CARYOLOGIA Vol. 55, no. 3: 181-188, 2002

Cytogenetic analysis of five species of the subfamily Tetragonopterinae (Teleostei, Characiformes, Characidae)

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Abstract - The subfamily Tetragonopterinae is composed by a large number of species distributed in South and Central America. This subfamily has many taxonomic and phylogenetic problems, being considered by several authors as an artificial group. With the objective to better understanding the relationships among the components of this fish group, cytogenetic studies were conduced on five species of Tetragonopterinae. *Astyanax janeiroensis* had 2*n*=50 chromosomes (6M+14SM+14ST+16A), *Hyphessobrycon reticulatus* had 2*n*=50 chromosomes (14M+20SM+16ST), *Hollandichthys multifasciatus* had 2*n*=50 chromosomes (10M+12SM+28ST), *Ctenobrycon hauxwellianus* had 2*n*=50 chromosomes (10M+6SM+34ST), and *Phenacogaster* cf. *pectinatus* had 2*n*=46 chromosomes (12M+2ST+32A). Only *A. janeiroensis* had multiple NORs, while all other species had simple NORs. Small heterochromatic blocks were observed in the chromosomes of all species in a pericentromeric position. *A. janeiroensis* also had some chromosomes with large heterochromatic blocks in a terminal position and a pair with an interstitial block. The karyotypic evolution of each genus is discussed.

Key Words: C-band, Evolution, Fish chromosomes, Tetragonopterinae.

INTRODUCTION

The subfamily Tetragonopterinae is composed of a large number of fish species distributed in South and Central America. They are small size fishes and live under several different environmental conditions. According to BRITSKI (1972), about 400 species occur in Brazil. EIGENMANN (1917), in his work on American characids, considers the subfamily Tetragonopterinae to be one of the most primitive in the Characidae, with the genus *Astyanax* probably being the most primitive. According to BOHLKE *et al.* (1978), small characids such as those of the genus *Astyanax*, *Hyphessobrycon*, *Hemigrammus*, and allies, have a high rate of speciation and probably there is a large number of species still unknown due to the high degree of endemism of this fish group. Considering the existing taxonomic and phylogenetic problems, this subfamily has been considered by several authors (WEITZMAN and FINK 1983; LUCENA 1993) as an artificial group, with new studies being necessary for a better understanding of the relationships among these organisms.

Cytogenetic studies carried out on representatives of this subfamily have shown a wide karyotypic diversity among genera, with diploid number ranging from 2*n*=36 to 2*n*=54, and 2*n*=50 and 52 chromosomes being considered the modal values for the groups (KLINKHARDT *et al.* 1995). This divergence of chromosome number and karyotype structure among the different species probably reflects the artificial nature of the group. In the present study the karyotype of five species of Tetragonopterinae were analyzed with the main objective to increase the amount of information that could be of help for a better understanding of the diversity and the relationships within the group.

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MATERIALS AND METHODS

Cytogenetic analyses were performed on five species belonging to the subfamily Tetragonopterinae collected in different hydrographic basins. Table 1 summarizes the information about the species, collection sites, and the number of specimens analyzed. All specimens were identified and deposited in the fish collection of the Laboratório de Biologia de Peixes of the Departamento de Morfologia, Instituto de Biociências, UNESP, campus de Botucatu, SP, Brazil.

Chromosome preparation and staining followed the techniques described by FORESTI *et al.* (1993). At least 30 metaphases were analyzed for each specimen. Chromosome morphology was determined on the basis of arm ratios as proposed by LEVAN *et al.* (1964) and the chromosomes were classified according to the morphology and size in metacentrics (M), submetacentrics (SM), subtelocentrics (ST), and acrocentrics (A).

