Cytogenetic Studies in Some *Apareiodon* Species (Pisces, Parodontidae)

Célia Maria de Jesus* and Orlando Moreira-Filho

Departamento de Genética e Evolução, Universidade Federal de São Carlos Rodovia Washington Luis,
km 235, Caixa Postal 676, CEP 13565–905, São Carlos, SP, Brasil

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Summary Cytogenetic studies in 6 species of *Apareiodon* (Pisces, Parodontidae) from different Brazilian hydrographic basins showed a diploid number equal to $2n=54$ chromosomes and the absence of morphologically differentiated sex chromosomes. Although the diploid and the fundamental numbers had been constant, some differences were observed concerning the karyotypic structure of the species. *A. ibitiensis*, *Apareiodon* sp. A and *Apareiodon* sp. B presented 50M/SM+4ST chromosomes, while *A. piracicabae*, *A. vittatus* and *Apareiodon* sp. C presented 52M/SM+2ST ones. *Apareiodon piracicabae* showed a polymorphism in relation to the number and position of the nucleolar organizer regions (NORs), while *A. vittatus* showed a variation in the NORs size. The available data indicate that the Parodontidae family have been submitted to a chromosomal diversification during their species differentiation process, despite the maintenance of the same diploid number.

Key words *Apareiodon*, Karyotypic evolution, NOR, C-band.

The Characiformes fish are widely distributed in the neotropical region, showing a wide variation in the diploid number. Two general trends in the chromosomal evolution can be observed in this order. In fact, some groups show a heterogeneous evolutionary with a diversity in the chromosome number and the karyotypic structure. In contrast, a more homogenous pattern can be observed in other groups, leading to a relatively stable karyotypes. Thus, Prochilodontidae, Anostomidae, Hemiodontidae, Curimatidae and Parodontidae families, have shown few or none variation in respect to the diploid number and the macrokaryotypic structure. On the other hand, Erythrinidae and Characidae have shown a wide numerical and structural variation in their karyotypes (Galetti Jr. *et al.* 1994). In the present work, 6 species of the *Apareiodon* genus were studied, with the description and discussion of their karyotypic patterns.

Materials and methods

Chromosome analyses were performed in six species of the *Apareiodon* genus from different Brazilian hydrographic basins. Some species are still awaiting a more precise identification and were temporarily designated as A, B and C. *Apareiodon ibitiensis* was collected in the Passa-Cinco river, and *A. piracicabae* in the Passa-Cinco and Mogi-Guaçu rivers, both belonging to upper Paraná basin. *Apareiodon* sp. A and *Apareiodon* sp. B were collected in the Frio and Barreiro Grande streams, respectively, both belonging to the São Francisco basin. *A. vittatus* was caught in the Timbó river, and *Apareiodon* sp. C in the Araguaia river, belonging to the Iguacu and Araguaia basins, respectively.

Mitotic chromosomes were obtained according to Bertollo *et al.* (1978). The detection of the nucleolar organizer regions (Ag-NORs), C-banding and the staining with distamycin/chromomycin A₃ (DA/CMA₃), followed the basic procedures of Howell and Black (1980), Sumner (1972) and

* Corresponding author, e-mail: pcej@iris.ufscar.br

Schweizer (1980), respectively. The chromosomes were arranged in 2 major groups in the karyotypes—the metacentric/submetacentric (M/SM) and the subtelocentric (ST) ones—and paired in a decreasing order of size.

Results

All the species showed a diploid number equal to 54 chromosomes and a fundamental number (FN) equal to 108, both for females and males. Chromosomal sex differences were not found.

Fig. 1. Giemsa stained karyotypes and NOR-bearing chromosomes (Ag-NORs). a) *Apareiodon* sp. A, b) *Apareiodon* sp. B, c) *A. ibitiensis*. Length bar corresponds to 20 μ m.

Apareiodon sp. A, *Apareiodon* sp. B and *A. ibitiensis* present 25 meta/submetacentric chromosome pairs plus 2 subtelocentric pairs (Fig. 1). *A. vittatus*, *A. piracicabae* and *Apareiodon* sp. C showed 26 meta/submetacentric chromosome pairs plus only one subtelocentric pair (Fig. 2).

The NOR is located in the terminal region of the long arm of a subtelocentric pair, correspondent to the number 26 in the *Apareiodon* sp. A, *Apareiodon* sp. B and *Apareiodon ibitiensis* karyotypes (Fig. 1) and to the number 27 in the *A. vittatus* karyotype (Fig. 2a). Although *A. piracicabae* also show the NOR in the subtelocentric pair 27, inter- and intraindividual variations can be ob-

Fig. 2. Giemsa stained karyotypes and NOR-bearing chromosomes (Ag-NORs). a) *Apareiodon vittatus*, b) *A. piracicabae*, c) *Apareiodon* sp. C. Ag-NORs are not showed for *A. piracicabae* and *Apareiodon* sp. C. Length bar corresponds to 20 μ m.

served in relation to its number, position and size, as follows: (a) a double NOR in the 2 homologous chromosomes, (b) a double NOR in only one of the chromosomes of the pair, (c) a large, but undivided NOR in the 2 homologues, (d) a large undivided NOR in a chromosome and a small NOR in the homologue (Fig. 3a–d). Other NOR phenotypes were observed, although with a few frequency: a small NOR in the 2 homologous chromosomes, a large or a small NOR in only one of homologues, the other not showing a NOR and a small interstitial NOR in only one chromosome. Chromomycin A₃ stained chromosomes evidenced a correspondent phenotypic pattern to that obtained with silver staining (Fig. 3e–h).

