

Karyotype description of three species of Loricariidae (Siluriformes) and occurrence of the ZZ/ZW sexual system in *Hemiancistrus spilomma* Cardoso & Lucinda, 2003

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The neotropical freshwater systems have a high number of catfish species (Siluriformes), and many of those are denominated “cascudos” in Brazil. Cytogenetic data about three “cascudos” species fished in the rio Araguaia are described in the present study. The *Pterygoplichthys joselimaianus* showed $2n=52$, with 28 metacentrics (M) chromosomes, 16 submetacentrics (SM) and 8 subtelocentrics/acrocentrics (ST/A) in both sexes. *Hemiancistrus spinosissimus* showed $2n=52$, with karyotype formulae $26M+22SM+4ST$, in both sexes. *Hemiancistrus spilomma* also showed $2n=52$, but in this species a ZZ/ZW sex chromosome system ($25M+21SM+6ST$ in females and $24M+22SM+6ST$ in males) was observed. The cells from *H. spinosissimus* and *P. joselimaianus* showed one chromosome pair bearing Ag-NORs, while in the *H. spilomma* three chromosome pairs bearing Ag-NORs were detected. The data showed in this work reveal particular chromosomal characteristics, important for a good recognition of both *Hemiancistrus* species, and also show the importance of the insertion of cytogenetic data on taxonomic phylogenetic studies.

Os sistemas de água doce neotropicais possuem um alto número de espécies de peixes Siluriformes, muitas das quais popularmente conhecidas como cascudos no Brasil. Dados citogenéticos sobre três espécies de cascudos, capturadas na bacia do rio Araguaia são descritos no presente trabalho. *Pterygoplichthys joselimaianus* possui $2n=52$, com 28 cromossomos metacêntricos (M), 16 submetacêntricos (SM) e 8 subtelecêntricos/acrocêntricos (ST/A) em ambos os sexos. *Hemiancistrus spinosissimus* revelou $2n=52$, com fórmula cariotípica $26M+22SM+4ST$, nos dois sexos. *H. spilomma* também possui $2n=52$, porém, nesta espécie foi observado um sistema de cromossomos sexuais do tipo ZZ/ZW ($25M+21SM+6ST$ para fêmeas e $24M+22SM+6ST$ para machos). As células de *H. spinosissimus* e *P. joselimaianus* mostraram um par de cromossomos portadores de Ag-RONs. Em *H. spilomma*, três pares de cromossomos portadores de Ag-RONs foram observados. Os dados apresentados neste trabalho revelam características cromossômicas particulares, importantes para um bom reconhecimento de ambas as espécies de *Hemiancistrus* e mostram a importância da inserção dos dados citogenéticos nos estudos taxonômicos e filogenéticos.

Key words: Ancistrinae, cytogenetic, sexual chromosomes, Araguaia.

Introduction

The Siluriformes is the most diverse and widely distributed of ostariophysan groups, with approximately 30 families, 412 genera and over 2400 species (Nelson, 1994). Except for the families Ariidae and Plotosidae, is limited to the tropical freshwater systems (Burgess, 1989). The Loricariidae, regionally called “cascudos” or “acarís” is endemic to the Neotropical region and is the largest family of catfishes, with 646 species currently considered valid (Isbrucker, 1980; Armbruster, 2004). Nevertheless, the Loricariidae is not a very studied group. According to Oliveira

and Gosztonyi (2000) only 56 loricariid species have been investigated at the cytogenetic level, and a range of diploid number among $2n=36$ in *Rineloricaria latirostris* (Loricariinae) (Giuliano-Caetano, 1998) and $2n=80$ in *Hypostomus* sp. (Hypostominae) has been reported (Artoni & Bertollo, 1996).

Andreato *et al.* (1992, 1993) described a XX/XY sexual chromosome determination in *Pseudotocinclus tietensis* and a ZZ/ZW in *Microlepidogaster leucofrenatus*, respectively. Structural chromosome polymorphisms in several other species of Hypoptopomatinae (Loricariidae) related to NORs position and C-banding pattern at both species and popula-

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tion levels was detected (Andreato *et al.*, 1994). A ZZ/ZW system was also observed in *Loricariichthys platymetopon* (Scavone & Júlio Jr. 1995), *Hypostomus* sp. from the Rio Araguaia (Artoni *et al.*, 1998), and *Ancistrus* cf. *dubius* (Mariotto *et al.*, 2004).

