Folia biologica (Kraków), vol. **57** (2009), No 1-2
doi:10.3409/fb57_1-2.43-48

Short Note
Karyotypes of Two Species of the Genus *Pimelodus* (Siluriformes, Pimelodidae)

Fernando Rodrigo TRECO and Ana Lúcia DIAS

Accepted September 15, 2008

TRECO F. R., DIAS A. L. 2009. Karyotypes of two species of the genus *Pimelodus* (Siluriformes, Pimelodidae). Folia biol. (Kraków) **57**: 43-48.

A cytogenetic study was conducted on two species of the genus *Pimelodus* that were collected from the Piquiri river, Paraná, Brazil: *Pimelodus paranaensis* and *Pimelodus heraldoi*. Both had a diploid number of 2n=56 chromosomes and a fundamental number (FN) of 104. In *P. paranaensis,* the karyotype consisted of 22m+22sm+4st+8a chromosomes, whereas the karyotype of *P. heraldoi* consisted of 18m+24sm+6st+8a. The AgNORs were localized in the terminal region of the long arm in one pair of subtelocentric chromosomes, pair 24 in *P. paranaensis* and pair 23 in *P. heraldoi*. The latter species showed size heteromorphism of these regions between the chromosome homologues. Heterochromatin was distributed mainly in the terminal regions in the two species. CMA_3-positive staining was observed in some chromosomes, besides being associated with NORs, which were all DAPI-negative, in both species of *Pimelodus*. C-banding plus CMA_3 and DAPI showed that most of the heterochromatic regions were rich in AT bases in *P. paranaensis* and *P. heraldoi*.

Key words: Pimelodidae, *Pimelodus*, karyotype, chromosome banding.

Fernando Rodrigo TRECO, Ana Lúcia DIAS, Universidade Estadual de Londrina, Centro de Ciencias Biológicas, Departamento de Biologia Geral, 86051-970, Londrina, Paraná, Brazil. E-mail: anadias@uel.br

The ichthyofauna of the Piquiri river, one of the tributaries of the Paraná river basin, Brazil, consists of approximately 62 species, however, there is a predominance of some groups, with 57% belonging to the order Characiformes and 24% to Siluriformes (GUBIANI *et al*. 2006). With respect to cytogenetic studies, some species representing different families have already been examined in this region, for example, *Apareiodon vladii* (cited as *Apareiodon* sp.) of the family Parodontidae (ROSA *et al*. 2006) and *Oligosarcus pintoi* and *O. paranensis* of the family Characidae (RUBERT & MARGARIDO 2007).

The genus *Pimelodus* is the most specious of the family Pimelodidae, composed of 24 species (FERRARIS 2007), that live in the Neotropical region, distributed in the main basins of Central and South America (SHIBATTA 2003).). It is also the most often studied cytogenetically. Currently, 11 of the 24 species have at least the diploid number reported showing 2n=56 chromosomes in the majority of its representatives with some variations in karyotype formula (SWARÇA *et al*. 2007).

The aim of this study was to characterize and compare cytogenetically two species of *Pimelodus* from the Piquiri river, Paraná, Brazil, using different chromosome banding techniques. This is the first cytogenetic study of the family Pimelodidae in this region, and the first karyotypic description for *Pimelodus paranaensis*.

Material and Methods

Nine specimens of *Pimelodus paranaensis* (4 males and 5 females) and four specimens of *Pimelodus heraldoi* (3 males and 1 female) collected in the Piquiri river, Nova Laranjeiras, Paraná state, Brazil, were cytogenetically studied.

Mitotic chromosome preparations were obtained from kidney according to BERTOLLO *et al.* (1978). Chromosome morphology was determined on the basis of arm ratio (LEVAN *et al*. 1964). Chromosomes classified as metacentric (m), submetacentric (sm) and subtelocentric (st) and acrocentric (a). Nucleolar organizer regions

(AgNOR) and C-banding (CB) were performed using the methods of HOWELL and BLACK (1980) and SUMNER (1972), respectively. Chromomycin A_3 (CMA_3) and DAPI staining followed SCHWEIZER (1976).