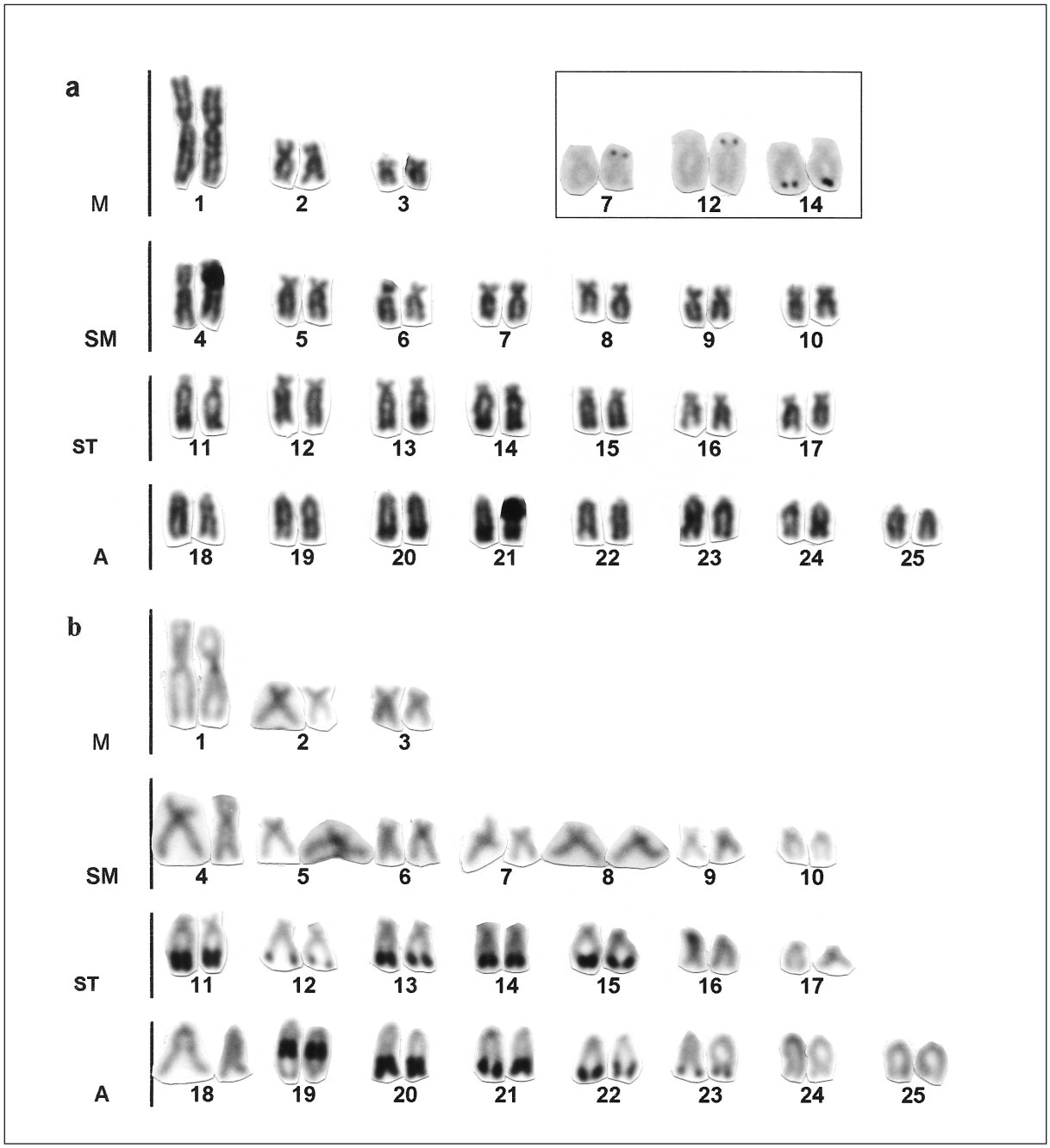

Fig. 1 – Karyotypes of *Astyanax janeiroensis* with 2*n*=50 chromosomes. (a) Giemsa stained; (b) C-banding. In the detail, the NOR-bearing pairs.

Table 1 – Summary of the fish species of the subfamily Tetragonopterinae analyzed, collection sites, number of specimens [males, females, and total (T)], diploid number (2n), fundamental number (NF), karyotype, and the number of chromosome pairs with nucleolus organizer regions (NORs).