Ancistrinae has been recognized as a well-defined monophyletic group (Schaefer, 1987), but a recent study showed that the species of this group are more related to those of the tribe *Pterygoplichthini* and were grouped in the tribe Ancistrini (Armbruster, 2004). Both these tribes now belong to the subfamily Hypostominae (Armbruster, 2004). Chromosome data from Ancistrini are showed in Table 1. Except for *Ancistrus* n.sp.1 from the rio São Francisco (Acre, Brazil), with $2n=38$ (Alves *et al.*, 2003), *Ancistrus* sp. from the rio Iguaçú (Paraná, Brazil) with $2n=48$ (Lara, 1998), and *Ancistrus* cf. *dubius* from the rio Paraguai basin (MT), with $2n=44$ (Mariotto *et al.*, 2004) all other species have a diploid number of $2n=52$. The only species of Pterygoplichthini karyotyped, *Liposarcus anisitsi*, also has $2n=52$ chromosomes (Artoni & Bertollo, 1996).

In the present work, the karyotypes and active rDNA nucleolar location of the three species of Loricariidae are presented, as well as the description of ZZ/ZW sexual chromosome system in *Hemiancistrus spilomma*.

Material and methods

In the present study, the taxonomy proposed by Armbruster (2004) who considers the genus *Glyptoperichthys* as synonyms with *Pterygoplichthys* was applied. In this way, *P. joselimaianus* (Weber, 1991) (three females, four males and one unidentified sex) were sampled in the lago Quatro Bocas ($15^{\circ}23'20.1''S$, $51^{\circ}42'45.9''W$), near the city of Araguaiana, Mato Grosso, Brazil. Voucher specimens were deposited in the fish collection of the Instituto de Ciências e Letras do Médio Araguaia, Universidade Federal de Mato Grosso, Brazil (ICLMA434). *Hemiancistrus spinosissimus* Cardoso & Lucinda, 2003 (two female and two males) and *H. spilomma* Cardoso & Lucinda, 2003 (eight females and two males) were caught in the rio Araguaia ($15^{\circ}50'15''S$ - $051^{\circ}58'43''W$), near the city of Pontal do Araguaia, Mato Grosso, Brazil. Voucher

specimens were deposited in the Museu de Ciências e Tecnologia of Pontifícia Universidade Católica do Rio Grande do Sul (MCP36689, MCP36686, MCP36685).

Chromosomal preparations were obtained either from kidney cells after *in vivo* colchicine treatment (Bertollo *et al.*, 1978), or from short-term cell culture (Fenocchio *et al.*, 1991). These were Giemsa stained for conventional analysis. The active rDNA nucleolar cistrons (Ag-NORs) were detected using AgNO₃ staining (Howell and Black, 1980). The chromosomes were classified as metacentric (M), submetacentric (SM), subtelocentric (ST), and acrocentric (A), according to the arm ratio criteria adopted by Levan *et al.* (1964).

Results

In both sexes of *Pterygoplichthys joselimaianus*, the diploid number was $2n=52$ and the karyotypic formulae $28M+16SM+8ST/A$ (Figure 1). In both sexes of *Hemiancistrus spinosissimus* the diploid number was $2n=52$ and the karyotypic formulae $26M+22SM+4ST$ (Figure 2a). *H. spilomma* also showed $2n=52$ but, a ZZ/ZW sex chromosome mechanism was observed, with a karyotypic formulae of $25M+21SM+6ST$ in females and $24M+22SM+6ST$ in males (Figure 2b). The ZZ chromosomes are represented by the first submetacentric pair whereas the W chromosome is morphologically similar to the third metacentric pair.

In the present study, it was observed that *P. joselimaianus* and *H. spinosissimus* cells showed one Ag-NOR-bearing chromosome pair (inset of Figure 1 and 2a, respectively). The Ag-NOR signals were subterminally located on the short arm of pair 9 in *P. joselimaianus* (Figure 1, inset) and terminally on the long arm of the pair 17 in *H. spinosissimus* (Figure 2a, inset). Three Ag-NOR-bearing pairs were detected in *H. spilomma*, one located in the terminal regions of the long arm in pairs 2 and 18 and other at the terminal end of the short arm of pair 3 (Figure 2b, inset).

