

Astyanax scabripinnis (PISCES, CHARACIDAE): A SPECIES COMPLEX

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ABSTRACT

Karyotypic studies were conducted on seven populations of *Astyanax scabripinnis* from different hydrographic basins. Six of these populations were also studied morphologically using canonical variable analysis. The combined karyotypic and morphological analyses permitted a better understanding of the diversity of this fish group. The group can be characterized as a complex of species isolated in the headwaters of small tributaries of large hydrographic systems.

INTRODUCTION

The genus *Astyanax* (Gery, 1977) is the largest unit of Tetragonopterinae and is one of the dominating genera in South America. Eigenmann (1921) considered the genus to consist of 74 species and subspecies living in continental Brazilian waters.

Only eight of the many related species have been studied cytogenetically thus far: *A. mexicanus* (Post, 1965; Kirby *et al.*, 1977); *A. bimaculatus* (Scheel, 1973; Jim and Toledo, 1975; Foresti *et al.*, 1977; Morelli *et al.*, 1983; Paganelli and Galetti Jr., 1986), *A. fasciatus* (Jim and Toledo, 1975; Foresti *et al.*, 1977; Morelli *et al.*, 1983; Paganelli and Moreira-Filho, 1986; Moreira-Filho and Bertollo, 1986), *A. schubarti* (Foresti *et al.*, 1977; Morelli *et al.*, 1983); *A. scabripinnis* (Moreira-Filho *et al.*, 1978; Morelli *et al.*, 1983; Martins *et al.*, 1984; Moreira-Filho and Bertollo, 1986; Stange *et al.*, 1986), *E. eigenmaniorum* (Stripeck *et al.*, 1985); *A. taeniatus* (Stange *et al.*, 1986);

Astyanax sp (Paganelli and Galetti Jr., 1986). Chromosome number ranges from $2n = 36$ to $2n = 50$. Another interesting feature is the occurrence of different diploid numbers among individuals considered to belong to the same species but originating from different hydrographic basins (Morelli *et al.*, 1983; Paganelli and Moreira-Filho, 1986; Moreira-Filho and Bertollo, 1986).

A. scabripinnis in particular is a small species inhabiting small streams with rapids and is limited to the headwaters of small tributaries (Gomes and Azevedo, 1960; Britski, 1972; Caramaschi, 1986). Fowler (1948) considers the species to have six subspecies: *A. scabripinnis scabripinnis* (Jenyns, 1842), *A. scabripinnis rivularis* (Lutken, 1874), *A. scabripinnis longirostris* (Steindachener, 1907), *A. scabripinnis intermedius* (Eigenmann, 1908), *A. scabripinnis laticeps* (Cope, 1984), and *A. scabripinnis paranae* (Eigenmann, 1927).

Considering that *A. scabripinnis* presents a pattern of distribution restricted to headwaters, with phenotypic plasticity and morphological differentiation within the same hydrographic basin (Caramaschi, 1986), we have given special treatment to this fish group since it may possibly represent a complex of species (Britski, personal communication). Thus, when we refer to this species we always use the term, "scabripinnis complex", followed by the collection site.

In view of the peculiarities of the "scabripinnis complex", the objective of the present study was to analyze the diversity observed in this group and to attempt to correlate it with (1) two distinct levels of analysis, i.e. karyotypic (chromosome number, shape, size and presence and distribution of chromosome bands) and morphological (morphometric and meristic characteristics) analyses, and (2) with small microbasins belonging to more extensive drainage systems. This approach permitted a better understanding of the nature and diversity of the "scabripinnis complex".

MATERIALS AND METHODS

The karyotypic study was conducted on 118 specimens of *A. scabripinnis* sampled from seven distinct populations (Table I), captured from rivers belonging to three large Brazilian hydrographic basins (Paranapanema, São Francisco and Tietê rivers). Figure 1 shows the collection sites and Figure 2 the specimens of each populations studied.

Mitotic chromosomes were obtained from kidney cells by the air-drying technique of Bertollo *et al.* (1978), and C banding was obtained by the technique of Sumner (1972).

Table I - Collection sites and number of specimens of the *scabripinnis* complex analyzed.

Collection sites	City	State	No. of specimens analyzed		Total
			♀	♂	
Centenário Stream	Maringá	PR	8	8	16
Marrecas Stream	Londrina	PR	6	14	20
Viveiro de Mudás Stream	Três Marias (CODEVASF)	MG	5	8	13
Barreiro Grande Stream	Três Marias	MG	7	4	11
Curral das Éguas Stream	Três Marias	MG	16	11	27
Claro River	Salesópolis	SP	6	8	14
Monjolinho Stream	São Carlos	SP	12	5	17

The chromosomes were analysed according to the measurement of short arm length, long arm length and total length, made with the help of a dry-tip compass and a pachymeter. The mean values were calculated for each chromosome pair. The relative length (%) of each chromosome pair in relation to the total length of the haploid lot was obtained from these values for the various populations studied.

The chromosomes were identified on the basis of the arm ratio (AR) criteria proposed by Levan *et al.* (1964) and classified as metacentric (M, AR = 1.00 to 1.70), submetacentric (SM, AR = 1.71 to 3.00), subtelocentric (ST, AR = 3.01 to 7.00) and acrocentric (A, AR > 7.01).

The idiograms were prepared on the basis of the measurements obtained, maintaining the same proportions of the arms and of the heterochromatin regions of each chromosome pair.

A total of 169 specimens from six of the populations studied were used for morphometric analysis. For the seventh population (from the Claro river, Salesópolis, SP) this analysis has not yet been possible. The following measurements (mm) were made with the help of a pachymeter: standard length (SL), rostrordorsal distance (RDD), rostro-anal distance (RAnD), body height (BH), head length (HL), snout length (StL), eye diameter (ED), interorbital width (IOW), and 3rd infraorbital width (IFOW). These nine morphometric traits were submitted to canonical variable analysis (CVA) according to Reis (1988) and Garavello and Reis (1988).

The specimens used for morphometric analysis were also employed in the study of some meristic parameters such as number of scales on the lateral line and number of rays of the anal fin.

