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 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.

**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, Glandium, 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 ucuvalensis, both from Catalão Lake (Santos & Nakayama, 2011), have the diploid number of 56 chromosomes. However, species from other genera that were cytogenetically studied, as Glandium 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 Araguai 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 chromosomal 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 (Raveduti & 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.](image)
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 galetautes (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. galetautes, 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. galetautes 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 telomeric interstitial 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. atra, 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. atra 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 subsides 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
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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