Discussion

In Ancistrini, pericentric and paracentric inversions probably were the most important chromosome rearrangements

Table 1. Cytogenetic data from the species of Ancistrini. References: 1. Lara (1998), 2. Alves *et al.* (2003), 3. Mariotto *et al.* (2004), 4. Artoni & Bertollo (2001), 5. Souza *et al.* 2004, 6. present study.

Species	Rivers / Brazilian states	2n	Karyotype formulae	Ag-NOR locations	Ref
<i>Ancistrus</i> sp.	Iguaçu / Paraná	48	18M+14SM+12ST+4A	(ST)p, terminal	1
<i>Ancistrus</i> n.sp.1	São Francisco / Acre	38	30M/SM+8ST	(M)5p, interstitial	2
<i>Ancistrus</i> n.sp.2	Betari / São Paulo	52	32M/SM+20ST/A	(ST)24p, terminal	2
<i>Ancistrus</i> cf. <i>dubius</i>	Pantanal / Mato Grosso	44	18M+10SM+8ST+8A (ZZ/ZW)	(SM)13p, interstitial	3
<i>Ancistrus multispinnis</i>	Itapocu / Santa Catarina	52	28M/SM+24ST/A	(ST)17p, terminal	2
<i>Hemiancistrus</i> sp.	Araguaia / Mato Grosso	52	20M+20SM+8ST+4A	(SM)q, terminal	4
<i>Hemiancistrus spinosissimus</i>	Araguaia / Mato Grosso	52	26M+22SM+4ST	(SM)17q, terminal	6
<i>Hemiancistrus spilomma</i>	Araguaia / Mato Grosso	52	Female 25M+21SM+6ST(ZW) Male 24M+22SM+6ST(ZZ)	(M)2q, terminal; (SM)18q, terminal; (M)3p, terminal	6
<i>Megalancistrus aculeatus</i>	Paraná / Paraná	52	26M+26SM	(SM)p, interstitial	1
<i>Panaque</i> cf. <i>nigrolineatus</i>	Araguaia / Mato Grosso	52	26M+20SM+6ST	(A)p terminal	4
<i>Baryancistrus</i> aff. <i>niveatus</i>	Xingu / Pará	52	16M+32SM+4A	(M) 3p, interstitial	5