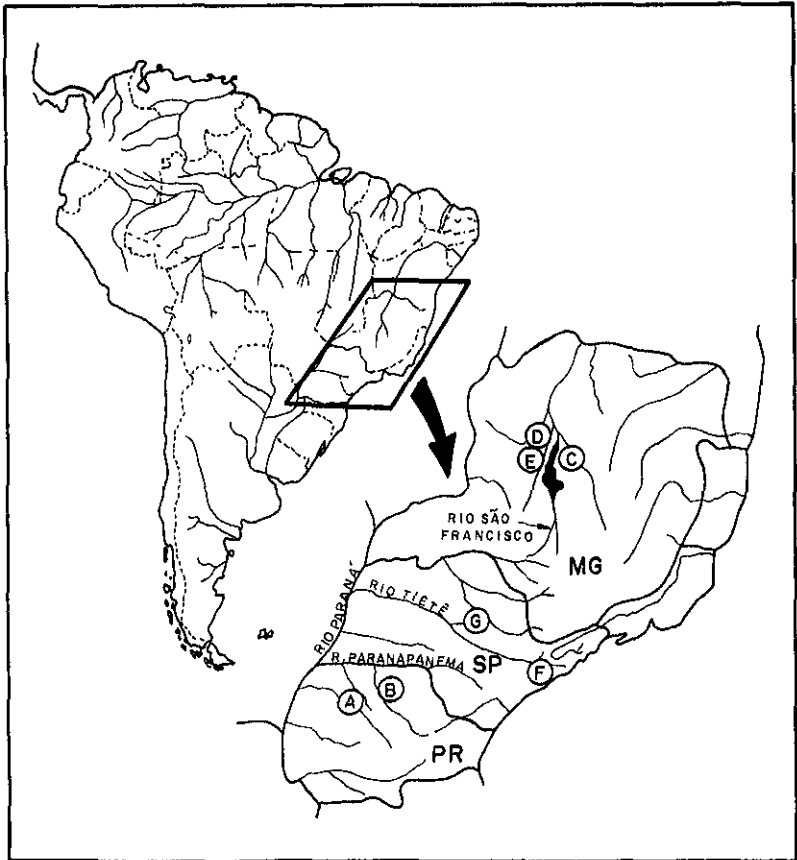


Figure 1 - Collection sites of the *scabripinnis* complex studied: A, Centenário stream (Maringá, PR); B, Marrecas stream (Londrina, PR); C, Viveiro de Mudas stream of CODEVASF (Três Marias, MG); D, Barreiro Grande stream (Três Marias, MG); E, Curral das Éguas stream (São Gonçalo do Abaeté, MG); F, Claro river (Salesópolis, SP); G, Monjolinho stream (São Carlos, SP).

RESULTS

Chromosome analyses

The data show that several populations differ in terms of chromosome number and type, as shown in Table II and in Figures 3 to 15.

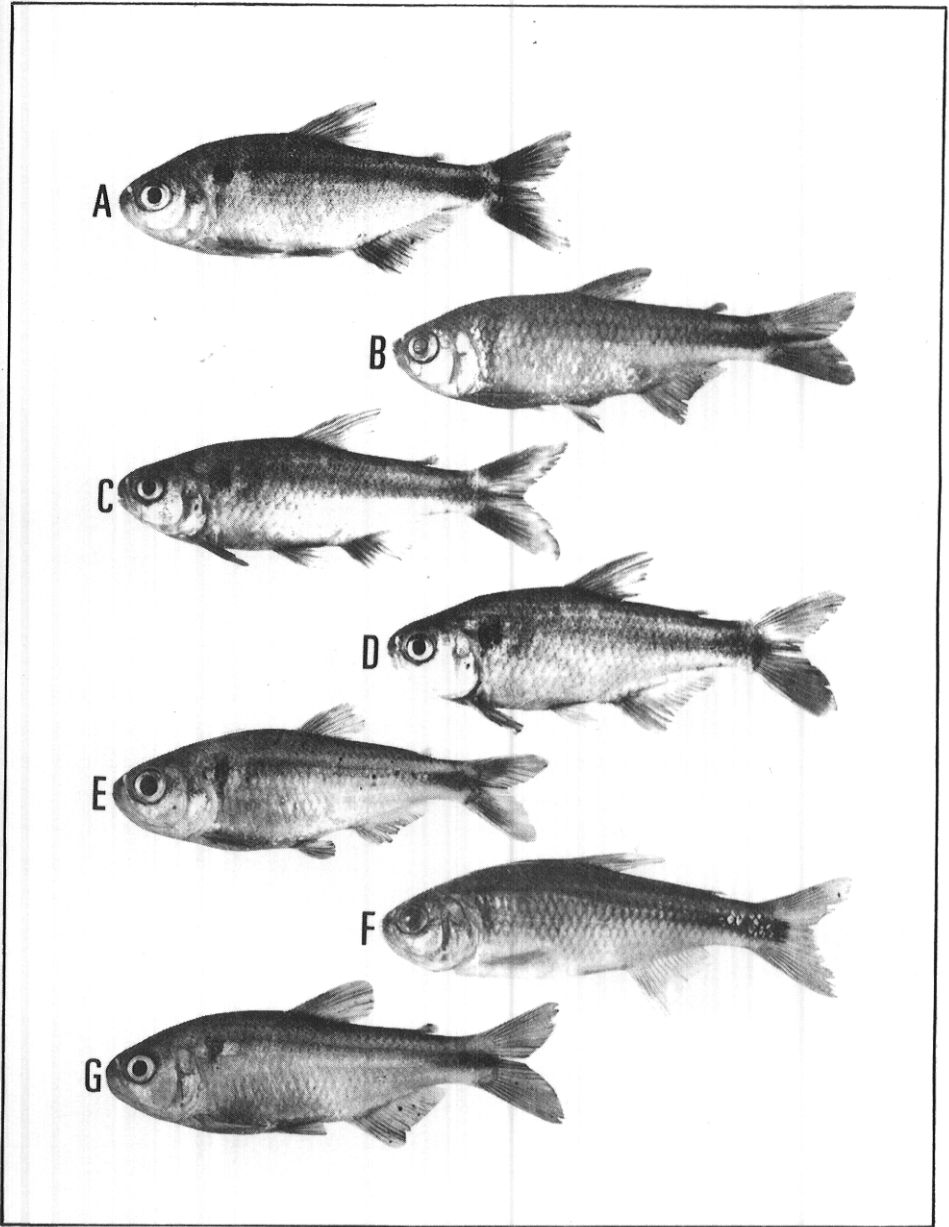


Figure 2 - Representatives of the *scabripinnis* complex studied, obtained from: A, Centenário stream; B, Marrecas stream; C, Viveiro de Mudás stream of CODEVASF; D, Barreiro Grande stream; E, Curral das Éguas stream; F, Claro river; G, Monjolinho stream.

Table II - Chromosome numbers and types of the *scabripinnis* complex analyzed, grouped by collection sites.

Hydrographic basin	Collection sites (streams)	No. of cells analyzed		2n	FN	Chromosome Types			
		♀	♂			M	SM	ST	A
Paranapanema	Centenário	387	373	50	84	3	10	4	8
Paranapanema	Marrecas	124	345	48	86	3	10	6	5
São Francisco	Viveiro de Mudanças (CODEVASF)	192	214	50	94	3	15	4	3
São Francisco	Barreiro Grande	214	97	50	94	3	15	4	3
São Francisco	Curral das Éguas	456	280	46	82	3	11	4	5
Tietê	Claro (River)	190	233	50	88	3	12	4	6
Tietê	Monjolinho	280	150	50	88	3	12	4	6

Figure 16 illustrates the idiograms of the specimens studied, with the C-banding pattern presented by site of collection. Except for the specimens from the Viveiro de Mudanças and Barreiro Grande streams (Três Marias, MG) which presented similar patterns of heterochromatin distribution (C-idiogram) the remaining populations can be characterized, in principle, by their C-banding patterns.

Using the karyotypic data obtained by chromosome measurement, some comparative analyses were carried out between specimens grouped by site of collection (Figure 17). Thus, the frequency of each chromosome type (metacentric, submetacentric, subtelocentric and acrocentric) in the karyotypes, the chromosome frequencies by relative size class, as well as the percent participation of each chromosome type in the haploid lot were compared.

The chromosomes were divided into eight size classes corresponding to the following percent values of the haploid lot: class 1 = 2.01 to 3.00%; class 2 = 3.01 to 4.00%; class 3 = 4.01 to 5.00%; class 4 = 5.01 to 6.00%; class 5 = 6.01 to 7.00%; class 6 = 7.01 to 8.00%; class 7 = 8.01 to 9.00%, and class 8 = 9.01 to 10.0%.

