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Research Article

Chromosomal diversity in three species of electric fish (Apteronotidae, Gymnotiformes) from the Amazon Basin

Fernando Henrique Ramos Silva[1], Julio Cesar Pieczarka[1], Adauto Lima Cardoso[1], Patrícia Corrêa da Silva[1], Jonas Alves de Oliveira[2] and Cleusa Yoshiko Nagamachi[1]

[1]*Instituto de Ciências Biológicas, Universidade Federal do Pará, Belém, PA, Brazil.*
[2]*Instituto de Desenvolvimento Sustentável Mamirauá, Tefé, AM, Brazil.*

Abstract

Cytogenetic studies were carried out on samples of *Parapteronotus hasemani*, *Sternarchogiton preto* and *Sternarchorhamphus muelleri* (Apteronotidae, Gymnotiformes) from the Amazon basin. The first two species exhibited both a 2n = 52 karyotype, but differed in their karyotypic formulae, distribution of constitutive heterochromatin, and chromosomal location of the NOR. The third species, *Sternarchorhamphus muelleri*, was found to have a 2n = 32 karyotype. In all three species the DAPI and chromomycin A3 staining results were consistent with the C-banding results and nucleolar organizer region (NOR) localization. The 18S rDNA probe confirmed that there was only one pair of ribosomal DNA cistron bearers per species. The telomeric probe did not reveal interstitial telomeric sequences (ITS). The karyotypic differences among these species can be used for taxonomic identification. These data will be useful in future studies of these fishes and help understanding the phylogenetic relationships and chromosomal evolution of the Apteronotidae.

Keywords: fluorochromes, FISH, chromosomal rearrangements, biodiversity.

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Introduction

Apteronotidae is the Gymnotiformes family with the largest number of formally described species: 86 species divided into 15 genera (De Santana, 2007; De Santana and Vari, 2009, 2010a,b; Albert and Crampton, 2009; De Santana and Crampton, 2010). The species of this family are found in rivers from Panama to northern Argentina, including rivers that flow into the Pacific Ocean (eastern Colombia), the Orinoco, Maracaibo, Magdalena, Guyana shield, the Amazon, and the Paraná-Paraguay and San Francisco basins (Mago-Leccia, 1994). Intra- and interspecific variations in size and shape of the head have been observed, probably related to trophic specialization and/or aggression between males (Cox-Fernandes, 1998; Albert, 2001; Cox-Fernandes *et al.*, 2002).

A few species of the order Gymnotiformes have been cytogenetically analyzed and have shown highly diverse karyotypes, with differences in both chromosome structure and number (Artoni *et al.*, 2000). Among the studied members of this order, the chromosome number varies from 2n = 24 in *Apteronotus albifrons* (Howell, 1972; Almeida-Toledo *et al.*, 1981; Mendes *et al.*, 2012) to 2n = 54 in *Gymnotus carapo*, *G. mamiraua, G. inaequilabiatus* and *G. paraguensis* (Lacerda and Maistro, 2007; Milhomem *et al.*, 2007, 2008, 2012a, 2012b; Scacchetti *et al.*, 2011).

Although Apteronotidae is the Gymnotiformes family with the largest number of described species, chromosomal information is available only for *Apteronotus albifrons*, which has 2n = 24 chromosomes (Howell, 1972; Almeida-Toledo *et al.*, 1981; Mendes *et al.*, 2012). Reportedly, *Apteronotus albifrons* from the Parana River also has B chromosomes, which were found as microchromosomes (Mendes *et al.*, 2012).

Here, we made the cytogenetic characterization of three additional Apteronotidae species, *Sternarchorhamphus muelleri*, *Parapteronotus hasemani* and *Sternarchogiton preto*, in an effort to increase the amount of chromosomal information available for representatives of this family, to allow comparative analyses and provide new insights into the possible mechanisms underlying the diversification of these species.

