High intraspecific karyological conservation in four species of *Gymnotus* (Pisces: Gymnotiformes) from Southeastern Brazilian basins

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SUMMARY – Four species of the genus *Gymnotus* from Southeastern Brazilian basins were extensively studied in relation to their karyology. A high intraspecific conservatism was detected and the cytotypes were species-specific, in relation to diploid number, NOR-bearing chromosomes and C-band pattern. No geographic chromosome races were found. The differences detected in the distribution of NOR- associated heterochromatin were considered as a good evidence of the heterochroma- tin loss that seems to have occurred in the evolutionary story of this group of fishes. *Gymnotus* is a complex of species which the variation is still poor understood. The cytogenetic data here described allowed the reinterpretation of previous karyological data already available in literature and is an attempt to contribute to a better under- standing of this group of Neotropical freshwater fishes.

Key words: cytogenetics, C-bands, NOR, *Gymnotus*, Gymnotidae.

INTRODUCTION

Gymnotus is the only genus belonging to the family Gymnotidae (Pisces: Gymnotiformes) which presents a wide Neotropical distribution. This genus encompasses weakly electric fishes, with pulse-type emission of electric organ discharges (EOD). Fifteen nominal species from this group have already been described (Ax.aERT and CRAMvvow, unpubl. obs; Ax.aERT et al. 1998), however many undescribed species still probably exist (NEr.sow 1994). Fishes of this group present nocturnal habits, are extremely territorial and aggressive (Lulvo- BERG et al. 1987). They take out a very important ecological hole, once they are considered the main source of food to the large freshwater fishes in some basins of South America (Bvr.Locv. et al. 1979). They are also used as alive baits in fishery. Some species of *Gymnotus* have been studied from the viewpoint of EOD features, systematics (Ar.vEs-GoMEs 1996; TRigvEs 1996; Ar.aERv et al. 1998;

Ax.aEar and CAMvos-DA-PAz 1998), and cytogenetics (Ar.MEmA-Tor.EDO 1978; FOREsn *et al.* 1984; FERxANDEs-MAvior.t *et al.* 1997). When it comes to the karyological analysis, very few data are available about this group. *G. carapo* is the only species for which more extensive karyological information is available, involving chromosome number and formula (Ar.MEtoA-Tor.Eno 1978; FORESTI *et aL* 1984; FERNANDES-MATIOLI *et al.* 1997), C-banding pattern (Foa.Esvi 1987) and chromosome evolution based on nucleolus organizer regions (NORs) polymorphism (FERNANovs-MATtor.t *et al.* 1997). An extensive chromosome variability was described for *G. carapo,* includ- ing chromosome numbers varying from *2n=52,* 2n=48, 2n=46 to 2e=42, that were described for different populations of this species sampled from Amazon basin, the Parana basin and from the East basin (Ar.MvmA-Tor.EDo 1978; FOREsTi *et al.* 1984; FOREsTr 1987). Nevertheless, the attribution of this chromosome variability to *G. carapo* is questionable, since a comprehensive phylogenetic revision of this group is needed, and according to Ar.acRv *et al.* (1999), the phylogenetic revision of this group has been hindered by a poor understanding of species level diversity and intraspecific variation. The purpose of this paper is to describe the diploid number, chromosome formula, constitutive heterochromatin pattern, and NOR location of four species of *Gymnotus,* and provide a preliminary examination of the karyotype evolution in the genus *Gymnotus.* Adequate samples of *G. carapo, G. inaequi-labiatus, G. sylvius* and *G. pantherinus,* collected from tributaries of the upper Parana river system in the States of Sao Paulo (SP) and one from Parana (PR), and also from the East basins in the Southeastern State of Sao Paulo, Brazil. The present data are a step towards the improvement of the understanding of the karyotype evolution and species diversity in the order Gymnotiformes. In addition, the new results empower a reinterpretation of the data already available in literature since the samples analyzed are morphologically identi- fied.

MATERIALS AND METHOD$

All 166 specimens analyzed in this study were collected from 21 natural popula- tions. The samples are shown in the Table 1, and the locations of populations studied are shown in Figure 1. *Chromosome Preparation.* – Animals were injected with 0.01-0.02% colchicine (0.75 mV100g bodyweight) 50 min before sacrifice. Cephalic kidney was extracted and minced in a 0.075M KC1 solution, placed in an incubator at 37»C for 30 min and then centrifuged. The supernatant was discarded and the cell pellet was fixed twice in a methanol:acetic acid (3:1) solution, resuspended in fresh fixative and dropped on heated slides (FORESTI *et al.* 1981). Chromosome preparations were stained with a 3%

TABLE 1 Specimens of *Gymnotus* analyzed.