Species	Collection site	Specimens ♂♂/♀♀/T	2*n*	NF	Karyotype	Pairs with NORs
Astyanax janeiroensis	Betari river, Apiaí, São Paulo	5/7/12	50	84	6M+14SM+14ST+16A	3
Hyphessobrycon reticulatus	Juquiá river, São Lourenço da Serra, São Paulo	2/7/9	50	100	14M+20SM+16ST	1
Hollandichthys multifaciatus	Grande river, Paranapiacaba, São Paulo	3/4/7	50	100	10M+12SM+28ST	1
Ctenobrycon hauxwellianus	São Francisco stream, Rio Branco, Acre	2/6/8	50	100	10M+6SM+34ST	1
Phenacogaster cf. *pectinatus*	São Francisco stream, Rio Branco, Acre	2/0/2	46	60	12M+2ST+32A	1

RESULTS

Astyanax janeiroensis had 2*n*=50 chromosomes (6M+14SM+14ST+16A), for both sexes (Fig. 1a). The NORs are multiple, being distributed in three chromosome pairs, at a terminal position on the short arm of pairs 7 and 12 and on the long arm of pair 14 (Fig. 1a). Small heterochromatic blocks were observed in a pericentromeric position in all chromosomes and large heterochromatic blocks were observed in a terminal position in seven chromosome pairs. A large heterochromatic segment was also observed on the long arm of an acrocentric pair in an interstitial position (Fig. 1b).

Hyphessobrycon reticulatus had 2*n*=50 chromosomes (14M+20SM+16ST) for both sexes (Fig. 2a). The NORs were identified on the long arm of pair 20 in a pericentromeric position (Fig. 2a).

