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The role of chromosomal fusion in the karyotypic evolution of the genus *Ageneiosus* (Siluriformes: Auchenipteridae)

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Ageneiosus is the most widely distributed genus of the family Auchenipteridae among South American river basins. Although chromosome studies in the family are scarce, this genus has the largest number of analyzed species, with 2n = 54 to 56 chromosomes, differing from the rest of the family (2n = 58). This study aimed to analyze *Ageneiosus inermis* from the Araguaia River basin. The diploid number found was of 56 chromosomes. Heterochromatin was allocated in terminal region of most chromosomes, plus a pericentromeric heterochromatic block in pair 1, a pair distinguished by size in relation to other chromosomes pairs. AgNORs were detected in only one submetacentric chromosome pair, which was confirmed by FISH. 5S rDNA was present in only one metacentric chromosome pair. Hybridization with $[TTAGGG]_n$ sequence marked the telomeres of all chromosomes, in addition to an ITS in the proximal region of the short arm of pair 1. The repetitive $[GATA]_n$ sequence was dispersed, with preferential location in terminal region of the chromosomes. *Ageneiosus* has a genomic organization somewhat different when compared to other Auchenipteridae species. Evidences indicate that a chromosomal fusion originated the first metacentric chromosome pair in *A. inermis*, rearrangement which may be a basal event for the genus.

Ageneiosus é o gênero da família Auchenipteridae mais amplamente distribuído em bacias da América do Sul. Apesar dos estudos cromossômicos nesta família serem escassos, este gênero tem o maior número de espécies analisadas, com número diploide variando de 54 a 56 cromossomos, o que difere do restante da família (2n = 58). Este estudo objetivou analisar *Ageneiosus inermis* da bacia do rio Araguaia. O número diploide encontrado foi de 56 cromossomos. A heterocromatina se mostrou localizada na região terminal da maioria dos cromossomos, além de um bloco heterocromático pericentromérico no par 1, um par facilmente distinguível no cariótipo pelo seu maior tamanho quando comparado aos outros pares do complemento. AgRONs foram detectadas em somente um par de cromossomos submetacêntricos, que foi confirmado pela FISH. 5S rDNA se mostrou presente em somente um par de cromossomos metacêntricos. A hibridização com a sequência $[TTAGGG]_n$ marcou os telômeros de todos os cromossomos, além de um ITS (sequência telomérica intersticial) na região proximal do braço curto do par 1. A sequência repetitiva $[GATA]_n$ se mostrou dispersa, com localização preferencial na região terminal dos cromossomos. *Ageneiosus* apresenta uma organização genômica um pouco diferente quando comparada a outras espécies de Auchenipteridae. As evidências indicam que uma fusão cromossômica originou o primeiro par de cromossomos metacêntricos de *A. inermis*, rearranjo que parece ser um evento basal para o gênero.

Key words: C banding, Cytogenetic Markers, $[GATA]_n$ sequence, rDNA-FISH, $[TTAGGG]_n$ sequence.

Introduction

Among Siluriformes, Auchenipteridae includes a group of fish endemic to the Neotropical region, specifically rivers of Central and South America hydrographic basins. According to Ferraris (2007), this family includes approximately 90 species distributed in 20 genera, 74 of which have been cataloged for the Brazilian territory (Akama & Sarmento-Soares, 2007). Furthermore, an increasing number of descriptions of new species for this family have been occurring recently, like the recent description of *Ageneiosus uranophthalmus* from the rivers of Central Amazonia (Ribeiro & Py-Daniel, 2010) and other six species that formally described for this genus (Ribeiro, 2011).

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Cytogenetic studies in Auchenipteridae are still scarce, comprising species just from the *Ageneiosus*, *Auchenipterus*, *Glanidium*, and *Parauchenipterus* genera. *Ageneiosus inermis* from Catalão Lake presents a diploid number of 54 chromosomes (Celeste M. Nakayama, pers. commun.), whereas another population of this species and *Ageneiosus atronasus* (cited as *A. brevifilis*), both from Solimões River (Fenocchio & Bertollo, 1992), and *Ageneiosus brevis* and *Ageneiosus ucayalensis*, both from Catalão Lake (Santos & Nakayama, 2011), have the diploid number of 56 chromosomes. However, species from other genera that were cytogenetically studied, as *Glanidium ribeiroi*, *Parauchenipterus galeatus* and *Auchenipterus osteomystax* (cited as *A. nuchalis*) have 58 chromosomes (Fenocchio & Bertollo, 1992; Ravedutti & Júlio Jr., 2001; Fenocchio *et al*., 2008; Lui *et al*., 2009; Lui *et al*., 2010).

