Acta Scientiarum. Biological Sciences
ISSN: 1679-9283
eduem@uem.br
Universidade Estadual de Maringá
Brasil

Endo, Kátia Sayuri; Monteiro Martinez, Emanuel Ricardo; Zawadzki, Claudio Henrique; de Souza Paiva, Luiz Ricardo; Ferreira Júlio Júnior, Horácio
Karyotype description of possible new species of the Hypostomus ancistroides complex (Teleostei: Loricariidae) and other Hypostominae
Acta Scientiarum. Biological Sciences, vol. 34, núm. 2, 2012, pp. 1-9
Universidade Estadual de Maringá
.png, Brasil

Acta Scientiarum
http://www.uem.br/acta
ISSN printed: 1679-9283
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v34i2.9318

Karyotype description of possible new species of the *Hypostomus ancistroides* complex (Teleostei: Loricariidae) and other Hypostominae

Kátia Sayuri Endo[1], Emanuel Ricardo Monteiro Martinez[2], Claudio Henrique Zawadzki[1*], Luiz Ricardo de Souza Paiva[2] and Horácio Ferreira Júlio Júnior[1]

*[1]Departamento de Biologia, Centro de Ciências Biológicas, Universidade Estadual de Maringá, Av. Colombo, 5790, 87020-900, Maringá, Paraná, Brazil. [2]Departamento de Morfologia, Instituto de Biociências, Universidade Estadual Paulista "Júlio de Mesquita Filho", Botucatu, São Paulo, Brazil. *Author for correspondence. E-mail: chzawadzki@hotmail.com*

ABSTRACT. Cytogenetic analyses were performed in four species of the Hypostominae subfamily, three from *Hypostomus* (Hypostomini) genus and *Rhinelepis aspera* (Rhinelepini). Three populations of *Hypostomus ancistroides* were analyzed, which had 2n=68 chromosomes, but presented different karyotype formulas. *Hypostomus regani* and *H. strigaticeps*, both from Ivaí river, showed 2n=72 chromosomes with two distinct cytotypes. In turn, *R. aspera* of the upper Paraná river basin presented 2n=54 chromosome. Multiple Nucleolar Organizer Regions (NORs) have been evidenced by silver nitrate staining in species of *Hypostomus* and single NOR in *R. aspera*. The observed variation in the chromosome number and the marked variability in karyotype formulas and NORs reveal a certain amount of karyotype variation in the genus *Hypostomus* suggesting the probable existence of cryptic species with independent chromosome traits. Therefore, our data can be of great value in discriminating species and understanding their chromosomal evolution.

Keywords: chromosomal evolution, cryptic species, Neotropical fishes, NOR.

Descrição cariotípica de possíveis novas espécies do complexo *Hypostomus ancistroides* e outros Hypostominae

RESUMO. Foram analisadas três populações de peixes identificadas como *Hypostomus ancistroides*, as quais apresentaram 2n=68 cromossomos, com distintas fórmulas cariotípicas. *Hypostomus regani* e *H. strigaticeps*, ambas do rio Ivaí, apresentaram 2n=72 cromossomos com citótipos distintos. *Rinelepis aspera* da bacia do alto rio Paraná apresentou 2n=54 cromossomos. Nucleolar Organizer Regions (NORs) múltiplas foram evidenciadas por nitrato de Prata para as espécies do gênero *Hypostomus* e NOR simples para *R. aspera*. A variação de número cromossômico observada, como também a acentuada variação nas fórmulas cariotípicas e nas NORs, são discutidas, sugerindo a existência de possíveis espécies crípticas com caracteres cromossômicos independentes. Portanto, nossos dados podem ser de grande valia na discriminação das espécies e no entendimento de sua evolução cromossômica.

Palavras-chave: evolução cromossômica, espécies crípticas, peixes Neotropicais, NOR.

Introduction

Siluriformes is an extremely large, diverse and widely distributed order of fish inhabiting tropical regions around the world (FERRARIS, 2007; TEUGELS, 1996). The number of species within Siluriformes is about 3100 and may be even higher (FERRARIS, 2007; NELSON, 2006; REIS et al., 2003; TEUGELS, 1996). Among the Siluriformes, the family Loricariidae has around 680 species, distributed into seven subfamilies: Delturinae, Hypoptomatinae, Loricariinae, Hypostominae, Neoplecostominae, Lithogeneinae and Otothyrinae (CHIACHIO et al., 2008; REIS et al., 2003, 2006).

Armbruster (2004) proposes Ancistrini and Hypostomini as tribes of the subfamily Hypostominae and created three new tribes in this subfamily: Corymbophanini, Pterygoplichthini, Rhinelepini. He also suggested the removal of *Delturus*, *Hemipsilichthys*, *Isbrueckerichthys*, *Kronichthys*, *Pareiorhina* and *Upsilodus* from the subfamily Hypostominae. Then, the subfamily Hypostominae is recognized as monophyletic. Armbruster (2004) recognized the genus *Hypostomus* as the only representative of Hypostomini.

Cytogenetically, the subfamily Hypostominae is the most well studied of the family Loricariidae, but it is also the most complex comprising a variation of the diploid number from 2n=38 chromosomes in *Ancistrus* sp. (ALVES et al., 2005a) to 2n=84 chromosomes in *Hypostomus* sp. (CEREALI et al., 2008). A very interesting feature in Hypostominae, particularly among *Hypostomus*, is the inverse relationship between the diploid number and the number of chromosomes with two arms, suggesting the occurrence of many events of centric fusion/fission during the development of the group (ARTONI; BERTOLLO, 2001).

According to Artoni and Bertollo (1996) these fish exhibit not conservative characteristics on the diploid number, karyotypic macrostructure and chromosome banding (ARTONI; BERTOLLO, 1996). Currently, the majority of cytogenetic data on *Hypostomus* is related to the diploid number, karyotype formula and location of NOR (RUBERT et al., 2008).

