



Comparative cytogenetics of *Carnegiella marthae* and *Carnegiella strigata* (Characiformes, Gasteropelecidae) and description of a ZZ/ZW sex chromosome system

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Abstract

Comparative cytogenetic analyses of hatchetfishes *Carnegiella marthae* and *Carnegiella strigata* (Gasteropelecidae) from the Rio Negro basin were performed using conventional Giemsa staining, silver (Ag) -staining and C-banding. The diploid chromosome numbers of both species equaled $2n = 50$ but their karyotypes were distinct. We found evidence for sex chromosomes in *C. marthae* since karyotype of males presented $20 M + 12 SM + 4 ST + 14 A$ and ZZ ST chromosomes while the females presented $20 M + 12 SM + 4 ST + 14 A$ and ZW ST chromosomes of distinct size. Conversely, *C. strigata* presented $4 M + 4 SM + 2 ST + 40 A$ chromosomes without sex chromosome heteromorphism. Karyotypes of both species had two NOR-bearing SM chromosomes of distinct size indicating the presence of multiple NOR phenotypes. The sex chromosome pair had specific C-banding pattern allowing identification of both Z and W. This heteromorphic system has previously been described for the gasteropelecids.

Key words: fish cytogenetics, karyotype differentiation, NOR phenotypes, heteromorphic sex chromosome system, cytotaxonomy.

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The family Gasteropelecidae (Characiformes) is a group of Neotropical fishes that includes three genera: *Thoracocharax* (two species), *Gasteropelecus* (three species), and *Carnegiella* (four species). Gasteropelecids inhabit major river basins of Central and South America, except those from southeastern Brazil. Almost all gasteropelecids have commercial value being very popular among fish-keeping hobbyists and mainly the *Carnegiella* species are widely exported throughout the world. They are commonly known as hatchetfish due to their ability to propel themselves out of the water, flapping their pectoral fins in flight either to evade predators or to catch insects (Weitzman, 1954; Weitzman, 1960; Géry, 1977; Weitzman and Palmer, 2003).

Karyology in gasteropelecids is very scarce and mostly is restricted to the record of chromosome number. Overall, only a single species of each genus (*Gasteropelecus sternicla*, *Thoracocharax stellatus* and *Carnegiella*

strigata) has been the subject of cytogenetic studies. Apparently, *Thoracocharax* and *Gasteropelecus* possess higher diploid number ($2n = 54$ and $2n = 52$) while *Carnegiella* possesses lower ($2n = 50$ and $2n = 48$). In addition, a ZZ/ZW sex chromosome system was described for *T. cf. stellatus* (Carvalho *et al.*, 2002).

Taking into account that understanding the genetic features of gasteropelecids might be of fundamental importance either for future plans related to conservation genetics or even to reveal aspects related to their evolution, this paper describes and compares the karyotypes of two *Carnegiella* species with other gasteropelecids and reports the occurrence of a ZZ/ZW sex chromosome system.

Cytogenetic analyses were performed on 22 specimens of blackwinged hatchetfish *C. marthae* (10 males and 12 females) and on 25 specimens of marbled hatchetfish *C. strigata* (10 males and 15 females) sampled in the middle Rio Negro basin, Amazonas, Brazil. Specimens were collected from opposite tributaries as follows: in Rio Preto (left margin - $0^{\circ} 9' 10''$ S, $63^{\circ} 52' 58''$ W) three females and three males of *C. marthae* and five females and four males of *C. strigata*; in Igarapé Zamula (left margin - $0^{\circ} 50' 12''$ S, $62 45' 58''$ W) six females and four males of *C. marthae* and

five females and three males of *C. strigata*; in Rio Unini (right margin - 01° 42' 04" S, 61° 38' 21" W) three females and three males of *C. marthae* and five females and three males of *C. strigata*. The fish were registered and deposited in the collection of the Fish Genetics Laboratory at the Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Amazonas, Brazil. Chromosomal preparations were obtained from kidney cells, following the air-drying technique of Bertollo *et al.* (1978). Constitutive heterochromatin was identified according to Sumner (1972) and nucleolar organizing regions (NORs) were detected using the technique described by Howell and Black (1980). Whenever possible, slides were stained sequentially with Giemsa, C-banding and silver nitrate solution (Ag-NOR) according to the procedures described in Centofante *et al.* (2002). Chromosome morphology was classified as metacentric (M), submetacentric (SM), subtelocentric (ST), and acrocentric (A). We scored metacentric, submetacentric and subtelocentric chromosomes as biarmed and acrocentric as uniarmed to determine the chromosome arm number or fundamental number (FN).