The main chromosomal rearrangement that can lead to decreased chromosome number is fusion. The occurrence of a centric fusion event between two chromosomes, telocentric or acrocentric, creating a metacentric chromosome is called a Robertsonian fusion (Robertson, 1916). These rearrangements occur altering an extremely important structure of the chromosome, the telomere. This specialized structure, located in the terminal portion of the chromosome, is considered important for chromosomal stability and integrity (Zakian, 1997), and for this kind of rearrangement to occur, it is fundamental either the elimination or inactivation of the telomeres (Slijepcevic, 1998). According to the aforementioned author, the explanation of such rearrangements has three possible hypotheses as consequence: 1) inactivation of the telomerase enzyme, 2) chromosomal breakage in the satellite sequence adjacent to the telomere, or 3) inactivation of the telomere. It is notable that only if the latter explanation occurs it would be possible to maintain the structure of the telomeric sequence in an interstitial region (ITS). These rearrangements are among the most important events in karyotype evolution of mammals (Holmquist & Dancis, 1979), and some interesting examples are found in the evolution of fish groups (Giuliano-Caetano, 1998; Margarido & Moreira-Filho, 2008). In Auchenipteridae, only *Ageneiosus* has species with diploid number different from 58 (*i.e*., 54 or 56), thus it is possible that Robertsonian rearrangements may be involved with the chromosomal evolution of the group.

Ageneiosus is the genus that presents the highest amount of chromosomal data in Auchenipteridae. *Ageneiosus inermis* is the species with the largest distribution in South America, being in almost every portion east of the Andes (Ribeiro, 2011), and it is the species that presents more chromosomal studies (*e.g*., Fenocchio & Bertollo, 1992; Santos & Nakayama, 2011), with 2n = 54 or 56 chromosomes, which is not found for any other genus of Auchenipteridae. Thus, *Ageneiosus* is an interesting model to study chromosomal evolution in Auchenipteridae. This study aimed to analyze *A. inermis* from the Araguaia river basin and test the hypothesis that chromosomal fusions can be related to the origin and diversification of *Ageneiosus*.

Material and Methods

Chromosomal analysis was performed on 19 specimens (6 males and 13 females) of *Ageneiosus inermis* from the Araguaia River basin, city of Aragarças - GO, Brazil (15°54'00.1"S 52°15'11.4"W). The specimens were deposited in the fish collection of the Museu de Zoologia, Universidade de São Paulo (MZUSP 109796).

Metaphasic chromosomes were obtained from the anterior kidney (Bertollo *et al*., 1978; Foresti *et al*., 1993) and classified as metacentric (m), submetacentric (sm), subtelocentric (st) and acrocentric (a), according to the ratio of arms (Levan *et al*., 1964). The fundamental number (FN) was calculated considering metacentric chromosomes (m), submetacentric (sm) and subtelocentric (st) as having two arms, and acrocentric chromosomes (a) as having only one chromosomic arm. The heterochromatic distribution pattern was obtained according to Sumner (1972), with modifications (Lui *et al*., 2012). The nucleolar organizing regions (AgNORs) were obtained using the method described by Howell & Black (1980). Both methods were applied sequentially, after conventional chromosomal staining with Giemsa (sequential analysis).

The fluorescence *in situ* hybridization (FISH) was performed according to Pinkel *et al*. (1986), using 18S rDNA (Hatanaka & Galetti Jr., 2004), 5S rDNA (Martins *et al*., 2000), $[TTAGGG]_n$ and $[GATA]_n$ probes which were amplified without DNA template for the reaction as described by Ijdo *et al*. (1991). The 18S rDNA probe was labeled with biotin-16-dUTP, by nick translation according to the manufacturer's instruction (Biotin Nick Translation mix - Roche). The 5S rDNA probe was labeled with digoxigenin 11-dUTP by Nick translation according to the manufacturer's instruction (Dig 11 Nick Translation mix - Roche). The $[TTAGGG]_n$ and $[GATA]_n$ sequences were labeled by Polymerase Chain Reaction (PCR), using biotin-16-dUTP (Roche Applied Science). All the hybridizations were performed with 77% stringency (200 ng from each probe, 50% deionized formamide, 10% dextran sulphate, 2xSSC; pH 7.0 - 7.2). The chromosomes were analyzed using an Olympus BX51 epifluorescence microscope. The software DP2-BSW (Olympus) was used for image capture.

Results

The diploid number found for *A. inermis* was 56 chromosomes (32m + 16sm + 4st + 4a, NF = 108) (Fig. 1a). Heterochromatin was observed in most of the terminal regions of chromosomes, with heterochromatic blocks showing themselves strongly labeled in some chromosomes (Fig. 1b). In addition, a heterochromatic block was detected in the pericentromeric region in pair 1 (Figs. 1b-3b). This pair stands out among the other chromosome pairs of the complement due to its significantly larger size.

The silver nitrate staining demonstrated simple NORs allocated in the terminal region of the short arm of submetacentric pair 20, coincident with a heterochromatic block (Fig. 1, in box). FISH with 18S rDNA probe confirmed

the results revealed by silver nitrate staining (Fig. 2a) and the hybridizations with 5S rDNA probe showed only a pair with this marker, present in the short arm of metacentric pair 4, also coincident with the heterochromatic block (Fig. 2a). FISH with telomeric probe revealed all the telomeric regions marked, in addition to an Interstitial Telomeric Site (ITS) in the proximal region of the short arm of pair 1 (Fig. 2b). The hybridization with the repetitive sequence $[GATA]_n$ showed that this marker is dispersed throughout the genome of the species, with a preferential location in the terminal region of the chromosomes; however, a lesser amount was also present in the interstitial regions (Fig. 2d).