The Hypostominae, showing high adaptive performance, inhabit several freshwater environments from headwaters (ALVES et al., 2005a) to major hydrographic basins (JEREP et al., 2007). This distribution pattern could lead to distinct groups without apparent geographic barriers, which makes the cytogenetic and molecular studies useful tools for identification of cryptic species (BICKFFORD et al., 2007; IRWIN, 2002). The Karyotype description of *Hypostomus ancistroides* from different localities showing different karyotype constitutions seems to reinforce such a view. In this study, three species of the genus *Hypostomus*, *H. ancistroides* (Ihering), *H. regani* (Ihering) and *H. strigaticeps* (Regan), and *Rhinelepis aspera* Spix & Agassiz are karyotyped aiming to enlarge the knowledge on patterns of diversity and karyotype evolution in catfishes of the upper Paraná river basin.

Material and methods

Three species of the genus *Hypostomus* and one of the *Rhinelepis* were collected (Table 1 and Figure 1). The specimens were collected under license from the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA), protocol number 11360-1. They were anesthetized with benzocaine (5%) and then sacrificed for subsequent cytogenetic analysis. The collected specimens were fixed in 10% formalin and are stored in 70% alcohol for further taxonomic studies. The preserved specimens were deposited in the collection at the Núcleo de Pesquisas em Limnologia, Ictiologia e Aqüicultura (Nupélia) of the 'Universidade Estadual de Maringá', Maringá, Paraná State, Brazil. *Hypostomus ancistroides* – NUP 4556; *H. regani* – NUP 4360; *H. strigaticeps* – NUP 7512; *Rhinelepis aspera* – NUP 1726.

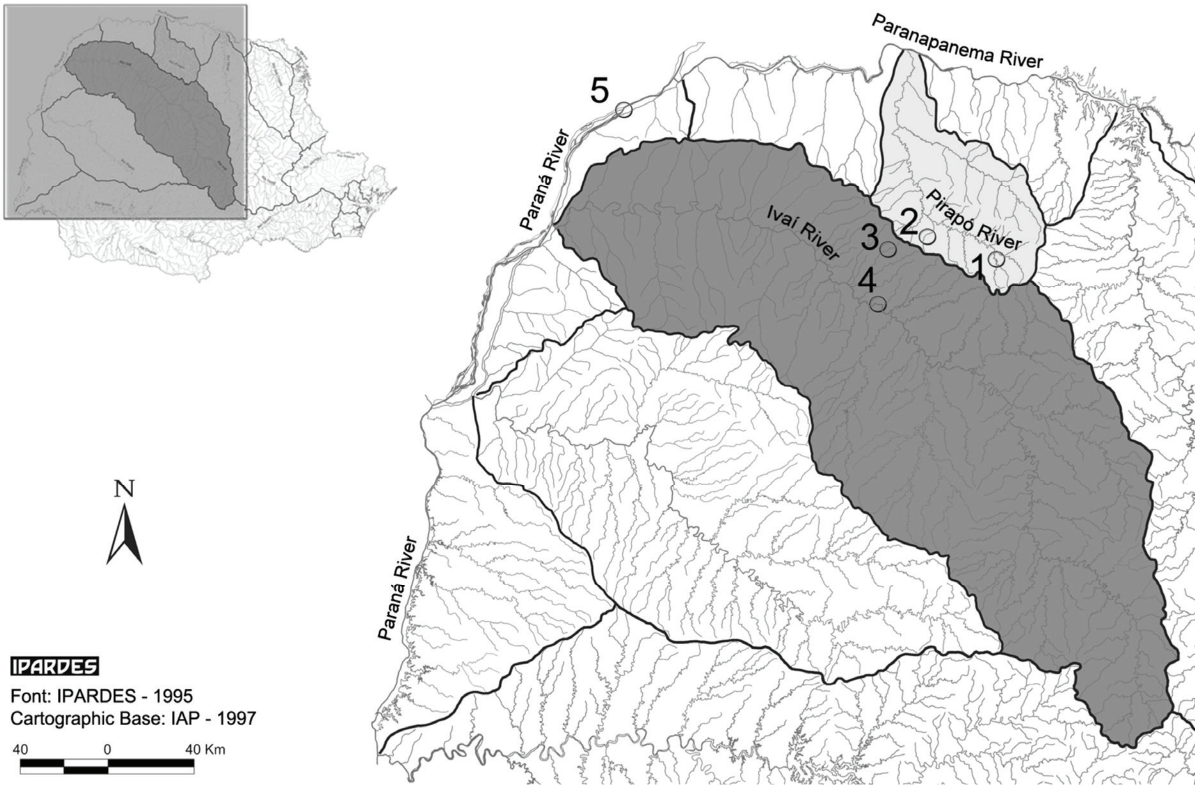

Figure 1. Hydrographic map of Paraná state showing the main rivers of the upper Paraná river basin. Numbers refers to the collecting sites: - Pirapó river basin: 1) Dourados stream; 2) Maringá stream; - Ivaí river basin: 3) Ximbaúva stream; 4) Ivaí river; - upper Paraná river basin: 5) Paraná river.

Table 1. Species, collecting sites and sex of analyzed specimens.

Species	Collection sites	Sex
Hypostomus ancistroides	Dourados stream, municipality of Mandaguari, Paraná State. (1)	4 males and 2 females
Hypostomus ancistroides	Maringá stream, municipality of Maringá, Paraná State. (2)	3 males and 1 female
Hypostomus ancistroides	Ximbaúva stream, municipality of Ourizona, Paraná State. (3)	2 males and 2 females
Hypostomus regani	Ivaí river, municipality of Floresta, Paraná State. (4)	2 males and 2 females
Hypostomus strigaticeps	Ivaí river, municipality of Floresta, Paraná State. (5)	3 males and 2 females
Rhinelepis aspera	Paraná river, Municipality of Porto Rico, Paraná State. (6)	3 males and 1 female

Metaphasic preparations of chromosomes were performed through the technique of air drying (FORESTI et al., 1981) and the detection of the nucleolus organizer regions (NOR) by the Silver Impregnation technique of Howel and Black (1980). Chromosome morphology was established on the basis of arm ratio as proposed by Levan et al. (1964), and the chromosome nomenclature commonly attributed to fish as metacentric (m), submetacentric (sm), subtelocentric (st) and acrocentric (a) was used. For the description of the chromosomes bearing NORs the karyotypes were sequentially assembled for all species.