Material and Methods

We analyzed two males of species *Sternarchorhamphus muelleri* (MPEG 22759) from the Anequara river (Abaetetuba - PA; 1°40'42.6" S and 49°00'16.6" W), 15 specimens (10 males and five females) of *Parapteronotus hasemani* (IDSMIctio 059, IDSMIctio

Send correspondence to Cleusa Y. Nagamachi. Instituto de Ciências Biológicas, Universidade Federal do Pará, Campus Guamá, Avenida Perimetral, sn., Guamá, 66075-900 Belém, PA, Brazil E-mail: cleusanagamachi@gmail.com.

0637, IDSMIctio 0746, IDSMIctio 0747, IDSMIctio 0754, IDSMIctio 0758, IDSMIctio 01001, IDSMIctio 01869, IDSMIctio 02047, IDSMIctio 02056, IDSMIctio 02057, IDSMIctio 02058, IDSMIctio 02095, IDSMIctio 02129, MPEG 22757) from the rivers of the Reserva de Desenvolvimento Sustentável Mamirauá (RDSM) - AM (3°2'50,2" S e 64°51'26,6" W), and five specimens of *Sternarchogiton preto* (one male, one female and three of unidentified sex; MPEG 22758) collected in the Caripetuba river (Abaetetuba - PA; 1°37'23,49"S e 48°55'33"W) (Figure 1). The specimens were deposited in the Instituto de Desenvolvimento Sustentável Mamirauá (IDSM) and the Museu Paraense Emílio Goeldi (MPEG). Figure 2 shows an example of each species studied.

Mitotic chromosomes were obtained according to the method described by Bertollo *et al.* (1978) and analyzed by conventional staining (Giemsa), C-banding (Sumner, 1972), Ag-NOR (Howell and Black, 1980), CMA_3 (Schweizer, 1980), and DAPI (Pieczarka *et al.*, 2006) staining. Fluorescent *in situ* hybridization (FISH) was performed with telomeric probes (All Telomere, Oncor) and 18S rDNA probes obtained from species *Prochilodus argentus* (Hatanaka and Galetti Jr, 2004) and labeled with biotin or digoxigenin by nick translation. Hybridization was detected with avidin-(Cy3 or FITC) or anti-digoxigenin-(Cy3 or FITC). The morphological classification of the chromosomes was made as described by Levan *et al.* (1964).

Results

The species *Sternarchorhamphus muelleri* was found to have a karyotype of 2n = 32 (28m/sm+4st/a) and a fundamental number (FN) of 60. C-banding showed the presence of constitutive heterochromatin (CH) in the centromeric regions of most chromosomes, except for pairs 7, 11, 12, 13, 14 and 16, where the banding was almost imperceptible (suggesting that there was relatively little CH). Pairs 1, 3 and 10 had pericentromeric heterochromatic blocks, and pair 15 had a heterochromatic block in the proximal region of the long arm. A nucleolar organizer region (NOR) was found in the interstitial region of the long arm of pair 15, and showed heteromorphism between the homologs (Figure 3A, B).

The karyotype of *Parapteronotus hasemani* was 2n = 52 (36m/sm+16st/a) and FN = 88. CH was observed in the centromeric regions of all chromosomes. Pairs 1, 22 and 23 also had heterochromatic blocks in the proximal regions of their long arms. Pairs 2, 3, 5 and 6 had large heterochromatic blocks that spanned nearly the entire length of their short arms. Pairs 4 and 12 had heterochromatic blocks in their pericentromeric regions. A NOR was found on the distal short arm of pair 3; it coincided with a secondary constriction and showed heteromorphism between the homologs (Figure 3C, D).