Species	Sample size	Localities
G. carapo	104	**Upper Paraná System** (Jundiaí, Rio Claro, Americana, Botucatu, Paula Souza, Salto Grande, Primeiro de Maio, Piraçununga, Mococa, São Simão, Santa Maria da Serra, Jacareí)
G. inaequilabiatus	8	**Upper Paraná System** (Rio Claro, Represa de Porto Primavera)
G. sylvius	31	**Upper Paraná System** (Americana, Represa de Capivara, São Simão, Santa Maria da Serra, Corumbataí, Jacareí, Paraibuna) **East Basins** (Miracatu)
G. pantherinus	23	**Upper Paraná System** (Paranapiacaba, Taguaruçu) **East Basins** (Itanhaém, Serra da Juréia)

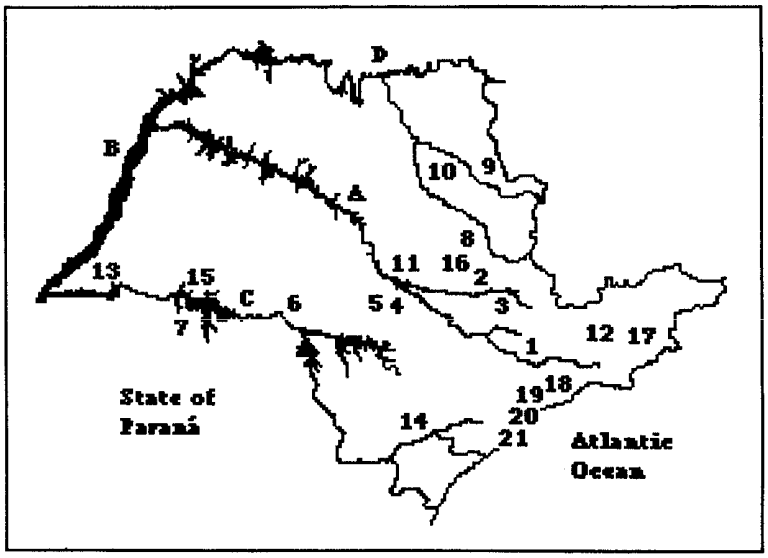

Fig. 1. - Map of State of Silo Paulo showing the localities where the specimens were collected. 1.Jundiai, 2. Rio Claro, 3. Americana, 4. Botucatu, 5. Paula Sousa, 6. Salto Grande, 7. Primeiro de Maio, 8. Piral;ununga, 9. Mococa, 10. Silo Sim1io, 1.1. Santa Mariada Serra, 12. Jacarei, 13. Rep. Porto Primavera, 14. Miracatu, 15. Rep. Capivara, 16. Corumbatai, 17. Paraibuna, 18. Paranapiacaba, 19. Taguarul;u, 20. Itanhem, 21. Serra daJureia. A. Tiete river, B. Paran& river, C. Paranapanema river, D.*r:ron,1p ri"pr* 1 .1 ? 5<00 000

Giemsa staining solution. NORs were silver stained according to the procedure of HoWELL and BLACK (1980). C-bands were obtained according to SUMNER (1972). Karyograms were analyzed for centromeric placement and arm ratios. The chromosomes were arranged in decreasing order of size in three groups: metacentrics (M), submetacentrics (SM), and subtelocentrics/acrocentrics (ST/ A). Secondary constrictions were not considered in the measure of the chromosome arms. The total amount of constitutive heterochromatin was calculated by the mesureament of at least five C-band stained metaphases of each species. The schemes representing NORs locations and C-bands distribution were done using Color-it! software (PINKERTON *et at.* 1992).

RESULTS

The four species analyzed showed specific diploid numbers, chromoSome formulae, C-band distribution, and NOR locations, as summarized in T able 2. In all species analyzed, the NOR was found in a single chromoSome pair . Figures 2-5 ShoW the Giemsa-stained and C-band stained karyotypes, and the NOR-bearing chromoSome pairs stained by the Ag-NOR technique in the four species of *Gymnotus* analyzed.

The schematic drawing of constitutive heterochromatin distribution for the haploid set and the NOR-bearing chromoSomes in the species analyzed are shown in the figures 6- 7.

TABLE 2 Karyological results obtained in four species of *Gymnotus.*

Species	2*n*	Formulae	NOR-bearing chromosome	C-bands distribution (~ %)
G. carapo	54	44M.8SM.2ST/A	1p (M)	45
G. inaequilabiatus	52	40M.10SM.2ST/A	23p (SM)	45
G. sylvius	40	28M.10SM.2ST/A	18p (SM)	20
G. pantherinus	52	38M.8SM.6ST/A	24p (ST/A)	31

DISCUSSION

The four species here analyzed showed a high intraspecific conservatism of karyotype features, with no geographically located chromosomal races, differently of what was observed in other Gymnotiformes (see, for example ALMEIDA- TOLEDO *et at.* 1993). Regardless the site of origin, all specimens of G. *carapo* presented *2n=54* chromosomes, G. *inaequitabiatus* and G. *pantherz'nus, 2n=52,* and G. *sytvius, 2n=40.* The NORs were detected in a single homologue pair per karyotype. In spite of presenting interspecific differences concerning the NOR-bearing chromosome morphology or its location in the karyotype, it was observed that the NOR regions in these species share some common characteristics. They are euchromatic, present size heteromorphism, are 10-