Results and discussion

Cases of sex chromosomes were found in the literature for some Hypostominae, as ZZ/ZW in *Hypostomus* sp. (ARTONI et al., 1998) and XX/X0 in *Ancistrus* sp. 1 (ALVES et al., 2006), which was not observed in this study, showing that chromosomal changes related to gender/sex should be a variable character in the group.

Specimens of *Hypostomus ancistroides* analyzed showed 2n=68 chromosomes, however, variations in the karyotype formula were observed.

The population of Dourados stream presented a karyotypic number with 14m, 12sm, 18st and 24a and fundamental number (NF) = 56 (Figure 2a, Table 2), the population of Maringá stream showed 16m, 12sm, 18st and 22a and NF = 57 (Figure 2b, Table 2), and the population of Ximbaúva stream, 8m, 10sm, 18st and 32a and NF = 52 (Figure 2c, Table 2).

Differences in karyotype formulas have also been described for other populations of *H. ancistroides* by Michele et al. (1977), Artoni and Bertollo (1996) and Alves et al. (2006) (Table 2). These differences in the karyotype formula between submetacentric and subtelocentric may be involved in the karyotype assembly since chromosomes are small, with size not more than 5μm, but herein the variation that occurs between metacentric and acrocentric chromosomes, does not allow assembly errors, ensuring the karyotypic formula as unique to each species.

These results suggest that the group known as *H. ancistroides* form a complex of species not so far diagnosed by morphological characteristics. The impregnation by silver nitrate revealed multiple NORs in the three populations of *Hypostomus ancistroides* analyzed, differing only in the type of chromosome pairs where they were found (Figure 2). In the population of Dourados stream (Figure 2a), the stained regions were observed in the terminal portion of short arms of pair nine and only one chromosome of pair 10, while for the population of Maringá stream (Figure 2b) the markings were in the terminal regions of short arms of two pairs to chromosomes 11 and 15 and only in a single chromosome of pair five. The population of Ximbaúva stream (Figure 2c) showed three markings in the terminal regions of short arms of only single chromosomes to pairs tree (metacentric), five and nine (submetacentric).

Hypostomus ancistroides is a widely distributed species throughout the upper Paraná river basin, inhabiting streams and creeks, specially under marginal vegetation (CASATTI, 2005). The fact of its preference for streams, the sedentary habits of most *Hypostomus* species and its wide distribution in this basin, make the many populations of *H. ancistroides* prone to show some degree of genetic divergence to other conspecific populations.

The maintenance of this variation could be tentatively explained by the ‘dilution gene flow cascate’, a model for non migratory species (Figure 3), based in the works of Irwin (2002) and Bickfford et al. (2007). In this model, the conspecific populations inhabiting their extremity ranges could present some karyotypic differences despite being morphologically similar. In such a scenario one population with fixed chromosome alterations (population a = Pa), has immediate contact with a subsequent population (b = Pb), and this has contact with another subjacent population (c = Px), and so on. Only a few individuals could maintain a gene flow between adjacent populations, however in the extreme edges of the species range we will probably detect differences among Pa and Px. The gene flow should occur in both directions when free of barriers. Such a kind of differentiation is very common in fishes (DE AGUIAR et al., 2009; CAPISTANO et al., 2008; NIRCHIO; OLIVEIRA, 2006; PANSONATO-ALVES et al., 2010). However, sometimes, the genetic variation does not origin immediate phenotypic differentiation in the external morphology of an organism. Herein, chromosome alterations as pericentric inversions support the reported karyotype structure due to the significant alterations that occurred in the metacentric and acrocentric chromosomes. Thus, we highlight to the possibility of two cryptic species when comparing the populations of *H. ancistroides* from the Pirapó river basin and the Ivaí river basin.