Heterochromatic blocks are seen in the centromeric regions of all chromosomes and also in the telomeric regions of some others, after C-banding (Fig. 4). In *A. ibitiensis* and *Apareiodon* sp. B heterochromatin is coincident with the NOR, while in *A. piracicabae* and *Apareiodon* sp. A it is adjacent to the NOR.

Fig. 3. NOR-bearing chromosomes of *Apareiodon piracicabae* stained by silver nitrate: Ag-NORs (a–d) and by DA/CMA₃ (e–h).

Discussion

Two karyotypic groups can be considered among the analysed species, according to the meta/submetacentric and subtelocentric chromosome numbers: *A. ibitiensis*, *Apareiodon* sp. A and *Apareiodon* sp. B show 50M/SM+4ST and *A. piracicabae*, *A. vittatus* and *Apareiodon* sp. C show 52M/SM+2ST chromosomes. In all the species the heterochromatic pattern is conserved, with C-bands preferentially located in the centromeric and some telomeric regions. These results are similar to that described for *Apareiodon affinis* (Jesus *et al.* 1999) and *Parodon* species (Moreira-Filho *et al.* 1993, Jesus and Moreira-Filho 2000).

NORs were already analysed in seven species of *Apareiodon*, including the six ones in the present paper (Moreira-Filho *et al.* 1984, Jorge 1995, Jesus *et al.* 1999). Except for *A. affinis* from the Cuiabá river (Jesus *et al.* 1999) and the low Paraná river (Jorge 1995), that present NORs in both subtelocentric and acrocentric chromosomes, the remaining species show NORs only located in subtelocentric chromosomes. Although *A. piracicabae* may present a double NOR on the same chromosomal arm, the other species show a single undivided NOR, which probably represents an ancestral condition in the Parodontidae family.

Variation in the size among homologous NOR was also frequently observed, although this fact was more accentuated in *A. vittatus*. According to Foresti *et al.* (1981) NORs present a polymorphic nature in fish, which has been confirmed by several other studies in the last years (Phillips *et al.* 1989, Bertollo and Cavallaro 1992, Galetti *et al.* 1995, Jesus *et al.* 1999). This occurrence can result from structural rearrangements in the chromosomes as duplications, deletions, and unequal exchanges (Gold *et al.* 1990). In particular, *A. piracicabae* showed an inter- and intraindividual polymorphism regarding the number, position and size of the NORs, where were observed since a single NOR placed in only one of the homologues, until a double NOR placed in both chromosomes of the pair 27 (Fig. 3). Prior studies indicate that the double NOR probably results from a paracentric in-

Fig. 4. a) C-banded karyotypes of *Apareiodon* sp. B, b) *A. piracicabae* showing the constitutive heterochromatin pattern. Length bar corresponds to 20 μ m.

version in an ancestral chromosome with a heterochromatic region adjacent to a single and terminal NOR, illustrating a derived condition among the Parodontidae (Moreira-Filho *et al.* 1984).

The species now analysed did not show any evidence of morphologically differentiated sex chromosomes. Nevertheless, distinct sex chromosome systems were already described in 2 Parodontidae species, showing two divergent differentiation process. Thus, in *A. affinis* from the upper Paraná a ZZ/ZW₁W₂ system appears to be originated through centric fission and pericentric inversions while in *P. hilarii* from the São Francisco basin the differentiation of a ZZ/ZW system was accomplished by a heterochromatinization process (Moreira-Filho *et al.* 1980, 1993, Jesus *et al.* 1999).

The present work demonstrates that the prior assumed premise, according to Parodontidae fish show a marked stability in karyotype macrostructure, with little or none variation in relation to the diploid number and chromosomal morphology (Galetti Jr. *et al.* 1994), is not totally correct. In contrast, the karyotypic evolution of this fish family presents structural divergences among their species. Besides the sex chromosomes differences in *A. affinis* and *P. hillarii*, above related, some *A. affinis* populations from Argentina and Cuiabá river (Jorge 1995, Jesus *et al.* 1999) show a polymorphic system due to pericentric inversions, originating acrocentric chromosomes only observed in these fishes. Moreover, the double NOR system found in *A. piracicabae* and the NOR location on biarmed chromosomes in *Parodon* sp. and *P. hilarii* (Jesus and Moreira-Filho 2000) point also to structural rearrangements, as paracentric inversions and translocations, respectively.

Thus, the results thus far obtained indicate for chromosomal divergences in the Parodontidae

family. Even maintaining a same diploid number, the Parodontidae probably have undergone some different ways of chromosomal diversification in their speciation process.

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