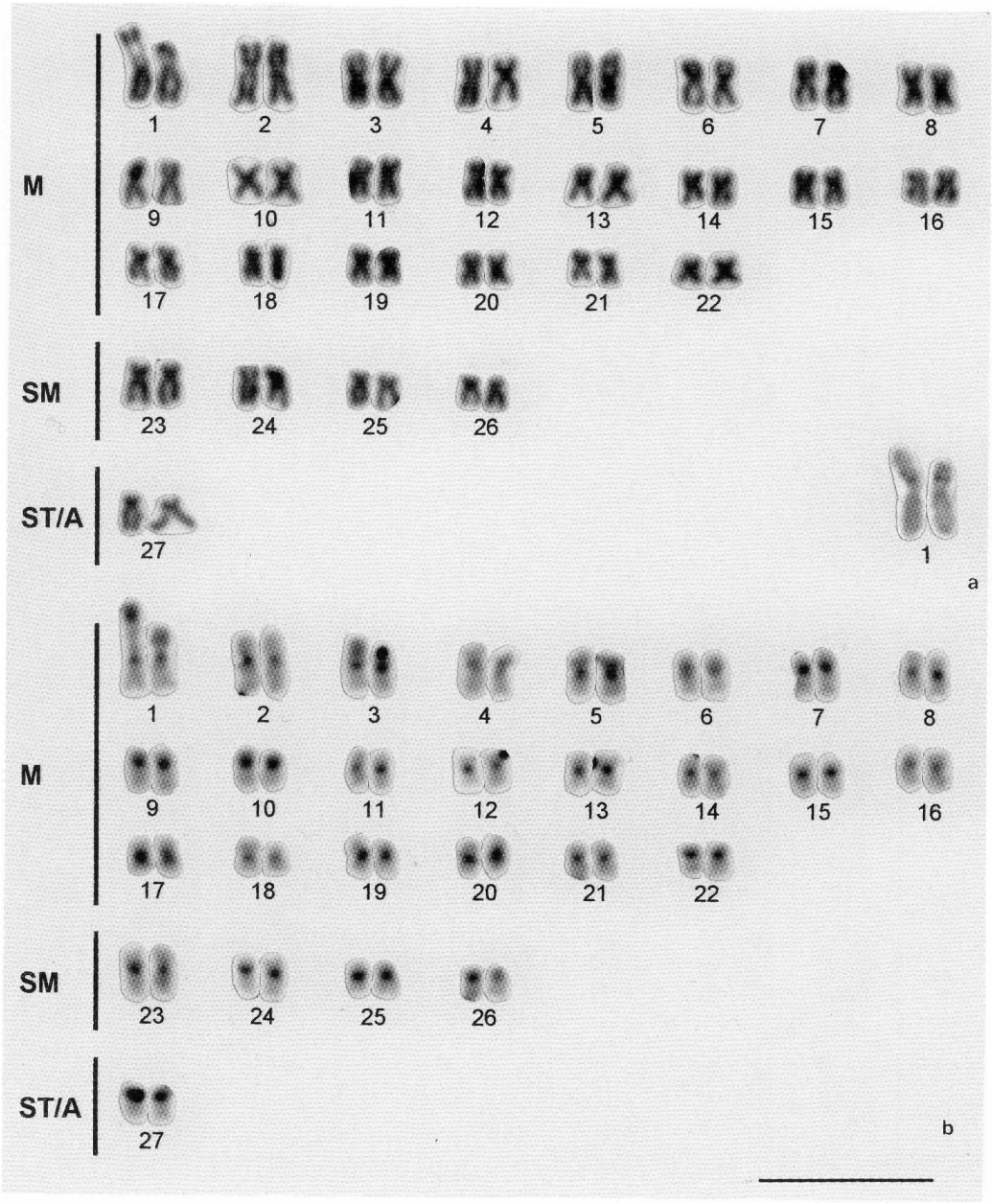

Fig. 2 - *C;ymnotus carapo* a) Giemsa-stained karyotype, and the NOR bearing chromosome pair stained by the Ag-NOR technique. b) C-band stained karyotype. Bar=10~m.