Morphological analyses

Canonical variable analysis applied to the morphometric data revealed that the first variable evaluates 92% of the morphometric variation of the six samples

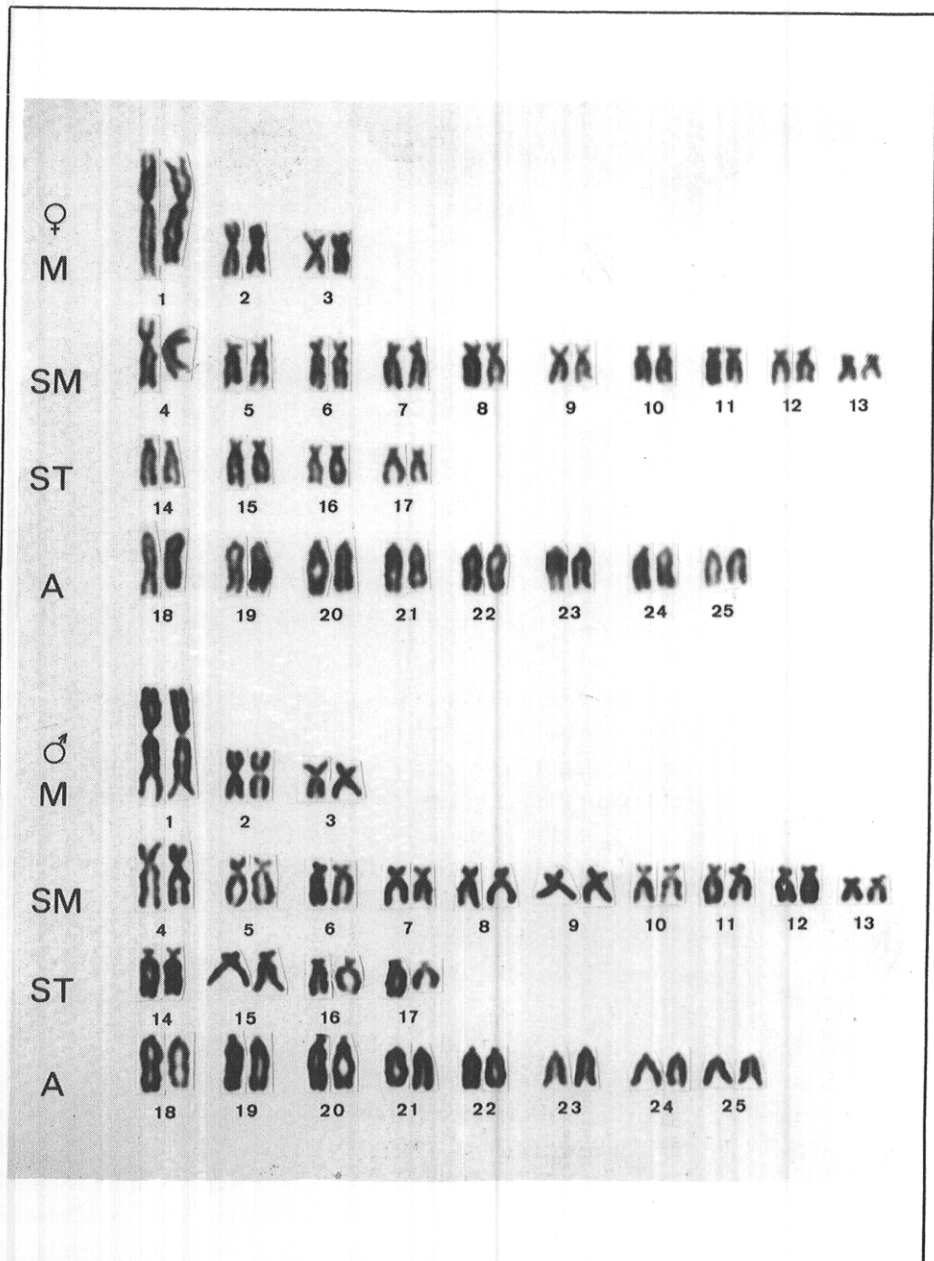


Figure 3 - Karyotype of females and males of the *scabripinnis* complex from the Centenário stream (Maringá, PR).

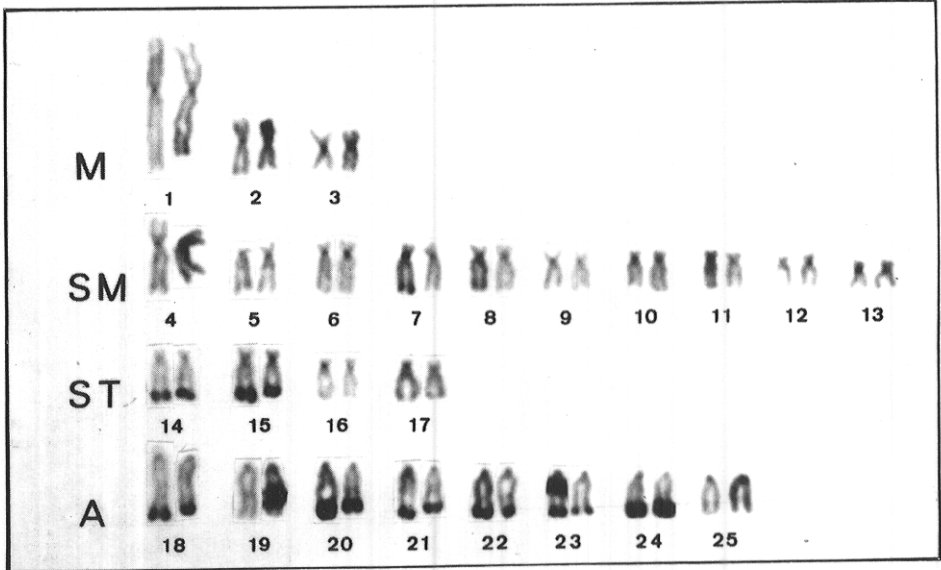


Figure 4 - C-banding pattern detected in specimens of the *scabripinnis* complex from the Centenário stream (Maringá, PR).

studied, while the second canonical variable evaluates 8.2%. Of the 30 individuals of the population from the Marrecas stream (Londrina, PR), 28 were grouped normally, revealing high canonical correlation. The same occurred for 29 of the 30 individuals of the population from the Curral das Éguas stream (São Gonçalo do Abaeté, MG), for 27 of the 30 individuals from the Monjolinho stream (São Carlos, SP), for 24 of the 30 specimens from the Centenário stream (Maringá, PR), for 19 of the 20 specimens from the Barreiro Grande stream (Três Marias, MG), and for 24 of the 29 individuals from the Viveiro de Mudas stream (Três Marias, MG) (Table III).

Four of the six populations, i.e., those from Curral das Éguas, Monjolinho, Centenário and Barreiro Grande, were fully discriminated on the basis of the first canonical variable (X) (Figure 18). Standard length (SL), body height (BH) and snout length (StL) yielded the highest coefficients in the first canonical variable. In the second variable (Y), rostradorsal distance (RDD), snout length (StL) and body height (BH) yielded the highest coefficients (Table IV). Thus, these four dimensions proved to be the most important ones for the discrimination of the samples studied.