Sternarchogiton preto had a 2n = 52 (38m/sm+14st/a) karyotype and FN = 90. CH was observed in the centromeric region of most chromosomes, except for pairs 5, 9, 12, 16 and 20, where the banding was almost im-

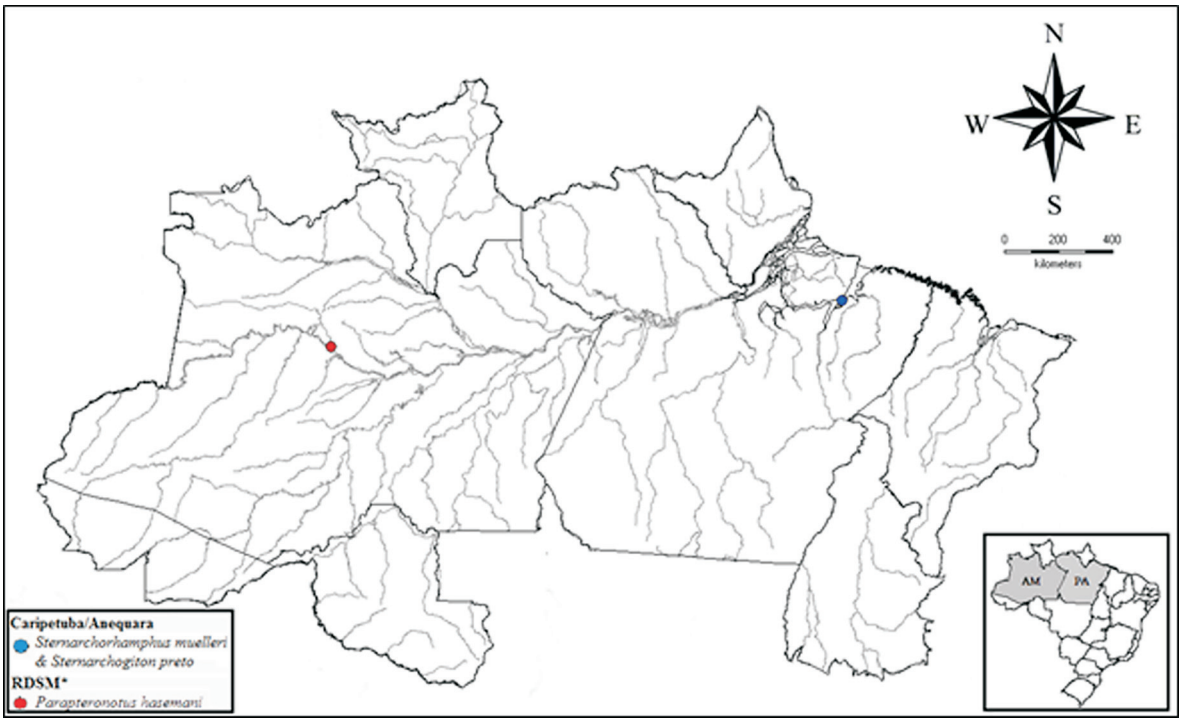

Figure 1 - Map of the collection sites of the *Sternarchorhamphus muelleri, Parapteronotus hasemani* and *Sternarchogiton preto* specimens (*Reserva de Desenvolvimento Sustentável Mamirauá, AM).

Figure 2 - Apteronotidae specimens preserved in 70% ethanol: (a) *Sternarchorhamphus muelleri,* (b) *Parapteronotus hasemani,* and (c) *Sternarchogiton preto* (Source: Laboratório de Citogenética – UFPA). Bar = 0.5 cm.

perceptible. In addition, pair 1 had a heterochromatic block throughout the long arm, pair 2 had a heterochromatic block in the proximal region of the long arm, and pair 3 had a heterochromatic block on the long arm. Pairs 4, 8, 21, 23 and 24 had heterochromatic blocks on their short arms. A NOR was observed in the distal region of the long arm of pair 3, within the heterochromatic block, and showed heteromorphism between the homologs (Figure 3E, F).

The three studied species showed similar results in the fluorochrome, CMA_3 and DAPI staining, as well as FISH analysis with 18S rDNA and telomere repeat probes. CMA_3 labeled the NORs in all three species, showing that the ribosomal genes were interspersed with GC-rich sequences. DAPI staining revealed that the CH was AT-rich, and FISH with 18S rDNA probes showed that these sites were localized in a single pair per species, coincident with the NOR. Finally, FISH using the telomeric probes did not indicate the presence of any interstitial telomeric sequences (Figure 4A-L).

