

**CYTOGENETIC CHARACTERIZATION OF TWO SPECIES OF *Hypostomus*  
(SILURIFORMES, LORICARIIDAE) FROM TRIBUTARIES OF THE  
VERMELHO RIVER, UPPER PARAGUAY RIVER BASIN**

Quizzi Maria Cordova Becker<sup>1</sup>  
Reinaldo José de Castro<sup>2</sup>  
Adriana Magalhães da Silva<sup>1</sup>  
Patrícia Cristina Vizzotto<sup>2\*</sup>

**ABSTRACT** – Cytogenetic data are presented for two fish species of the genus *Hypostomus* from two streams belonging to the upper Paraguay river basin in the state of Mato Grosso, Brazil. There are no reports in the literature on the cytogenetic features of *Hypostomus* species from these localities. *Hypostomus* aff. *cochliodon* presented a diploid number of  $2n=64$  chromosomes (18M+20SM+26ST/A) and *Hypostomus* sp. showed  $2n=74$  (12M+20SM+42ST/A) chromosomes. Multiple nucleolar organizer regions were evidenced by silver nitrate staining in analyzed species. *Hypostomus* aff. *cochliodon* exhibited large heterochromatin segments at the terminal regions of three chromosome pairs. These results confirm previous data concerning the *Hypostomus* genus and reinforce the importance of studying the ichthyofauna of Mato Grosso, providing perspectives for future research in the area of cytogenetic and molecular biology to elucidate unresolved issues of evolution and taxonomy.

**Key-words:** Neotropical fishes, NORs, C-band, chromosomal evolution.

**CARATERIZAÇÃO CITOGÊNÉTICA DE DUAS ESPÉCIES DE *Hypostomus*  
(SILURIFORMES, LORICARIIDAE) DE TRIBUTÁRIOS DO RIO VERMELHO,  
BACIA DO ALTO RIO PARAGUAI**

**RESUMO** – Dados citogenéticos de duas espécies de peixes do gênero *Hypostomus* de dois pequenos tributários da bacia do Alto Rio Paraguai no estado de Mato Grosso, Brasil, são apresentados. Não há relatos na literatura sobre as características citogenéticas das espécies de *Hypostomus* destas localidades. *Hypostomus* aff. *cochliodon* apresentou o número diplóide de  $2n=64$  cromossomos (18M+20SM+26ST/A) e *Hypostomus* sp.  $2n=74$  (12M+20SM+42ST/A) cromossomos. Várias regiões organizadoras de nucléolos foram evidenciadas pela coloração de nitrato de prata nas espécies analisadas. *Hypostomus* aff. *cochliodon* exibiu grandes segmentos de heterocromatina nas regiões terminais de três pares de cromossomos. Estes resultados confirmam dados anteriores relativos ao gênero *Hypostomus* e reforçam a importância dos estudos da ictiofauna de riachos do estado de Mato Grosso, de forma a proporcionar perspectivas para pesquisas futuras na área de biologia molecular e citogenética com a finalidade de elucidar questões não resolvidas de evolução e taxonomia.

**Palavras-chave:** Peixes Neotropicais, NORs, Banda C, evolução cromossômica.

---

<sup>1</sup> Graduado em Ciências Biológicas/UFMT/CUR/UFMT - Rondonópolis, MT.

<sup>2</sup> Docente do Departamento de Ciências Biológicas. ICEN/CUR/UFMT. MT-270, Km 6, 78735-901, Rondonópolis, Mato Grosso, Brazil. (\*) pcvizzotto@ufmt.br, reinaldocastro@ufmt.br, quizzi\_becker@hotmail.com, adriana\_ufmt@hotmail.com

## INTRODUCTION

Mato Grosso state harbors the headwaters of some major river basins of South America, where the Cerrado, Pantanal and Amazon are closely linked and dependent on these macro-basins and have taxonomically complex fish fauna. According to the latest surveys, the Pantanal has approximately 270 fish species (Britski *et al.*, 2007). Although some groups of fish in this region, mainly those with commercial importance, have been studied in terms of their biology and taxonomy, the genetic data are still scarce, especially for the small fishes, which are practically unknown to science.