We found that *C. marthae* had a modal chromosome number of $2n = 50$, $FN = 86$ (20 M + 12 SM + 4ST + 14A) without population differentiation. However, different karyotypes were observed between the sexes since karyotype of males presented ZZ ST chromosomes while the females presented ZW ST chromosomes of distinct size. Heterochromatin was located in the centromeric and terminal regions in the majority of the chromosomes. The sex chromosome pair showed a specific C-banding pattern allowing identification of both Z and W. The Z sex chromosome presented a large block of heterochromatin in the terminal position and the W showed heterochromatin in centromeric and terminal positions. Ag-NORs were analyzed in 41 metaphases. Interphasic nuclei showed a minimum of one and a maximum of three nucleoli and a maximum of two chromosomes bearing the NORs. After Giemsa/silver nitrate sequential staining the Ag-NORs were identified on the terminal position of the short arm of nonhomologous submetacentrics (pairs 11 and 15), suggesting multiple NORs (Figure 1).

Carnegiella strigata has a modal chromosome number of $2n = 50$, $NF = 60$ (4 M + 4 SM + 2 ST + 40 A) without evidence of sexual dimorphism or population differentiation for this species. The constitutive heterochromatin appeared distributed mainly at the centromeric and terminal positions. Ag-NORs analyzed in 52 metaphases and interphasic nuclei showed a minimum of one and a maximum of three nucleoli, and a maximum of two chromosomes bearing the NORs. After Giemsa/silver nitrate sequential staining the Ag-NORs were identified on the terminal position of the short arm of nonhomologous submetacentrics (pairs 3 and 4), again suggesting multiple NORs (Figure 2).

The gasteropelecids are characterized by a non-conservative karyotype, with diploid number varying between

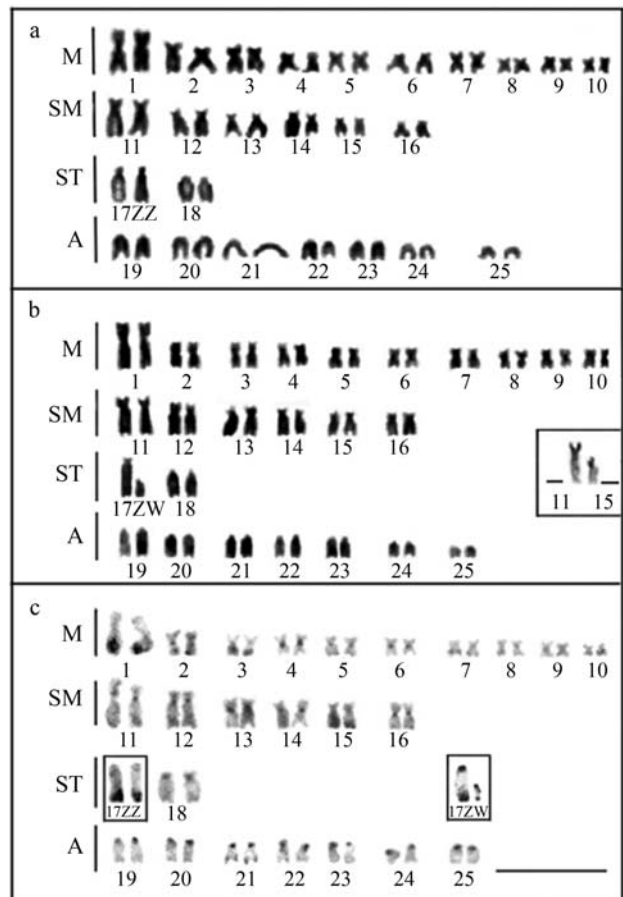


Figure 1 - Giemsa-stained (a) and C-banded (b) karyotypes of *Carnegiella marthae*. The Ag-NOR-bearing chromosomes are shown in the insert (c). Bar - 10 μ m.

$2n = 48$ and $2n = 54$ constituted by a large number of subtelocentric and acrocentric chromosomes (Carvalho *et al.*, 2002). The two *Carnegiella* species studied here presented very distinct karyotypes. *Carnegiella strigata* presented many uni-armed chromosomes while *C. marthae* has mainly bi-armed chromosomes demonstrating that several chromosomal rearrangements have occurred in the course of their karyoevolution. Previous cytogenetic studies conducted in *C. strigata* found different haploid numbers. In this study *C. strigata* presented a diploid number ($2n = 50$) that corroborates the findings of Hinegardner and Rosen (1972) who found a haploid number of $n = 25$, but did not corroborate the findings of Scheel (1973) who found a haploid number of $n = 24$. Unfortunately we have not yet been able to determine the karyotypes of *Carnegiella myersi* and *Carnegiella shერი*, and this is necessary to better understand chromosome evolution in the genus.