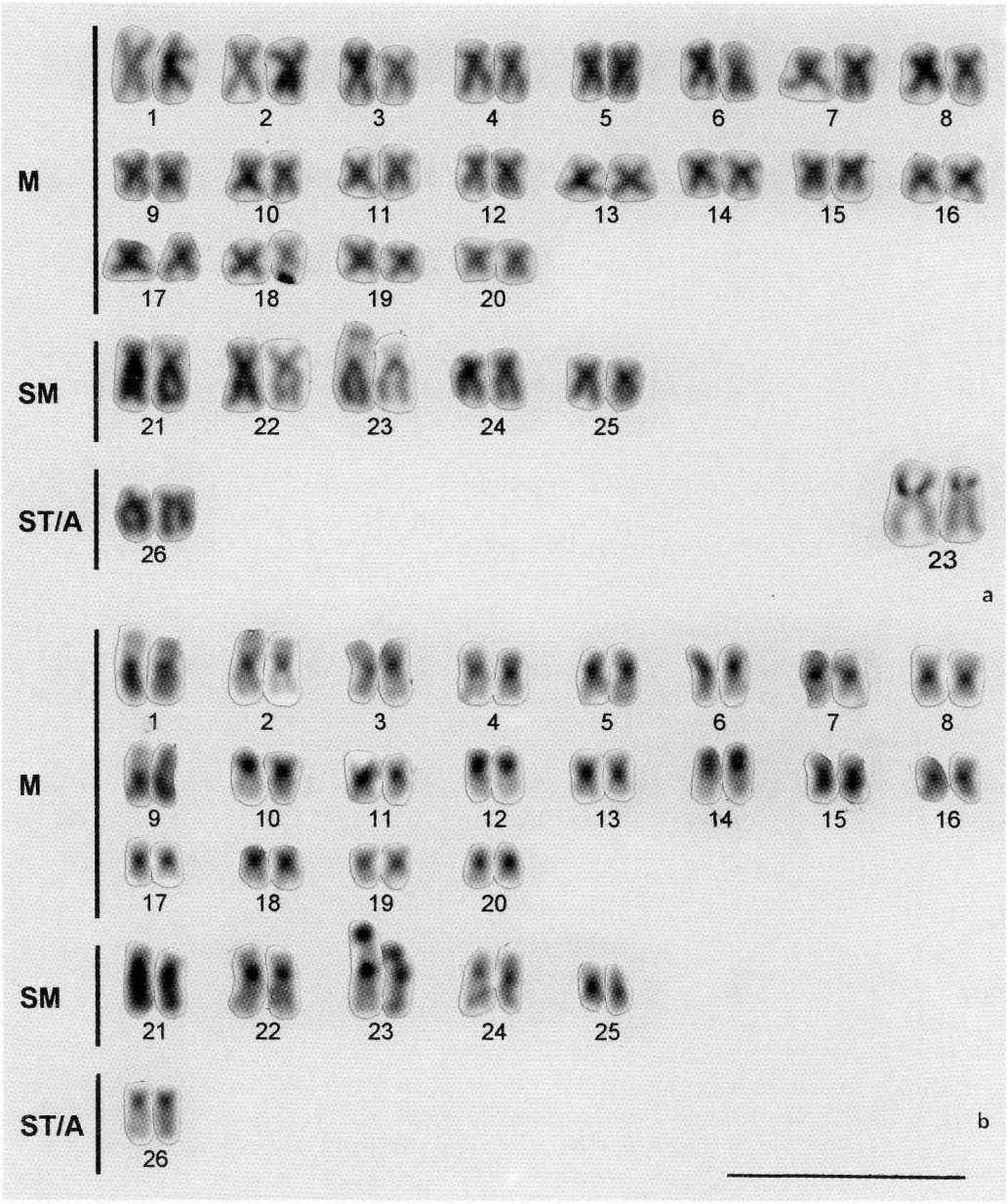

Fig. 3 - *Gymnotus inaequilabiatus* a) C;iemsa-stained karyotype, and the NOR-bearing chromosome pair stained by the AgNOR technique. b) C-band stainedkaryotype. Bar=10~m.