The populations from the Marrecas (Londrina, PR) and Viveiro de Mudas (Três Marias, MG) streams revealed extreme morphometric similarity according to the first canonical variable and were discriminated from the other populations on the basis of the traits described above.

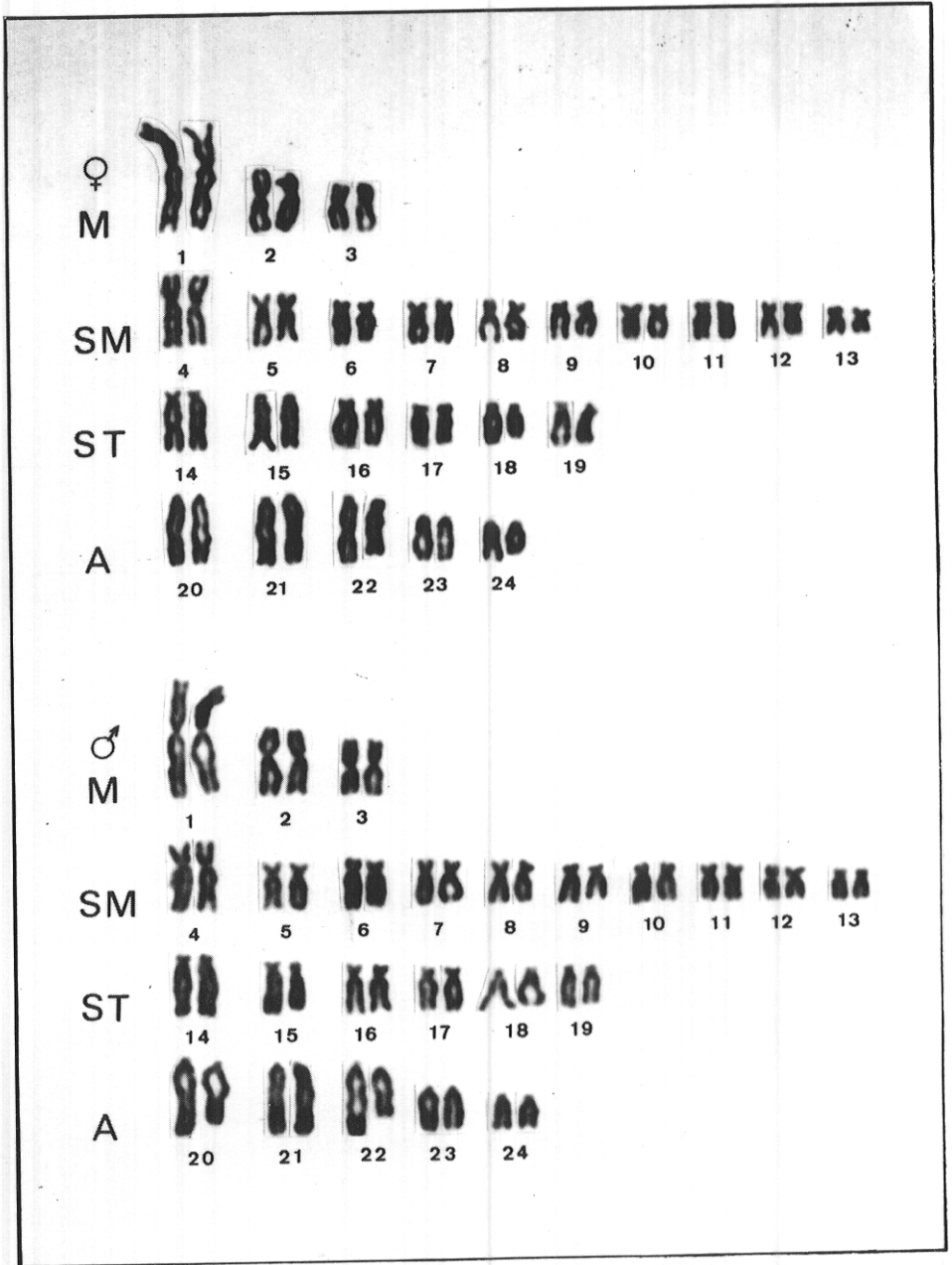


Figure 5 - Karyotype of females and males of the *scabripinnis* complex from the Marrecas stream (Londrina, PR).

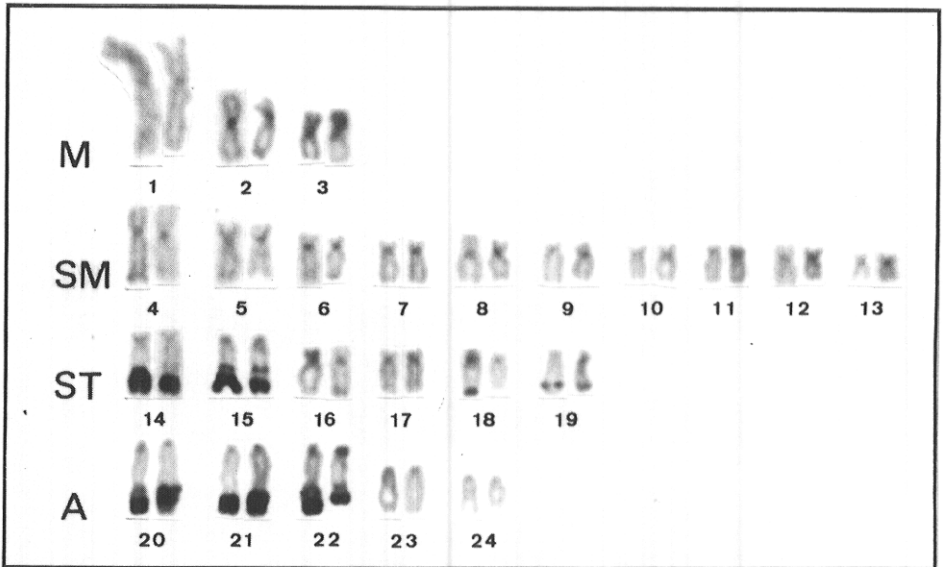


Figure 6 - C-banding pattern detected in specimens of the *scabripinnis* complex from the Marrecas stream (Londrina, PR).

The data obtained for the meristic parameters (no. of scales on the lateral line and no. of rays of the anal fin) for the six populations were submitted to comparative graphic analysis. Although the samples from the Marrecas stream (Londrina, PR) and for Curral das Éguas (São Gonçalo do Abaeté, MG) differed from the remaining ones in amplitude of variation of scale number on the lateral line and the sample from the Centenário stream (Maringá, PR) differed from the others in terms of number of rays of the anal fin, we believe that, taken as a whole, the meristic traits analyzed were not quite decisive for a good characterization of each population and therefore were not included in the present paper.