A review of published cytogenetic studies shows that chromosome data have provided valuable information about the relationships among fish groups, the occurrence of cryptic species and complexes of species, the mechanisms of sex determination and sex evolution of chromosomes, the distribution of nucleolus organizer regions, the existence of supernumerary chromosomes and the relationship between polyploidy and evolution (Oliveira *et al.*, 2009).

Although the Loricariidae family is considered the largest family of catfishes in the world, the number of Loricariidae species with known karyotypes is limited. In Loricariidae, the subfamily Hypostominae is the best studied but is also the most complex. In Hypostominae, *Hypostomus* is the most species-rich genus, having 126 described species (Reis *et al.*, 2003; Zawadzki *et al.*, 2010). The fishes of this genus are commonly known as armored catfishes and are widely distributed throughout the Neotropical region, with a great diversity in color patterns and morphology, making it difficult to identify species (Armbruster, 2004). Britski *et al.* (2007), studying fishes from the Pantanal, considered the species of the genus *Hypostomus* to pose such a complex taxonomic problem that they were unable to confidently assign any species names.

*Hypostomus* species are benthic fishes that are mainly herbivorous or detritivorous (Casatti, 2002) and exhibit non-migratory and nest/brood guard behavior (Suzuki *et al.*, 2000). Such behavioral traits make these fishes good candidates for approaches that account for both genetic variation and genetic structure because very little gene flow between populations is expected (Zawadzki *et al.*, 2005).

Considering the high number of species of *Hypostomus*, there are few cytogenetic data available in the literature (Bueno *et al.*, 2012). The cytogenetic data on the genus

*Hypostomus* show that the diploid number varies from 52 chromosomes in *Hypostomus emarginatus* (Artoni and Bertollo, 2001) to 84 in *Hypostomus* sp.2 (Cereali *et al.*, 2008), and the most common diploid number among the studied species is 72 chromosomes.

The aim of this study was to characterize cytogenetically two species of *Hypostomus* collected from streams belonging to the upper Paraguay river basin, in the municipality of Rondonópolis, Mato Grosso state, Brazil. There are no reports in the literature on the cytogenetic features of the species of *Hypostomus* from this locality, and these new data can help clarify the evolutionary history of the group.

## MATERIALS AND METHODS

Two species of *Hypostomus* were collected for cytogenetic analysis from the Esparramo stream (16°30.326'S/054°40.526'W) and the Pitaluga stream (16°28.635'S/054°32.138'W), small tributaries of the Vermelho river, which is an affluent of the São Lourenço river in the upper Paraguay river basin in the municipality of Rondonópolis, Mato Grosso, Brazil. A total of forty-nine specimens were analyzed: twenty-one *Hypostomus* aff. *cochliodon* from the Pitaluga stream, nineteen *Hypostomus* aff. *cochliodon* from the Esparramo stream and nine *Hypostomus* sp. from the Esparramo stream. The fishes were identified and deposited in the Laboratório de Zoologia, UFMT, and Rondonópolis, MT, Brazil.

Chromosome spreads and staining procedures were performed following the methods of Foresti *et al.* (1981). Active nuclear organizer regions (NORs) were detected by silver nitrate staining (Howell and Black, 1980), and constitutive heterochromatin was visualized by C-banding (Sumner, 1972). Karyotypes were made based on the arm ratio as proposed by Levan *et al.* (1964), and chromosomes were classified as metacentric (M), submetacentric (SM) and subtelocentric/acrocentric (ST/A). The fundamental number (NF) was determined by considering the metacentric and submetacentric chromosomes to have two arms and the subtelocentric/acrocentric chromosomes to have one arm.