Discussion

Family Apteronotidae is not only more diverse in its number of genera, species and morphological forms, it also displays the greatest variation in diploid number among the Gymnotiformes, ranging from 2n = 24 (16m/sm+8st/a) in *Apteronotus albifrons* (Howell, 1972; Almeida-Toledo *et al.*, 1981; Mendes *et al.*, 2012) to 2n = 52 in *Parapteronotus hasemani* (36m/sm+16st/a) and *Sternarchogiton preto* (38m/sm+14st/a) (this work). *Sternarchorhamphus muelleri* (2n = 32; 28m/sm+4st/a) has an intermediate diploid number (this work).

Parapteronotus hasemani and *Sternarchogiton preto* have the same diploid number (2n = 52), but differ in their karyotypic formulae. This difference can be explained by chromosomal inversions, which can change the morphology of chromosomes while maintaining the diploid number (Alves *et al.*, 2003; De Oliveira *et al.*, 2006). Karyotypes of 2n = 52 are also found in other Apteronotidae species (*Sternarchorhynchus cramptoni, S. oxyrhynchus, Platyurosternachus macrostomus, Apteronotus bonapartii*; unpublished data), while 2n = 50 is seen in two species of genus *Adontosternarchus* (*A. clarkae* and *A. balaenops*; unpublished data). This indicates that a higher diploid number is common in the family. Although they share the same diploid number, *P. hasemani* and *S. preto* display considerable differences in their C-banding patterns and the location of their NORs. The latter may be due to translocations of the NOR-bearing chromosomes.

The karyotype of *Sternarchorhamphus muelleri* (2n = 32) is quite different from those of the other two species in chromosome number and morphology, CH distribution and NOR location. The diploid number reduction appears to have occurred through chromosomal fusion events. Such events are believed to explain the reduction of 2n in genus *Ancistrus* (Loricariidae) (Alves *et al.*, 2003; De Oliveira *et al.*, 2009), and have been suggested for other groups of fishes (Cipriano *et al.*, 2008; Margarido and Moreira-Filho, 2008). Similarly, fusion events could also explain the diploid number decrease in *Apteronotus albifrons*. The location of the NORs in this species is quite different from that seen in other species, as it typically appears in the interstitial region of the long arm of an acrocentric chromosome. This variation in the NOR-bearing chromosome may have arisen, in this case, through a pericentric inversion that moved the NOR to the middle of the chromosome arm.

Nagamachi *et al.* (2010) used chromosome painting to show that the degree of chromosomal rearrangement between karyotypes of two cryptic species of *Gymnotus* cf *carapo* (2n = 40 and 2n = 42) was much greater than that estimated using classical cytogenetics (Milhomem *et al.*, 2008). Thus, the pericentric inversions and centric fusions that were believed to differentiate the karyotypes of these species may be an underestimate of the actual degree of genomic rearrangement at work.

CH was found in the centromeric regions of virtually all chromosomes of the analyzed species. DAPI staining revealed that this CH is AT-rich, which is consistent with reports concerning other Gymnotiformes (Milhomem *et al.*, 2007, 2008, 2012a,b; Silva *et al.*, 2008, 2009; Cardoso *et al.*, 2011). The three species analyzed in the present study showed differing CH patterns, with several additional blocks in non-centromeric regions. Processes related to the dynamics of repetitive DNA, such as amplifications and translocations, might have been involved in the develop-