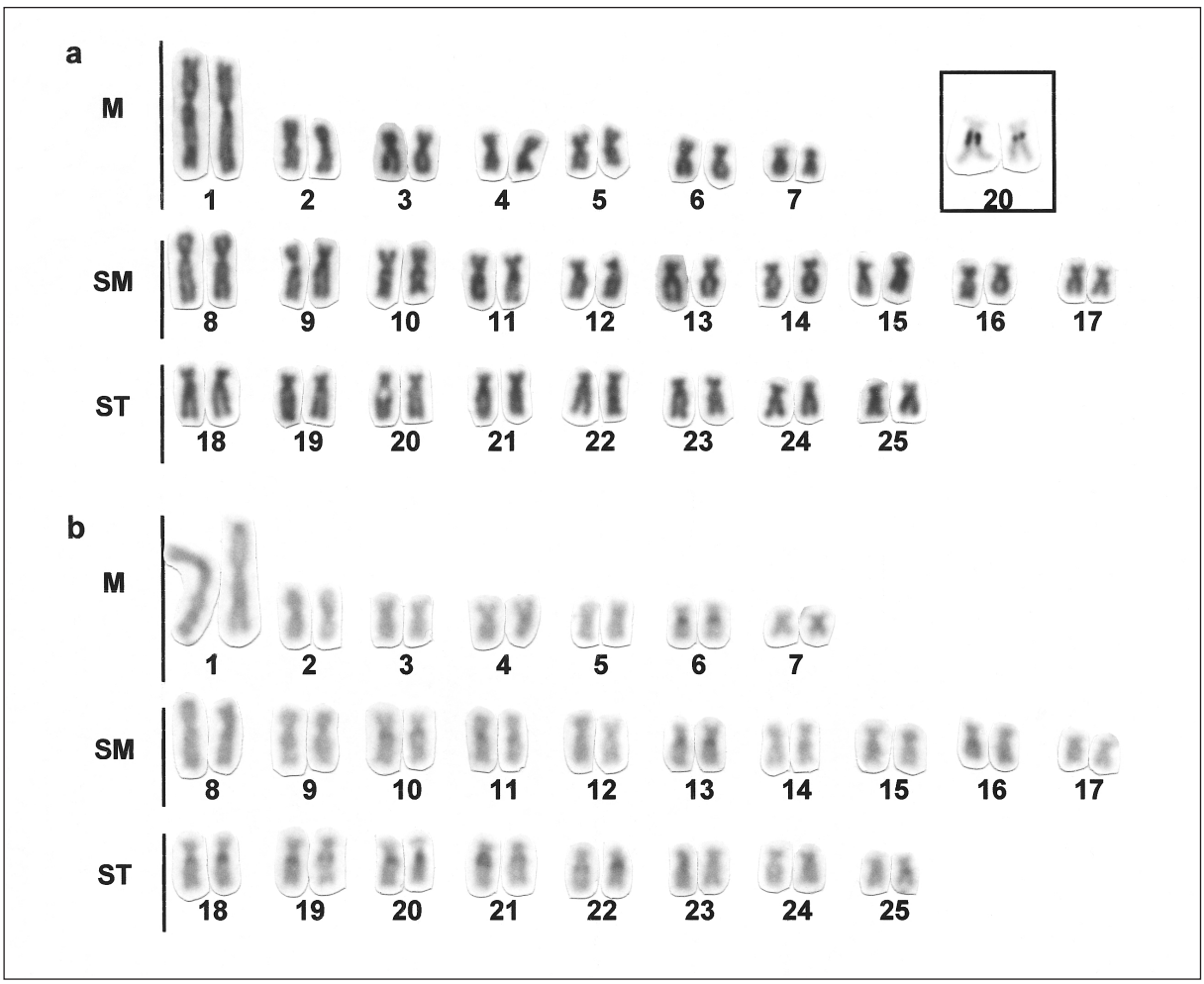

Fig. 2 – Karyotypes of *Hyphessobrycon reticulatus* with 2*n*=50 chromosomes. (a) Giemsa stained; (b) C-banding. In the detail, the NOR-bearing pair.

Small heterochromatic blocks were observed in all chromosomes in a pericentromeric position (Fig. 2b).

The specimens of *Hollandichthys multifasciatus* had 2*n*=50 chromosomes (10M+12SM+28ST) for both sexes (Fig. 3a). The NORs were observed in an interstitial position on the long arm of the fourth chromosome pair (Fig. 3a). Small heterochromatic blocks were observed in all chromosomes in a pericentromeric position (Fig. 3b).

Ctenobrycon hauxwellianus had 2*n*=50 chromosomes (10M+6SM+34ST), for both sexes (Fig. 4a). The NORs were observed in a terminal position on the short arm of pair number 9 (Fig. 4a). Small heterochromatic blocks were observed in almost all chromosomes, mainly in a pericentromeric position (Fig. 4b).

Phenacogaster cf. *pectinatus* had 2*n*=46 chromosomes (12M+2ST+32A) (Fig. 5a). The NORs were observed in a terminal position on the short arm of the pair number 7 (Fig. 5a). Small heterochromatic blocks were observed in most chromosomes in a pericentromeric position (Fig. 5b).

