Original article

Cytogenetics of two *Farlowella* species (Loricariidae: Loricariinae): implications on the taxonomic status of the species

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Farlowella is one of the most diverse genera of the Loricariinae, restricted to South America rivers. The taxonomic and phylogenetic relationships among its species are contentious and, while genetic studies would contribute to the understanding of their relationships, the only available *datum* refer to the karyotype description of only one species. In the present study two Amazonian species, *Farlowella* cf. *amazonum* and *F. schreitmuelleri*, were analyzed using conventional and molecular cytogenetic procedures. Both species had diploid chromosome number 58, but different fundamental numbers (NF) 116 and 112, respectively, indicative of chromosomal rearrangements. C-banding is almost poor, especially in *F.* cf. *amazonum*, and occurs predominantly in the centromeric and in some telomeric regions, although genome of *F. schreitmuelleri* possessed a much larger heterochromatin amount then those of *F.* cf. *amazonum*. The chromosomes bearing the NOR sites were likely the same for both species, corresponding to the 1st metacentric pair in *F.* cf. *amazonum* and to the 28th acrocentric in *F. schreitmuelleri*. The location of the 5S rDNA was species-specific marker. This study expanded the available cytogenetic data for *Farlowella* species and pointed the remarkable karyotype diversity among species/populations, indicating a possible species complex within genus.

Key words: Amazon region, Chromosomal markers, Karyotypic characterization, Ribosomal DNA, Twig catfish.

Farlowella é um dos gêneros mais diversos de Loricariinae, restrito aos rios da América do Sul. As relações taxonômicas e filogenéticas entre suas espécies são contenciosas e, enquanto os estudos genéticos contribuem para a compreensão dessas relações, o único dado disponível refere-se à descrição cariotípica de apenas uma espécie. No presente estudo, foram analizadas duas espécies amazônicas *Farlowella* cf. *amazonum* e *F. schreitmuelleri*, empregando procedimentos citogenéticos convencionais e moleculares. Ambas as espécies apresentaram número diploide igual a 58 cromossomos, mas com números fundamentais diferentes (NF) de 116 e 112, respectivamente, indicando rearranjos cromossômicos. Bandas C são poucas, especialmente em *F.* cf. *amazonum*, e ocorre predominantemente nas regiões centroméricas e em algumas regiões teloméricas, embora *F. schreitmuelleri* apresenta uma quantidade de heterocromatina muito maior que *F.* cf. *amazonum*. Os cromossomos carreadores dos sítios da NOR foram provavelmente os mesmos para ambas as espécies, correspondendo ao primeiro par metacêntrico em *F.* cf. *amazonum* e ao 28º acrocêntrico em *F. schreitmuelleri*. A localização do DNAr 5S foi espécie-específico. Este estudo expandiu os dados citogenéticos disponíveis para espécies de *Farlowella* e apontou uma remarcável diversidade cromossômica entre espécies/populações, indicando um possível complexo de espécies neste gênero.

Palavras-chave: Bagre-vara, Caracterização cariotípica, DNA ribossômico, Marcadores cromossômicos, Região amazônica.

Introduction

The Loricariidae is one of the world's most diverse families of freshwater fish (Vari, Malabarba, 1998; Nelson *et al.*, 2016; Reis *et al.*, 2016). Endemic to the Neotropical region, approximately 970 valid loricariid species are known to exist, in eight recognized subfamilies, the Lithogeneinae, Delturinae, Rhinelepinae, Hypoptopomatinae, Neoplecostominae, Hypostominae, Otothyrinae, and Loricariinae, and two *incertae sedis* lineages (Chiachio *et al.*, 2008; Roxo *et al.*, 2014; Lujan *et al.*, 2015; Reis *et al.*, 2016; Eschmeyer, Fong, 2017) where Rhinelepinae and Otothyrinae were created based on molecular data only.

The Loricariinae is a group of armored catfishes divided into two tribes, Harttiini and Loricariini (Covain *et al.*, 2016), with a total of 34 recognized genera, of which, 15 are

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monotypic. The genera *Loricaria* (17 species), *Loricariichthys* (18 species), *Harttia* (23 species), *Farlowella* (29 species), and *Rineloricaria* (63 species) are the most diverse, but also the most contentious in taxonomic and phylogenetic terms (Covain, Fisch-Muller, 2007; Ballen *et al*., 2016).

The species of the genus *Farlowella* are widely distributed in the rivers of South America, including the Amazon, Orinoco, Paraná/Paraguay, and Essequibo basins, and the coastal basins of the Guianas (Ferraris, 2003). These catfish are relatively small, reaching a length of only 26.5 cm, with an extremely thin and elongated body, a bony snout, and prominent tail filaments. The elongated shape and wood-like appearance of these animals has earned them the common name of twig catfish, and their unique morphology makes them extremely popular as ornamental fish (Berra, 2001; Ferraris, 2003; Covain, Fisch-Muller, 2007).

Cytogenetic studies in *Farlowella* species are still scarce. Only one of the 29 recognized species, *F. amazonum*, has been analyzed up to now, with individuals from two locations on the Paraguay River being studied using conventional cytogenetic procedures. In the present study, we describe the karyotype and other chromosomal markers of two Amazonian species of the genus *Farlowella*.

Material and Methods

Sampling sites. We analyzed five individuals of *Farlowella* cf. *amazonum* from the paraná do Piloto in the municipality of Barcelos, Amazonas, Brazil, Negro River basin (0°56'04.8"S, 62°58'01.6"W). We also analyzed 14 individuals of *Farlowella schreitmuelleri* collected from the Jundiá Stream in the municipality of Manaus, Amazonas, Brazil, Cuieiras River basin (2°19'43.8"S, 60°04'40.4"W).