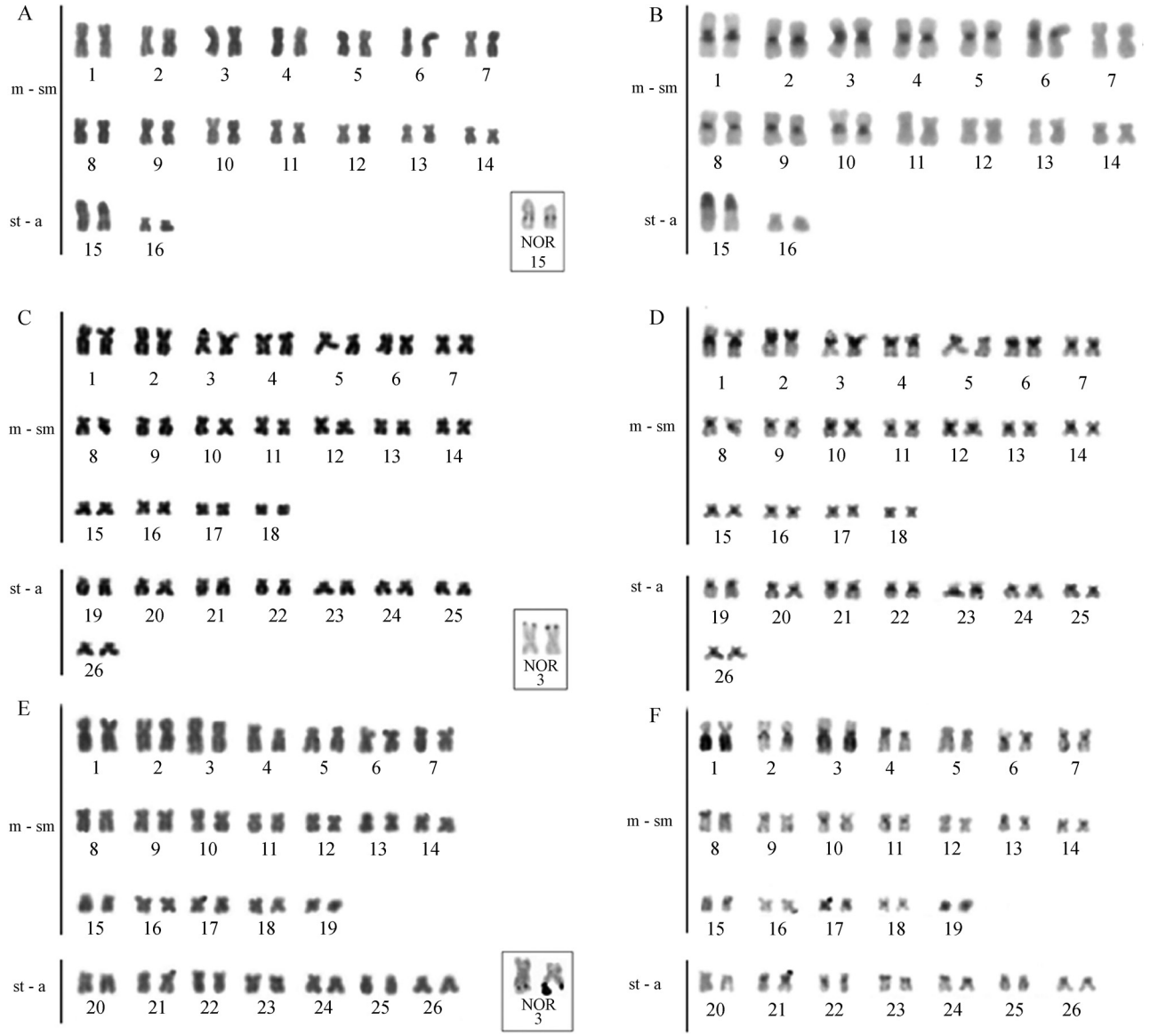

Figure 3 - Karyotypes submitted to conventional staining and C-banding: A and B - *Sternarchorhamphus muelleri*, C and D -*Parapteronotus hasemani*; and E and F - *Sternarchogiton preto*. Abbreviations: m-sm = metacentric-submetacentric; st-a = subtelocentric-acrocentric.

ment of these blocks. Using microarray analysis, Lippman *et al.* (2004) showed that in *Arabidopsis* the heterochromatin is determined by transposable elements and related to tandem repeats. Transposons have been found in the heterochromatin of several fish groups, including *Cichla kelberi* (Teixeira *et al.*, 2009), *Hisonotus leucofrenatus* (Ferreira *et al.*, 2011), and Antarctic fishes of the suborder Notothenioidei (Ozouf-Costaz *et al.*, 2004). Furthermore, transposons have been associated with the karyotypic variation observed in *Erythrinus erythrinus* (Cioffi *et al.*, 2010), and the formation of the Y chromosome in *Chionodraco roseofuscus* (Capriglione *et al.*, 2000).