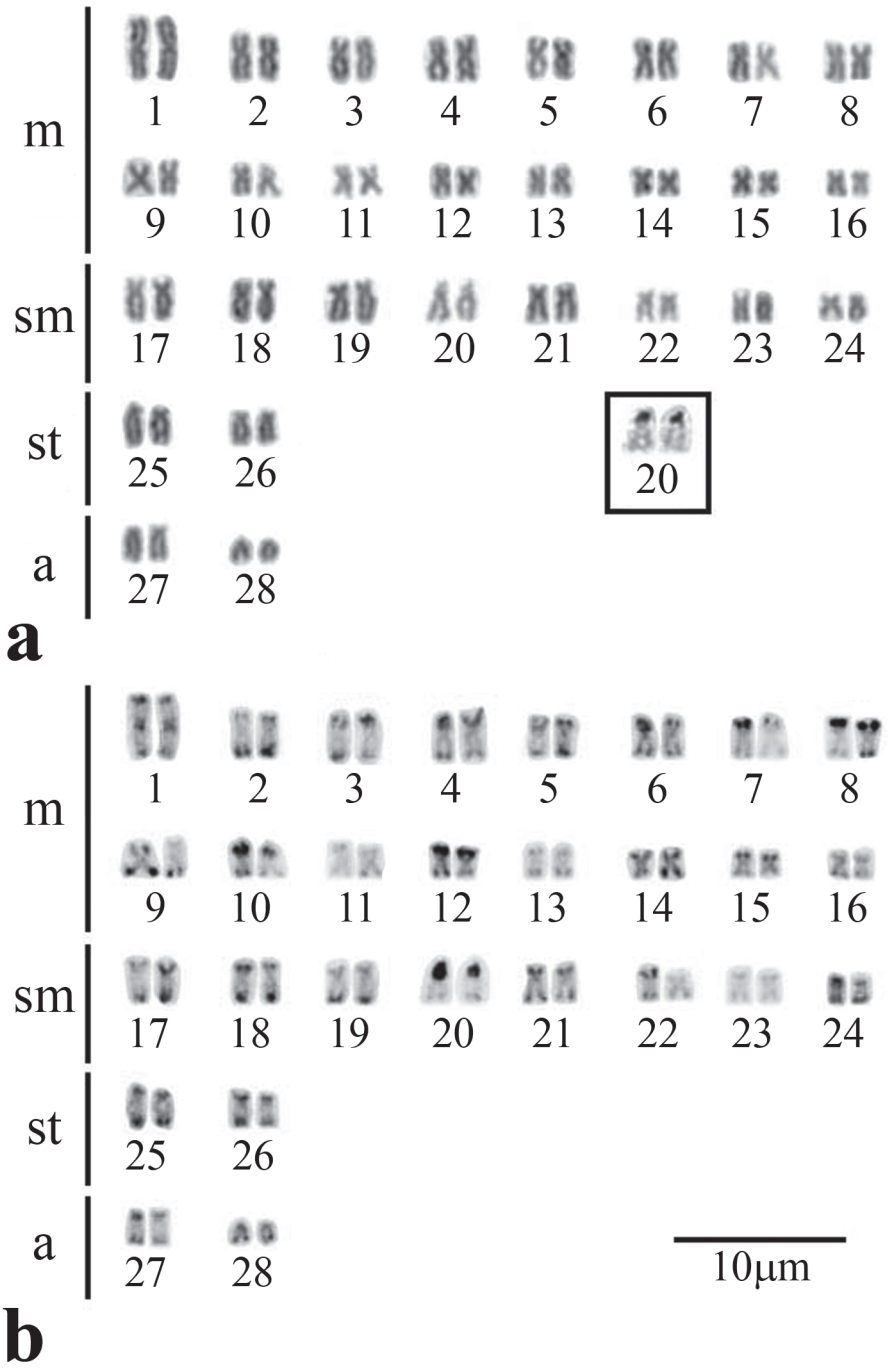

Fig. 1. Karyotypes of *Ageneiosus inermis* stained with Giemsa (**a**) and sequentially C-banded (**b**). The AgNORs bearing chromosomes pair is presented in the box.

Discussion

According to Ribeiro (2011), *Ageneiosus* is the more diverse and widely distributed genus of Auchenipteridae, and *A. inermis* is the species of this genus that has the largest distribution in the river basins of South America. The diploid number (2n = 56) found for the population of *A. inermis* analyzed in this work is equal to the one found for a population of the Solimões River (Fenocchio & Bertollo, 1992) (Amazon basin); however, in the population of Catalão Lake (Amazon basin), a lower diploid number (2n = 54) was found (Santos & Nakayama, 2011). Despite the conservation of the diploid number between the population of the Solimões River and the population of the present study, there are some small differences related to the karyotypic constitution that may be due to translocations and/or pericentric inversions, a situation commonly observed in other fish species, for example *Rhamdia quelen* (Heptapteridae) (Garcia *et al*., 2010; Martinez *et al*., 2011), *Parauchenipterus galeatus* (Auchenipteridae) (Lui *et al*., 2010), *Hoplias malabaricus* (Erythrinidae) (Blanco *et al*., 2010), *Pimelodus maculatus* (Pimelodidae) (Mazzuchelli *et al*., 2007; Treco *et al*., 2008), *Hypostomus ancistroides* (Loricariidae) (Bueno *et al*., 2012, 2013), among others.