## RESULTS

The cytogenetic results for the analyzed specimens are summarized in Table 1.

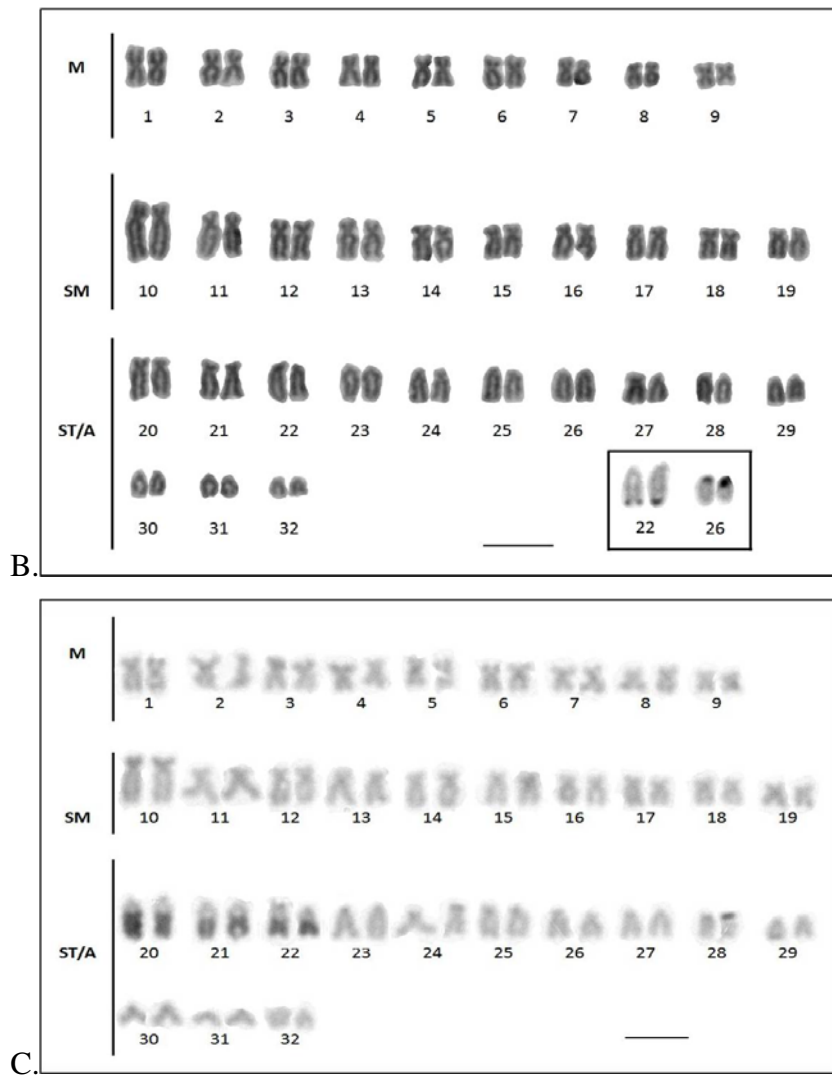
**TABLE 1. Summary of the cytogenetic data for the *Hypostomus* species analyzed in the present study.**

Species	Sampling Site	Diploid Number	Karyotype	NF	NOR Pair
<i>H. aff. cochliodon</i>	Esparramo stream	2n=64	18M+20SM+26ST/A	102	22, 26
<i>H. aff. cochliodon</i>	Pitaluga stream	2n=64	18M+20SM+26ST/A	102	22, 26
<i>Hypostomus</i> sp.	Esparramo stream	2n=74	12M+20SM+42ST/A	106	8, 24

NF: Fundamental number, M: metacentric, SM: submetacentric, ST: subtelocentric, A: acrocentric.