The collection of individuals was authorized by the Brazilian Institute for the Environment and Renewable Natural Resources (IBAMA: Instituto Brasileiro do Meio Ambiente and Recursos Naturais Renováveis) through SISBIO license number 28095-1. Voucher specimens were deposited in the fish collection of the Instituto Nacional de Pesquisas da Amazônia (INPA-ICT 057606, INPA-ICT 057615). This study followed the ethical guidelines established by the INPA Ethics Committee for Animal Research, which approved the research through protocol number 006/2016.

Cytogenetic analyses. The mitotic chromosomes were obtained using the *in vitro* protocol of Gold *et al*. (1990), with the RPMI medium (Cultilab). The chromosomes were stained with Giemsa to determine the diploid number and the chromosome morphology. The chromosomes were classified according to Levan *et al*. (1964). Were applied the C-banding technique (Sumner, 1972) to characterize the heterochromatic band pattern, and silver nitrate impregnation (Ag-NOR) to visualize the nucleolus organizer regions (Howell, Black, 1980).

The probes used to locate the ribosomal genes were obtained by extracting the total DNA from the *Farlow-*

ella muscle tissue using a Wizard® Genomic Purification kit (Promega), according to the manufacturer's protocol. We used a GoTaq Colorless Master Mix kit (Promega) for the Polymerase Chain Reaction (PCR), and the amplification of the 18S rDNA (Gross *et al*., 2010) and 5S rDNA (Martins, Galetti, 1999) genes, and the (TTAGGG)n telomeric sequences (Ijdo *et al*., 1991). The fluorescence *in situ* hybridization (FISH) was based on Pinkel *et al*. (1986), with modifications. The 18S rDNA and telomeric probes were marked with digoxigenin-11 dUTP using a DIG-Nick Translation Mix kit (Roche), while the 5S rDNA probe was marked with biotin-14-dATP using a Biotin-Nick Translation Mix kit (Roche). The FISH had a stringency of 77%. We counterstained the chromosomes with 4', 6-5 diamidino-2-phenylindole dihydrochloride (DAPI, 2 mg/ml) in VECTASHIELD® Mounting Media (Vector).

Results

***Farlowella* cf. *amazonum* (Günther, 1864).** *Farlowella* cf. *amazonum* had diploid chromosome number 58 and karyotype composed of 14metacentric+30submetacentric+14subtelocentric, with fundamental number (FN) = 116 (Fig. 1a). No evidence was found of the presence of differentiated sex. The first chromosome pair had a secondary constriction on the long arm with a subtle difference between the two homologs. This region corresponded to the NOR (Fig. 1c) and the 18S rDNA sites (Fig. 2c).

C-bands are pale, distributed mainly in the centromeric position, although differing in amount among chromosomes. Additionally, some other conspicuous signals were also found, such as in the long arms of the 1st pair and the short arms of the 20th pair that were completely heterochromatic, and in both telomeric regions of pairs No. 13, 21, 26, 27, and 28 (Fig. 1b).

Hybridization with the 5S rDNA probe revealed signals only on the short arms of sm pair No. 20, which was completely heterochromatic (Fig. 2d). The mapping of the telomeric sequences revealed signals in the terminal regions of all the chromosomes, in addition to an accumulation of these sequences in the terminal portion of the long arms of pairs No. 16, 19, 21, 22, 26 and 27, no Interstitial Telomeric Sequences (ITS) were observed (Fig. 2a).

***Farlowella schreitmuelleri* Arnold, 1936.** *Farlowella schreitmuelleri* also had a diploid chromosome number 58 and karyotype composed of 10m+30m+14st+4a, and FN = 112 (Fig. 1d). A secondary constriction was observed in the distal region of the long arms of the acrocentric pair No. 28, together with size heteromorphism, corresponding to the Ag-NOR (Fig. 1f), the ribosomal 18S DNA (Fig. 2e), and positive C-banding sites.