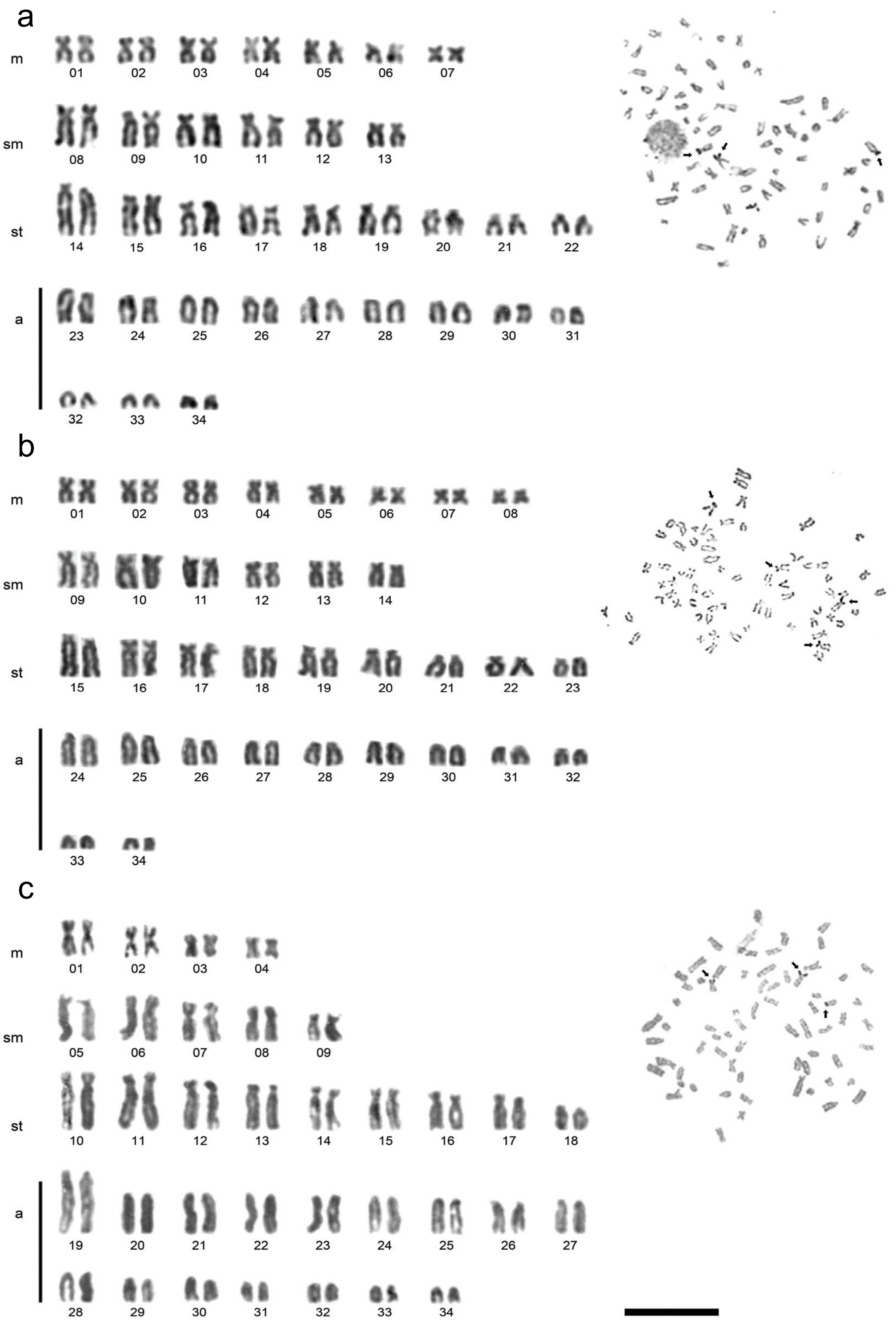

Figure 2. Karyotype showing chromosome morphology and NOR-silver staining dots of populations of *Hypostomus ancistroides* from: a) Dourados stream; b) Maringá stream, and; c) Ximbaúva stream. Bar = 5 μm. Obs.: m = metacentric; sm = submetacentric; st = subtelocentric; a = acrocentric.

Table 2. Summary of cytogenetic data from *Hypostomus* and *Rhinelepis* genera. Obs.: 2n = diploid number; m = metacentric; sm = submetacentric; st = subtelocentric; a = acrocentric; NOR = number of chromosome pairs with nucleolar organizer regions.

Species	Location	2n	Karyotype	NOR	Reference
Hypostomini					
Hypostomus affinis	Jacuí stream, São Paulo State (Paraíba do Sul river basin)	66	14m+14sm+12st+26a	3	Kavalco et al. (2004)
H. aff. *auroguttatus*	Mogi-Guaçu river, Pirassununga, São Paulo State (Mogi-Guaçu river basin)	76	8m+30sm+38st/a	1	Artoni and Bertollo (1996)
H. albopunctatus	Mogi-Guaçu river, Pirassununga, São Paulo State (Mogi-Guaçu river basin)	74	10m+20sm+44st/a	3	Artoni and Bertollo (1996)
H. ancistroides	Monjolinho stream, São Carlos, São Paulo State (Piracicaba river basin)	68	16m+18sm+34st/a	3	Artoni and Bertollo (1996)
H. ancistroides	-	68	10m+28sm+30st/a	-	Michele et al. (1977)
H. ancistroides	Araquá river, Botucatu, São Paulo State (Tietê river basin)	68	18m+10sm+12st+28a	3	Alves et al. (2006)
H. ancistroides	Dourados stream, Mandaguari, Paraná State (Pirapó river basin)	68	14m+12sm+18st+24a	3	Present study
H. ancistroides	Maringá stream, Maringá, Paraná State (Pirapó river basin)	68	16m+12sm+18st+22a	4	Present study
H. ancistroides	Ximbaúva stream, Ourizona, Paraná State (Ivaí river basin)	68	8m+10sm+18st+32a	3	Present study
H. emarginatus	Araguaia river, Barra do Garças, Mato Grosso State (Araguaia river basin)	52	16m+30sm+6st	1	Artoni and Bertollo (2001)
H. goyazensis	Vermelho river, Goiás Velho, Goiás State (Araguaia river basin)	72	10m+16sm+10st+36a	1	Alves et al. (2006)
H. nigromaculatus	Mogi-Guaçu river, Pirassununga, São Paulo State (Mogi-Guaçu river basin)	76	8m+20sm+48st/a	1	Rubert et al. (2008)
H. nigromaculatus	Três Bocas stream, Londrina, Paraná State (Tibagi river basin)	76	6m+20sm+50st/a	2	Rubert et al. (2008)
H. nigromaculatus	Apertados stream, Londrina, Paraná State (Tibagi river basin)	76	8m+20sm+48st/a	2	Rubert et al. (2008)
H. paulinus	-	74	10m+ 20sm+44st/a	-	Michele et al. (1977)
H. macrops	-	68	10m+14sm+44st/a	-	Michele et al. (1977)
H. regani	Ivaí river, Paraná State (Ivaí river basin)	72	12m+ 14sm+26st+20a	2	Present study
H. regani	Mogi-Guaçu river, Pirassununga, São Paulo State (Mogi-Guaçu river basin)	72	10m+20sm+42st/a	-	Artoni and Bertollo (1996)
H. regani	Araquá river, Botucatu, São Paulo State (Tietê river basin)	72	12m+18sm+26st+16a	1	Alves et al. (2006)
Hypostomus sp. 2	Perdido river, Plateau of Bodoquena, Mato Grosso do Sul State (Paraguai river basin)	84	6m+16sm+62st/a	1	Cereali et al. (2008)
Hypostomus sp. 3	Salobrinha stream, Plateau of Bodoquena, Mato Grosso do Sul State (Paraguai river basin)	82	6m+12sm+64st/a	1	Cereali et al. (2008)
Hypostomus sp. 3	Salobrinha stream, Plateau of Bodoquena, Mato Grosso do Sul State (Paraguai river basin)	84	6m+12sm+66st/a	1	Cereali et al. (2008)
Hypostomus sp. A	Rincão river, Rincão, São Paulo State. (Piracicaba river basin)	70	18m+14sm+38st/a	2	Artoni and Bertollo (1996)
Hypostomus sp. B	Mogi-Guaçu river, Pirassununga, São Paulo State (Mogi-Guaçu river basin)	72	12m+18sm+42st/a	1	Artoni and Bertollo (1996)
Hypostomus sp. C	Mogi-Guaçu river, Pirassununga, São Paulo State (Mogi-Guaçu river basin)	72	10m+18sm+44st/a	2	Artoni and Bertollo (1996)
Hypostomus sp. D1	Mogi-Guaçu river, Pirassununga, São Paulo State (Mogi-Guaçu river basin)	72	10m+26sm+36st/a	2	Artoni and Bertollo (1996)
Hypostomus sp. D2	Mogi-Guaçu river, Pirassununga, São Paulo State (Mogi-Guaçu river basin)	72	14m+20sm+38st/a	2	Artoni and Bertollo (1996)
Hypostomus sp. E	Mogi-Guaçu river, Pirassununga, São Paulo State (Mogi-Guaçu river basin)	80	8m+16sm+56st/a	2	Artoni and Bertollo (1996)
H. strigaticeps	Mogi-Guaçu river, Pirassununga, São Paulo State (Mogi-Guaçu river basin)	74	8m+4sm+62st/a	-	Michele et al. (1977)
H. strigaticeps	Ivaí river, Paraná State (Ivaí river basin)	72	10m+ 14sm+18st+30a	4	Present study
Pterygoplichtyni					
Pterygoplichthys anisitsi	Mogi-Guaçu river, Pirassununga, São Paulo State (Mogi-Guaçu river basin)	52	16m+24sm+8st+4a	1	Artoni et al. (1999)
Rhinelepini					
Rhinelepis aspera	Paraná river, Porto Rico, Paraná State (Paraná river basin)	54	24m+18sm+12st	1	Present study
R. aspera	Paraná river, Porto Rico. Paraná State (Paraná river basin)	54	20m+26sm+8st	1	Artoni and Bertollo (2001)
Pogonopoma wertheimeri	Mucuri river, Taquarinha, Bahia State	54	20m+30sm+4st	1	Artoni and Bertollo (2001)