No karyotypic differences were observed among the individuals of *Hypostomus* aff. *cochliodon* collected from different localities. The studied samples of *Hypostomus* aff. *cochliodon* (Figure 2A) showed a diploid number of 2n=64 chromosomes, distributed into a karyotype of 18M+20SM+26ST/A, with a NF of 102 (Figure 2B). NORs appeared in two pairs of subtelocentric/acrocentric chromosomes at the terminal regions of the long arms of pair 22 and the short arms of pair 26 (Figure 2B, inset). The chromosomes of the individuals analyzed exhibited small amounts of C-band-positive heterochromatin, but large heterochromatin segments were observed at the terminal regions on the long arms of pairs 20, 21 and 22 (Figure 2C).

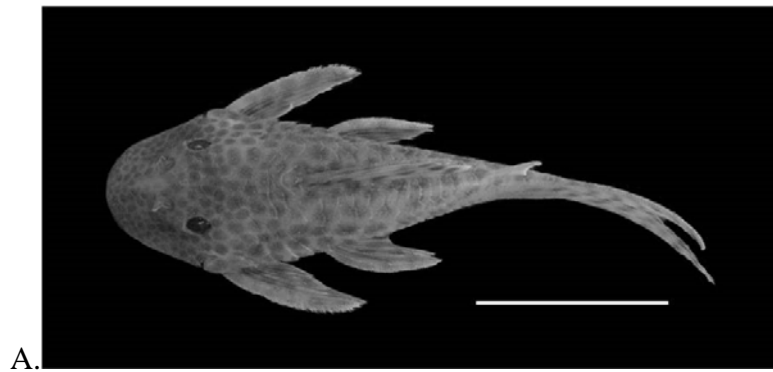




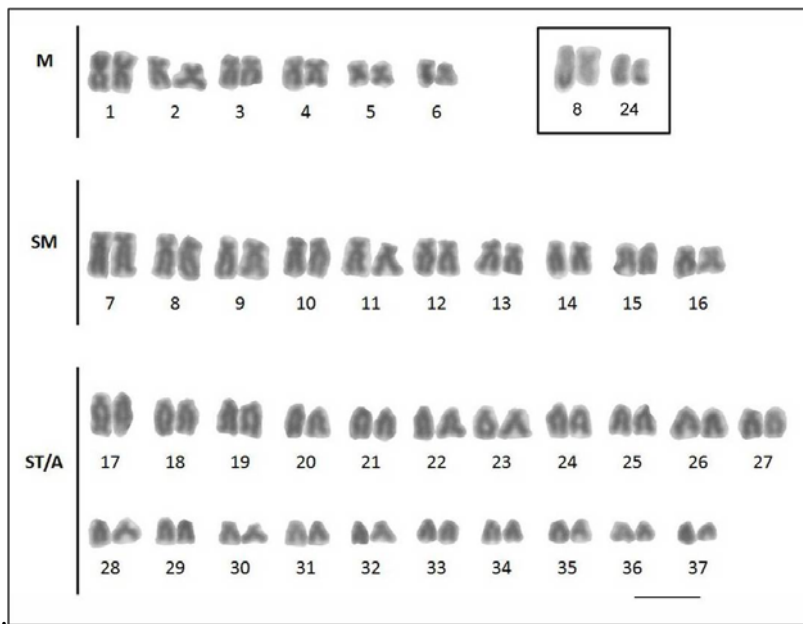
**FIGURE 2.** Specimen (A), Giemsa-stained karyotype (B) and C-band karyotype (C) of *Hypostomus aff. cochliodon* with  $2n=64$  chromosomes. The inset in (B) shows silver-stained chromosomes with the nucleolar organizer region. Scale bars: 3 cm (specimen), 10  $\mu\text{m}$  (karyotype). M=metacentric, SM=submetacentric and ST/A=subtelocentric/acrocentric chromosomes.

The species *Hypostomus* sp. (Figure 3A) showed  $2n=74$  chromosomes, arranged in a karyotype of  $12M+20SM+42ST/A$ , and a  $NF=106$  (Figure 3B). This species showed NORs at the terminal regions of the long arms of submetacentric pair 8 and the long arms of subtelocentric/acrocentric pair 24 (Figure 3B, inset).