Although considered a less speciose fish family, when compared to other characiforms, the relationships within the Gasteropelecidae and their relationship with other characiforms are not fully resolved. Weitzman (1960)

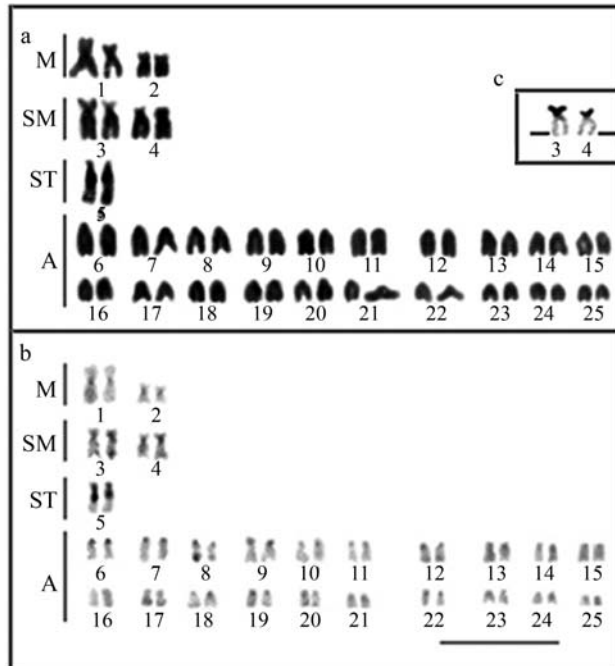


Figure 2 - Male (a) and female (b) Giemsa-stained karyotypes of *Carnegiella strigata*. In (b) the Ag-NOR-bearing chromosomes are shown in the insert. C-banded female karyotype showing the ZZ and ZW chromosomes in the insert (c). Bar - 10 μ m.

putatively considered *Thoracocharax* the least specialized genus while *Gasteropelecus* and *Carnegiella* would be more closely related to each other, with *Carnegiella* considered a neotenic form derived from *Gasteropelecus*. If this is true, then a scenario for chromosome evolution in gasteropelecids would consider $2n = 54$ chromosomes a plesiomorphic character, as found in *Thoracocharax* and *Gasteropelecus*, and giving rise to $2n = 50$ chromosomes of *Carnegiella*. It appears that chromosomal evolution in each genus followed its own path, basically reducing chromosome number. However, further studies will be necessary since *Thoracocharax securis*, *Gasteropelecus maculatus*, *Gasteropelecus levis*, *C. myersi* and *C. schereri* were not yet cytogenetically studied.

Regarding sex chromosomes, it is well known that Neotropical fish display an amazing variety of sex chromosome systems ZZ/ZW being the most common (Moreira-Filho *et al.*, 1993). When the heteromorphic ZZ/ZW system was discovered in the gasteropelecid *T. cf. stellatus* (Carvalho *et al.*, 2002) it was noticed that the ZW chromosomal pair presented both different morphology and different sizes (a large SM and a small A). In the genus *Carnegiella*, *C. marthae* presented its ZW chromosomal pair as subtelocentrics of distinct sizes (a large and a small). Thus, since we are reporting the second ZZ/ZW sex chromosome system for gasteropelecids, the occurrence of a ZZ/ZW system in two out of three gasteropelecid genera indicates that sex chromosome differentiation is not an isolated case as previously thought.

The evolution of sex chromosomes has been discussed at length and it is possible that cytogenetic differences result from structural rearrangements, such as inversions, translocations, duplications, and addition of heterochromatin (Ayling and Griffin, 2002). Considering that less than half of the gasteropelecids were cytogenetically analyzed, there is no *a priori* reason to assume that the ZZ/ZW sex chromosome system has a common origin in this clade.

With respect to the nucleolar organizing region, it was evident that there are two positive Ag-NORs occurring on nonhomologous chromosomes. This leads us to putatively suggest a case of multiple NORs for both *Carnegiella* species. Variation in copy number of the rDNA repeating units or even inactivation of transcription in some rDNA clusters may explain this behavior. Heterozygous combinations where only one of the homologues bears NORs is not uncommon in cytogenetics literature on fish, as described for the Neotropical fish groups Curimatidae (Feldberg *et al.*, 1992), Serrasalminae (Nakayama *et al.*, 2002) and Tetragonopterinae (Gross *et al.*, 2004). However, an analysis of nucleolus organizer regions (NORs) by Fluorescence *In Situ* Hybridization (FISH) with 18S rDNA will be needed to confirm how many ribosomal cistrons are in fact involved in the organization of NORs.

Overall, the present results should be useful for further cytotaxonomic and cytosystematic studies within the family Gasteropelecidae taking into account the level of chromosomal differentiation between species, the second description of a ZZ/ZW sex chromosome system in gasteropelecids, the evidences for multiple NORs in *Carnegiella* and the fact that the study samples did not differ across the tributaries in the Rio Negro basin.

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