DISCUSSION

Karyotypic comparisons among the populations of the scabripinnis complex

A comparative chromosome analysis of the different populations immediately led to differentiation of the Londrina and São Gonçalo do Abaeté populations both from one another and from all other populations, not only in terms of the different chromosome numbers they presented ($2n = 48$ and 46 , respectively), but

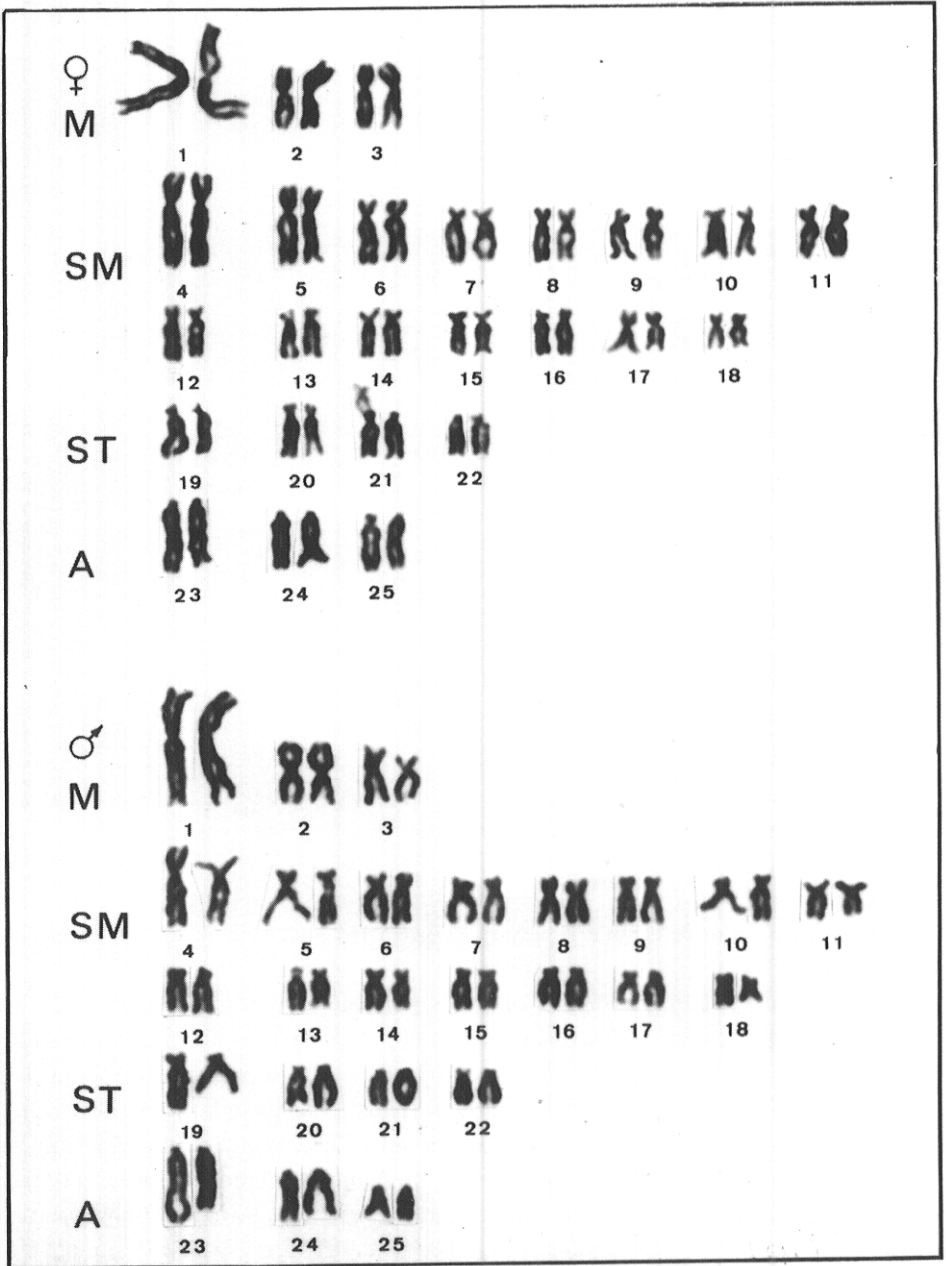


Figure 7 - Karyotype of females and males of the *scabripinnis* complex from the Viveiro das Mudas stream of CODEVASF (Três Marias, MG).

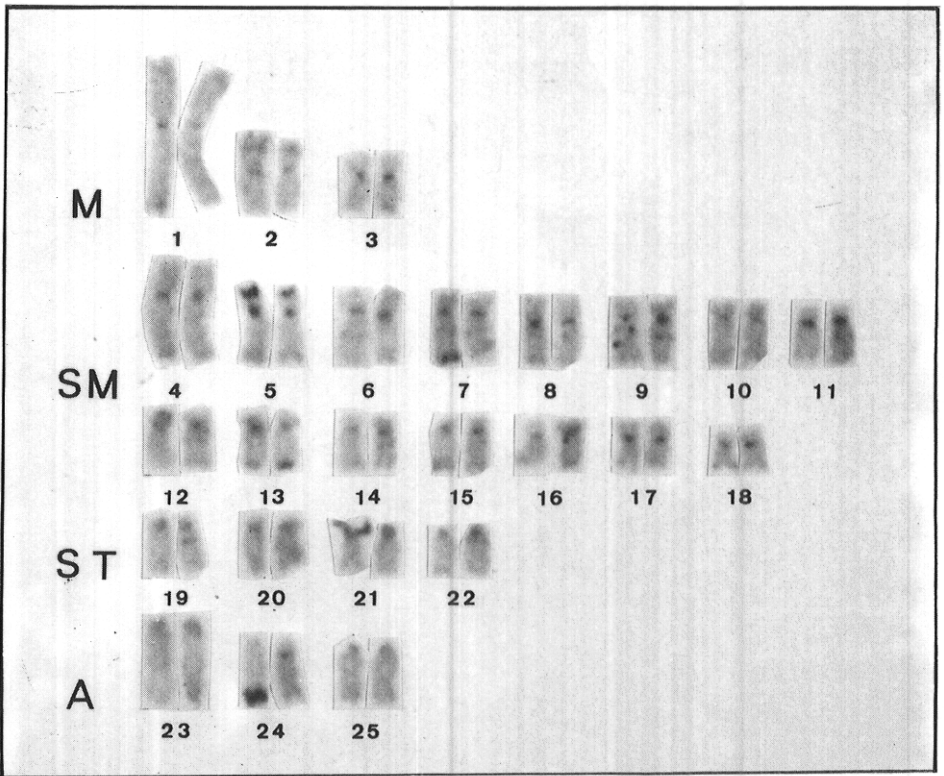


Figure 8 - C-banding pattern detected in specimens of the *scabripinnis* complex from the Viveiro das Mudas and Barreiro Grande streams (Três Marias, MG).

also in terms of heterochromatin pattern (Figure 16) and other karyotype characteristics (Figure 17).

Similarly, when the populations with the same chromosome number ($2n = 50$), i.e., the population from Maringá, the two populations from Três Marias and the two from Salesópolis/São Carlos, were compared, there were no doubts about the degree of chromosome diversity among them. Thus, even though the number of M and ST chromosomes was homogeneous in all of the populations, SM and A chromosomes were sufficiently distinct to divide them into three well-differentiated groups of karyotypes (corresponding to the three localities above, not only in terms of the number of these chromosomes present in each group (Figure 17), but also in terms of heterochromatin distribution (Figure 16) and of percent participation of chromosome types in the haploid lot (Figure 17).