The few chromosomal studies in Auchenipteridae suggest that the diploid number of 58 chromosomes is a characteristic of the group (Ravedutti & Júlio Jr., 2001). Except for species of *Ageneiosus*, which have a diploid number different from 58, the other genera that were analyzed until this moment (*Auchenipterus*, *Glanidium* and *Parauchenipterus*) confirm the greater occurrence of this diploid number in the group. According to Pinna (1998), the Doradidae family is considered sister group of Auchenipteridae. Although the diploid number in Doradidae vary from 56 to 66 chromosomes (Eler *et al*., 2007), the modal diploid number is 58 chromosomes, found in 14 out of the 16 previously analyzed species (Eler *et al*., 2007; Milhomem *et al*., 2008). According to Milhomem *et al*. (2008), the diploid number of 58 should be considered basal to Doradidae. Thus, it is likely that the same diploid number (58) should be also considered basal for Auchenipteridae.

The Auchenipteridae species of the *Auchenipterus*, *Glanidium* and *Parauchenipterus* genera analyzed by C-banding showed a pattern of heterochromatin distribution preferentially in the terminal regions, which seems to be a feature of the family. However, the *Ageneiosus* genus differs from this pattern, being that two aspects can be highlighted: 1) the heterochromatic regions of the *Ageneiosus* species

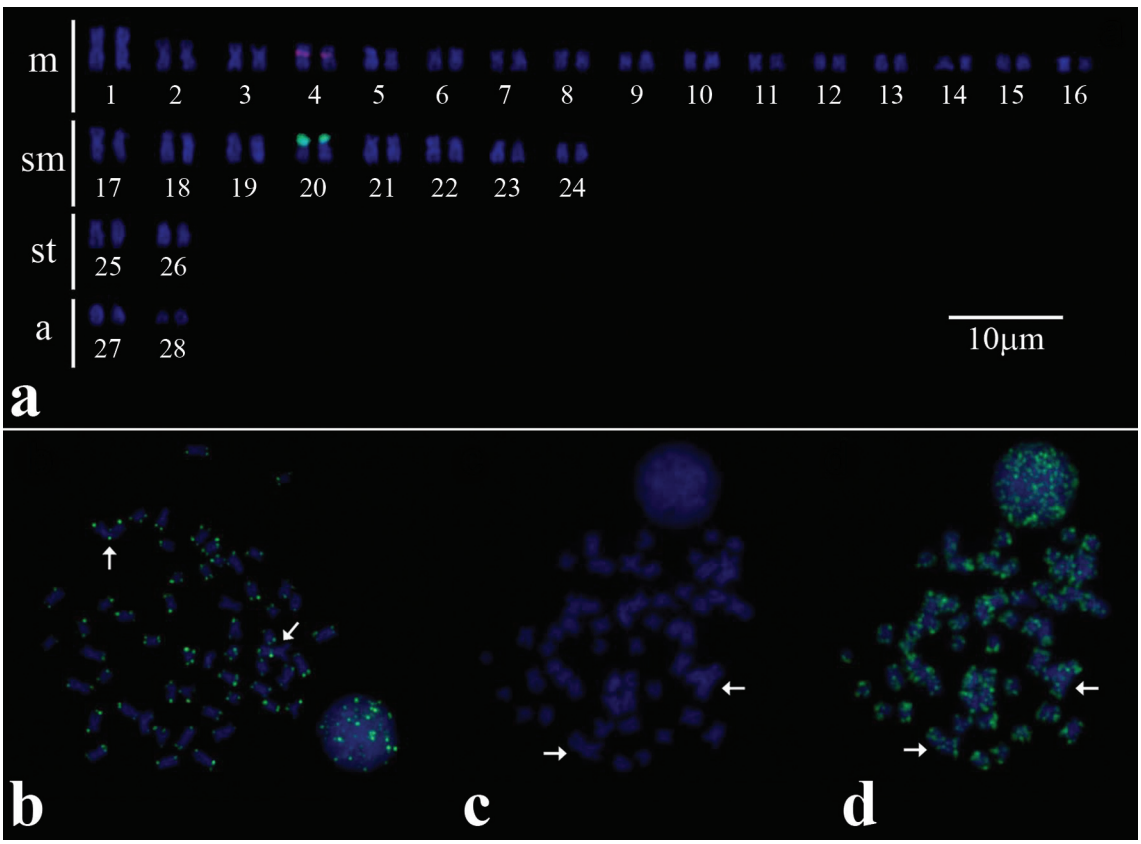

Fig. 2. Karyotype of *Ageneiosus inermis* hybridized with (**a**) 5S rDNA (digoxigenin, red) and 18S rDNA (FITC, green). Metaphases of *Ageneiosus inermis* hybridized with (**b**) $[TTAGGG]_n$ telomeric sequence and with (**d**) $[GATA]_n$ repeats. The arrows indicate the metacentric chromosomal pair 1, which was originated by fusion.

showed themselves strongly labeled, unlike other species of Auchenipteridae, where they are normally shown pallid, and 2) the first metacentric pair shows a strongly marked pericentromeric block (Fig. 1). Although these heterochromatic blocks are more conspicuous, the localization preferentially in the terminal regions of most chromosomes of the karyotype (which is a characteristic of Auchenipteridae) was maintained.