Souza *et al.* (2009) analyzed species of genus *Peckoltia* (Siluriformes: Loricariidae) and proposed possible homeologies among some pairs with similar C-banding patterns, morphologies and NOR localizations. In the karyotypes of *P. hasemani*, *S. muelleri* and *S. preto*, we identified chromosomes (pair 1 in the first two species and pair 2 in the latter) that resembled one another in chromosome morphology, size and HC distribution (Figure 5).

Heteromorphisms in NOR size (by 2-fold or more) have been described in some Gymnotiformes species, and the NORs of *Eigenmannia* sp and *E. virescens* were found to be larger than those of the other examined species. Tandem duplications, unequal crossovers involving repeated regions, and/or accidental duplications might explain these variations (Foresti *et al.*, 1981).

The NOR regions of the tested species stained positive with CMA_3, revealing that the ribosomal genes are interspersed with GC-rich sequences (Pendás *et al.*, 1993).

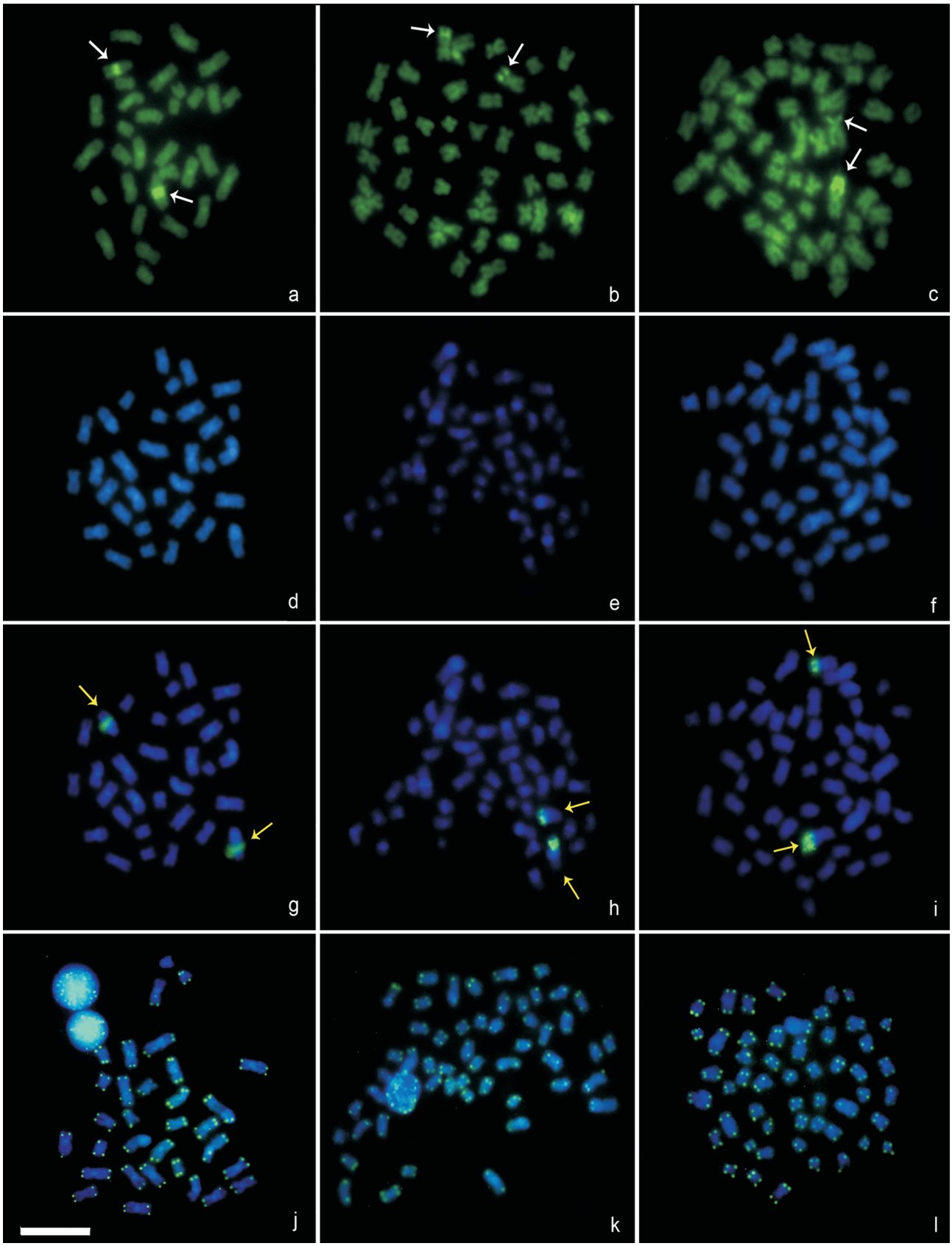

Figure 4 - Fluorescent staining of *Sternarchorhamphus muelleri* (first column), *Parapteronotus hasemani* (second column) and *Sternarchogiton preto* (third column) metaphases, respectively, with CMA_3 (a, b, c), DAPI (d, e, f), 18S rDNA probe (g, h, i), and telomeric probe (j, k, l). White arrows indicate CMA3-marked NORs; yellow arrows indicate NORs marked by 18S rDNA probe. Bar = 5 μm.

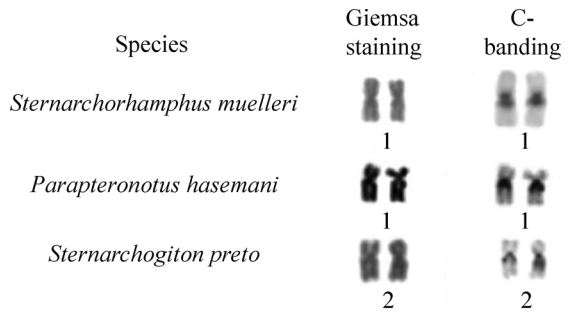

Figure 5 - Possible homeologies among *Sternarchorhamphus muelleri, Parapteronotus hasemani* and *Sternarchogiton preto* (conventional Giemsa staining and C-banding).

Our results resemble those found in other Gymnotiformes and additional fish species (Artoni and Bertollo, 1999; Milhomem *et al.*, 2007, 2008, 2012a,b; Silva *et al.*, 2008, 2009; Cardoso *et al.*, 2011).