Results and Discussion

The two species studied presented a diploid number of 56 chromosomes and FN of 104, but differed in karyotypic macrostructure. *Pimelodus paranaensis* showed a formula of 22m+22sm+4st+8a (Fig. 1a) and *P. heraldoi* 18m+24sm+6st+8a (Fig. 1b). In the latter species, a secondary constriction was observed in the terminal region of the long arm of the subtelocentric chromosome 23.

The diploid number of 56 chromosomes is very conserved in *Pimelodus*, identified in the majority of species of this genus, with the exception of *P. blochi* with 2n=58 (DELLA-ROSA *et al*. 1980) and *P. fur* with 2n=54 (GARCIA & MOREIRA-FILHO 2005). OLIVEIRA and GOSZTONYI (2000) suggested that 2n=56 chromosomes is the basic number in the order Siluriformes and that this diploid number could represent a plesiomorphic character of the genus *Pimelodus*, whereas 2n=54 and 2n=58 found in this group represent an apomorphic characteristic, as proposed by GARCIA and MOREIRA-FILHO (2005).

SOUZA *et al*. (2004) studied *P. heraldoi* from the Tibagi river and observed a karyotypic formula different than that found in the present work, with 22m+22sm+6st+6a. This observed karyotypic variability within the same species, among different populations, may indicate that chromosome rearrangements, such as pericentric inversions and/or translocations, are involved in the process of karyotype evolution in this group of fishes.

Heterochromatin was distributed mainly in the terminal regions of various chromosomes in the two species (Fig. 1c, d). In *P. paranaensis,* metacentric chromosomes 4, 8 and 11 and submetacentric chromosomes 14, 16 and 17 showed heterochromatin in both terminal regions (Fig. 1c). *P. heraldoi* had a greater number of chromosomes with bands at both ends of metacentric chromosomes 2, 3, 6, 7 and 8; submetacentric chromosomes 12, 14 and 16, being more evident than in *P. paranaensis*; and one pair of subtelocentric chromosomes (24), with stronger staining at both ends (Fig. 1d). In *P. heraldoi*, the secondary constriction in pair 23 (st) was heterochromatic, where a size heteromorphism was observed between the homologues.

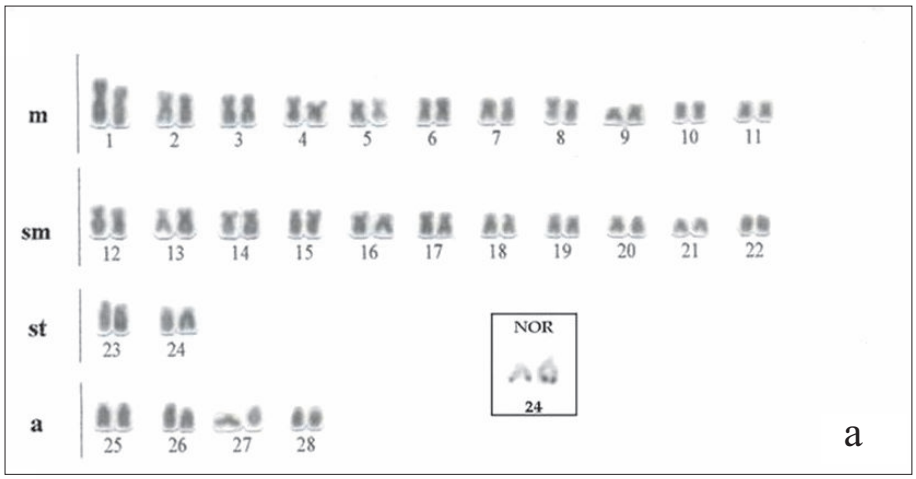
SOUZA *et al*. (2004) also identified heterochromatic bands mainly in the terminal regions in *P. heraldoi* from the Tibagi river, but only one pair of chromosomes with bands at both ends. Thus, it is possible that differences in heterochromatin location may differentiate the two populations.