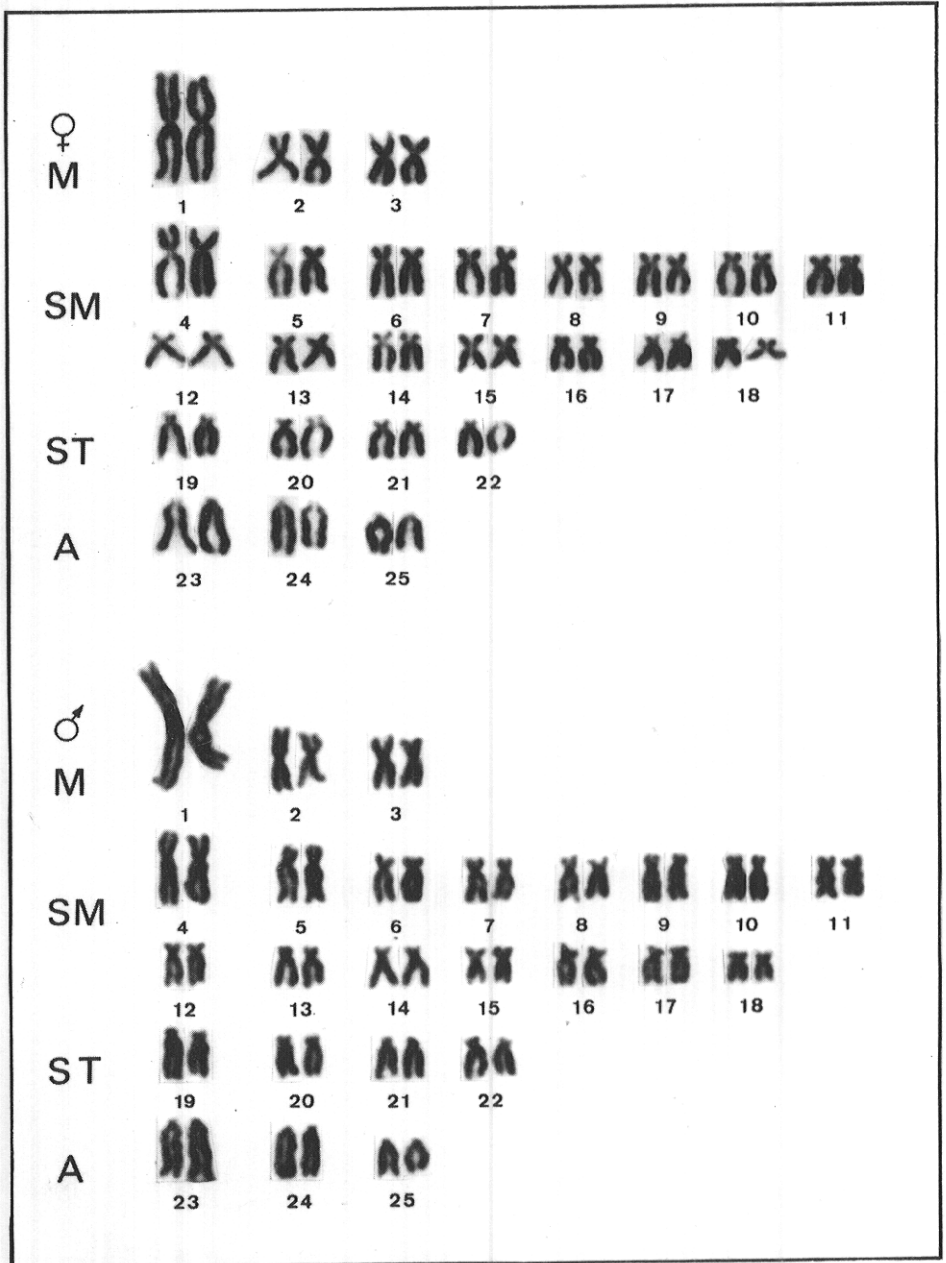


Figure 9 - Karyotype of females and males of the *scabripinnis* complex from the Barreiro Grande stream (Três Marias, MG).

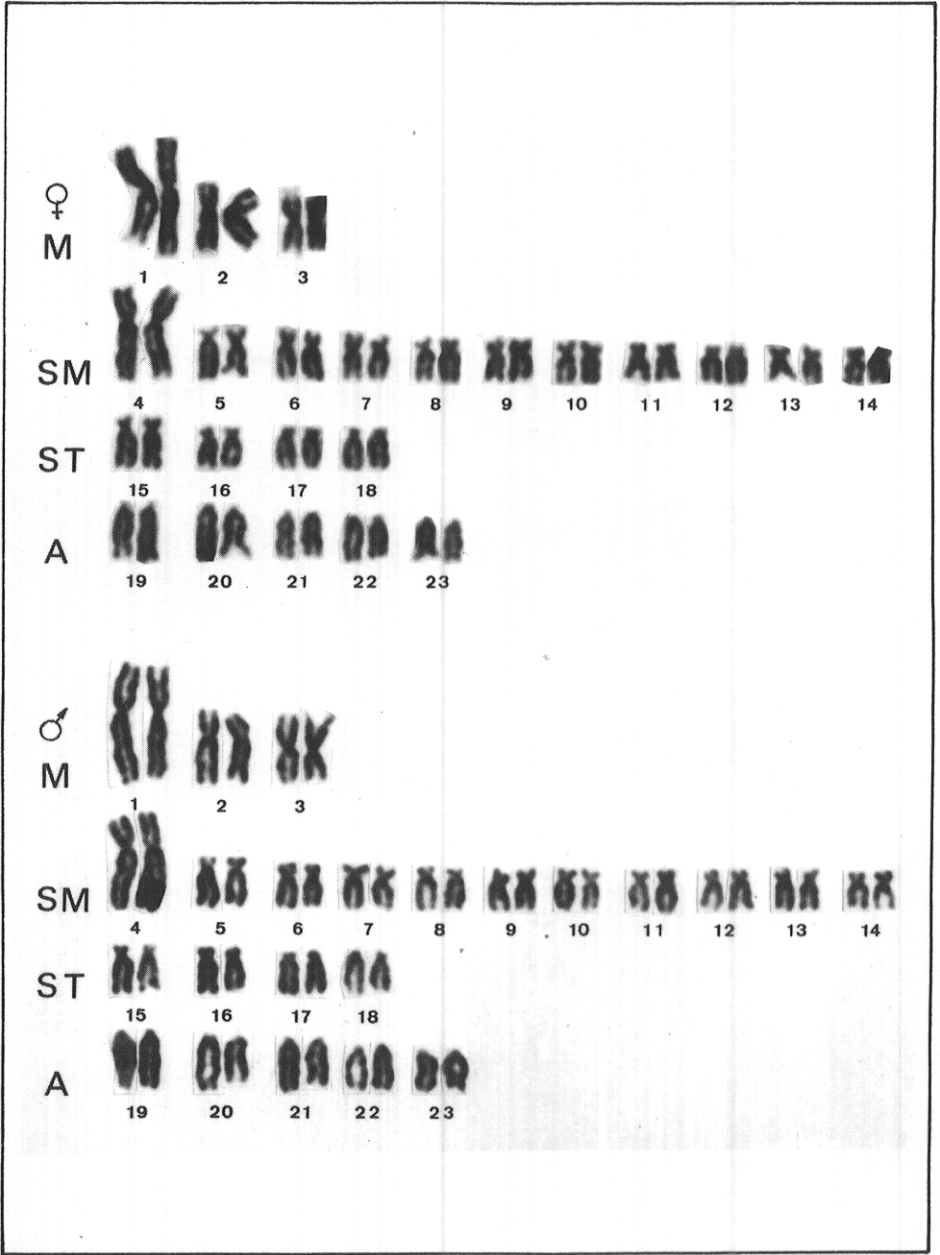


Figure 10 - Karyotype of females and males of the *scabripinnis* complex from the Curral das Éguas stream (São Gonçalo do Abaeté, MG).