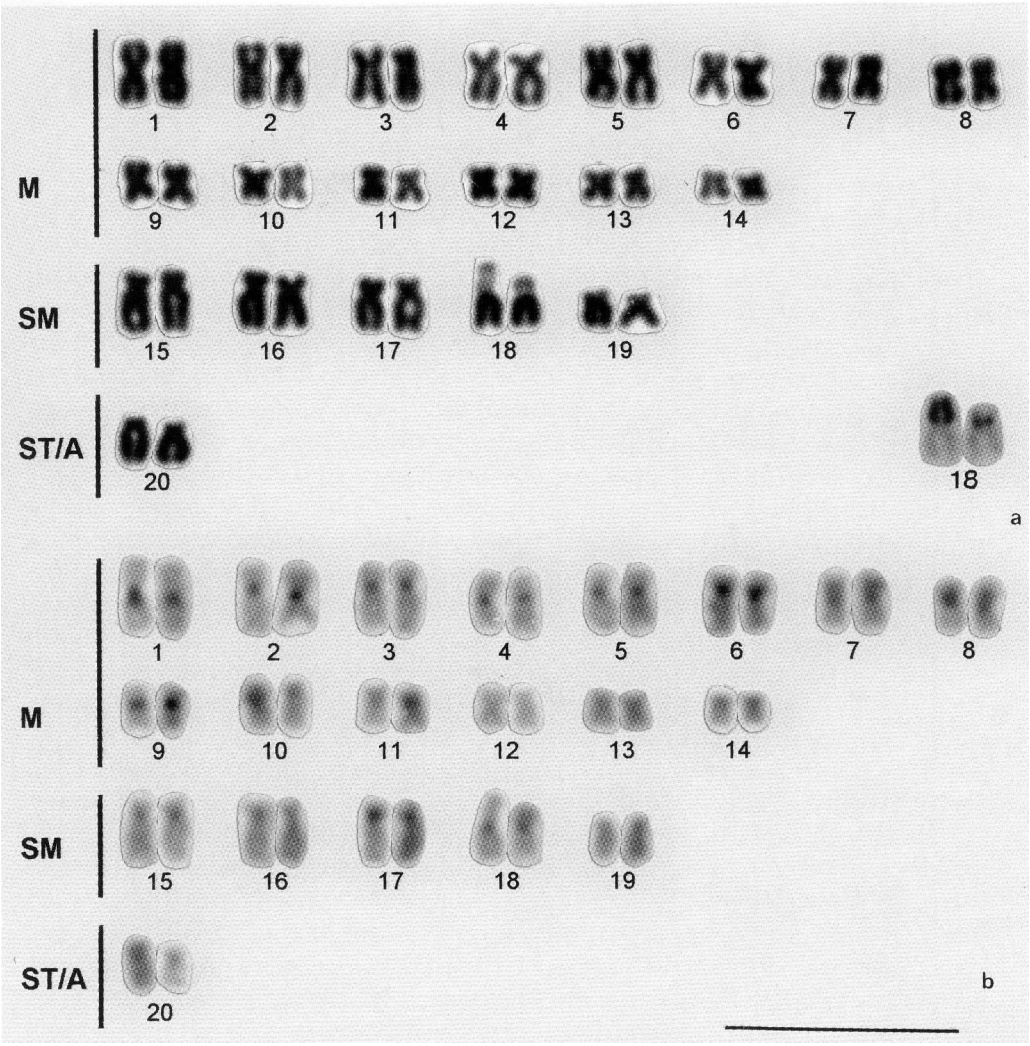

Fig. **4** - *Gymnotus sylvius* a) Giemsa-stained karyotype, and the NOR-bearing chromosome pair stained by the Ag-NOR technique. b) C-band stained karyotype. Bar=10~m.

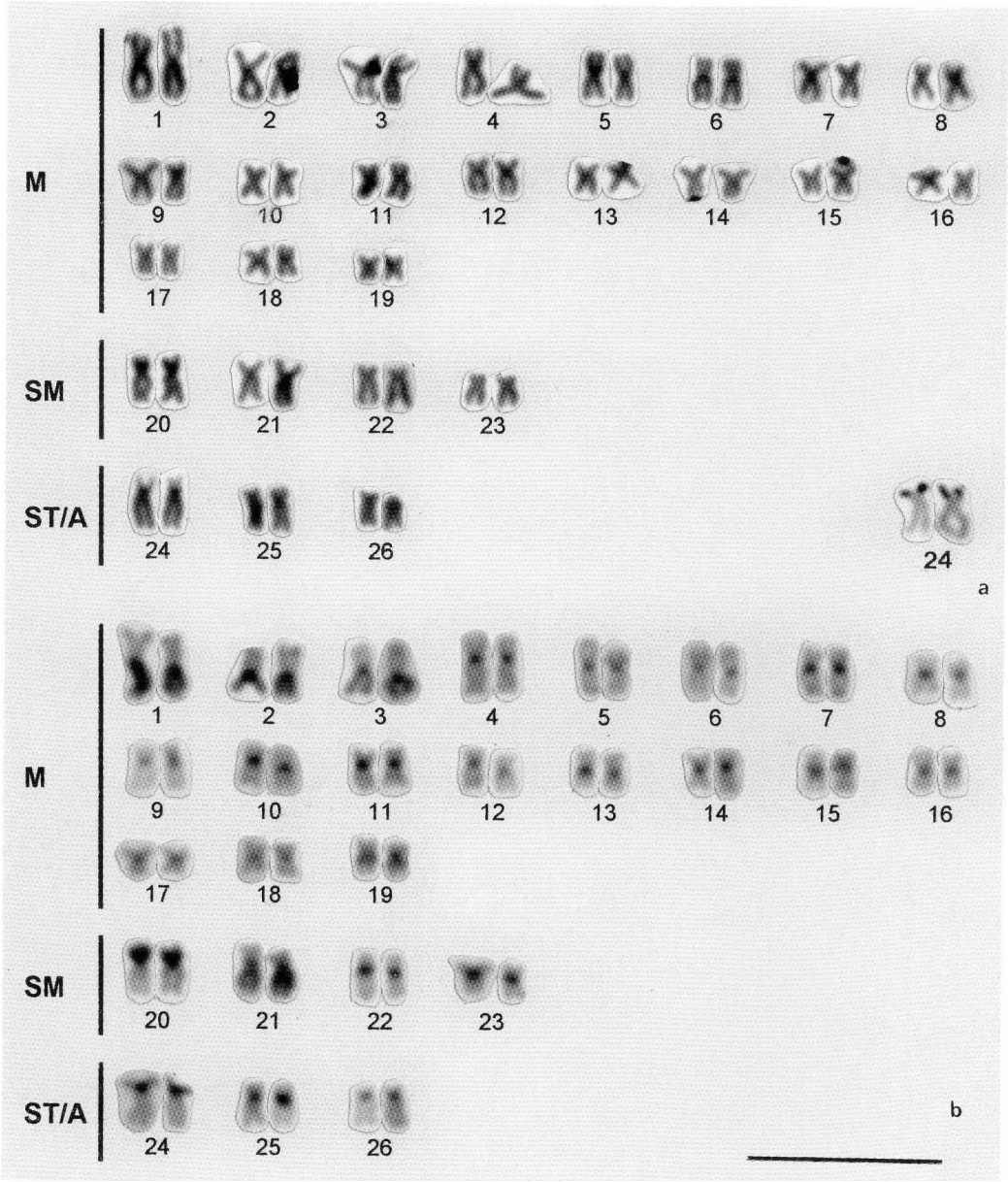

Fig. 5- *Gymrtotus pantherinus* a) Giemsastained karyotype, and the NOR-bearing chromosome pair stainedby the Ag-NOR technique. b) C-band stainedkaryotype. Bar=10~m.

cated on the short arm of the chromosomes, and are placed on secondary constrictions when stained by Giemsa (Figs. 2- 5) .