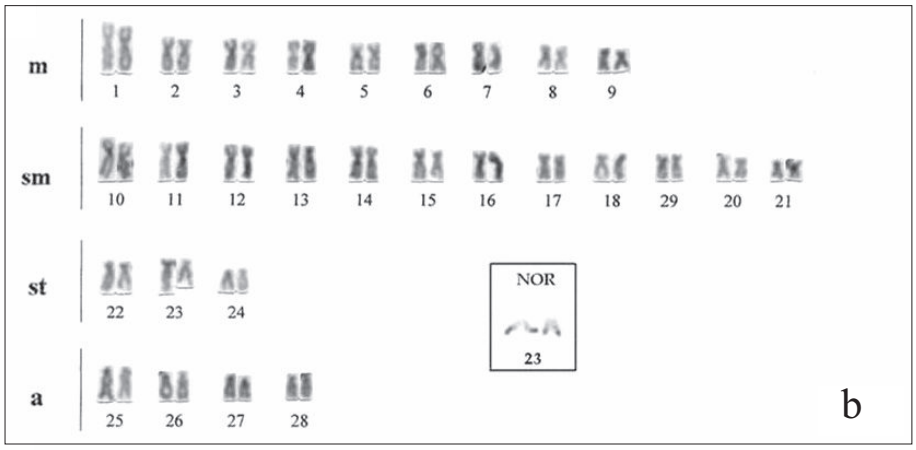
The presence of heterochromatin at both terminal regions, as shown here, appears to be a common characteristic of some species of this genus, such as *Pimelodus maculatus* from the Paraná river (BORIN & MARTINS-SANTOS 2002), *Pimelodus* sp. from the Iguaçu river (BORIN & MARTINS-SANTOS 2004; SOUZA *et al*. 2004), and *Pimelodus* sp. from the São Francisco river (GARCIA & MOREIRA-FILHO 2005) and may indicate a cytotaxonomic marker for the genus *Pimelodus*, and for the family Pimelodidae, thereby being an important tool in the understanding of phylogenetic relationships in this group of fishes, as suggested by SOUZA *et al*. (2004).

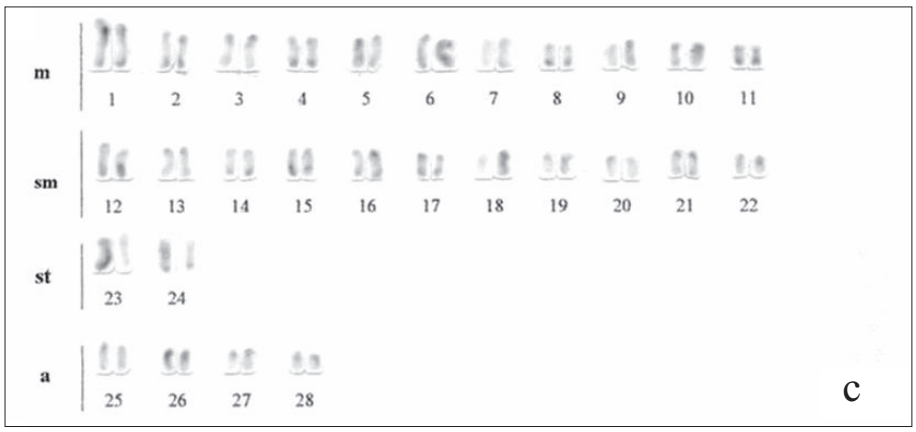
The AgNORs in the two species were located in the terminal region of the long arm in a pair of subtelocentric chromosomes: pair 24 in *P. paranaensis* (Fig. 1a, box) and pair 23 in *P. heraldoi* (Fig. 1b, box). In the latter species, a size heteromorphism was observed between these regions. In addition, heterochromatin appeared to be associated with NORs in both species (Fig. 1c,d). The findings obtained here for *P. heraldoi* differed from those reported by SOUZA *et al*. (2004) in the population from the Tibagi river, in which the NORs were identified on pair 25, without evidence of size heteromorphism.