The genome of this species possessed a much larger quantity of heterochromatin compared to *Farlowella* cf. *amazonum*, located in the centromeric region of all chromosomes, where some bands were more conspicuous than others. Inter-
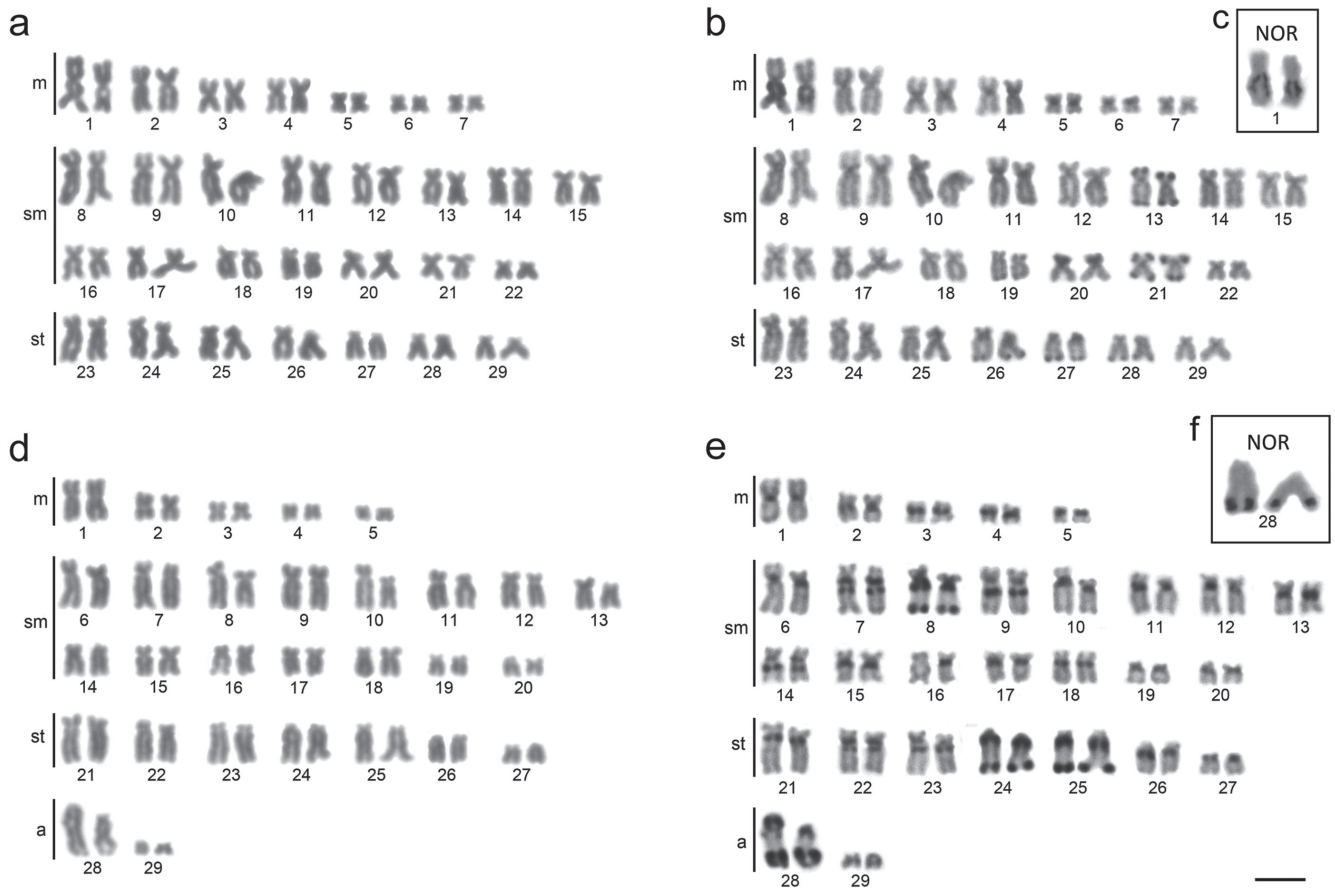
Fig. 1. Karyotype of *Farlowella* cf. *amazonum*: (a) Giemsa staining, (b) C-banding, and (c) NORs (highlighted). Karyotype of *Farlowella schreitmuelleri*: (d) Giemsa staining, (e) C-banding, and (f) NORs (highlighted). Scale bar = 10 μm.
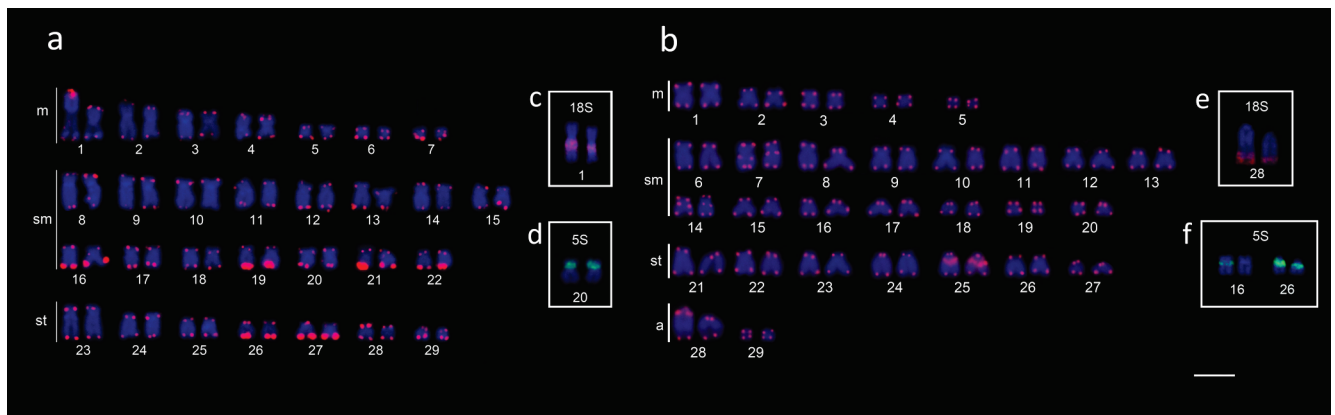

Fig. 2. Karyotype of *Farlowella* cf. *amazonum*: (a) telomeric sequences, (c) 18S rDNA, and (d) 5S rDNA. Karyotype of *Farlowella schreitmuelleri*: (b) telomeric sequences, (e) 18S rDNA, and (f) 5S rDNA. Scale bar = 10 μm.

stitial bands also occurred in the long arms of pairs Nos. 7, 9, 14, 22, and 23. Pairs Nos. 8, 24, 25, and 28 also had conspicuous bands in the terminal region of the long arms (Fig. 1e).

Multiple 5S rDNA sites were observed, in sm pair No. 16 (in only one of the homologs) and st pair No. 26. Both these sites were positive for C-banding (Fig. 2f). Hybridization with telomeric probes revealed terminal signals in all chromosomes and Interstitial Telomeric Sequences (ITSs)

in pairs Nos. 7, 14, 25, and 28, all of which corresponded with heterochromatin blocks. In pair No. 28, the ITS was observed in only one of the homologs (Fig. 2b).

Discussion

The hypothetical ancestral 2n suggested for the Loricariinae is 2n = 54 (Artoni, Bertollo, 2001). However, the

Loricariinae is characterized by a high degree of chromosomal diversity, in terms of both 2n and karyotype structure, as exemplified by the genus *Rineloricaria*, which has 2n varying from 36 to 70, indicating a large number of chromosomal rearrangements (Giuliano-Caetano, 1998; Alves *et al*., 2003; Mendes-Neto, 2008; Maia *et al*., 2010; Rodrigues, 2010; Porto *et al*., 2011; 2014; Rosa *et al*., 2012; Venturelli, 2014). While scarce, the cytogenetic data available for the genus *Farlowella* indicate a relative uniformity of the 2n = 58 chromosomes. However, the chromosomal markers, *i.e*., Ag-NOR sites, C-banding pattern, and karyotypes, does vary considerably among populations (Gindri, 2009; Fernandes *et al*., 2012; 2015; present study).