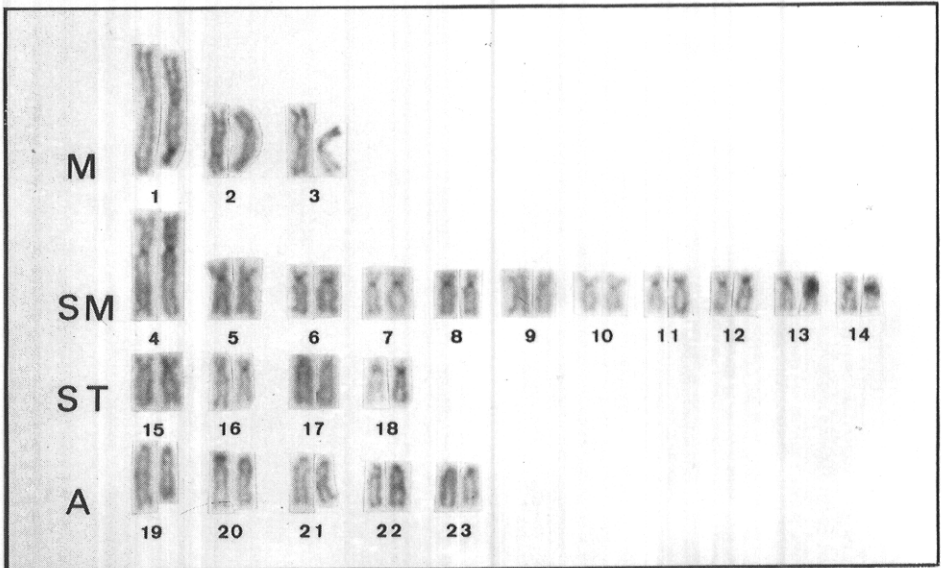


Figure 11 - C-banding pattern detected in specimens of the *scabripinnis* complex from the Curral das Éguas stream (São Gonçalo do Abaeté, MG).

The highest degree of chromosome similarity was observed between the specimens from the two Três Marias sites (Viveiro de Mudanças and Barreiro Grande streams) in which pair 21 presented a well-developed secondary constriction which was C-banding positive (Figures 7, 8, 9, 16 and 17).

The two populations from the Tietê river basin (Claro river - Salesópolis, SP and Monjolinho stream - São Carlos, SP) were generally similar, showing the same diploid number ($2n = 50$), the same number of meta-, submeta-, subtelo- and acrocentric chromosomes, as well as the same general karyotype pattern (Figures 12, 14 and 16). Some differences were chromosome frequency by size class and the participation of the different chromosome types in the haploid lot size (Figure 17), as well as the distribution of some telomeric constitutive heterochromatin blocks (Figure 16 E and F). However, we believe that the differences detected do not represent sufficiently conclusive characteristics to permit considering these two populations as distinct karyotypic forms. On this basis, until further information becomes available, we prefer to consider the two populations from the Tietê river to be cytogenetically similar.

It is interesting to point out that, regardless of the different arrangements accounting for the karyotypic diversity of the "*scabripinnis* complex", the metacentric

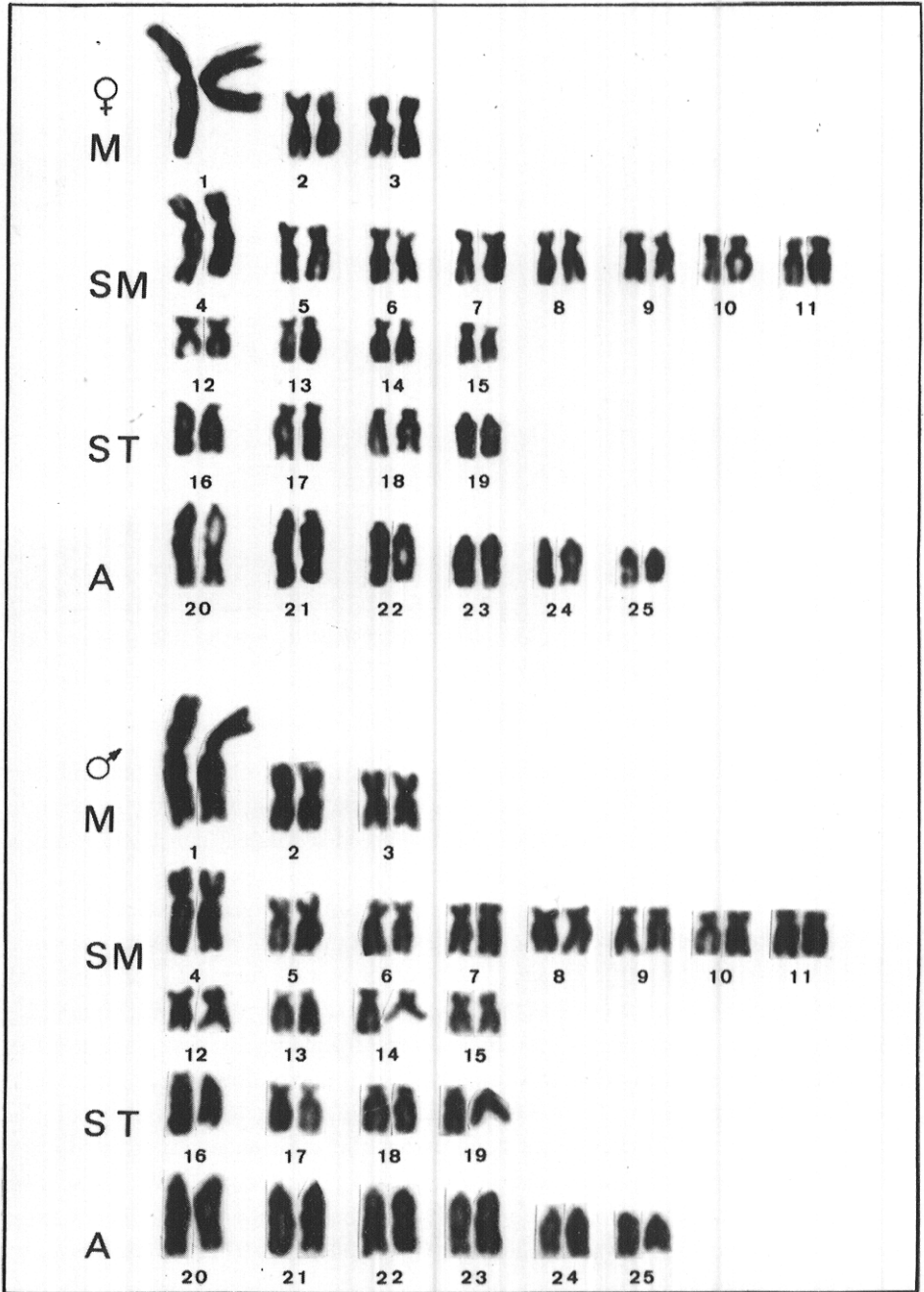


Figure 12 - Karyotype of females and males of the *scabripinnis* complex from the Claro river (Salesópolis, SP).