The presence of a single pair of NOR-bearing chromosomes has been proposed as a primitive condition in animal species (Hsu *et at.* 1975). Considering this hypothesis, the presents results suggest that Gymnotidae could be located at the basis of Gymnotiformes, as previously proposed by FORESTI *et at.* (1981) and FERNANDES- MA TIOLI *et at.* (1997) , and reinforced by taxonomic data of this group (FINK and FINK 1981; TRIQUES 1993; ALVES-GOMES *et at.* 1995). Size heteromorphism involving the NORs was detected both interspecific, intraspecific and intraindividually. In some cases the NOR was three times larger than its homologue (Fig. 2). Following GOLD (1979), the longitudinal increasing of the NOR size can be explained mainly by unequal recombination or randomic duplication. Events as these can be observed in chromosome regions Composed by repetitive DNA, as ribosomal genes. Regions of repetitive DNA seem to be more exposed to the occurrence of unequal recombination, and if this is the case, it is possible to speculate that the enlargement of the NOR size could be advantageous to the organisms for the consequent increasing of the ribosomal genes product (HANSCHE 1975).

As previously described by FERNANDES-MATIOLI *et at.* (1997) in G. *carapo,* we also found NOR phenotype polymorphisms in G. *inaequitahiatus,* but not So extensive as in G. *carapo.* The variability of NOR phenotype could be explained by the occurrence of paracentric inversions involving the short arm of the chromosome. NOR polymorphisms usually involve NOR size and is quite common in fishes (FORESTI *et at.* 1981). Till this time, this group of fishes is the only one presenting this extensive NOR variability, although no variation was observed in G. *.'\ytviu.\'* and in G. *pantherinus.*

In G. *carapo* (Figs. 2, 6) and G. *z'naequitahiatu.\'* (Figs. 3, 6), the NORs are flanked by constitutive heterochromatin. That was not observed in G. *sytvius* (Figs. 4, 7), and in G. *pantherinus* (Figs. 5, 7), whose NORs were always located interstitially in the short arm. Heterochromatic regions are known to contain highly repetitive DNA and to present heteromorphisms (SUMNER 1990). This fact allowed us to suggest that heterochromatic regions bordering NOR tends to favor chromosome rearrangements in these particular chromosome areas.

The C-band patterns are conserved both intraspecifically and intrapopulationally in the four species analyzed. G. *carapo* (Figs. 2, 6) and G. *z'naequitahiatus* (Figs. 3,6) showed the largest amount of constitutive heterochromatin, reaching about 45% of the karyotype. G. *pantherinu.\'* presented about 31% of heterochromatin in its karyotype (Figs. 5, 7), and in G. *sytviu.'* the smallest amount was noticed, being about 20% (Figs. 4, 7). According to the intrageneric phylogeny based on molecular data in preparation by FERNANDES-MATIOLI and her colleagues, G. *sytviu,'* seems to be the most derived species of *Gymnotus,* considering the four species here discussed. This observation al-