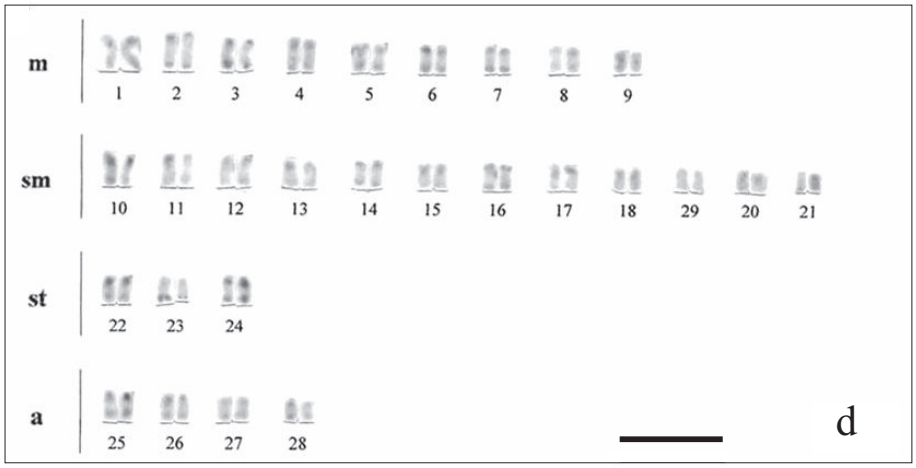
CMA_3 staining revealed positive fluorescent signals corresponding to Ag-NOR in *P. paranaensis* and *P. heraldoi* (Fig. 2a and 2b, respectively); other sites rich in GC base pairs were shown, characteristic for each of the species. In *P. paranaensis*, fluorescent regions were identified at the centromeres of some chromosome pairs (Fig. 2a), while in *P. heraldoi*, some terminal regions showed CMA_3-positive bands in which one chromosome was found to have bands at both ends (Fig. 2b). Reports of others fluorescent signals besides those corresponding to AgNORs have also been identified in other *Pimelodus*, such as in *P. heraldoi* and *Pimelodus* sp (SOUZA *et al*. 2004) and *P. fur* (GARCIA & MOREIRA-FILHO 2005). The combination CB+CMA_3 confirmed that the

Fig. 1. Karyotype of *Pimelodus paranaensis:* a – Giemsa, c – C-banding and *Pimelodus heraldoi*: b – Giemsa, d – C-banding. In the boxes the NOR-bearing pair of chromosomes. Scale bar=10μm.


m
1 2 3 4 5 6 7 8 9 10 11
sm
12 13 14 15 16 17 18 19 20 21 22
st
23 24
NOR
24
a
25 26 27 28
a


m
1 2 3 4 5 6 7 8 9
sm
10 11 12 13 14 15 16 17 18 29 20 21
st
22 23 24
NOR
23
a
25 26 27 28
b


m
1 2 3 4 5 6 7 8 9 10 11
sm
12 13 14 15 16 17 18 19 20 21 22
st
23 24
a
25 26 27 28
c


m
1 2 3 4 5 6 7 8 9
sm
10 11 12 13 14 15 16 17 18 29 20 21
st
22 23 24
a
25 26 27 28
d