No differences were found between the male and female karyotypes in either species studied in this work. Similarly, neither species showed B chromosomes.



A.



B.

**FIGURE 3.** Specimen (A) and Giemsa-stained karyotype (B) of *Hypostomus* sp. with  $2n=74$  chromosomes. The inset in (B) shows silver-stained chromosomes with the nucleolar organizer region. Scale bars: 3 cm (specimen), 10  $\mu\text{m}$  (karyotype). M=metacentric, SM=submetacentric and ST/A=subtelocentric/acrocentric chromosomes.

## DISCUSSION

In the present study, the diploid numbers observed in the analyzed specimens of *Hypostomus* aff. *cochliodon* ( $2n=64$  chromosomes) and *Hypostomus* sp. ( $2n=74$  chromosomes) are consistent with the chromosomal data previously reported for the *Hypostomus* genus, which show a wide variation in the diploid number, from 52 to 84 chromosomes.

Cereali (2006) described the karyotype formulae for *Hypostomus cochliodon* populations ( $2n=64$  chromosomes) from Planalto da Bodoquena, Mato Grosso do Sul, Brazil, to be  $16M+19SM+29ST/A$ ,  $NF=99$  in females and juveniles and

16M+20SM+28ST/A, NF=100 in one male, suggesting the occurrence of an XX/XY sex chromosome system in those populations. The karyotype formula of *Hypostomus* aff. *cochliodon* populations (2n=64 chromosomes) from the two tributaries of the Vermelho river, analyzed in the present work, was 18M+20SM+26ST/A, NF=102, demonstrating a strong similarity between this karyotype formula and those described by Cereali (2006) for the populations from Planalto da Bodoquena. In the present work, which analyzed a large number of juveniles, no differences were observed between the karyotypes of males and females. Rubert *et al.* (2011) observed that the available cytogenetic studies have reported some species that possess the same diploid number but have different karyotypes and fundamental numbers among different populations; the authors commented that this difference may be ascribed to the existence of different cytotypes in these species, the occurrence of cryptic species or problems with species identification or chromosomal classification.

*Hypostomus* sp. from the Esparramo stream, a tributary of the Vermelho river in the upper Paraguay river basin, seems to be an undescribed species, as it does not match any of the nominal *Hypostomus* species in the basin listed in Zawadzki *et al.* (2010). The karyotype formula of *Hypostomus* sp. was 12M+20SM+42ST/A, NF=106. This diploid number, 2n=74 chromosomes, is the same as in *H. paulinus*, *H. strigaticeps* (Michele *et al.*, 1977), *H. albopunctatus* (Artoni and Bertollo, 1996) and *Hypostomus* aff. *agna* (Martinez *et al.*, 2011), although these species can be differentiated by their karyotypic organization.

In the present study, *Hypostomus* aff. *cochliodon* (2n=64 chromosomes) showed a higher number of metacentric/submetacentric chromosomes, while in *Hypostomus* sp. (2n=74 chromosomes) subtelocentric/acrocentric chromosomes prevailed. In Loricariidae, the karyotypes with 2n=54 chromosomes could represent a plesiomorphic condition (Artoni and Bertollo, 2001; Kavalco *et al.*, 2005). Endo *et al.* (2012) propose that the higher number of chromosomes of most of species of *Hypostomus* could be a derived condition, and considering the karyotype analyses, *Hypostomus* species seem to be the most derivate members of the Loricariidae family. According to Artoni and Bertollo (2001), it is possible that chromosomal structure could be more conserved among *Hypostomus* species with smaller diploid numbers, while those with larger chromosomal numbers could be karyotypically derived, indicating that the occurrence of centric fissions could have been important in the derivation of species of this group. However, Bueno *et al.*

(2012) suggested that there is no correlation between chromosome numbers and percentages of subtelocentric and acrocentric chromosomes for most of the species, since there is considerable variation between these percentages even between species with the same diploid number, indicating that the proportion of chromosome types is not always associated to diploid numbers.

