Genetics and Molecular Biology, 31, 1 (suppl), 235-238 (2008)

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Short Communication

Karyotypic differentiation through chromosome fusion and number reduction in *Imparfinis hollandi* (Ostariophysi, Heptapteridae)

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Abstract

The Neotropical Heptapteridae fish *Imparfinis hollandi*, endemic to the Iguaçu River Basin (Brazil), was cytogenetically analyzed and the diploid chromosome number of 2n = 42 chromosomes was determined (22m + 10sm + 10st), the lowest diploid number in this genus and family. Like other Heptapteridae species, only one NOR-bearing chromosome pair was detected by silver nitrate staining. Dark heterochromatic blocks were visualized in only three chromosome pairs, and chromomycin A_3^+ bands were coincident with Ag-NORs. Although no intercalary $(TTAGGG)_n$ sequence was observed through FISH with a telomere probe, an asymmetric karyotype showing four large chromosome pairs with diploid chromosome number reduction suggests that tandem chromosome fusions probably occurred during the karyotypic differentiation of *Imparfinis hollandi*.

Key words: Siluriformes, chromosome banding, chromosomal rearrangements, $(TTAGGG)_n$.

Received: August 21, 2006; Accepted: May 22, 2007.

Comprising 1,548 valid species grouped into 15 families, Siluriformes is the most diversified and widely distributed Ostariophysi fish order, and its members are commonly known as catfishes (Reis *et al.*, 2003) and occur in all continents. The genus *Imparfinis* belongs to Heptapteridae, an endemic family of the Neotropics that includes 26 genera of small to medium-sized fishes with 186 valid species (Bockmann and Guazzelli, 2003). *Imparfinis hollandi* was previously identified as *Pariolius hollandi* (Julio *et al.*, 1997) or *Heptaterus hollandi* (Eschmeyer, 1998), thus reflecting the systematic difficulties of this group. Recent studies in Heptapteridae have been performed in order to discover the family phylogeny and to provide major rearrangements in the classification of the group, as well as to recognize the correct name of some taxa, since the species-level taxonomy is poorly developed in their genera (Pinna, 1998; Bockmann and Guazzelli, 2003).

Among Siluriformes, the diploid number ranges from 2n = 22 to 132 chromosomes, with a modal number of 58 chromosomes; this variation is due to chromosomal rearrangements associated with the speciation process (Oliveira *et al.*, 1988). LeGrande (1981) suggested an ancestral karyotype with 2n = 56 ± 2 and a relatively high fundamental number (up to 80). Moreover, Oliveira and Gosztonyi (2000), through the study of *Diplomystes mesembrinus*, a representative of the most primitive family of Siluriformes (Diplomystidae), revealed a diploid number of 56 chromosomes, which is considered as the most basal in this fish order. It is important to emphasize that the relative chromosomal condensation hinders the determination of the chromosome type as well as the organization of the fish karyotype, implying in some comparison difficulties among Siluriformes karyotypes (LeGrande, 1981). Among Heptapteridae fish, the diploid number ranges from 2n = 46 chromosomes in *Pimelodella avanhandavae* (Vissoto *et al.*, 1999) and *Pimelodella* aff. *meeki* (Dias and Giuliano-Caetano, 2002), to 58 chromosomes in *Cetopsorhamdia iheringi* (Vissoto *et al.*, 1999), *Pimelodella kronei* (Almeida-Toledo *et al.*, 1992), and *Rhamdia* species (*R. hillari*, Fenocchio and Bertollo, 1990; *R. branneri* and *R. voulezi*, Abucarma and Martins-Santos, 2001; *R. quelen*, Stivari and Martins-Santos, 2004). The *Imparfinis* species studied until now show a diploid number of 56 (*Imparfinis* cf. *piperatus*, Vissoto *et al.*, 2001) to 58 chromosomes (*Imparfinis mirini*, Vissoto *et al.*, 1997; *Imparfinis piperatus*, Vissoto *et al.*, 2001; *Imparfinis* aff. *shubarti*, Stolf *et al.*, 2004).