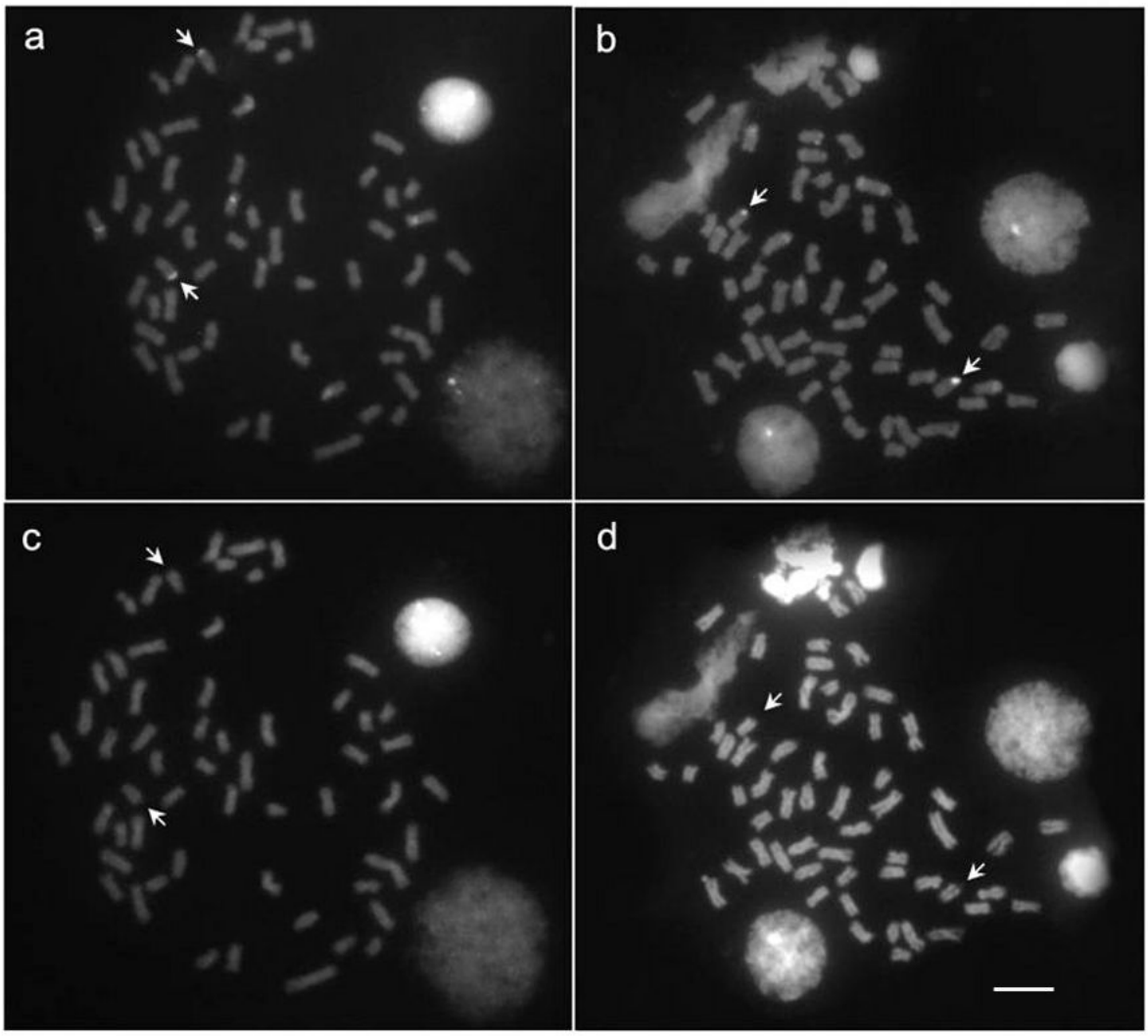


Fig. 2. Somatic metaphase plates of: *Pimelodus paranaensis*: a – CMA_3, c – DAPI and *Pimelodus heraldoi*: b – CMA_3, d – DAPI. The arrows indicate the NOR-bearing pair of chromosomes. Scale bar=10μm.

heterochromatin associated with NORs in the two species is GC-rich (Fig. 3a and 3b), while in *P. heraldoi,* more GC-rich heterochromatic regions were evident, including both ends on some chromosomes (Fig. 3b). SOUZA *et al*. (2004) also confirmed some GC-rich heterochromatic regions in *P. heraldoi* from the Tibagi river.

Staining with DAPI fluorochrome in the two species from the Piquiri river evidenced only pale regions coincident with the GC-rich regions (Fig. 2c and d), thereby being DAPI negative. However, the results of CB+DAPI revealed various fluorescent bands in *P. paranaensis* and *P. heraldoi* (Fig. 3c, d), mainly in the latter species, demonstrating a greater quantity of AT bases in the heterochromatic regions in both species. Recently, TRECO *et al.* (2008) analysed CB+CMA_3 and CB+DAPI in *Parapimelodus nigribarbis* and *Pimelodus maculatus* from Guaíba lake and showed that the composition of heterochromatin in these species includes GC bases and mainly AT bases.

The present study makes an important contribution to the cytogenetics of the genus *Pimelodus*, since it provides the first description of *P. paranaensis* and of the family Pimelodidae of the Piquiri river, confirming the conservation of the diploid number in this group and showing some differentiated characteristics in the two species in relation to microstructure.