Three different karyotypes have been described in *Farlowella amazonum* from the Paraguay River basin, of Mato Grosso do Sul: 18m+20sm+12st+8a from the Ribeirão Stream, a tributary of the Taquari River (Gindri, 2009); 6m+38sm+8st+6a from the Água Boa Stream on the Iguatemi River (Fernandes *et al*., 2012); and 12m+30sm+10st+6a from the Dourado Stream, also in the Iguatemi basin (Fernandes *et al*., 2015). In the present study, we described a fourth karyotype from the Negro River, in the Amazon basin.

However, the fourth karyotype was identified as *F*. cf. *amazonum*, and is distinct from the *F*. *amazonum* populations of the Paraguay basin, due primarily to the absence of acrocentric chromosomes, although the karyotype of the populations from the Paraguay basin is highly similar to that described for *F*. *schreitmuelleri*, given that it has an acrocentric pair, which contains Ag-NOR sites. This pair seems to be homeologous between these species/populations.

A number of chromosomal rearrangements are found in the genomes of *Farlowella* species analyzed in the present study, given that, while they have the same 2n, the FN value and the karyotypes are different. The reduction of the number of metacentric chromosomes and the appearance of acrocentric chromosomes in genome of *F*. *schreitmuelleri* indicates a probably process of pericentric inversion, in which the 1st metacentric pair (pair No. 1) of *F*. cf. *amazonum* would have undergone a pericentric inversion to become an acrocentric, corresponding to the 1st acrocentric pair of *F*. *schreitmuelleri* (pair No. 28), or vice versa.

This proposed chromosomal rearrangement is supported by the presence of a secondary constriction and other cytogenetic markers (NOR, C⁺ banding and the 18S rDNA) in these chromosome pairs. In addition, the karyotypes of two species have the same number of sm and st chromosomes, which are consistent with the view that centric fusion and fission events, associated with the inversions, played an important role in the karyotype diversification observed in *Farlowella* in our study, and in the Loricariinae in general (Giuliano-Caetano, 1998; Alves *et al*., 2003; Rosa *et al*., 2012; Porto *et al*., 2014; Takagui *et al*., 2014; Blanco *et al*., 2017; present study).

Most of the species of *Farlowella* present reduced vagility and inhabit specific portions of the river, which may lead to the formation of small, isolated populations, in which

independent processes of genetic differentiation may result in speciation. This type of process has been observed in both sympatric and allopatric populations of *Bunocephalus coracoideus*, for example, which presents innumerable cytotypes, resulting from chromosomal rearrangements fixed in small populations (Ferreira *et al*., 2017).

The morphological parameters of the individuals examined in the present study are not fully consistent with those of *F*. *amazonum*. This implies two possibilities, that either (i) the specimens identified as *F*. cf. *amazonum* in the present study are in fact representatives of a distinct species or (ii) the *F*. *amazonum* specimens analyzed by Gindri (2009) and Fernandes *et al*. (2011; 2014) are actual members of a different species. In particular, Retzer, Page (1997) considered *Farlowella amazonum* (Günther, 1864), *F*. *gladiolus* (Günther, 1864), *F*. *carinata* Garman, 1889, *F*. *oliveirae* Miranda-Ribeiro, 1939, *F*. *paranaense* Meinken, 1937 (incorrectly spelled *paranaensis*), *F*. *pleurotaenia* Miranda-Ribeiro, 1939, and *F*. *pseudogladiolus* Steindachner, 1910 to be junior synonyms of *F*. *amazonum*.

Farlowella amazonum was described from Santarém, Pará state, Brazil. *Farlowella paranaense* is the only junior synonym of *F*. *amazonum* from the Paraguay River, whereas all the others are from the Amazon basin. That certainly raises questions about the correct identity of the species found in the Paraguay River. Retzer (in Retzer, Page, 1997) remarks that he only examined one specimen of *F*. *paranaense* in Museu de Zoologia da USP – MZUSP (from the upper Paraguay River). Based on the karyotype diversity observed so far, we are most certainly dealing with different species. However, the taxonomy of this genus is not well resolved, and detailed comparative morphometric studies are needed to understand the variation observed in *F*. *amazonum*.

In the *Farlowella* species analyzed up to now, the NOR phenotype is simple, as confirmed by the 18S rDNA probe (Fernandes *et al*., 2011, 2014; present study). A single chromosome pair with NOR sites is thought to be a plesiomorphic characteristic of the Loricariinae (Ziemniczak *et al*., 2012).

While there is a certain amount of consistency in the number of NOR sites, there is considerable variation in the position and types of chromosome that carry these sites in the different *Farlowella* species/populations. In the karyotype of *Farlowella* cf. *amazonum*, the NOR site is located in the pericentromeric region of the long arms of the 1st metacentric pair, whereas in those of *F*. *schreitmuelleri* and the other species described in the literature, the NORs are found on the long arms of the 1st acrocentric pair, which appears to be a conserved pattern in this genus. The presence of these sites in these chromosome pairs reinforces the conclusion that the origin of the different karyotypes in the *Farlowella* species/populations has originated from pericentric inversions.

In the Loricariinae, most of the cytogenetic studies that have focused on the physical mapping of the 18S ribosomal gene have demonstrated the presence of these sequences in a single chromosome pair, which has thus been identi-

fied as a basal character for this group, given that in all the *Harttia* species analyzed, the 18S rDNA was restricted to a single pair (Kavalco *et al.*, 2005; Centofante *et al.*, 2006; Rodrigues, 2010; Blanco *et al.*, 2017). By contrast, the 5S rDNA are much more variable in number and location (Venturelli, 2014; Blanco *et al.*, 2017).