Due to the rare occurrence of *Imparfinis hollandi*, only three individuals (2 males and 1 female) were collected from the Salto Osório reservoir of the Iguaçu River,

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in Quedas do Iguaçu (Paraná State, Brazil). Mitotic chromosomes were obtained from kidney cells. Ag-NOR (Nucleolar Organizer Regions) sites were detected using silver nitrate staining. Chromomycin A_3 staining, using distamycin A as a counterstaining, was performed to investigate GC-rich isochores. C-banding was produced with barium hydroxide. The classification of chromosomes in metacentric (m), submetacentric (sm), and subtelocentric (st) followed the arm ratio criterion, and the chromosomes were organized into types and in a decreasing order of size in the karyotype. All these methods were carried out according to procedures found in Margarido and Galetti (2000). A telomere repeat probe was generated by PCR according to IJdo *et al.* (1991) using primers $(TTAGGG)_5$ and $(CCCTAA)_5$. The probe was marked with 14-dATP biotin by nick translation following the manufacturer's instructions (Bionick Labelling System – Invitrogen). Chromosomes were denatured in 0.05 N NaOH/2xSSC for 3 min. After overnight hybridization at 37 °C, the hybridization signal was detected using conjugated avidin-fluorescein (FITC) and biotinylated anti-avidin antibody. Chromosomes were counterstained with propidium iodide (50 µg/mL) and analyzed with an Olympus BX50 epifluorescence microscope. Chromosome images were captured with the use of the CoolSNAP-Pro software (Media Cybernetic).

The diploid chromosome number determined for *Imparfinis hollandi* (2n = 42; 22m + 10sm + 10 st) (Figure 1a) is different from the diploid number of all the other *Imparfinis* species studied until now (predominantly 2n = 58; Vissoto *et al.*, 1997; Vissoto *et al.*, 2001; Stolf *et al.*, 2004), and is the lowest diploid number in the Heptapteridae family. Until the present study, the lowest diploid chromosome number described for a heptapterid fish was 2n = 46 chromosomes in the genus *Pimelodella* (Vissoto *et al.*, 1999; Dias and Giuliano-Caetano, 2002). Even though *Pimelodella* shows 2n = 46 chromosomes, the karyotype of this species is symmetric. This stands in contrast with *Imparfinis hollandi* which shows an asymmetric karyotype with four outstandingly large chromosomes pairs (pairs 1m, 2m, 12sm, and 17st) that correspond to almost twice the size of the other chromosomes in the complement, a characteristic not shared by any studied heptapterid. This diploid chromosome number reduction suggests that tandem chromosome fusions may have occurred during the karyotypic differentiation of this species. Despite the fact that C-banding showed a reduced amount of heterochromatin (Figure 1b), like in other Heptapteridae (Abucarma and Martins-Santos, 2001; Swarça *et al.*, 2003; Stolf *et al.*, 2004), these results corroborate the idea of chromosome fusions. Although dark heterochromatic blocks were observed in only three chromosome pairs (numbers 8, 12, and 18), slightly differential longitudinal staining can be observed in the four larger chromosome pairs by both Giemsa staining and C-banding, in accordance with the suggestion