The available data in the literature for hybridization with 18S rDNA probe for Auchenipteridae are restricted to *Parauchenipterus galetaus* (Lui *et al*., 2009; Lui *et al*., 2010) and *Glanidium ribeiroi* (Fenocchio *et al*., 2008), which always confirmed the results presented by silver impregnation of only one marked pair. According to Ravedutti & Júlio Jr. (2001), simple NORs seem to be a feature of Auchenipteridae. Physical mapping of 5S rDNA data are restricted to *P. galetaus*, which has sites located in the interstitial position of two submetacentric pairs, being in the short arm of one pair and in the long arm of the other (Lui *et al*., 2010). The location in the interstitial region of the short arm of the metacentric pair 4 on *A. inermis* suggests that this pair may correspond to the chromosomal pair of *P. galetaus* that present the 5S rDNA site in the same position, that is, on the short arm. Furthermore, the region where the 5S rDNA site was detected was coincident with a heterochromatic block.

FISH with $[GATA]_n$ and $[TTAGGG]_n$ repetitive sequences provided interesting information about the genome of *A. inermis*. Regarding the first element, it was initially discovered by Epplen *et al*. (1982), and several subsequent studies showed that this sequence is conserved in different species, including humans (Srivastava *et al*., 2008), and seems to be associated to sex determination and evolution of sex chromosomes in snake groups (Jones & Singh, 1985). Although no sex chromosome system has been described in Auchenipteridae, this highly dispersed sequence found in *A. inermis* is a new factor to the group, which must be further exploited in other species. The hybridization with the $[GATA]_n$ sequence showed correspondence with the heterochromatin in the terminal region of almost all chromosomes, also being present in lower amount in interstitial regions, and coincident with the unique C-band in the pericentromeric region (pair 1). This situation in which repetitive elements have been found widely dispersed in the genomes is relatively common and has been observed in other fish species recently (*e.g*., Mazzuchelli & Martins, 2009; Teixeira *et al*., 2009; Ferreira *et al*., 2011).

Indications of chromosomal rearrangements like fusions are common in vertebrates, as already detected in a lot of different groups, and in most of cases it is possible to detect telomerics interstitials sites (Meyne *et al*., 1990), although not in others (*e.g*. in *Imparfinis hollandi*, Margarido & Moreira-Filho, 2008). The hybridizations with telomeric sequences marked terminal regions of all chromosomes, additionally detecting an interstitial site on the short arm of the metacentric pair 1 near the centromere (Fig. 2b; Fig. 3). The ITS found in the studied population is a strong evidence that a chromosomal fusion event is related to the diversification of Auchenipteridae, more specifically in the *Ageneiosus* genus. The metacentric pair that suffered fusion in *A. inermis* can be easily distinguished in the karyotype of this species because its size is almost double compared to the other chromosomes of the complement. Analyzing the karyotypes available in the literature for other species of the genus, *A. atronasus*, *A. inermis* (Fenocchio & Bertollo, 1992) and *A. dentatus*, *A. inermis*, *A. ucayalensis* (Santos & Nakayama, 2011), for example, it is also possible to identify a chromosome pair significantly larger than the others from the karyotype, which seems to be shared among the previously studied species of the *Ageneiosus* and the species of this paper.

According to Ribeiro (2011), the species belonging to this genus are divided into two clades containing, respectively, six and thirteen species. Among the species analyzed by cytogenetic methods, *A. atronasus* is present in the first branch (clade with six species), while *A. dentatus*, *A. inermis* and *A. ucayalensis* are present in the second branch (clade with thirteen species). This information from the phylogeny (showing that the species with chromosomal studies are present in the two major clades of the genus), in addition to the information that all species of *Ageneiosus* with chromosomal data have diploid numbers lower than 58 chromosomes, and contain the easily distinguishable large chromosome in the karyotype, provides subsidies to propose that this evident fusion event in *A. inermis* between two acrocentric pairs (Fig. 3) may represent a basal rearrangement for the genus.

There is a species of *Ageneiosus* present in drainages west of the Andes, *A. pardalis*, which is endemic to trans-Andine rivers. The existence of this species in this region suggests that the origin of the *Ageneiosus* genus must have occurred before the elevation of the northern portion of the Andes. According to Lundberg *et al*. (1998), the geomorphological events that would have originated these trans-Andine basins date from 8 and 11.8 million years ago, thus the cytogenetical analysis of specimens from this species could be very interesting to confirm this hypothesis.