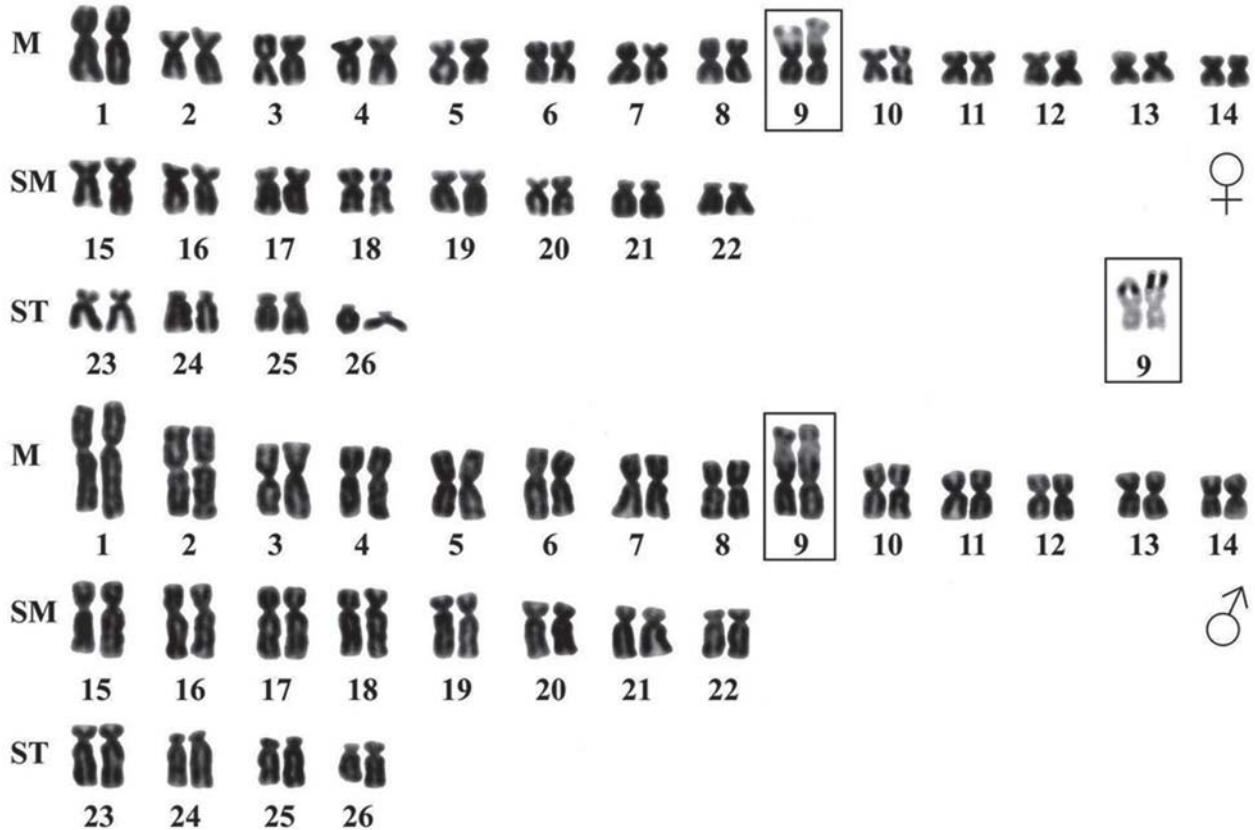


Fig. 1. Karyotypes of female and male of *Pterygoplichthys joselimaianus* with conventional Giemsa staining. The chromosome pair bearing Ag-NORs are in the inset.

that occurred in the karyotypic evolution of the group (Alves *et al.*, 2003). The present data reinforce this hypothesis since all species analyzed also have $2n=52$ chromosomes. These rearrangements keep the diploid number equal to $2n=52$ chromosomes, metacentric and submetacentric, in most of the studied species (Table 1). Ancistrini has a lower diploid number when compared with Loricariinae (Alves *et al.*, 2003), Hypostominae (Artoni & Bertollo, 1996), and Hypoptopomatinae (Andreato *et al.*, 1994). However, these conclusions are preliminary because Ancistrini is a very specious subfamily and these data are only related to a few species from five genera. The species *P. joselimaianus* also has $2n=52$, the same diploid number found in *Liposarcus anisitsi* (Artoni & Bertollo, 1996), other species of the tribe Pterygoplichthini. According to Armbruster (2004), the tribes Ancistrini and Pterygoplichthini are sister groups and compose a derived clade of the Hypostominae. The present cytogenetic data reinforce the putative relationship between these tribes suggesting that $2n=52$ could be a primitive diploid number for them.

A few years ago the presence of a sexual differentiation at a chromosome level was considered an uncommon occurrence in fishes. Advances in the studies of Neotropical fishes, however, have shown a great number of new occurrences in different species and families. Among those species with dif-

ferentiated sex chromosomes, 64% showed female heterogamety and 36% male heterogamety. Eighty percent of the sex chromosomes corresponds to simple systems (77% ZZ/ZW and 23% XX/XY) and twenty percent correspond to multiple sex chromosome mechanisms (Centofante *et al.*, 2002). The presence of a ZZ/ZW sex chromosome system in *H. spilomma* (present study) and in *Ancistrus cf. dubius* (Mariotto *et al.*, 2004) suggest that this system may have a wide distribution in Ancistrini.

Some fish groups have a predominance of one chromosome pair in their karyotype bearing just one active NOR, as some species of Pimelodidae (Dias & Foresti, 1993) and Loricariidae (Alves *et al.*, 2003). On the other hand, occurrence of multiple NORs can be observed in some species of Hypostominae (Artoni & Bertollo, 1996). Among the species examined, *Hemiancistrus spilomma* is the only species thus far to show multiple NORs, as described in Table 1 and confirmed by the nucleolus analysis (Figure 2b).

It is early to draw conclusions on the significance of the ZZ/ZW occurrence associated with the multiplicity of Ag-NORs in *Hemiancistrus spilomma*, as well the maintenance of $2n=52$ and single Ag-NOR in the majority of the Ancistrini species because this tribe is very specious (Fisch-Muller, 2003), and only a few species from five genus have been studied.