The two species analyzed in this study showed multiple NORs with markings at the terminal positions at different sites in each species.

*Hypostomus* aff. *cochliodon* presented NORs in two pairs of subtelocentric/acrocentric chromosomes at the terminal regions of the long arms of pair 22 and the short arms of pair 26. After the application of the C-banding method, the specimens showed large heterochromatin blocks at the terminal regions of the long arms of subtelocentric/acrocentric chromosomes pairs 20, 21 and 22. The occurrence of adjacent nucleolus organizer regions and positive heterochromatic bands has been described by Artoni and Bertollo (1999) in other species of the genus, and these occurred herein on pair 22 of *Hypostomus* aff. *cochliodon*.

*Hypostomus* sp. presented NORs at the terminal regions of the long arms of submetacentric pair 8 and subtelocentric/acrocentric pair 24. There was size heteromorphism between the NORs on homologous chromosomes of pair 8. In fishes, this characteristic is common and can be a consequence of unequal crossing-over between homologous chromosomes (Rubert *et al.*, 2008).

Several Hypostominae species have an extensive diversity of NOR phenotypes (Artoni and Bertollo, 2001; Kavalco *et al.*, 2005, Martinez *et al.*, 2011). Many Hypostominae species have a single NOR at a terminal position (Artoni and Bertollo, 2001; Cereali *et al.*, 2008; Milhomem *et al.*, 2010), but the presence of multiple NORs located at terminal positions is also constant in the *Hypostomus* genus (Artoni and Bertollo, 1996; Alves *et al.*, 2006; Rubert *et al.*, 2008; Martinez *et al.*, 2011), while interstitial NORs are less common (Kavalco *et al.*, 2005). Artoni and Bertollo (1996) proposed that NORs located terminally on the long arm of a single metacentric chromosomal pair represent the primitive condition in Hypostominae. Based on these hypotheses, the species studied here, with multiple NORs, would be derived in the Hypostominae group.

Based on geological and molecular data, Montoya-Burgos (2003) estimated that the origin of the main clade of *Hypostomus* was in the former Amazon River Basin. Milhomem *et al.* (2010) described different cytotypes in fishes of the genus *Hypostomus*



from the Xingu river. However, there are practically no available cytogenetic data from this region, and an adequate understanding of the karyotypic evolutionary history of *Hypostomus* requires detailed cytogenetic studies of Amazonian species and other constituents of this group.

Bitencourt *et al.* (2011a, 2011b) studied populations of *Hypostomus* from coastal hydrographic systems in eastern Brazil using C-banding and chromosomal digestion by several restriction enzymes to characterize the population differences and peculiarities in the genome organization of *Hypostomus*. The resulting data indicated the occurrence of cryptic species in this group.

Information found in the referenced works and in the present study reinforces the idea that chromosome evolution in the *Hypostomus* genus is non-conservative with respect to the diploid number and chromosomal macrostructure. Fish groups characterized by high mobility and by populations composed of a large numbers of individuals, show stable chromosome constitutions. In contrast, groups of low mobility and low population density usually show extensive interspecific and intraspecific karyotype variation (Oliveira *et al.*, 1988; Artoni and Bertollo, 1996). According this idea, the diversification in this group can be related to the occurrence of different chromosomal rearrangements during the evolutionary history of this genus and/or to mobility and group size.

This information reinforces the importance of studying the ichthyofauna of Mato Grosso to fill the gaps in the knowledge of the evolution and taxonomy of the *Hypostomus* genus and to provide perspectives for future research in the area of cytogenetic and molecular biology.