According to Slijepcevic (1998), there are three possible molecular events that would enable the occurrence of a chromosomal fusion, as mentioned in the introduction, however, only one includes the structural maintenance of the telomeric sequence (as observed in this work by ITS detection) which is the loss of telomere function. In this context, and according to the aforementioned author, there are at least three possible explanations for the loss of telomere function: 1) loss of function of telomere associated proteins, 2) loss of function as a result of changes in chromatin structure (*e.g*. decondensation of this region) and 3) temporary inactivation of the telomerase gene(s) in germ line cells. The investigation about the cause of this rearrangement would be extremely complex, however it is likely that the loss of telomere function, not mattering its

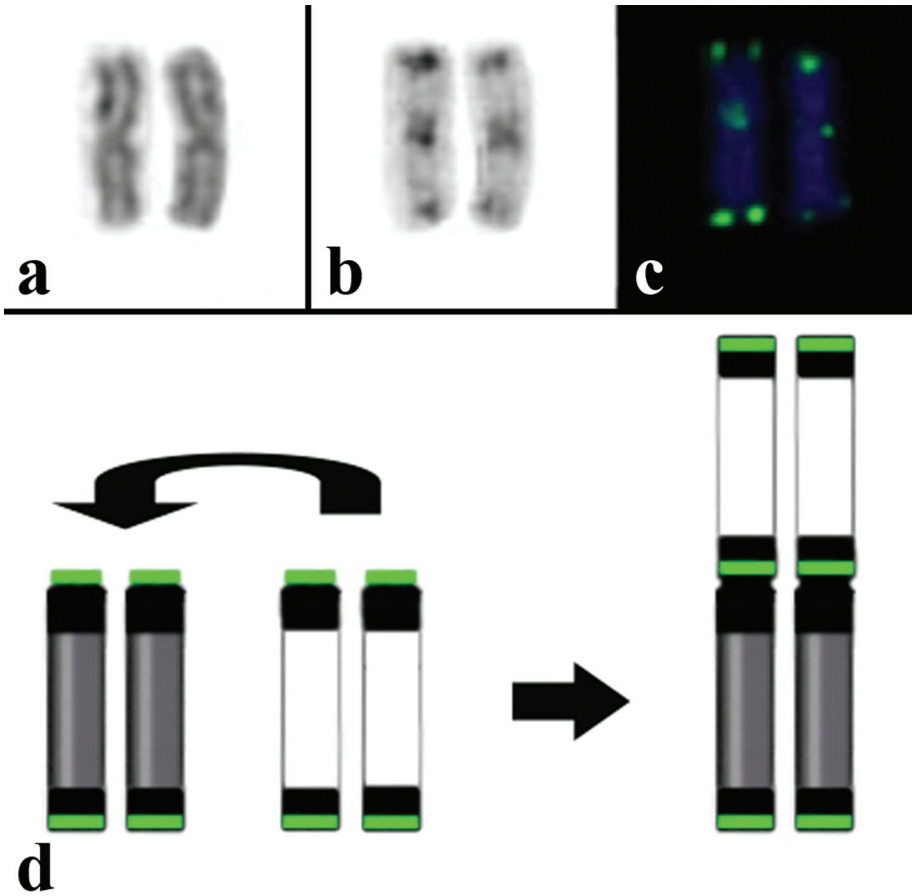

Fig. 3. Metacentric chromosomal pair 1 (**a**) stained with Giemsa, (**b**) C-banded and (**c**) hybridized with $[TTAGGG]_n$. The schematic illustration in (d) represents the possible fusion rearrangement which originated this pair.

specific origin, is the cause of the rearrangements found in *A. inermis*, since the ITS was observed.

Thus, the genome of the *Ageneiosus* species appears to have a little different organization when compared to other species of Auchenipteridae, due to the heterochromatin pattern, 5S rDNA and the lower diploid number. These factors added to the evidence of fusion in pair 1 and the derived condition of the genus in phylogenies based on morphological data suggest that the chromosomal evolutionary processes in *Ageneiosus* differs from the rest of the family, with a less conserved chromosomal evolution than the other studied genera. Furthermore, it is likely that the chromosomal fusion that originated pair 1 in *A. inermis* could be a basal event for the genus.

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Literature Cited

Akama, A. & L. M. Sarmento-Soares. 2007. Família Auchenipteridae. Pp. 116-120. In: Buckup, P. A., N. A. Menezes & M. S. Ghazzi (Eds.). Catálogo das espécies de peixes de água doce do Brasil. Série Livros 23. Rio de Janeiro, Museu Nacional, Universidade Federal do Rio de Janeiro.

Bertollo, L. A. C., C. S. Takahashi & O. Moreira-Filho. 1978. Cytotaxonomic consideration on *Hoplias lacerdae* (Pisces, Erythrinidae). Brazilian Journal of Genetics, 1: 103-120.

Blanco, D. R., R. L. Lui, L. A. C. Bertollo, V. P. Margarido & O. Moreira-Filho. 2010. Karyotype diversity between allopatric populations of the group *Hoplias malabaricus* (Characiformes: Erythrinidae): evolutionary and biogeographical considerations. Neotropical Ichthyology, 8: 361-368.

Bueno, V., C. H. Zawadzki & V. Margarido. 2012. 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.

Bueno, V., P. C. Venere, C. H. Zawadzki & V. Margarido. 2013. Karyotypic diversification in *Hypostomus* Lacépède, 1803 (Siluriformes, Loricariidae): biogeographical and phylogenetic perspectives. Reviews in Fish Biology and Fisheries, 23: 103-112.