Acknowledgments

The authors are grateful to CAPES and Fundação Araucaria for financial support. We are also thankful to Dr. Albert LEYVA for his help in the preparation of the manuscript and Dr. Vladimir Pavan MARGARIDO for helping in collecting the specimens analysed.

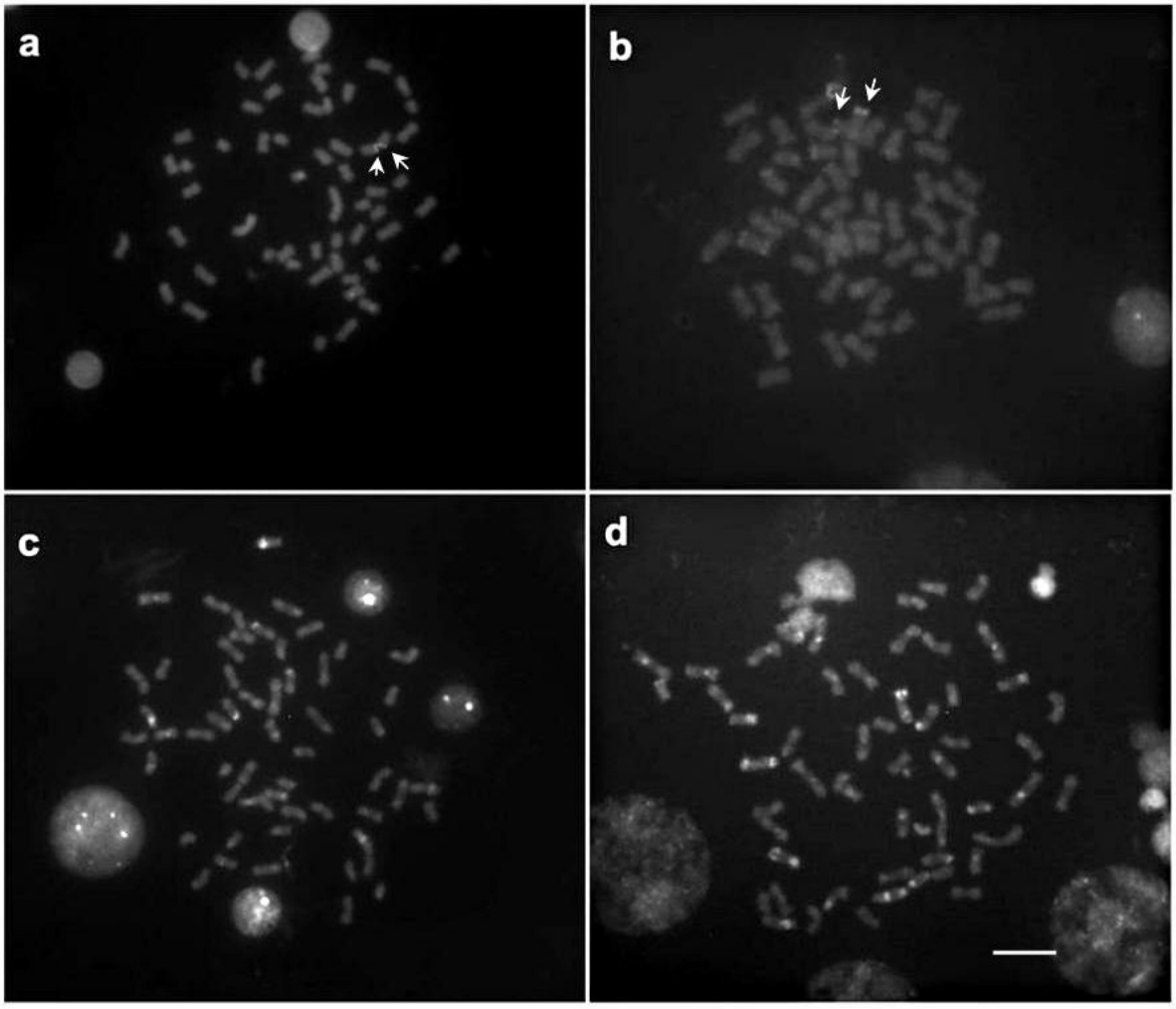


Fig. 3. Somatic metaphase plates of *Pimelodus paranaensis*: a – CB+CMA_3, c – CB+DAPI and *Pimelodus heraldoi*: b – CB+CMA_3, d – CB+DAPI. The arrows in (a) and (b) indicate the NOR-bearing pair of chromosomes. Scale bar=10μm.