In *Farlowella* cf. *amazonum*, the 5S rDNA is located in a single chromosome pair, whereas *F. schreitmuelleri* had three signals, including one site located on one of the homologs of the submetacentric pair No. 16, and the other two in subtelocentric pair No. 26. The absence of a 5S rDNA site on one of the homologs of pair 16 may have been the result of unequal crossing-over or the result of the displacement of the ribosomal sequence from pair 26 by mobile transposable elements, as observed in a number of different fish groups (Gross *et al.*, 2010; Ferreira *et al.*, 2011; Silva *et al.*, 2011; 2016; Schneider *et al.*, 2013).

In both species studied here, the 5S rDNA and 18S rDNA sites were associated with constitutive heterochromatin. The presence of these sequences in heterochromatic regions, and their association with transposable elements, were considered by Silva *et al.* (2014) to represent "hotspots" of chromosomal rearrangement, given that repetitive segments are more susceptible to rearrangement, due to their intrinsic structural organization (Ferreira *et al.*, 2014). This implies that the heterochromatin is an important element for the diversification of the genome, in particular in isolated populations (Elgin, 1996; Schneider *et al.,* 2013). In the present study, C-banding is almost poor, especially in *F.* cf. *amazonum*, and occurs an accentuated accumulation of heterochromatin in *F. schreitmuelleri*, with large, conspicuous bands, found in a number of different chromosome pairs was observed.

Comparing the distribution of the heterochromatin in genomes of the different *Farlowella* species, it is clear that *Farlowella* cf. *amazonum* (present study), *F. amazonum* (Fernandes *et al.,* 2015), and *F. amazonum* (Gindri, 2009) have less heterochromatin in comparison with *F. schreitmuelleri*. The distribution of the C-bands in *F.* cf. *amazonum* is also different from the patterns described by Gindri (2009) and Fernandes *et al.* (2015), with a much larger quantity of heterochromatin found in the former one. These differences may be associated with environmental factors, given that different environmental stressors may mediate epigenetic processes of heterochromatin regulation, although it seems more likely that these differences are related to the lack of migration in these species, which favors the accumulation of differential sequences in isolated populations (Guimarães *et al.*, 2017).

Present study performed the first physical mapping of the telomeric DNA in chromosomes of *Farlowella*. Whereas a stronger signal was recorded in some chromosome pairs in *Farlowella* cf. *amazonum*, *F. schreitmuelleri* presented more homogeneous telomeric signals, but also ITSs, which were not recorded in *F.* cf. *amazonum*. This variation in the intensity of the telomeric DNA signals is generally related to the variation in the length of the telomeric sequences (Pathak *et al.*, 1994, 1996; Multani *et al.*, 1999) and, the presence

of ITSs may indicate the past occurrence of rearrangements such as fusions, inversions and translocations, during the genome evolution of this genus (Holmquist, Dancis, 1979; Hastie, Allshire, 1989; Meyne *et al.*, 1990).

ITSs have been recorded in a number of different loricariine genera, including *Harttia*, *Loricaria*, *Loricarichthys*, and *Rineloricaria* (Rodrigues, 2010; Rosa *et al.*, 2012; Porto *et al.*, 2014; Blanco *et al.*, 2017; Primo *et al.*, 2017), as well as in *Farlowella schreitmuelleri* (present study), in which four chromosome pairs (Nos. 7, 14, 25 and 28) had ITSs in the pericentromeric region. In pair No. 28, the ITS was observed in only one of the homologs. In *F. schreitmuelleri*, the ITSs were observed in association with heterochromatin in all other pairs except pair No. 14. An association between intra-chromosomal telomeric DNA and constitutive heterochromatin has been recorded in many vertebrates, and this association is considered a component of the satellite DNA (Meyne *et al.*, 1990; Multani *et al.*, 2001; Rovatsos *et al.*, 2015; Viana *et al.*, 2016). These signals may also represent remnants of ancient chromosomal rearrangements (Metcalfe *et al.*, 2004), indicating potential hotspots of rearrangement (Scouarnec, Gribble, 2012).

Overall, then, the *Farlowella* species analyzed up to now present an uniform karyotype macrostructure in terms of the 2n, although the chromosomal markers indicated remarkable and dynamic evolutionary processes of their genomes. The presence of ITSs indicates the chromosomal rearrangements, which may have contributed to the diversification of their karyotype. These findings highlight the potential of the genus for further, integrated studies of taxonomy, ecology and genetics to a better knowledge of the systematics and evolutive relationships among the species of *Farlowella* and other Loricariidae.

Acknowledgments

References

Alves AL, Oliveira C, Foresti F. Karyotype variability in eight species of the subfamilies Loricariinae and Ancistrinae (Teleostei, Siluriformes, Loricariidae). Caryologia [serial on

the Internet]. 2003; 56(1):57-63. Available from: https://doi.or g/10.1080/00087114.2003.10589308

Artoni RF, Bertollo LAC. Trends in the karyotype evolution of Loricariidae fish (Siluriformes). Hereditas [serial on the Internet]. 2001; 134(3):201-10. Available from: https://doi. org/10.1111/j.1601-5223.2001.00201.x

Ballen GA, Urbano-Bonilla A, Zamudio JE. *Farlowella mitoupibo*, a new species of stick catfish from the upper Guaviare River, Orinoco basin, Colombia (Teleostei: Loricariidae). Ichthyol Explor Freshwat. 2016; 27(4):325-32.

Berra TM. Freshwater fish distribution. San Diego: Academic Press; 2001.