Eler, E. S., J. A. Dergam, P. C. Venere, L. C. Paiva, G. A. Miranda & A. A. Oliveira. 2007. The karyotypes of the thorny catfishes *Wertheimeria maculata* Steindachner, 1877 and *Hassar wilderi* Kindle, 1895 (Siluriformes: Doradidae) and their relevance in doradids chromosomal evolution. Genetica, 130: 99-103.

Epplen, J. T., J. R. McCarrey, S. Sutou & S. Ohno. 1982. Base sequence of a cloned snake W-chromosome DNA fragment and identification of a male-specific putative mRNA in the mouse. Proceedings of the National Academy of Sciences of the United States of America, 79: 3798-3802.

Fenocchio, A. S. & L. A. C. Bertollo. 1992. Karyotype, C-bands and NORs of the neotropical siluriform fish *Ageneiosus brevifilis* and *Ageneiosus atronases* (Ageneiosidae). Cytobios 72: 19-22.

Fenocchio, A. S., A. L. Dias, V. P. Margarido & A. C. Swarça. 2008. Molecular cytogenetic characterization of *Glanidium ribeiroi* (Siluriformes) endemic to the Iguaçu river, Brazil. Chromosome Science, 11: 61-66.

Ferraris Jr., C. J. 2003. Family Auchenipteridae. Pp. 470-482. In: Reis, R. E., S. O. Kullander & C. J. Ferraris Jr. (Eds.). Check List of the Freshwater Fishes of South and Central America. Porto Alegre, Edipucrs.

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

Ferreira, D. C., C. Oliveira & F. Foresti. 2011. Chromosome mapping of retrotransposable elements *Rex1* and *Rex3* in three fish species in the subfamily Hypoptopomatinae (Teleostei, Siluriformes, Loricariidae). Cytogenetic and Genome Research, 132: 64-70.

Foresti, F., C. Oliveira & L. F. Almeida-Toledo. 1993. A method for chromosome preparations from large fish specimens using in vitro short-term treatment with colchicines. Experientia, 49: 810-813.

Garcia, C., C. Oliveira & L. F. Almeida-Toledo. 2010. Karyotypic evolution trends in *Rhamdia quelen* (Siluriformes, Heptapteridae) with considerations about the origin and differentiation of its supernumerary chromosomes. Genetics and Molecular Research, 9: 365-384.

Giuliano-Caetano, L. 1998. Polimorfismo cromossômico Robertsoniano em populações de *Rineloricaria latirostris* (Pisces, Loricariidae). Unpublished Ph.D. Dissertation. Universidade Federal de São Carlos, São Carlos, 78p.

Hatanaka, T. & P. M. Galetti Jr. 2004. Mapping of the 18S and 5S ribosomal RNA genes in the fish *Prochilodus argenteus* Agassiz, 1829 (Characiformes, Prochilodontidae). Genetica, 122: 239-244.

Holmquist, G. P. & B. Dancis. 1979. Telomere replication, kinetochore organizers, and satellite DNA evolution. Proceedings of the National Academy of Sciences of the United States of America, 76: 4566-4570.

Howell, W. M. & D. A. Black. 1980. Controlled silver staining of Nucleolus Organizer Regions with protective colloidal developer: a one-step method. Experientia, 36: 1014-1015.

Ijdo, J. W., R. A. Wells, A. Baldini & S. T. Reeders. 1991. Improved telomere detection using a telomere repeat probe $[TTAGGG]_n$ generated by PCR. Nucleic Acids Research, 19: 4780.

Jones, K. W. & L. Singh. 1985. Snakes and the evolution of sex chromosomes. Trends of Genetics, 1: 55-61.

Levan, A., K. Fredga & A. A. Sandberg. 1964. Nomenclature for centromeric position on chromosomes. Hereditas, 52: 201-220.

Lui, R. L., D. R. Blanco, V. P. Margarido & O. Moreira-Filho. 2009. First description of B chromosomes in the family Auchenipteridae, *Parauchenipterus galeatus* (Siluriformes) of the São Francisco River basin (MG, Brazil). Micron, 40: 552-559.

Lui, R. L., D. R. Blanco, V. P. Margarido & O. Moreira-Filho. 2010. Chromosome characterization and biogeographic relations among three populations of the driftwood catfish *Parauchenipterus galeatus* (Linnaeus, 1766) (Siluriformes: Auchenipteridae) in Brazil. Biological Journal of the Linnean Society, 99: 648-656.

Lui, R. L., D. R. Blanco, O. Moreira-Filho & V. P. Margarido. 2012. Propidium iodide for making heterochromatin more evident in the C-banding technique. Biotechnic & Histochemistry, 87: 433-438.

Lundberg, J. G., L. G. Marshall, J. Guerero, B. Horton, M. C. S. L. Malabarba & F. Wesselingh. 1998. The stage for neotropical fish diversification: a history of tropical south american rivers. Pp. 13-48. In: Malabarba, L. R., R. E. Reis, R. P. Vari, Z. M. S. Lucena & C. A. S. Lucena (Eds.). Phylogeny and Classification of Neotropical Fishes. Porto Alegre, Edipucrs.