Hypostomus regani presented a diploid number of 72 chromosomes, showing 12m, 14sm, 26st and 20a (Figure 4a, Table 2), as found by Artoni and Bertollo (1996) and Alves et al. (2006) (Table 2), showing different shapes for chromosomes. *Hypostomus regani* also had multiple NORs, with stained regions found in the short arms of the subtelocentric chromosome pairs 12 and 19 (Figure 4a). Despite species of

Hypostomus are known to be sedentary (GARAVELLO; GARAVELLO, 2004), *H. regani* is one of the most widely-distributed species throughout Paraná-Paraguay river basin. Through alloenzymatic data, Zawadzki et al. (2008b) found genetically-structured populations of *H. regani* from the Manso reservoir (Paraguay river basin), Itaipu reservoir (lower portion of the upper Paraná river basin) and Corumbá reservoir (upper portion of the upper Paraná river basin). Therefore, differences in karyotype formulas between different populations of *H. regani* are somewhat expected, as well.

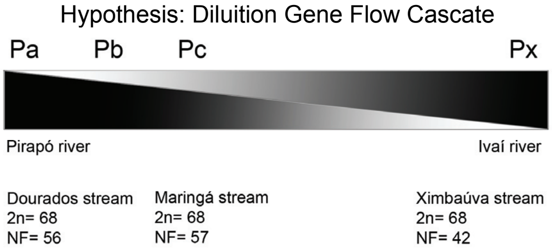

Figure 3. Hypothesis: Dilution Gene Flow Cascate. Pa, Pb, Pc and Px = populations throughout the basin.

Hypostomus strigaticeps presented a diploid number of 72 chromosomes, showing 10m, 14sm, 18st and 30a (Figure 4b, Table 2). *Hypostomus strigaticeps* (Figure 4b) showed multiple NORs due to markings found in the short arms on the subtelocentric chromosome pairs 10, 14 and 18, and in the acrocentric chromosome pair 29. However, Michele et al. (1977) found 2n=74 for *H. strigaticeps* in Mogi-Guaçu river. Two alternatives can be raised for these differences, a) once as the specimens of Michele et al. (1977) were not preserved, this identification may be questioned, given the known taxonomic difficulty of the genus *Hypostomus* (OYAKAWA et al., 2005; REIS et al., 1990; ZAWADZKI et al., 2008a); b) alternatively, they may be conspecific populations exhibiting polymorphisms on chromosome numbers. According to L. Giuliano-Caetano (Pers. Comm.), there are species differing in the number of chromosomes such as in the loricariine *Rineloricaria latirostris*, where the author found a variation of 2n=36 to 2n=48.

Rhinelepis aspera had a diploid number of 54 chromosomes, showing 24m, 18sm and 12st (Figure 4c, Table 2). *Rhinelepis aspera* was the only species to exhibit single NOR, located on the short arm of the first metacentric chromosome (Figure 4c).

This diploid number was also found in populations analyzed by Artoni and Bertollo (2001) of *R. aspera*, exhibiting a distinct karyotype formula (Table 2). Zawadzki et al. (2005) observed through alloenzymatic data that *R. aspera* is in a basal position when related to other Hypostominae from the Itaipu reservoir. Additionally, based on morphological data, Armbruster (2004) considered *R. aspera* basal for Hypostomini and Pterygoplychthini.

Artoni and Bertollo et al. (2001) indicate that the diploid number of 2n=54 chromosomes is a basal condition for Loricariidae. In *Neoplecostomus microps*, *N. paranensis* and *Hemipsilichthys steindachneri* (ALVES et al., 2005b) all the studied populations have 2n=54 chromosomes. In representatives of the subfamily Hypoptopomatinae as *Corumbataia cuestae* and *Hisonotus depressicauda* (FERREIRA et al., 2005) it was also found the same diploid number. The karyotype morphology of these species show a higher number of metacentric/submetacentric chromosomes, corroborating the hypothesis of Artoni and Bertollo (2001) that the inverse relationship between the diploid number and the number of chromosomes with two arms suggests the occurrence of many events of centric fusion/fission in the developments of the group. Thus, it is proposed that the higher number of chromosomes to most of the species of *Hypostomus* should be a derived condition, and *Hypostomus* seems to be one of the most derivative taxon in karyotypic terms within Loricariidae.