Blanco DR, Vicari MR, Lui RL, Traldi JB, Bueno V, Martinez JDF *et al.* Karyotype diversity and evolutionary trends in armored catfish species of the genus *Harttia* (Siluriformes: Loricariidae). Zebrafish [serial on the Internet]. 2017; 14(2):169-76. Available from: https://doi.org/10.1089/zeb.2016.1377

Centofante L, Bertollo LAC, Moreira-Filho O. Cytogenetic characterization and description of an XX/XY$_1$Y$_2$ sex chromosome system in catfish *Harttia carvalhoi* (Siluriformes, Loricariidae). Cytogenet Genome Res [serial on the Internet]. 2006; 112(3-4):320-24. Available from: https://doi. org/10.1159/000089887

Chiachio MC, Oliveira C, Montoya-Burgos JI. Molecular systematic and historical biogeography of the armored Neotropical catfishes Hypoptopomatinae and Neoplecostominae (Siluriformes: Loricariidae). Mol Phylogenet Evol [serial on the Internet]. 2008; 49(2):606-17. Available from: https://doi. org/10.1016/j.ympev.2008.08.013

Covain R, Fisch-Muller S. The genera of Neotropical armored catfish subfamily Loricariinae (Siluriformes, Loricariidae): a practical key and synopsis. Zootaxa [serial on the Internet]. 2007; 1462(1):1-40. Available from: http://dx.doi. org/10.11646/zootaxa.1462.1.1

Covain R, Fisch-Muller S, Oliveira C, Mol JH, Montoya-Burgos JI, Dray S. Molecular phylogeny of the highly diversified catfish subfamily Loricariinae (Siluriformes, Loricariidae) reveals incongruences with morphological classification. Mol Phylogenet Evol [serial on the Internet]. 2016; 94:492-517. Available from: https://doi.org/10.1016/j.ympev.2015.10.018

Elgin SC. Heterochromatin and gene regulation in *Drosophila*. Curr Opin Genetics Dev [serial on the Internet]. 1996; 6(2):193-202. Available from: https://doi.org/10.1016/S0959-437X(96)80050-5

Eschmeyer WN, Fong JD. Species by family/subfamily in the Catalog of Fishes. [Electronic version]. San Francisco (CA): California Academy of Sciences; 2017. [cited 2017 Nov 29]. Available from: http://researcharchive.calacademy.org/ research/ichthyology/ catalog/SpeciesByFamily.asp

Fernandes CA, Alves DS, Guterres ZR, Martins-Santos IC. Cytogenetic analysis of two locariid species (Teleostei, Siluriformes) from Iguatemi River (Parana River drainage) in Brazil. Comp Cytogenet [serial on the Internet]. 2015; 9(1):67-78. Available from: http://dx.doi.org/10.3897/CompCytogen. v9i1.8804

Fernandes CA, Damásio JF, Martins-Santos IC. Cytogenetic studies in species of family Loricariidae (Osteichthyes, Siluriformes) from Iguatemi River basin, Brazil. First cytogenetic report in *Farlowella amazonum* (Günther, 1864). Caryologia [serial on the Internet]. 2012; 65(4):276-80. Available from: https://doi.or g/10.1080/00087114.2012.752913

Ferraris CJ Jr. Subfamily Loricariinae (armored catfishes). In: Reis RE, Kullander SO, Ferraris CJ Jr., organizers. Check list of the freshwater fishes of South and Central America. Porto Alegre: Edipucrs; 2003. p.330-350.

Ferreira M, Garcia C, Matoso DA, de Jesus IS, Cioffi MDB, Bertollo LAC, Zuanon J, Feldberg E. The *Bunocephalus coracoideus* species complex (Siluriformes, Aspredinidae). Signs of a speciation process through chromosomal, genetic and ecological diversity. Front Genet [serial on the Internet]. 2017; 8:120. Available from: https://doi.org/10.3389/ fgene.2017.00120

Ferreira M, Kavalco KF, de Almeida-Toledo LF, Garcia C. Cryptic diversity between two *Imparfinis* species (Siluriformes, Heptapteridae) by cytogenetic analysis and DNA barcoding. Zebrafish [serial on the Internet]. 2014; 11(4):306-17. Available from: https://doi.org/10.1089/zeb.2014.0981

Ferreira DC, Oliveira C, Foresti F. Chromosome mapping of retrotransposable elements Rex1 and Rex3 in three fish species in the subfamily Hypoptopomatinae (Teleostei, Siluriformes, Loricariidae). Cytogenet Genome Res [serial on the Internet]. 2011; 132(1-2):64-70. Available from: https://doi. org/10.1159/000319620

Gindri BS. Contribuição ao estudo citogenético em Loricariinae (Siluriformes, Loricariidae) da região do alto Taquari. [Masters Thesis]. Maringá, PR. Universidade Estadual de Maringá; 2009.

Giuliano-Caetano L. Polimorfismo cromossômico Robertsoniano em populações de *Rineloricaria latirostris* (Pisces, Loricariidae). [Ph.D. Dissertation]. São Carlos, SP. Universidade Federal de São Carlos; 1998.

Gold JR, Li YC, Shipley NS, Powers PK. Improved methods for working with fish chromosomes with a review of metaphase chromosome banding. J Fish Biol [serial on the Internet]. 1990; 37(4):563-75. Available from: https://doi. org/10.1111/j.1095-8649.1990.tb05889.x

Gross MC, Schneider CH, Valente GT, Martins C, Feldberg E. Variability of 18S rDNA locus among *Symphysodon* fishes: chromosomal rearrangements. J Fish Biol [serial on the Internet]. 2010; 76(5):1117-27. Available from: http://dx.doi. org/10.1111/j.1095-8649.2010.02550.x

Guimarães EMC, Carvalho NDM, Schneider CH, Feldberg E, Gross MC. Karyotypic Comparison of *Hoplias malabaricus* (Bloch, 1794) (Characiformes, Erythrinidae) in Central Amazon. Zebrafish [serial on the Internet]. 2017; 14(1):80-89. Available from: https://doi.org/10.1089/zeb.2016.1283

Hastie ND, Allshire RC. Human telomeres: fusion and interstitial sites. Trends Genet. 1989; 5:326-31.

Holmquist GP, Dancis B. Telomere replication, kinetochore organizers, and satellite DNA evolution. Proc Natl Acad Sci. 1979; 76(9):4566-70.