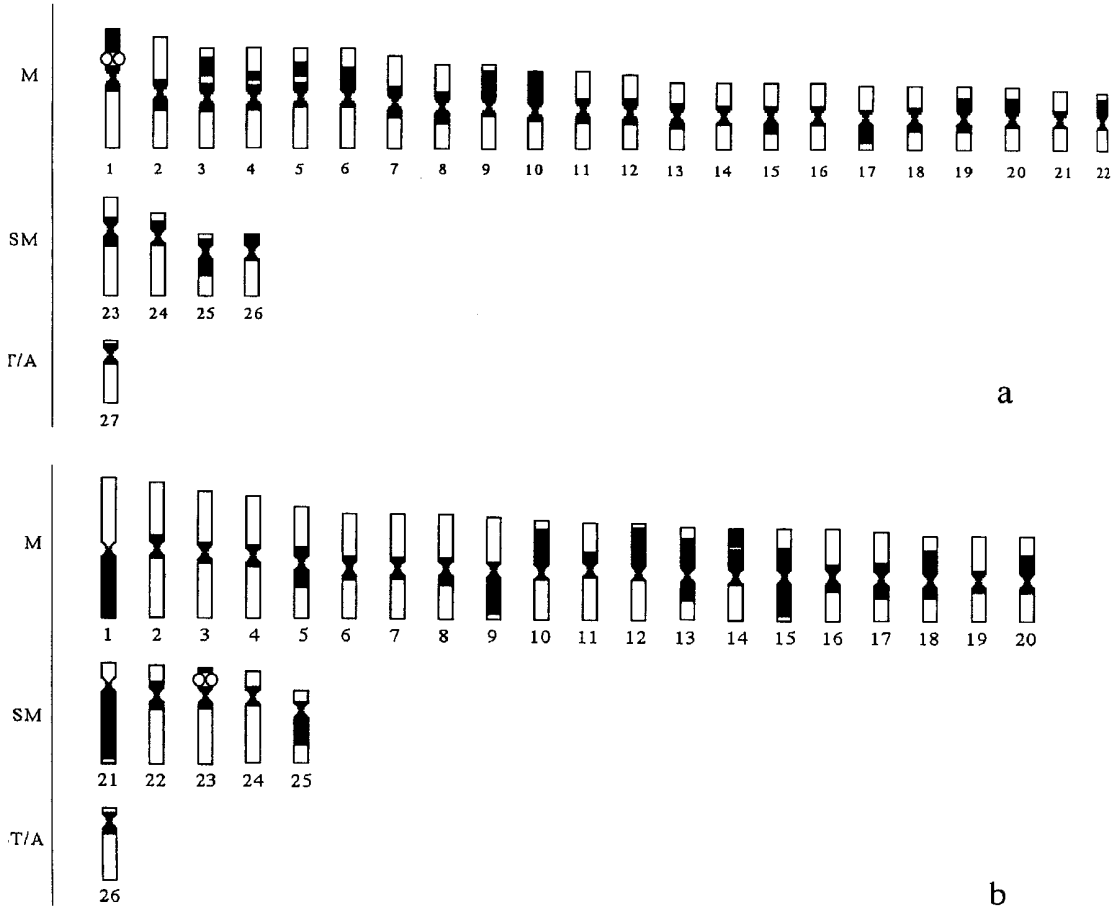

Fig. 6 - Schematic drawing of the haploid chromosome set representing the C-band pattern: a) *Gymnotus carapo*, approximately, 45% heterochromatic; b) *Gymnotus inaequilabiatus*, approximately, 45% heterochromatic.

lowed us to propose, preliminarily, that probably a gradual loss of constitutive heterochromatin has occurred along the evolutionary story of this group of fishes.

Other possible evidences of the loss of heterochromatin is the composition of the NOR-bearing chromosomes in the four species analyzed. In G. *carapo* NOR-bearing chromosomes are metacentrics and in G. *inaequitahiatus*

they are submetacentrics. In both cases, NOR-bearing chromoSOmeS presented large heterochromatic blocks bordering the NOR regions (Figs. 2,3). In G*pantherinus,* NOR-bearing chromoSOmeS are subtelocentric/acrocentrics ones, and the NORs were always detected in interstitial position (Fig. 5). The presence ofpericentromeric heterochromatin flanking only one side of the NOR could be an evidence of apericentric inversion associated with the loss of heterochromatin if compared with NOR-bearing chromoSOmeS of G. *carapo* and G. *z'naequitahiatus.* The same could be true to G. *sytvius,* whose NORbearing chromosomes are submetacentric, and NORs were always detected in interstitial position (Fig. 4) . These observations reinforce our hypothesis ofheterochromatin loss along the evolutionary time in this group of fishes. Gradual deletion of theheterochromatin block adjacent to the NOR was already described in Gymnotiformes, in the familySternopygidae (ALMEIDATOLEDO *et at.* 1993).

In the same way, the reduction ofchromoSOme numbers might be interpreted as an evolutionary tendency in this group of fishes, since G. *carapo,* which presents *2n=54,* seems to be more primitive than the other species here analyzed. *2n=54* chromoSOmeS is the highest diploid number described in the family Gymnotidae. Reduction in chromoSOme number could be explained by chromoSOme fusion. However, the subject of mutational variation in chromsomes is still very complex, due to the large variability of meiotic processes and chromoSOmal behavior in plants and animals (STEBBINS 1971; WHITE 1973; SWANSON *et at.* 1981). The techniques here employed do not allow a good inference about mutational events involving chromoSOme numbers.

The present results of G. *carapo* are in agreement with those ones in the literature, which describe *2n=54* chromoSOmes in this species (ALMEIDATOLEDO 1978; FORESTI *et at.* 1984; FORESTI 1987; FERNANDES-MATIOLI *et at.* 1997). However, the specimens considered as G. *carapo* and presenting *2n=52* (FORESTI *et at.* 1984; FORESTI 1987) maybe understood as G. *inaequitahiatus* or G. *pantherinus,* more probably as G. *z'naequitahiatus* once they were collected in a site near the population ofCarumbatai, here extensively visited, and where only G. *inaequitahiatus* was found (Fig. 1). *Gymnotus* sp., described by FORESTI *et at.* (1984) with *2n=52,* was collected near the coast rivers ofsao Paulo, where we only found G. *pantherinus (2n=52).* This allowed us to suggest that those specimens studied by FORESTI *et at.* (1984) were in true G. *pantherinus.* The other karyotypes described in the literature, with *2n=52, 2n=48, 2n=46,* and *2n=42* are probably characteristics of other species notanalyzed here, and this assumption is reinforced by the fact that these forms were originated from the Amazon basin.