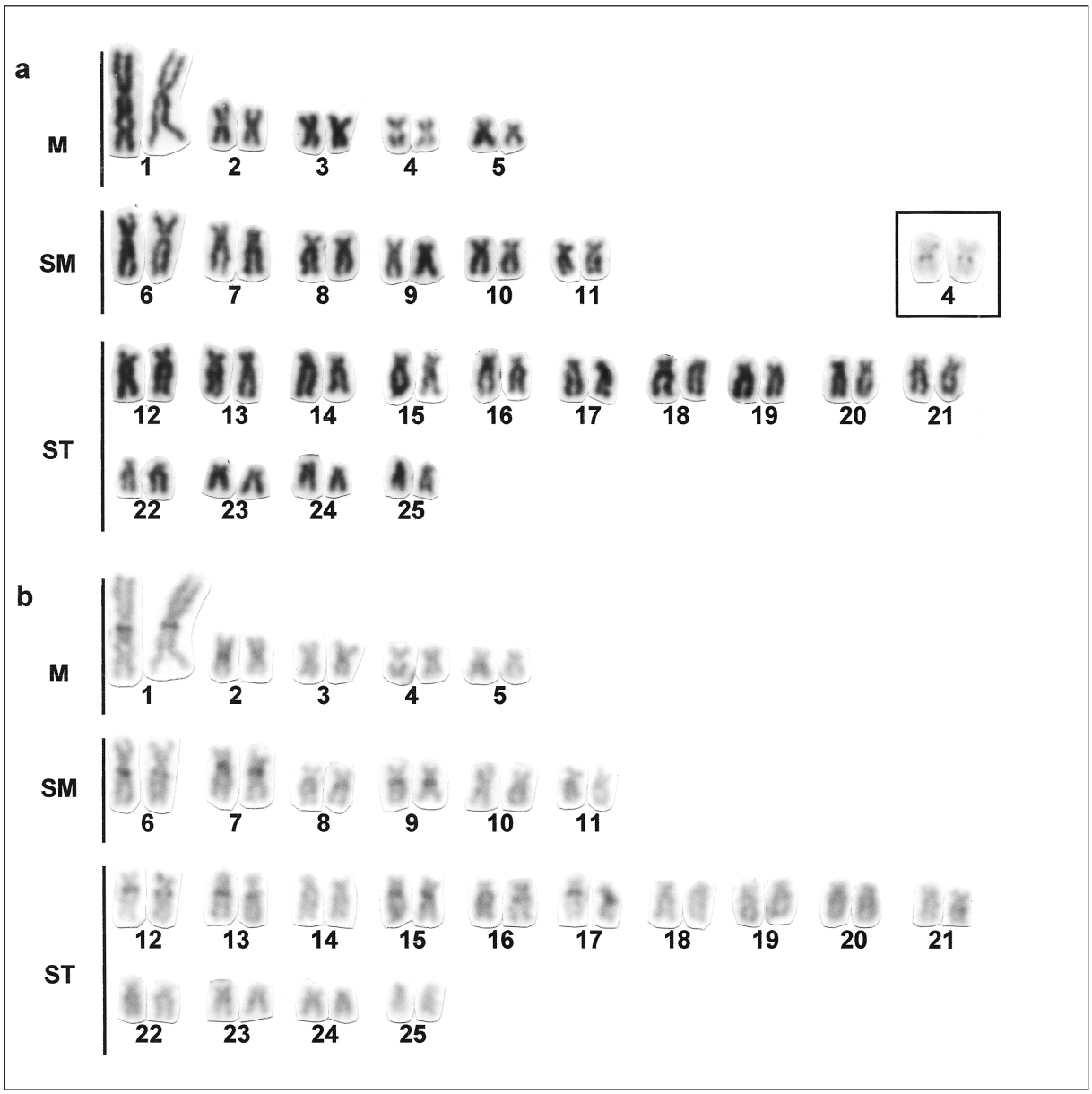

Fig. 3 – Karyotypes of *Hollandichthys multifasciatus* with 2*n*=50 chromosomes. (a) Giemsa stained; (b) C-banding. In the detail, the NOR-bearing pair.

DISCUSSION

Based on the analysis of karyotype structure and chromosome number, it is possible to divide the species studied into three groups. The first comprises *Astyanas janeiroensis* with 2*n*=50 and a karyotype consisting of all types of chromosomes; the second group comprises *Hyphessobrycon reticulatus*, *Hollandichthys multifasciatus*, and *Ctenobrycon hauxwellianus* with 2*n*=50 chromosomes of M, SM, and ST types, and the third comprises *Phenacogaster* cf. *pectinatus*, with 2*n*=46 chromosomes and karyotypic formulae consisting of chromosomes of the M, ST, and A types. The species of the first and second groups, in addition to having the same diploid number, also have chromosome pair number 1 composed by two large metacentrics, a common characteristic of several species of the family Characidae.