The diploid number in *Hypostomus* varies from 2n=52 in *H. emarginatus* (ARTONI; BERTOLO, 2001) to 84 in *Hypostomus* sp. 3 (CEREALI et al., 2008). Some studied species have distinct karyotype formulas and the chromosome variation is accompanied by an increase in the number of subtelo/acrocentric chromosome types. According to Artoni and Bertollo (1996), chromosomal rearrangements as centric fission and pericentric inversions play an important role in karyotype evolution of this group of fish.

The karyotyped species of the tribes of Hypostominae show the same relationship proposed by Artoni and Bertollo (2001) on the amount of chromosomes and the variation of karyotype morphology. The representatives of the tribe Ancistrini as *Ancistrus* sp. 2 (2n=52 chromosomes) exhibits karyotype formula equal to 32m/sm and the 20st/a and *Ancistrus multispinnis* (2n=52 chromosomes) shows 28m /sm and 24st/a (ALVES et al., 2005a); the Corymbophanini, *Corymbophanes* sp. (2n=54 chromosomes) has 20m, 20sm and 14st (ALVES et al., 2005b); the Pterygoplichthini, *Pterygoplichthys joselimaianus* shows 2n=52 chromosomes (OLIVEIRA et al., 2006) and *Hemiancistrus* sp. (2n=52 chromosomes) 20m, 20sm and 12st/a; whereas the Rhinelepini, *Rhinelepis aspera* (2n=54 chromosomes) has 20m, 26sm and 8st (ARTONI; BERTOLLO, 1996).

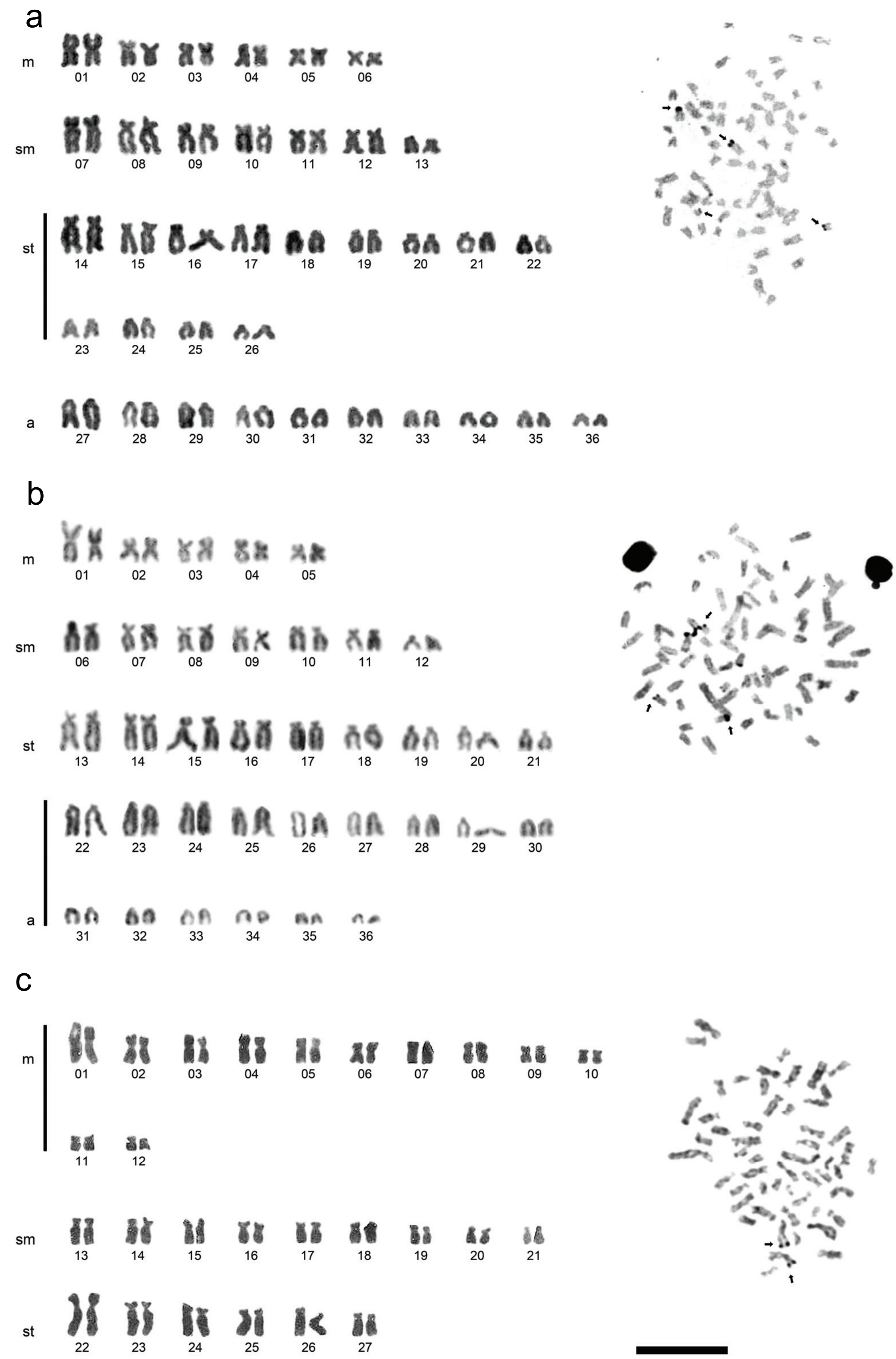

Figure 4. Karyotype showing chromosome morphology and NOR-silver staining arrowhead of: a) *Hypostomus regani* from Ivaí river; b) *H. strigaticeps* from Ivaí river and; c) *Rhinelepis aspera* from Paraná river. Bar = 5 μm. Obs.: m = metacentric; sm = submetacentric; st = subtelocentric; a = acrocentric.