FISH with telomeric probes failed to show any evidence of interstitial telomeric sequences (ITSs). This may reflect the absence of chromosomal rearrangements involving the telomeres (Silva *et al.*, 2009). Alternatively, telomeric sequences away from the chromosomal ends may have undergone sequence changes that hinder probe hybridization (Albuín *et al.*, 1996).

Accumulating evidence suggests that variability in diploid number and karyotype formula can be explained by chromosome rearrangements, such as fusions (which can decrease the diploid number) and inversions. According to the phylogenies of Alves-Gomes *et al.* (1995) and Albert (2001), the family Gymnotidae, represented by *Gymnotus* and *Electrophorus*, occupies the basal position among the other Gymnotiformes. The species in this family have diploid numbers ranging from 34 to 54, with a higher occurrence of 2n above 50 (for review, see Milhomem *et al.*, 2012a,b). Thus, we believe that the higher diploid numbers (2n = 50 or 52) are basal in Apteronotidae and that the karyotypes of *Apteronotus albifrons* and *Sternarchorhamphus muelleri* underwent rearrangements that decreased their 2n values. Their CH patterns are also quite variable, showing additional blocks that are characteristic to each species. The NORs varied in size and location on the chromosome, but all three species had a single NOR.

It is therefore likely that some homeologies exist among these species. Together, the results presented herein add new information that may prove valuable in future studies of this group, facilitating taxonomic identification, and increasing our understanding of the chromosomal evolution and phylogenetic relationships of the Apteronotidae.

Acknowledgments

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