#### **ACKNOWLEDGMENTS**

The authors are grateful to Cláudio Henrique Zawadzki for identifying the specimens, for critical reading of the manuscript and for valuable suggestions for its improvement; to the Financiadora de Estudos e Projetos (FINEP) for financial support for the purchase of equipment; and to the Universidade Federal do Mato Grosso (UFMT) for logistical support.

## REFERENCES

- ALVES A.L, OLIVEIRA C., NIRCHIO M., GRANADO A. & FORESTI F. **Karyotypic relationships among the tribes of Hypostominae (Siluriformes, Loricariidae) with description of XO sex chromosome system in a Neotropical fish species.** *Genetica* 128:1-9. 2006.
- ARMBRUSTER J.W. **Phylogenetic relationships of the suckermouth armored catfishes (Loricariidae) with emphasis on the Hypostominae and the Ancistrinae.** *Zoological Journal of the Linnean Society* 141:1-80. 2004.
- ARTONI R.F. & BERTOLLO L.A.C. **Cytogenetic studies on Hypostominae (Pisces, Siluriformes, Loricariidae). Considerations on karyotype evolution in genus *Hypostomus*.** *Caryologia* 49:81-90. 1996.
- ARTONI R.F. & BERTOLLO L.A.C. **Nature and distribution of constitutive heterochromatin in fishes, genus *Hypostomus* (Loricariidae).** *Genetica* 106(3):209-214. 1999.
- ARTONI R.F. & BERTOLLO L.A.C. **Trends in Karyotype evolution of Loricariidae fish (Siluriformes).** *Hereditas* 134:201-210. 2001.
- BITENCOURT J.A., AFFONSO P.R.A.M., GIULIANO-CAETANO L. & DIAS A.L. **Identification of distinct evolutionary units in allopatric populations of *Hypostomus* cf. *wuchereri* Günther, 1864 (Siluriformes: Loricariidae): karyotypic evidence.** *Neotropical Ichthyology* 9(2):317-324. 2011a.
- BITENCOURT J.A., AFFONSO P.R.A.M., GIULIANO-CAETANO L. & DIAS A.L. **Heterochromatin heterogeneity in *Hypostomus prope unae* (Steindachner, 1878) (Siluriformes, Loricariidae) from Northeastern Brazil.** *Comparative Cytogenetics* 5:329-344. 2011b.
- BRITSKI H.A., SILIMON K.Z.S. & LOPES B.S. **Peixes do Pantanal: manual de identificação.** Embrapa Informação Tecnológica, Brasília. 2007.
- BUENO V., ZAWADZKI C.H. & MARGARIDO V.P. **Trends in chromosome evolution in the genus *Hypostomus* Lacépède, 1803 (Osteichthyes, Loricariidae): a new perspective about the correlation between diploid number and chromosomes types.** *Reviews in Fish Biology and Fisheries* 22:241-250. 2012.
- CASATTI L. **Alimentação dos peixes em um riacho do Parque Estadual Morro do Diabo, bacia do alto Rio Paraná, sudoeste do Brasil.** *Biota Neotropica* 2(2):1-14. 2002.
- CEREALI S.S. **Estudos citogenéticos de Loricariidae (Siluriformes) do Planalto da Bodoquena - Mato Grosso do Sul.** Master's thesis, Universidade Estadual de Londrina, Londrina. 2006.
- CEREALI S.S., POMPINI E., ROSA R., ZAWADZKI C.H., FROEHLICH O. & GIULIANO-CAETANO L. **Karyotype description of two species of *Hypostomus***

**(Siluriformes, Loricariidae) of the Planalto da Bodoquena, Brazil.** *Genetics and Molecular Research* 7(3):583-591. 2008.

ENDO K.S., MARTINEZ E.R.M., ZAWADZKI C.H., PAIVA L.R.S. & JÚLIO-JÚNIOR H.F. **Karyotype description of possible new species of the *Hypostomus ancistroides* complex (Teleostei: Loricariidae) and other Hypostominae.** *Acta Scientiarum. Biological Sciences* 34(2):181-189. 2012.