This relatively conserved karyotype number of 52 and 54 among the representatives of different Loricariidae subfamilies corroborates the hypothesis that these karyotype numbers should be a basal condition for Loricariidae. Therefore, in the light of the results herein obtained the specimens of these three species of *Hypostomus* presented a more derived karyotype structure within Loricariidae.

Conclusion

NORs on Loricariidae present a great phenotypic diversity. However, single NOR in terminal position on the chromosome presents itself as a basal character, which is usually found in Hypostominae and Hypoptopomatinae (ARTONI; BERTOLLO, 1996; OLIVEIRA; GOSZTONYI, 2000). In sum, the high amount of variation in karyotype and NOR structure draw a karyotypically fluctuating evolutive condition for this group. Additionally, many species of *Hypostomus* are taxonomically undefined and there is still a lack of characters enabling nominate them as distinct species.

Acknowledgements

The authors thank Carlos Alexandre Fernandes and Luciana A. Borin due to the critical reading and valuable suggestions to the manuscript; to Izabel Cristina Martins-Santos and Ana Luiza B. P. Castro for the aid in the laboratorial procedures to the Núcleo de Pesquisas em Limnologia, Ictiologia e Aqüicultura (Nupélia) for logistical support; to the Coordenadoria de Aperfeiçoamento de Ensino Superior (CAPES) and to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support (to CHZ process 306066/2009-2).

References

ALVES, A. L.; OLIVEIRA, C.; FORESTI, F. Comparative cytogenetic analysis of eleven species of subfamilies Neoplecostominae and Hypostominae (Siluriformes: Loricariidae). **Genetica**, v. 124, n. 2-3, p. 127-136, 2005a.

ALVES, A. L.; OLIVEIRA, C.; FORESTI, F. Karyotype variability in eight species of the subfamilies Loricariinae and Ancistrinae (Teleostei, Siluriformes, Loricariidae). **Caryologia**, v. 56, n. 1, p. 57-63, 2005b.

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**, v. 128, n. 1, p. 1-9, 2006.

ARMBRUSTER, J. W. Phylogenetic relationships of the sucker-mouth armored catfishes (Loricariidae) with particular emphasis on the Hypostominae and the Ancistrinae. **Zoological Journal of the Linnean Society**, v. 141, n. 1, p. 1-80, 2004.

ARTONI, R. F.; BERTOLLO, L. A. C. Cytogenetic studies on Hypostominae (Pisces, Siluriformes, Loricariidae). Considerations on karyotype evolution in the genus *Hypostomus*. **Caryologia**, v. 49, n. 1, p. 81-90, 1996.

ARTONI, R. F.; BERTOLLO, L. A. C. Trends in the Karyotype evolution of Loricariidae fish (Siluriformes). **Hereditas**, v. 134, n. 3, p. 201-210, 2001.

ARTONI, R. F.; VENERE, P. C.; BERTOLLO, L. A. C. A heteromorphic ZZ/ZW sex chromosome system in fish, genus *Hypostomus* (Loricariidae). **Cytologia**, v. 63, n. 4, p. 421-425, 1998.

ARTONI, R. F.; MOLINA, W. F.; BERTOLLO, L. A. C.; GALETTI J. P. M. Heterochromatin analysis in the fish species *Liposarcus anisitsi* (Siluriformes) and *Leporinus elongatus* (Characiformes). **Genetics and Molecular Biology**, v. 22, n. 1, p. 39-44, 1999.

BICKFFORD, D.; LOHMAN, D. J.; SODHI, N. S.; NG, P. K. L.; MEIER, R.; WINKER, K.; INGRAM, K. K.; DAS, I. Cryptic species as a window on diversity and conservation. **Trends in Ecology and Evolution**, v. 22, n. 3, p. 148-155, 2007.

CAPISTANO, T. G.; PORTELA-CASTRO, A. L. B.; JULIO-JUNIOR, H. F. Chromosome divergence and NOR polymorphism in *Bryconamericus* aff. *iheringii* (Teleostei, Characidae) in the hydrographic systems of the Paranapanema and Ivaí Rivers, Paraná, Brazil. **Genetics and Molecular Biology**, v. 31, n. 1, p. 203-207, 2008.

CASATTI, L. Fish assemblage structure in a first order stream, southeastern Brazil: longitudinal distribution, seasonality, and microhabitat diversity. **Biota Neotropica**, v. 5, n. 1, 2005. Avaliable from: <http://www.biotaneotropica.org.br/v5n1/pt/abstract?article+BN02505012005>. Access on: 22 Jan., 2010.

CEREALI, S. S.; POMINI, 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**, v. 7, n. 3, p. 583-591, 2008.

CHIACHIO, M. C.; OLIVEIRA, C.; MONTOYA-BURGOS, J. I. Molecular systematic and historical biogeography of the armored Neotropical catfishes Hypoptopomatinae and Neoplecostominae (Siluriformes: Loricariidae). **Molecular Phylogenetics and Evolution**, v. 49, n. 2, p. 606-617, 2008.

DE AGUIAR, M. A. M.; BARANGER, M.; BAPTESTINI, E. M.; KAUFMAN, L.; BAR-YAM, Y. Global patterns of speciation and diversity. **Nature**, v. 460, n. 7253, p. 384-388, 2009.

FERRARIS, C. J. Checklist of catfishes, recent and fossil (Osteichthyes: Siluriformes), and catalogue of siluriform primary types. **Zootaxa**, v. 1418, p. 1-628, 2007.

FERREIRA, D. C.; CHIACHIO, M. C.; TAKAKO, A. K.; ANDREATA, A. A.; FORESTI, F.; OLIVEIRA, C. Comparative cytogenetics of nine species of Hypoptopomatinae (Teleostei: Siluriformes: Loricariidae): the importance of structural rearrangements in chromosome evolution. **Caryologia**, v. 58, n. 4, p. 387-395, 2005.