Howell WM, Black DA. Controlled silver staining of nucleolus organizer region with a protective colloidal developer: a 1-step method. Experientia [serial on the Internet]. 1980; 36(8):1014-15. Available from: https://doi.org/10.1007/BF01953855

Ijdo JW, Wells RA, Baldini A, Reeders ST. Improved telomere detection using a telomere repeat probe (TTAGGG)$_n$ generated by PCR. Nucleic Acids Res. 1991; 19(17):4780.

Kavalco KF, Pazza R, Bertollo LAC, Moreira-Filho O. Karyotypic diversity and evolution of Loricariidae (Pisces, Siluriformes). Heredity [serial on the Internet]. 2005; 94(2):180-86. Available from: https://doi.org/10.1038/sj.hdy.6800595

Levan A, Fredga K, Sandberg AA. Nomenclature for centromeric position on chromosomes. Hereditas [serial on the

L. Marajó, P. F. Viana, M. Ferreira, L. H. Rapp Py-Daniel & E. Feldberg

Internet]. 1964; 52(2):201-20. Available from: https://doi.org/10.1111/j.1601-5223.1964.tb01953.x

Lujan NK, Armbruster JW, Lovejoy NR, López-Fernández H. Multilocus molecular phylogeny of the suckermouth armored catfishes (Siluriformes: Loricariidae) with a focus on subfamily Hypostominae. Mol Phylogenet Evol [serial on the Internet]. 2015; 82:269-88. Available from: https://doi.org/10.1016/j.ympev.2014.08.020

Maia TPA, Giuliano-Caetano L, Rodrigues MS, Rubert M, Takagui FH, Dias AL. Chromosomal banding in three species of the genus *Rineloricaria* (Siluriformes, Loricariidae, Loricariinae). Ichthyol Res [serial on the Internet]. 2010; 57(2):209-13. Available from: https://doi.org/10.1007/s10228-009-0145-7

Martins C, Galetti PM Jr. Chromosomal localization of 5S rDNA genes in *Leporinus* fish (Anostomidae, Characiformes). Chromosome Res. 1999; 7(5):363-67.

Mendes-Neto EO. Estudos citogenéticos em algumas espécies de Loricariidae (Teleostei, Siluriformes) da região de transposição do rio Piumhi para o rio São Francisco. [Masters Thesis] São Carlos, SP: Universidade Federal de São Carlos; 2008.

Metcalfe CJ, Eldridge MD, Johnston PG. Mapping the distribution of the telomeric sequence (T_2AG_3)n in the 2n = 14 ancestral marsupial complement and in the macropodines (Marsupialia: Macropodidae) by fluorescence in situ hybridization. Chromosome Res [serial on the Internet]. 2004; 12(4):405-14. Available from: http://dx.doi.org/10.1023/B:CHRO.0000034133.77878.88

Meyne J, Baker RJ, Hobart HH, Hsu TC, Ryder OA, Ward OG, Wiley JE, Wurster-Hill DH, Yates TL, Moyzis RK. Distribution of non-telomeric sites of the (TTAGGG)n telomeric sequence in vertebrate chromosomes. Chromosoma [serial on the Internet]. 1990; 99(1):3-10. Available from: https://doi.org/10.1007/BF01737283

Multani AS, Li C, Ozen M, Imam AS, Wallace S, Pathak S. Cell-killing by paclitaxel in a metastatic murine melanoma cell line is mediated by extensive telomere erosion with no decrease in telomerase activity. Oncol Rep [serial on the Internet]. 1999; 6(1):39-83. Available from: https://doi.org/10.3892/or.6.1.39

Multani AS, Ozen M, Furlong C, Zhao YJ, Hsu TC, Pathak S. Heterochromatin and interstitial telomeric DNA homology. Chromosoma [serial on the Internet]. 2001; 110(3):214-20. Available from: http://dx.doi.org/10.1007/s004120100133

Nelson JS, Grande TC, Wilson MVH. Fishes of the world. Hoboken: John Wiley & Sons; 2016.

Pathak S, Multani AS, Amoss MS. Telomere, telomerase and malignant melanomas in human and domestic mammals. Arch Zootec. 1996; 45:141-49.

Pathak S, Risin S, Brown NM, Berry K. Telomeric association of chromosomes is an early manifestation of programmed cell death. Int J Oncol [serial on the Internet]. 1994; 4(2):323-28. Available from: https://doi.org/10.3892/ijo.4.2.323

Pinkel D, Straume T, Gray JW. Cytogenetic analysis using quantitative, high sensitivity, fluorescence hybridization. Proc Natl Acad Sci [serial on the Internet]. 1986; 83(9):2934-38. Available from: https://doi.org/10.1073/pnas.83.9.2934

Porto FE, Portela-Castro ALB, Martins-Santos IC. Chromosome polymorphism in *Rineloricaria pentamaculata* (Loricariidae, Siluriformes) of the Paraná River basin. Ichthyol Res [serial on the Internet]. 2011; 58(3):225-31. Available from: https://doi.org/10.1007/s10228-011-0215-5

Porto FE, Vieira MM, Barbosa LM, Borin-Carvalho LA, Vicari MR, Portela-Castro ALB, Martins-Santos IC. Chromosomal

polymorphism in *Rineloricaria lanceolata* Günther, 1868 (Loricariidae: Loricariinae) of the Paraguay Basin (Mato Grosso do Sul, Brazil): Evidence of fusions and their consequences in the population. Zebrafish [serial on the Internet]. 2014; 11(4):318-24. Available from: https://doi.org/10.1089/zeb.2014.0996

Primo CC, Glugoski L, Almeida MC, Zawadzki CH, Moreira-Filho O, Vicari MR, Nogaroto V. Mechanisms of Chromosomal Diversification in Species of *Rineloricaria* (Actinopterygii: Siluriformes: Loricariidae). Zebrafish [serial on the Internet]. 2017; 14(2):161-8. Available from: https://doi.org/10.1089/zeb.2016.1386

Reis RE, Albert JS, Di Dario F, Mincarone MM, Petry P, Rocha LA. Fish biodiversity and conservation in South America. J Fish Biol [serial on the Internet]. 2016; 89(1):12-47. Available from: http://dx.doi.org/10.1111/jfb.13016

Retzer ME, Page LM. Systematics of the stick catfishes, *Farlowella* Eigenmann & Eigenmann (Pisces, Loricariidae). Proc Acad Nat Sci Philadelphia. 1997; 147:33-88.