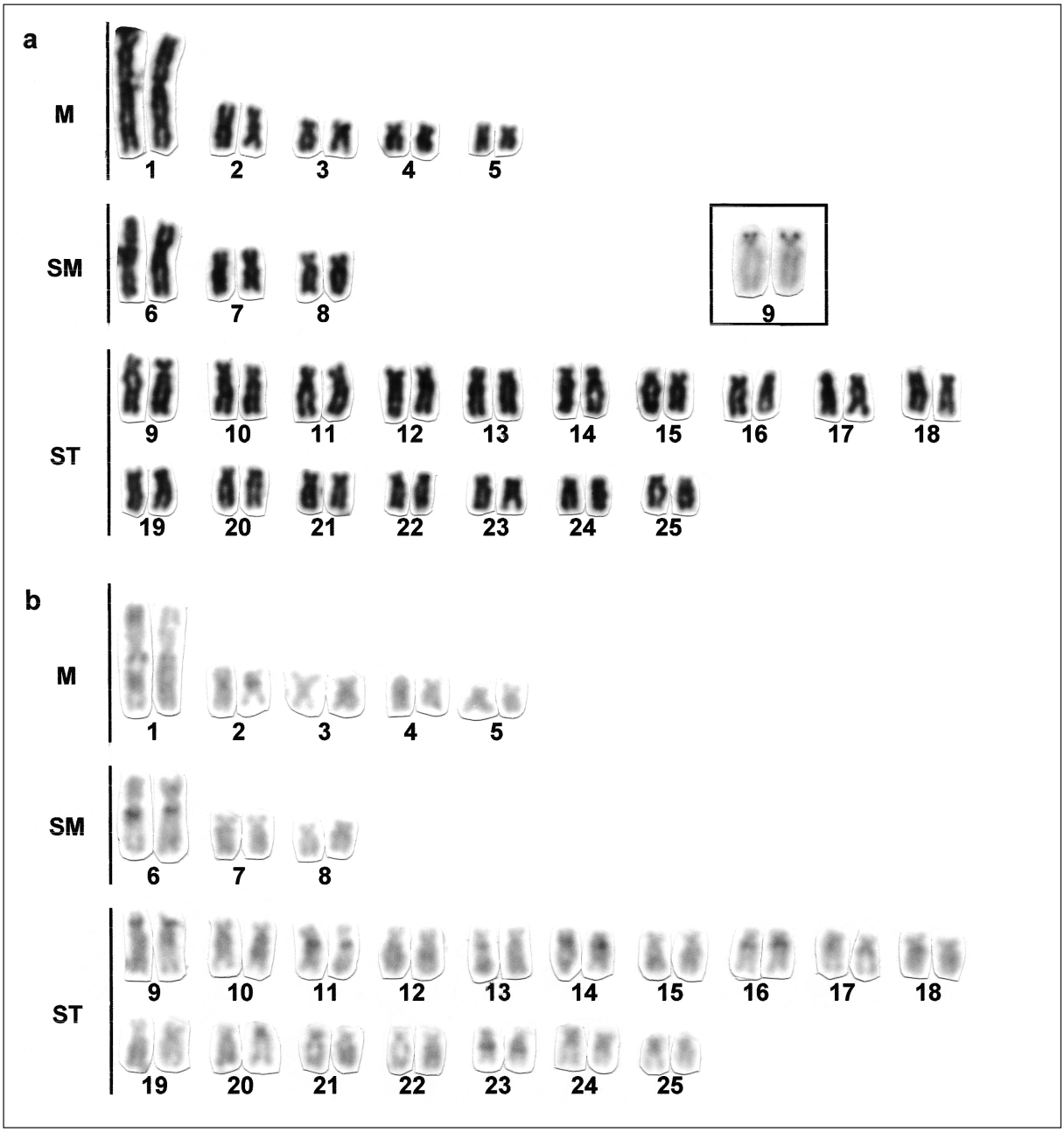

Fig. 4 – Karyotypes of *Ctenobrycon hauxwellianus* with 2*n*=50 chromosomes. (a) Giemsa stained; (b) C-banding. In the detail, the NOR-bearing pair.