FORESTI, F.; ALMEIDA-TOLEDO, L. F.; TOLEDO, S. A. Polymorphic nature of nucleolus organizer regions in fishes. **Cytogenetics and Cell Genetics**, v. 31, n. 3, p. 137-144, 1981.

GARAVELLO, J. C.; GARAVELLO, J. P. Spatial distribution and interaction of four species of the catfish genus *Hypostomus* Lacépède with bottom of rio São Francisco, Canindé do São Francisco, Sergipe, Brazil (Pisces, Loricariidae, Hypostominae). **Brazilian Journal of Biology**, v. 64, n. 3B, p. 591-598, 2004.

HOWELL, W. M.; BLACK, D. A. Controlled silver staining of Nucleolus Organizer Regions with a protective colloidal developer: a 1-step method. **Experientia**, v. 36, n. 8, p. 1014-1015, 1980.

IRWIN, D. E. Phylogeographic Breaks without Geographic Barriers to Gene Flow. **Evolution**, v. 56, n. 12, p. 2383-2394, 2002.

JEREP, F. C.; SHIBATTA, O. A.; ZAWADZKI, C. H. A new species of *Hypostomus* Lacépède, 1803 (Siluriformes: Loricariidae) from the upper rio Paraná basin, Southern Brazil. **Neotropical Ichthyology**, v. 5, n. 4, p. 435-442, 2007.

KAVALCO, K. F.; PAZZA, R.; BERTOLLO, L. A. C.; MOREIRA-FILHO, O. Heterochromatin characterization of four fish species of the family Loricariidae (Siluriformes). **Hereditas**, v. 141, n. 3, p. 237-242, 2004.

LEVAN, A.; FREGDA, K.; SANDBERG, A. A. Nomenclature for centromeric position on chromosomes. **Hereditas**, v. 52, n. 2, p. 201-220, 1964.

MICHELE, J. L.; TAKAHASHI, C. S.; FERRARI, I. Karyotypic study of some species of the family Loricariidae (Pisces). **Cytologia**, v. 42, n. 3-4, p. 539-546, 1977.

NELSON, J. S. **Fishes of the World**. 4th ed. New Jersey: John Wiley and Sons, 2006.

NIRCHIO, M.; OLIVEIRA, C. **Citogenética de Peces**. 1st ed. Caracas: Universidad de Oriente, 2006. v. 1.

OLIVEIRA, C.; GOSZTONYI, A. E. A cytogenetic study of *Diplomystes mesembrinus* (Teleostei, Siluriformes, Diplomistidae) with a discussion of chromosome evolution in siluriforms. **Caryologia**, v. 53, n. 1, p. 31-37, 2000.

OLIVEIRA, C.; ALMEIDA-TOLEDO, L. F.; FORESTI, F. Karyotypic evolution in Neotropical fishes. In: PISANO, E.; COZOUF-COSTAZ, C.; FORESTI, F.; KAPOOR, B. G. (Org.). **Fish Cytogenetic**. 1st ed. Endfield: Enfield Science Publisher Inc., 2006. p. 111-164.

OYAKAWA, O. T.; AKAMA, A.; ZANATA, A. M. Review of the genus *Hypostomus* Lacépède, 1803 from rio Ribeira de Iguape basin, with description of a new species (Pisces, Siluriformes, Loricariidae). **Zootaxa**, v. 921, p. 1-27, 2005.

PANSONATO-ALVES, J. C.; PAIVA, L. R. S.; OLIVEIRA, C.; FORESTI, F. Interspecific chromosomal divergences in the genus *Characidium* (Teleostei: Characiformes: Crenuchidae). **Neotropical Ichthyology**, v. 8, n. 1, p. 77-86, 2010.

REIS, R. E.; KULLANDER, S. O.; FERRARIS-JR, C. J. **Check list of the freshwater fishes of South and Central**. Porto Alegre: Edipucrs, 2003.

REIS, R. E.; PEREIRA, E. H. L.; ARMBRUSTER, J. H. Delturinae, a new loricariid catfish subfamily (Teleostei, Siluriformes), with revisions of *Delturus* and *Hemipsilichthys*. **Zoological Journal of the Linnean Society**, v. 147, n. 2, p. 277-299, 2006.

REIS, R. E.; WEBER, C.; MALABARBA, L. R. Review of the genus *Hypostomus* Lacépède, 1803 from Southern Brazil, with descriptions of three new species (Pisces, Siluriformes, Loricariidae). **Revue suisse de Zoologie**, v. 97, n. 3, p. 729-766, 1990.

RUBERT, M.; ZAWADZKI, C. H.; GIULIANO-CAETANO, L. Cytogenetic characterization of *Hypostomus nigromaculatus* (Siluriformes: Loricariidae). **Neotropical Ichthyology**, v. 6, n. 1, p. 93-100, 2008.

TEUGELS, G. G. Taxonomy, phylogeny and biogeography of catfishes (Ostariophysi, Siluroidei); an overview. **Aquatic Living Resources**, v. 9, p. 9-34, 1996.

ZAWADZKI, C. H.; BIRINDELLI, J. L. O.; LIMA, F. C. T. A new pale-spotted species of *Hypostomus* Lacépède (Siluriformes: Loricariidae) from the rio Tocantins and rio Xingu basins in central Brazil. **Neotropical Ichthyology**, v. 6, n. 3, p. 395-402, 2008a.

ZAWADZKI, C. H.; RENESTO, E.; PERES, M. D.; PAIVA, S. Allozyme variation among three populations of the armored catfish *Hypostomus regani* (Ihering, 1905) (Siluriformes, Loricariidae) from the Paraná and Paraguay river basins, Brazil. **Genetics and Molecular Biology**, v. 31, n. 3, p. 767-771, 2008b.

ZAWADZKI, C. H.; RENESTO, E.; REIS, R. E.; MOURA, M. O.; MATEUS, R. P. Allozyme relationships in hypostomines (Teleostei: Loricariidae) from the Itaipu Reservoir, Upper Rio Paraná basin, Brazil. **Genetica**, v. 123, n. 3, p. 271-283, 2005.

Received on February 1, 2010.
Accepted on May 11, 2010.