SM), subtelocentrics (ST) and acrocentrics (A) according arm ratios and arranged in decreasing order size in the karyotypes.

Results and discussion

The populations of *Rhamdia hilarii* and *R. quelen* here analyzed present a basic karyotype composed by 2n=58 chromosomes, being 26 M, 16 SM, 8 ST and 8 A (NF=108) (Fig. 1A, B). However, some reports show slight variations around this karyotypic formula, probably due to differences in chromosome condensation. On the other hand, both species can present a B chromosome system (Hochberg and Erdtmann 1988, Fenocchio and Bertollo 1990), ranging the diploid number until 2n=63 in *Rhamdia hilarii* (Fenocchio and Bertollo 1990, Fenocchio et al. 2000), but in this case species analyzed have not show these chromosomes. NORs are located on an acrocen-
tric chromosome pair, in a terminal position on the short arm. These regions can show a strong size heteromorphism and frequent chromosomal associations.

*Cetopsorhamdia iheringhi* presents $2n=58$, with a karyotype composed by 11 M, 8 SM, 5 ST and 5 A chromosome pairs. NORs are located in an interstitial position on the long arm of a ST chromosome (Fig. 2A).

*Cetopsorhamdia* sp. shows $2n=58$ chromosomes, distributed among 11 M, 8 SM, 5 ST and 5 A pairs. NORs are interstitially located on the long arm of the first M chromosome (Fig. 2B).

*Imparfinis* aff. *schubarti*. also presents $2n=58$ and a karyotype composed by 11 M, 9 SM, 5 ST and 4 A chromosome pairs. NORs are located on the long arm of the first M chromosome, on a

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**Fig. 2.** Karyotypes and nucleolar organizer regions (Ag-NORs) of the Rhamdiinae species studied. A) *Cetopsorhamdia iheringhi*; B) *Cetopsorhamdia* sp.; C) *Imparfinis* aff. *schubarti*; D. *Imparfinis* cf. *piperatus*. In the boxes NOR bearing chromosomes.
secondary constriction that frequently appear extremely decondensed (Fig. 2C).

*Imparfinis cf. piperatus* is the only species showing 2n=56 chromosomes, with a karyotype entirely composed by biarmed chromosomes: 12 M, 6 SM and 10 ST pairs. NORs are also located in an interstitial position on the long arm of a ST chromosome (Fig. 2D).

In view of the present results and other available data it is evident that Rhamdiinae species share some general karyotypic features, *i.e.*, 2n=58 chromosomes, a fundamental number (number of chromosomal arms) beyond to 100, only one pair of NORs (Toledo and Ferrari 1976, LeGrande 1981, Hochberg and Erdmann 1988, Fenocchio and Bertollo 1990, Abucarma and Martins-Santos 2001, Vissoto et al. 2001). Two exceptions could be seen, the first is represented by *Imparfinis cf. piperatus* that shows 2n=56, as well as the absence of acrocentric chromosomes which occur in the other species, indicating that this karyotype is a derivative one. The second is *Heptapterus longicauda* with only 52 chromosomes and single terminal NORs.

Lundberg *et al.* (1991) suggested that some species could constitute subgroups within the Rhamdiinae subfamily (Group B), on the basis of their synapomorphies, occupying different branches in the proposed phylogenetic tree. *Rhamdia* and some other related genera are seen on the same branch of the proposed cladogram, while *Cetopsorhamdia* and *Imparfinis* occupy another one, together with other genera (Fig. 3). The branch including Rhamdia shows other seven nominal genera but is only partly resolved. Likewise, in the other, (Lundberg *et al.* 1991) included *Heptapterus*, but
some morphological evidences suggest that this genus represents an independent basal taxon, originating the family Heptapteridae (de Pinna 1998).

In accordance, cytogenetic data can also support this proposition. Thus, independently of the diploid numbers, NORs allow to set apart these 2 fish groups. Indeed, all *Rhamdia* species/populations and related genera studied thus far show a single NOR pair located in a terminal position. In opposition, *Cetopsorhamdia* and *Imparfinis* species show these regions in an interstitial position on the chromosomes (Hochberg and Erdtmann 1988, Fenocchio and Bertollo 1990, Fenocchio *et al.* 2000, Abucarma and Martins-Santos 2001, Vissotto *et al.* 1999, 2001).

It is possible to observe that the species included by Lundberg *et al.* (1988) on the basis of morphological characters into the Group C had 2n=54 (*Lophiosilurus*, *Microglanis*) and almost all the cytogenetically studied species of the Group A 56 chromosomes (Swarça *et al.* 2000).

The present study makes clear that cytogenetic data can corroborates with the morphological ones in phylogenetic hypothesis offering additional support. However, although these data represent and interesting approach, specially in a very specious family as Pimelodidae it is necessary to study a larger number of species in each group in order to confirm the observations now reported.

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**References**