Astyanax janeiroensis had 2*n*=50 chromosomes and multiple NORs as observed in many species of the genus *Astyanax* already karyotyped (KLINKHARDT *et al.* 1995). The presence of large heterochromatic blocks in terminal and interstitial positions observed in this species was also found in some local populations of *A. scabripinnis* (MOREIRA-FILHO and BERTOLLO 1991; SOUZA and MOREIRA-FILHO 1995; MAISTRO *et al.* 1998), suggesting that these species could be related.

Hyphessobrycon is a Tetragonopterinae genus with a high number of species karyotyped, although for several species only the haploid number has been described. In this genus, the number of chromosomes ranges from 2*n*=42 to 2*n*=52, with most species having 2*n*=50 or 52 (KLINKHARDT *et al.* 1995). A sample of *Hyphessobrycon reticulatus* from Uruguay also showed 2*n*=50 chromosomes (WLASIUK and GARCIA 1996), but with a karyotype (20M+14SM+16ST,A)

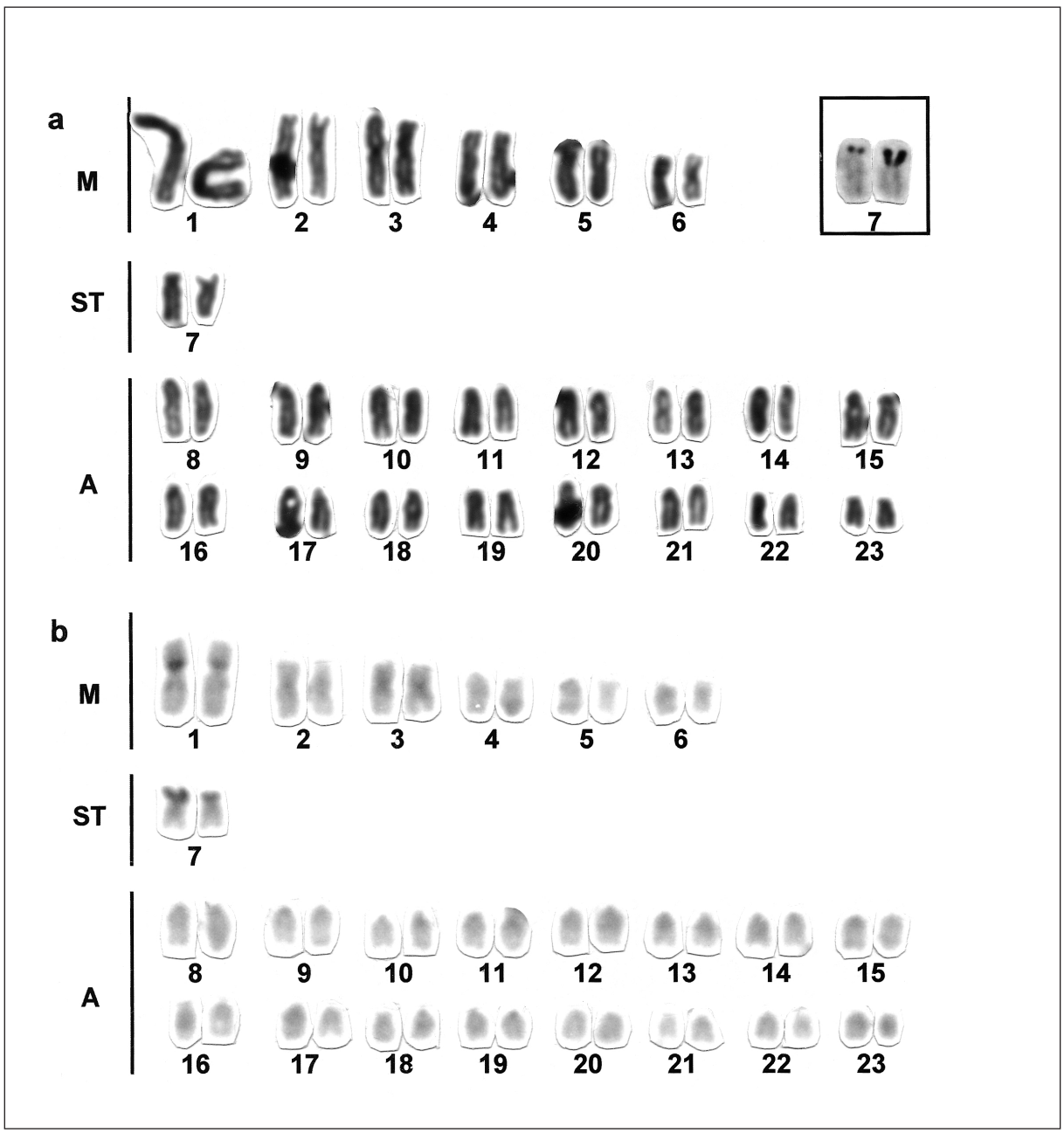

Fig. 5 – Karyotypes of *Phenacogaster* cf. *pectinatus* with 2*n*=46 chromosomes. (a) Giemsa stained; (b) C-banding. In the detail, the NOR-bearing pair.

very different from that found in the present study. Information about the number and distribution of the NORs in this genus is limited to the description of the occurrence of multiple NORs in *H. bifasciatus* (MIYAZAWA and BONATTO 1992) and *H.* aff. *santae* (MIYAZAWA *et al.* 1994; CENTOFANTE and MOREIRA-FILHO 2000), which differ from the results obtained in the present study since the sample of *H. reticulatus* had single NORs. The pattern of constitutive heterochromatin distribution found in *H. reticulatus* is similar to that described for *H.* aff. *santae* (CENTOFANTE and MOREIRA-FILHO 2000). The diversity of chromosome number, karyotypic constitution and number and distribution of NORs suggests that the species of this genus had a high karyotypic divergence or that this species belongs to different natural groups.