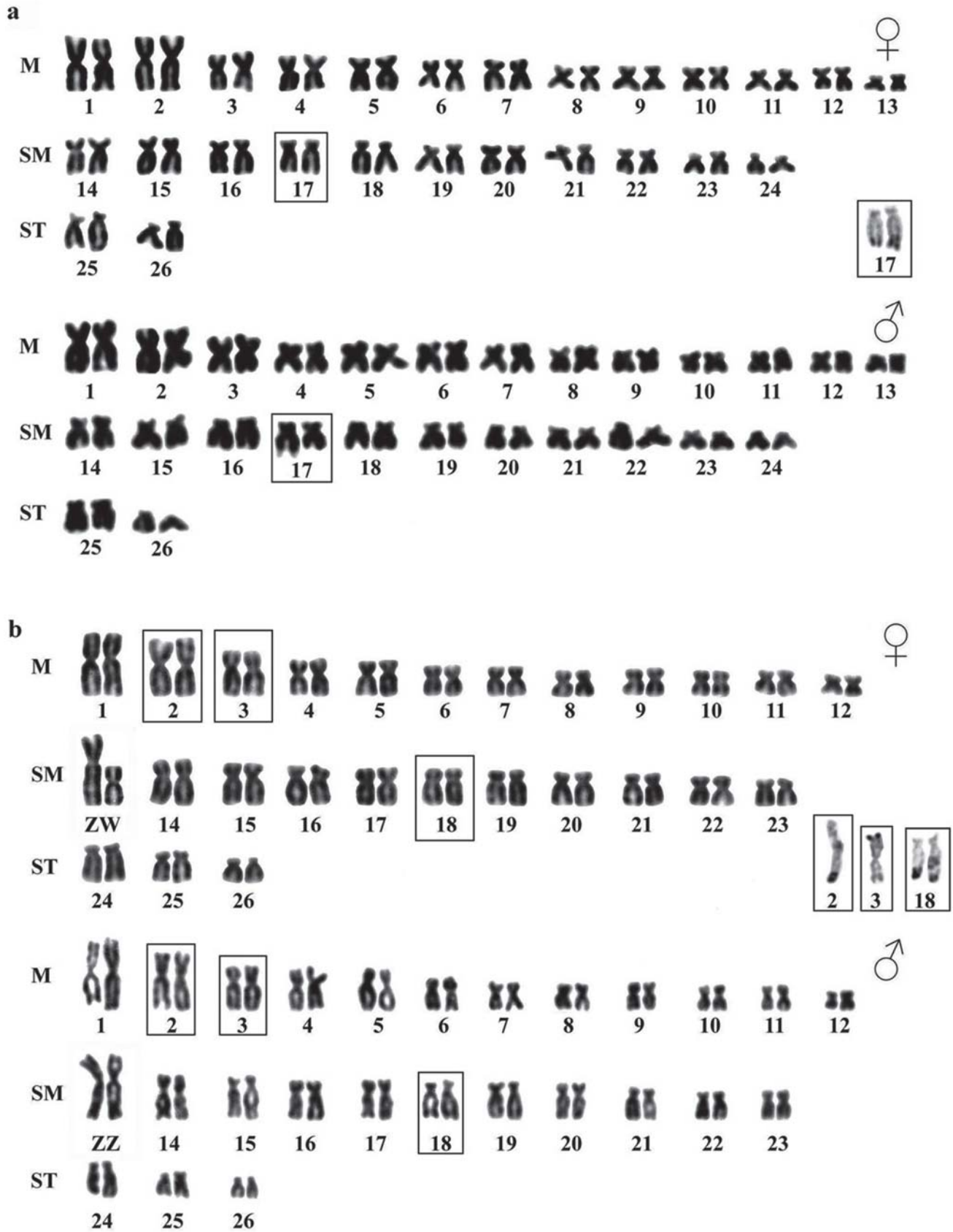


Fig. 2. Karyotypes of female and male of *Hemiancistrus spinosissimus* (a) and *Hemiancistrus spilomma* (b), with conventional Giemsa staining. The chromosome pair bearing Ag-NORs are in the inset.

Nevertheless, our research contributes to new karyological information that can aid in understanding of the evolutionary history of this group of fish. *Hemiancistrus spilomma* and *Hemiancistrus spinosissimus* are morphologically very similar and can easily be confused. Since these species have particular chromosomal characteristics the present cytogenetic data may be used as markers for differentiating the two species in the middle rio Araguaia.

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