Margarido, V. P. & O. Moreira-Filho. 2008. Karyotypic differentiation through chromosome fusion and number reduction in *Imparfinis hollandi* (Ostariophysi, Heptapteridae). Genetics and Molecular Biology, 31: 235-238.

Martinez J. F., R. L. Lui, D. R. Blanco, J. B. Traldi, L. F. Silva, P. C. Venere, I. L. Souza & O. Moreira-Filho. 2011. Comparative cytogenetics of three populations from the *Rhamdia quelen* species complex (Siluriformes, Heptapteridae) in two Brazilian hydrographic basins. Caryologia, 64: 121-128.

Martins, C., A. P. Wasko, C. Oliveira & J. M. Wright. 2000. Nucleotide sequence of 5S rDNA and localization of the ribosomal RNA genes to metaphase chromosomes of the Tilapiine cichlid fish, *Oreochromis niloticus*. Chromosome Research, 133: 39-46.

Mazzuchelli, J., A. C. Swarça & A. L. Dias. 2007. Structural chromosome polymorphism in a *Pimelodus maculatus* La Cépède, 1803 Population (Siluriformes, Pimelodidae) from the Paranapanema River Basin, PR. Brazilian Journal of Biology, 67: 631-633.

Mazzuchelli, J. & C. Martins. 2009. Genomic organization of repetitive DNAs in the cichlid fish *Astronotus ocellatus*. Genetica, 136: 461-469.

Meyne, J., R. J. Baker, H. H. Hobart, T. C. Hsu, O. A. Ryder, O. G. Ward, J. E. Wiley, D. H. Wurster-Hill, T. L. Yates & R. K. Moyzis. 1990. Distribution of non-telomeric sites of the $(TTAGGG)_n$ telomeric sequence in vertebrate chromosomes. Chromosoma, 99: 3-10.

Milhomem, S. S. R., A. C. P. Souza, A. L. Nascimento, J. R. Carvalho Jr., E. Feldberg, J. C. Pieczarka & C. Y. Nagamachi. 2008. Cytogenetic studies in fishes of the genera *Hassar*, *Platydoras* and *Opsodoras* (Doradidae, Siluriformes) from Jarí and Xingú rivers, Brazil. Genetics and Molecular Biology, 31: 256-260.

Pinkel, D., T. Straume & J. W. Gray. 1986. Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. Proceedings of the National Academy of Sciences of United States of America, 83: 2934-2938.

Pinna, M. C. C. 1998. Phylogenetics relationships of Neotropical Siluriformes: historical overview and synthesis of hypothesis. Pp. 279-330. In: Malabarba L. R., R. E. Reis, R. P. Vari, Z. M. S. Lucena & C. A. S. Lucena (Eds.). Phylogeny and classification of Neotropical Fishes. Porto Alegre, Edipucrs.

Ravedutti, C. G. & H. F. Júlio Jr. 2001. Cytogenetic Analysis of Three Species of the Neotropical Family Auchenipteridae (Pisces, Siluriformes) from the Paraná River Basin, Brazil. Cytologia, 66: 65-70.

Ribeiro, F. R. V. 2011. Sistemática do gênero *Ageneiosus* La Cépède (Siluriformes; Auchenipteridae). Unpublished Ph.D. Dissertation, Instituto Nacional de Pesquisas da Amazônia Manaus, 355p.

Ribeiro, F. R. V. & L. H. R. Py-Daniel. 2010. *Ageneiosus uranophthalmus*, a new species of auchenipterid catfish (Osteichthyes: Siluriformes) from river channels of the central Amazon basin, Brazil. Neotropical Ichthyology, 8: 97-104.

Robertson, W. M. R. B. 1916. Chromosome studies. I. Taxonomic relationships shown in the chromosomes of Tettegidae and Acrididiae: V-shaped chromosomes and their significance in Acrididae, Locustidae and Grillidae: chromosomes and variations. Journal of Morphology, 27: 179-331.

Slijepcevic, P. 1998. Telomeres and mechanisms of Robertsonian fusion. Chromosoma, 107: 136-140.

Srivastava, J., S. Premi, S. Kumar & S. Ali. 2008. Organization and differential expression of the GACA/GATA tagged somatic and spermatozoal transcriptomes in Buffalo *Bubalus bubalis*. BMC Genomics, 9: 132.

Sumner, A. T. 1972. A simple technique for demonstrating centromeric heterocromatin. Experimental Cell Research, 75: 304-306.

Teixeira, W. G., I. A. Ferreira, D. C. Cabral-de-Mello, J. Mazzuchelli, G. T. Valente, D. Pinhal, A. B. Poletto, P. C. Venere & C. Martins. 2009. Organization of repeated DNA elements in the genome of the cichlid fish *Cichla kelberi* and its contributions to the knowledge of fish genomes. Cytogenetic and Genome Research, 125: 224-234.

Treco, F. R., L. R. Malabarba, L. Giuliano-Caetano & A. L. Dias. 2008. Cytogenetic study of two species of the family Pimelodidae (Siluriformes) collected in lago Guaíba, Rio Grande do Sul, Brazil. Neotropical Ichthyology, 6: 87-92.

Zakian, V. A. 1997. Life and cancer without telomerase. Cell, 91: 1-3.

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