FORESTI F., ALMEIDA-TOLEDO L.F. & TOLEDO S.A. **Polymorphic nature of nucleolus organizer regions in fishes.** *Cytogenetics and Cell Genetics* 31(3):137-144. 1981.

HOWELL W.M. & BLACK D.A. **Controlled silver staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method.** *Experientia* 36:1014-1915. 1980.

KAVALCO K.F., PAZZA R., BERTOLLO L.A.C. & MOREIRA-FILHO O. **Karyotypic diversity and evolution of Loricariidae (Pisces, Siluriformes).** *Heredity* 94:180-186. 2005.

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

MARTINEZ E.R.M., ZAWADZKI C.H., FORESTI F. & OLIVEIRA C. **Cytogenetic analysis of five *Hypostomus* species (Siluriformes, Loricariidae).** *Genetics and Molecular Biology* 34(4):562-568. 2011.

MICHELE J.L., TAKAHASHI C.S. & FERRARI I. **Karyotypic study of some species of the family Loricariidae (Pisces).** *Cytologia* 42:539-546. 1977.

MILHOMEM S.S.R., CASTRO R.R., NAGAMACHI C.Y., SOUZA A.C.P., FELDBERG E. & PIECZARKA J.C. **Different cytotypes in fishes of the genus *Hypostomus* Lcépède, 1803, (Siluriformes: Loricariidae) from Xingu river (Amazon region, Brazil).** *Comparative Cytogenetics* 4:45-54. 2010.

MONTOYA-BURGOS J.I. **Historical biogeography of the catfish genus *Hypostomus* (Siluriformes, Loricariidae), with implications on the diversification of Neotropical ichthyofauna.** *Molecular Ecology* 12:1855-1867. 2003.

OLIVEIRA C., ALMEIDA-TOLEDO L.F., FORESTI F., BRITSKI H.A. & TOLEDO-FILHO S.A. **Chromosome formulae of Neotropical freshwater fishes.** *Brazilian Journal of Genetics* 11(3):577-624. 1988.

OLIVEIRA C., FORESTI F. & HILSDORF A.W.S. **Genetics of neotropical fish: from chromosomes to populations.** *Fish Physiology and Biochemistry* 35:81-100. 2009.

REIS R.E., KULLANDER S.O. & FERRARIS-JUNIOR C.L. **Check list of the freshwater fishes of South and Central America.** EDIPUCRS, Porto Alegre. 2003.

RUBERT M., ROSA R., JEREP F.C., BERTOLLO L.A.C. & GIULIANO-CAETANO L. **Cytogenetic characterization of four species of the genus *Hypostomus* Lacépède, 1803 (Siluriformes, Loricariidae) with comments on its chromosomal diversity.** Comparative Cytogenetics 5:397-410. 2011.

RUBERT M., ZAWADZKI C.H. & GIULIANO-CAETANO L. **Cytogenetic characterization of *Hypostomus nigromaculatus* (Siluriformes, Loricariidae).** Neotropical Ichthyology 6:93-100. 2008.

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

SUZUKI H.I., AGOSTINHO A.A. & WINEMILLER K.O. **Relationship between oocyte morphology and reproductive strategy in loricariidae catfishes of the Paraná River, Brazil.** Journal of Fish Biology 57:791-807. 2000.

ZAWADZKI C.H., RENESTO E., PAIVA S. & LARA-KAMEI C.S. **Allozyme relationships in hypostomines (Teleostei: Loricariidae) from the Itaipu Reservoir, upper Rio Paraná basin, Brazil.** Genetica 123:271-283. 2005.

ZAWADZKI C.H., WEBER C. & PAVANELLI C.S. **A new dark-saddled species of *Hypostomus* (Siluriformes: Loricariidae) from the upper rio Paraguay basin.** Neotropical Ichthyology 8(4):719-725. 2010.