The karyotype of *Hollandichthys multifasciatus* (2*n*=50, 10M+12SM+28ST) is similar to that found in specimens of *H. multifasciatus* collected in Itanhaém (São Paulo, Brazil) (2*n*=50, 12M+24SM+12ST+2A) (VIEIRA *et al.* 1988). Moreover, both samples had one chromosome pair with interstitial NORs and small heterochromatic blocks in a pericentromeric position in all chromosomes (VIEIRA *et al.* 1988; present study). The difference between these cytotypes seems to be related to the isolation of these two populations, since the sample studied in the present paper was collected in a tributary of the Tiete river and the sample described by VIEIRA *et al.* (1988) was collected in a small coastal river.

To date, the only information available for the genus *Ctenobrycon* was the description of the haploid number (*n*=25) and of the fundamental number (FN=100) of *C.* aff. *hauxwellianus* (SCHEEL 1973). The data obtained in the present study coincide with this karyotypic description. The presence of a large metacentric pair in *C. hauxwellianus* suggests that this species is related to other members of the subfamily Tetragonopterinae which also have this characteristic.

Of the Tetragonopterinae species analyzed in the present study, *Phenacogaster* cf. *pectinatus* had the high divergence in diploid number, with 2*n*=46 chromosomes (12M+2SM+32A). These results are in contrast with those described by SCHEEL (1973), who found a haploid number of *n*=25 and 26 chromosomes for two samples of *P.* aff. *microstictus*. Moreover, pair number 1 of *P.* cf. *pectinatus* also is not composed by two large metacentrics, as found in several Tetragonopterinae groups. New data are necessary for a better understanding of the relations between this species and the other Tetragonopterinae.

Acknowledgments – The authors are grateful to Dr. Heraldo A. Britski for taxonomic identification of the specimens, to R. Devidé for technical assistance, and to Drs. L.A.C. Bertollo, L. Giuliano-Caetano and Dr. C. Martins for a critical review of the original manuscript. Funds supporting this study were provided by FAPESP, CAPES, CNPq, and FUNDUNESP.

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Received January 9, 2002; accepted March 26, 2002