In respect to the geographic distribution of the species hereanalyzed, G. *pantherinus* was exclusively signed in the coastal basins. The other species are distributed along the countryside basins. G. *carapo* showed the most wide distribution. In the population of RioClaro (Table 1, Fig. 1) we found G.

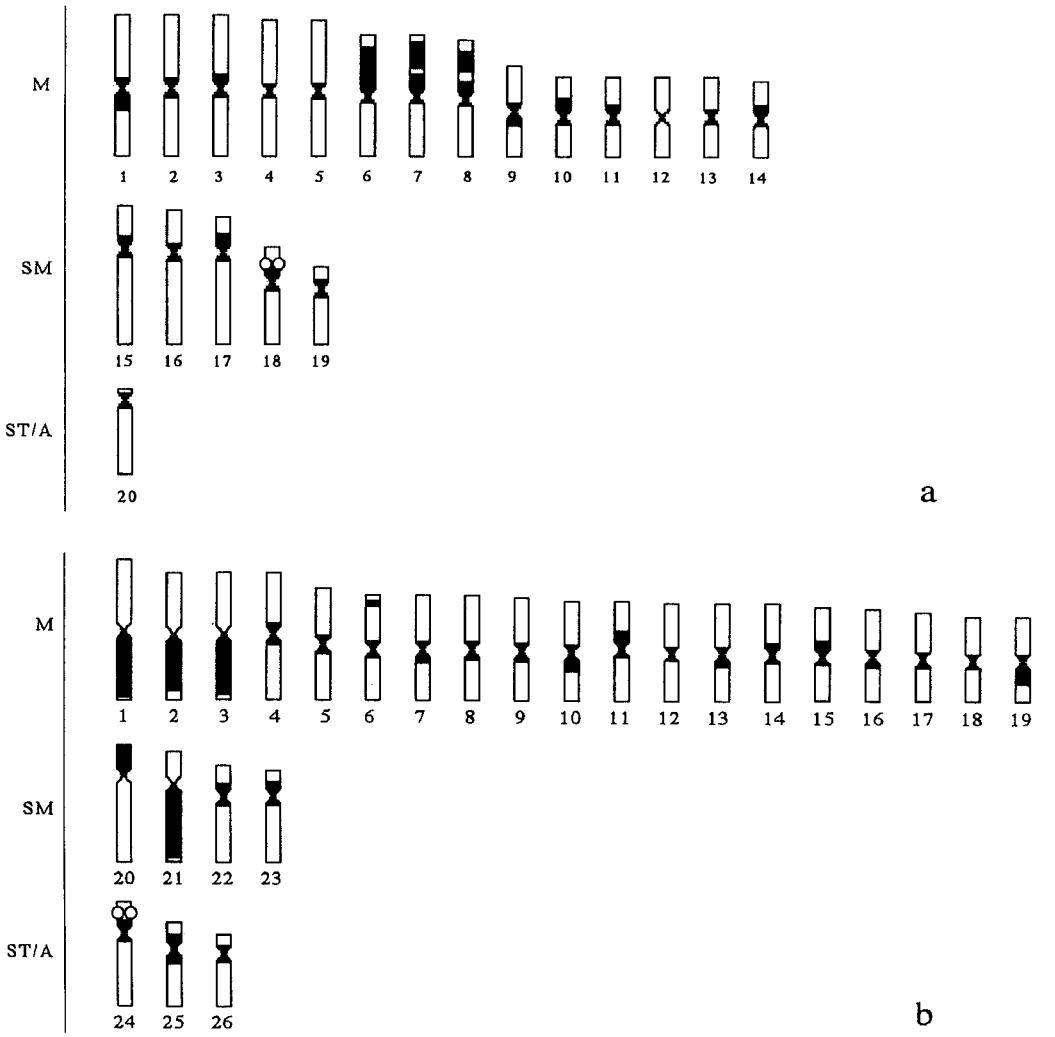

Fig. 7 - Schematic drawing of the haploid chromosome set representing the C band pattern. a) *Gymnotus sylvius*, approximately, 20% heterochromatic; b) *Gymnotuj pantherinuj*, approximately, 31% heterochromatic.

carapo and G. *inaequitahiatus* in simpatry, and in Jacarei G. *carapo* and G. *sylvius* were observed in simpatry (Table 1, Fig. 1). The more reasonable hypothesis to explain both cases could be the occurrence of a contact zone, which generates an overlap of the species due to a secondary invasion (T AUBER and TAUBER 1989).

Simpatry was yet observed in other populations: sao Simao, Americana, and Santa Maria da Serra (Table 1, Fig. 1). However, in these localities specimens of *Gymnotus* are usually sold as baits, So that is possible to consider a mixture of species of *Gymnotus* in these sites. In this regard, the sympatry in these locations could be false.

The total sample here studied is a very good representation of *Gymnotus* fauna, at least of State of sao Paulo basins. Although we can not turn down the hypothesis of the existence of other species in these basins, till this time this is the most complete karyologycal study in the genus *Gymnotus* in this geographic region of Brazil.

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