References

BERTOLLO L. A. C., TAKAHASHI C. S., MOREIRA-FILHO O. 1978. Cytotaxonomic considerations on *Hoplias lacerdae* (Pisces, Erythrinidae). Rev. Brasil. Genet. **1**: 103-120.

BORIN L. A., MARTINS-SANTOS I. C. 2002. Cytogenetic aspects in species of the genus *Pimelodus* (Pisces, Siluriformes, Pimelodidae) of the river Paraná Basin. Cytologia. **67**: 199-204.

BORIN L. A., MARTINS-SANTOS I. C. 2004. Study on karyotype and occurrence of B chromosomes in two endemic species of the genus *Pimelodus* (Siluriformes, Pimelodidae) from the river Iguaçu. Hereditas **140**: 201-209.

GARCIA C., MOREIRA-FILHO O. 2005. Cytogenetical analyses in three fish species of the genus *Pimelodus* (Siluriformes: Pimelodidae) from Rio São Francisco: Considerations about the karyotypical evolution in the genus. Neotrop. Ichthyol. **3**: 285-289.

GUBIANI E. A., HOLZBACH A. J., BAUMGARTNER G., NETO l. B. R., BERGMANN F. 2006. Fish, Piquiri River, upper Paraná River Basin, Paraná State, Brazil. Check List. **2**: 9-14.

HOWELL W. M., BLACK D. A. 1980. Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a I-step method. Experientia **36**: 1014-1015.

LEVAN A., FREDGA K., SANDBERG A. A. 1964. Nomenclature for centromeric position on chromosomes. Hereditas **52**: 201-220.

OLIVEIRA C., GOSZTONYI A. E. 2000. A cytogenetic study of *Diplomystes mesembrinus* (Teleostei, Siluriformes, Diplomystidae) with a discussion of chromosome evolution in siluriforms. Caryologia **53**: 31-37.

ROSA R., BELLAFRONTE E., MOREIRA-FILHO O., MARGARIDO V. P. 2006. Constitutive heterochromatin, 5S and 18S rDNA genes in *Apareiodon* sp. (Characiformes, Parodontidae) with a ZZ/ZW sex chromosome system. Genetica **128**: 159-166.

RUBERT M., MARGARIDO V. P. 2007. Cytogenetic studies in three species of the genus *Oligosarcus* (Pisces, Characidae, Acestrorhynchinae). Brazil. Arch. Biol. Technol. **50**: 127-135.

SCHWEIZER D. 1980. Simultaneous fluorescent staining of R-bands and specific heterochromatic regions (DA-DAPI bandas) in human chromosomes. Cytogenet. Cell. Genet. **27**: 190-193.

SOUZA L., GIULIANO-CAETANO L., DIAS A. L. 2004. Banding chromosome pattern of two species of *Pimelodus* (Siluriformes, Pimelodidae) from the Paraná river basin of Brazil. Folia biol. (Kraków) **52**: 165-169.

SUMNER A. T. 1972. A simple technique for demonstrating centromeric heterochromatin. Exp. Cell Res. **75**: 304-306.

SWARÇA A. C., FENOCCHIO A. S., DIAS A. L. 2007. An update cytogenetic review for species of the families Pseudomelodidae, Pimelodidae and Heptapteridae (Pisces, Siluriformes). Suggestion of a cytotaxonomical classification. Caryologia **60**: 338-348.

TRECO F. R., MALABARBA L.R., GIULIANO-CAETANO L., DIAS A. L. 2008. Cytogenetic study of two species of the family Pimelodidae (Siluriformes) collected in lago Guaíba, Rio Grande do Sul, Brazil. Neotrop. Ichthyol. **6**: 87-92.