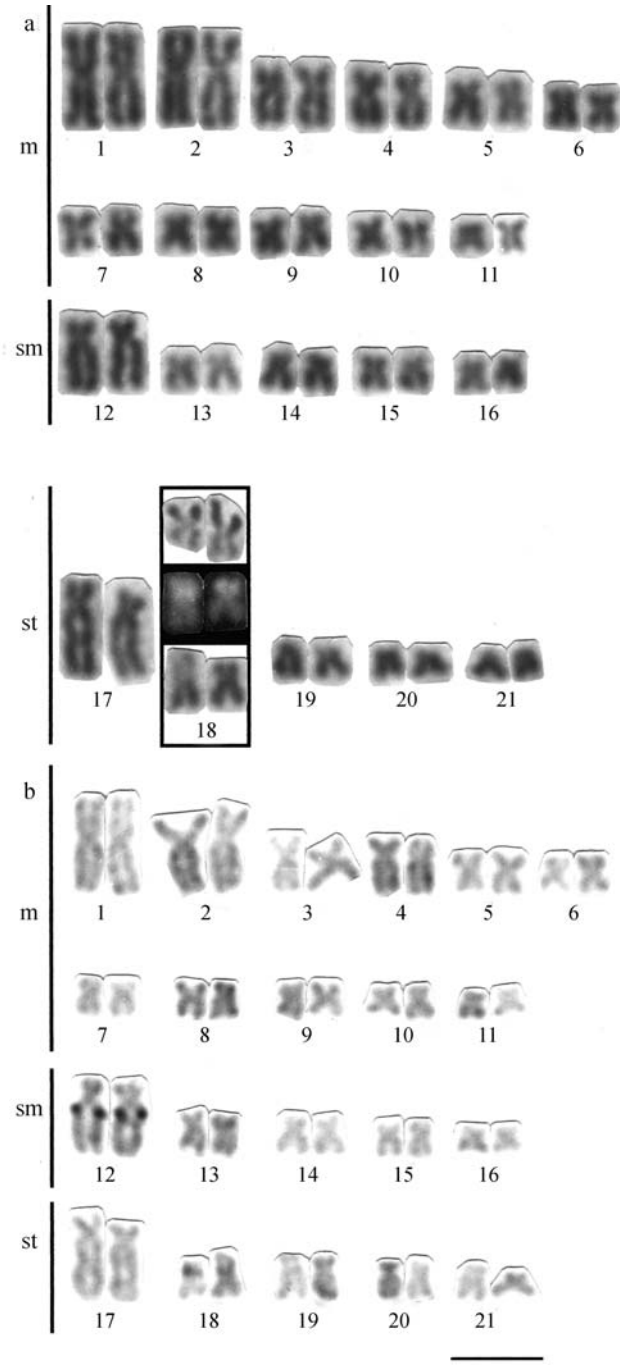

Figure 1 - Karyotypes of *Imparfinis hollandi* Giemsa-stained (a), and C-banded (b). The NOR bearing pair chromosomes (pair 18) are detailed in the box (a), stained by silver nitrate (above), Chromomycin A_3 (center), and Giemsa (below). Bar = 5 µm.

of their origin though the fusion of ancestral minor chromosomes.

A prerequisite for fusion should either be elimination or inactivation of telomeres (Slijepcevic, 1998), since telomeres are specialized structures at chromosome ends required for maintaining chromosome stability and integrity (Zakian, 1997). The absence of intercalary

$(TTAGGG)_n$ sites in the four larger chromosome pairs of *Imparfinis hollandi* indicates the elimination of telomeres during fusion of ancestral minor chromosomes (Figure 2). Another explanation is the high stringency used in the FISH technique since the hybridization solution consisted of 50% formamide. Abuín *et al.* (1996) reported the verification (presence/absence) of interstitial telomeric sequences by varying the formamide concentration (low/high, respectively). On the contrary, Meyne *et al.* (1990) verified the occurrence of interstitial telomeric sites from a number of vertebrate species, suggesting fusion without telomere loss. In fish, chromosome fusion has been reported in processes involved in the differentiation of multiple sex chromosome systems mainly through centric fusion of acrocentric chromosomes, such as in *Eigenmannia* sp. (Almeida-Toledo *et al.*, 2000a), *Brachyhypopomus pinnicaudatus* (Almeida-Toledo *et al.*, 2000b), *Erythrinus eyrthrinus* (Bertollo *et al.*, 2004), *Gymnotus pantanal* (cited as *Gymnotus* sp., Silva and Margarido, 2005), and *Harttia carvalhoi* (Centofante *et al.*, 2006), although fusion between metacentric and submetacentric chromosomes had already been well documented in *Hoplias malabaricus* (Bertollo *et al.*, 1997).

In conclusion, silver and CMA_3^+ staining were neither good tools to detect rearrangements in karyotype structure nor good cytogenetic markers, since only pair 18 was evidenced by both techniques (Figure 1a). A single chromosome pair bearing either NORs or CMA_3^+ bands is a common feature shared by several Heptapteridae (Swarça *et al.*, 2003; Stolf *et al.*, 2004). Furthermore, the data presented in this study confirm that karyotypic evolution in Heptapteridae is more divergent than conservative and that more studies, especially morphological and cytogenetic analyses, would be of great value in order to better understand the family systematics and evolution and the chromosomal mechanisms involved in the speciation process.

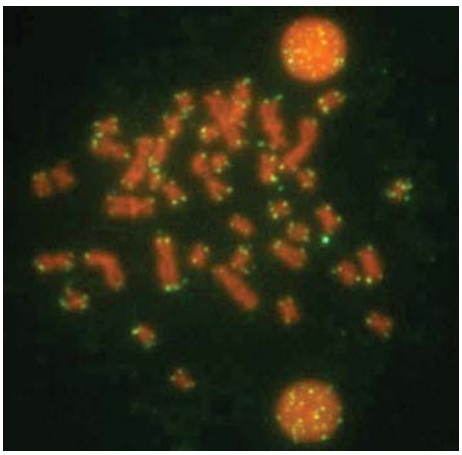

Figure 2 - Metaphase of *Imparfinis hollandi* after fluorescence in situ hybridization with telomere probe $(TTAGGG)_n$.

Acknowledgments

The authors are grateful to Marcos Paulo Roman for his help in the fish capture. This work was supported by Unioeste-*Campus* Cascavel.

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Associate Editor: Lurdes Foresti de Almeida-Toledo