Rodrigues RM. Estudos cromossômicos em Loricariinae com ênfase em espécies de *Rineloricaria* (Siluriformes, Loricariidae): uma abordagem evolutiva. [Master Thesis]. São Paulo, SP: Universidade de São Paulo; 2010.

Rosa KO, Ziemniczak K, Barros AV, Nogaroto V, Almeida MC, Cestari MM, Artoni RF, Vicari MR. Numeric and structural chromosome polymorfism in *Rineloricaria lima* (Siluriformes: Loricariidae): fusions points carrying 5S rDNA or telomere sequence vestiges. Rev Fish Biol Fish [serial on the Internet]. 2012; 22(3):739-49. Available from: https://doi.org/10.1007/s11160-011-9250-6

Rovatsos M, Kratochvíl L, Altmanová M, Pokorná MJ. Interstitial telomeric motifs in squamate reptiles: when the exceptions outnumber the rule. PloS One [serial on the Internet]. 2015; 10(8):e0134985. Available from: https://doi.org/10.1371/journal.pone.0134985

Roxo FF, Albert JS, Silva GSC, Zawadzki CH, Foresti F, Oliveira C. Molecular Phylogeny and Biogeographic History of the Armored Neotropical Catfish Subfamilies Hypoptopomatinae, Neoplecostominae and Otothyrinae (Siluriformes: Loricariidae). PLoS One [serial on the Internet]. 2014; 9(8):e105564. Available from: https://doi.org/10.1371/journal.pone.0105564

Schneider CH, Gross MC, Terencio ML, do Carmo EJ, Martins C, Feldberg E. Evolutionary dynamics of retrotransposable elements Rex 1, Rex 3 and Rex 6 in Neotropical cichlid genomes. BMC Evol Biol [serial on the Internet]. 2013; 13(1):152. Available from: https://doi.org/10.1186/1471-2148-13-152

Scouarnec SL, Gribble SM. Characterizing chromosome rearrangements: recent technical advances in molecular cytogenetics. Heredity [serial on the Internet]. 2012; 108(1):75-85. Available from: http://dx.doi.org/10.1038/hdy.2011.100

Silva M, Barbosa P, Artoni RF, Feldberg E. Evolutionary dynamics of 5S rDNA and recurrent association of transposable elements in electric fish of the family Gymnotidae (Gymnotiformes): The case of *Gymnotus mamiraua*. Cytogenet Genome Res [serial on the Internet]. 2016; 149(4):297-303. Available from: https://doi.org/10.1159/000449431

Silva M, Matoso DA, Vicari MR, de Almeida MC, Margarido VP, Artoni RF. Physical mapping of 5S rDNA in two species of knifefishes: *Gymnotus pantanal* and *Gymnotus paraguensis* (Gymnotiformes). Cytogenet Genome Res [serial on the

Internet]. 2011; 134(4):303-07. Available from: https://doi.org/10.1159/000328998

Silva M, Ribeiro ED, Matoso DA, Sousa LM, Hrbek T, Py-Daniel LR, Feldberg E. Chromosomal polymorphism in two species of *Hypancistrus* (Siluriformes: Loricariidae): an integrative approach for understanding their biodiversity. Genetica [serial on the Internet]. 2014; 142(2):127-39. Available from: https://doi.org/10.1007/s10709-014-9760-y

Sumner AT. A simple technique for demonstrating centromeric heterochromatin. Exp Cell Res [serial on the Internet]. 1972; 75(1):304-06. Available from: https://doi.org/10.1016/0014-4827(72)90558-7

Takagui FH, Venturelli NB, Dias AL, Swarça AC, Vicari MR, Fenocchio AS, Giuliano-Caetano L. The importance of pericentric inversions in the karyotypic diversification of the species *Loricariichthys anus* and *Loricariichthys platymetopon*. Zebrafish [serial on the Internet]. 2014; 11(4):300-05. Available from: https://doi.org/10.1089/zeb.2014.0985

Vari RP, Malabarba LR. Neotropical Ichtyology: an overview. In: Malabarba LR, Reis RE, Vari RP, Lucena ZMS, Lucena CAS, editors. Phylogeny and Classification of Neotropical Fishes. Porto Alegre: Edipucrs; 1998. p.1-11.

Venturelli NB. Mapeamento dos genes ribossômicos e cromossomos marcadores em nove espécies de *Rineloricaria* (Siluriforme, Loricariidae, Loricariinae) de distintas bacias hidrográficas. [Masters Thesis]. Londrina, PR: Universidade Estadual de Londrina; 2014.

Viana PF, Ribeiro LB, Souza GM, Chalkidis HM, Gross MC, Feldberg E. Is the karyotype of neotropical boid snakes really conserved? Cytotaxonomy, chromosomal rearrangements and karyotype organization in the Boidae family. PLoS One [serial on the Internet]. 2016; 11(8):e0160274. Available from: https://doi.org/10.1371/journal.pone.0160274

Ziemniczak K, Barros AV, Rosa KO, Nogaroto V, Almeida MC, Cestari MM, Moreira-Filho O, Artoni RF, Vicari MR. Comparative cytogenetics of Loricariidae (Actinopterygii: Siluriformes): emphasis in Neoplecostominae and Hypoptopomatinae. Ital J Zool [serial on the Internet]. 2012; 79(4):492-501. Available from: https://doi.org/10.1080/11250003.2012.676677