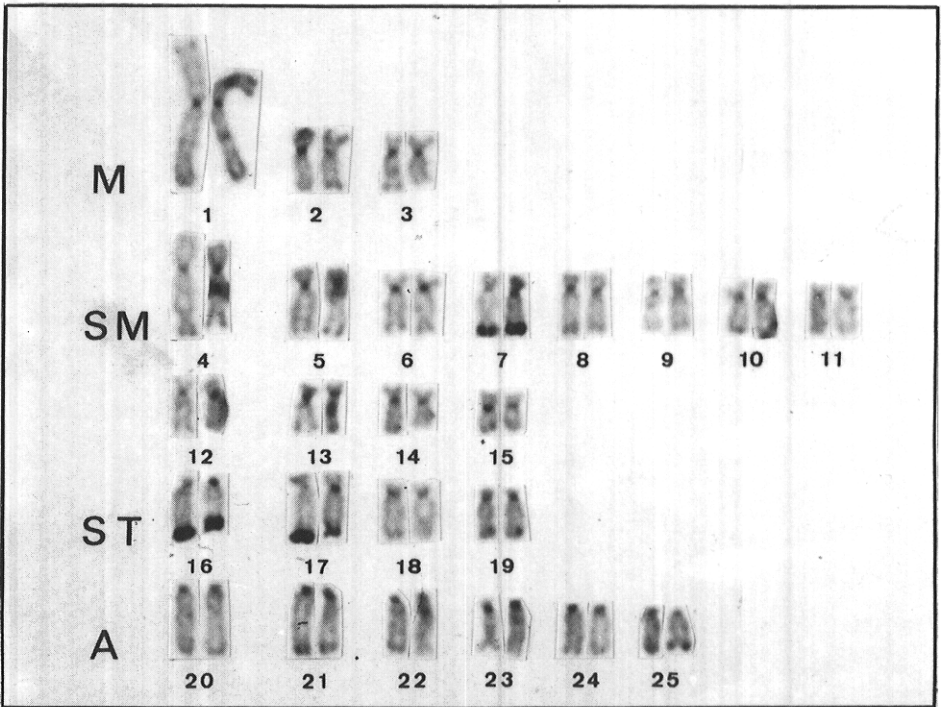


Figure 13 - C-banding pattern detected in specimens of the *scabripinnis* complex from the Claro river (Salesópolis, SP).

chromosome group was relatively the most stable among the various karyotypes identified (Figure 16), representing a conservative trait in relation to the variation detected in the sumeta-, subtelo- and acrocentric chromosomes.

Another interesting fact concerns constitutive heterochromatin, whose effect on the karyotypic diversification of the group analyzed can be observed in Figure 16. Some populations clearly present larger amounts of constitutive heterochromatin. The role of heterochromatin in the evolution of fish karyotypes has been described in several studies. It also is important for sex chromosome differentiation in fish of the genera *Leporinus* (Galetti Jr. and Foresti, 1986) and *Triporthus* (Bertollo *et al.*, 1984; Falcão, 1988).

In summary, the karyotypic data obtained for the set of populations of the "*scabripinnis* complex" analyzed here were sufficient to differentiate five cytogenetically distinct entities, i.e., those from the Centenário (Maringá, PR) (1), Marrecas (Londrina, PR) (2), Barreiro Grande/Viveiro de Mudás (Três Marias, MG) (3),

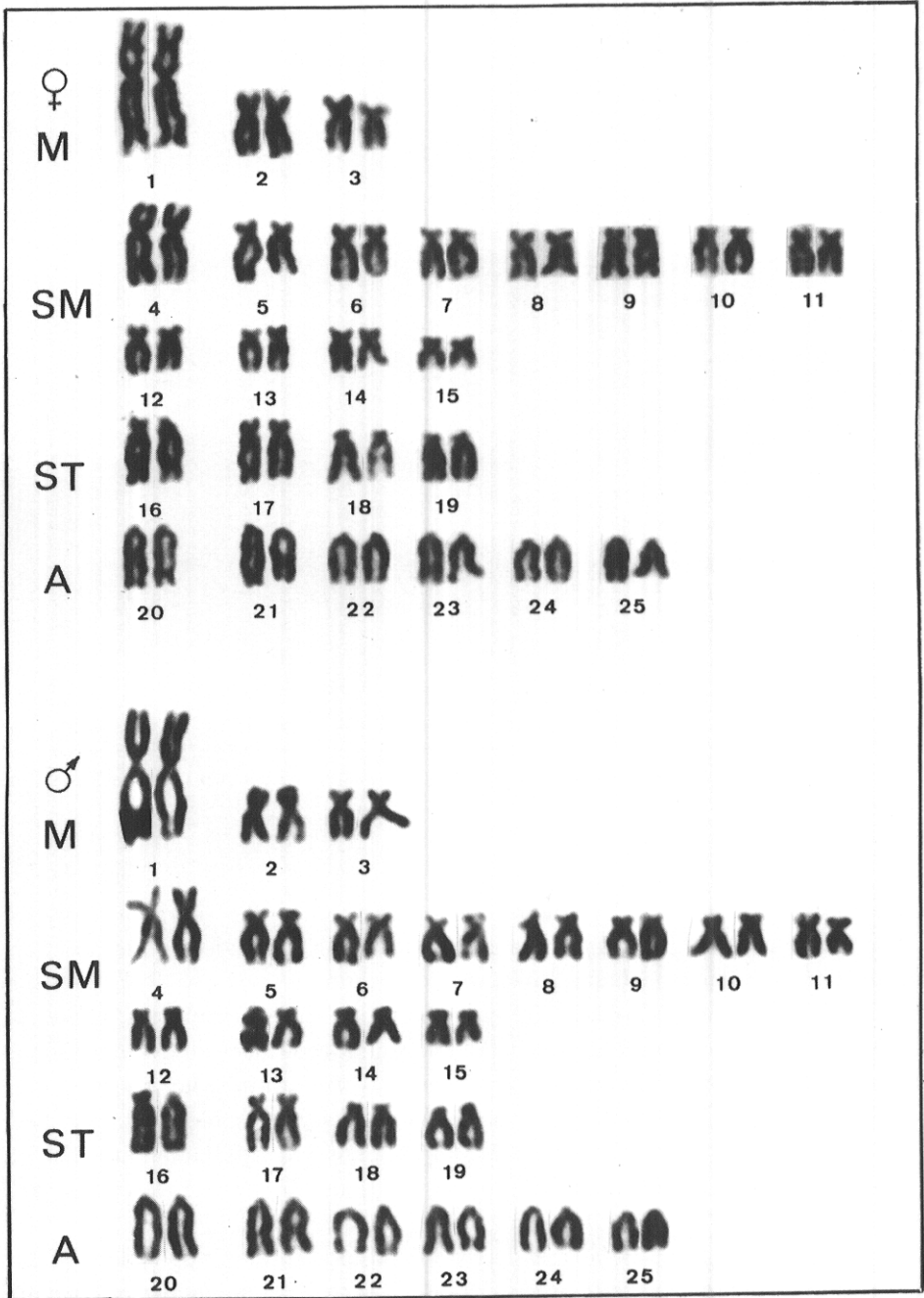


Figure 14 - Karyotype of females and males of the *scabripinnis* complex from the Monjolinho stream (São Carlos, SP).

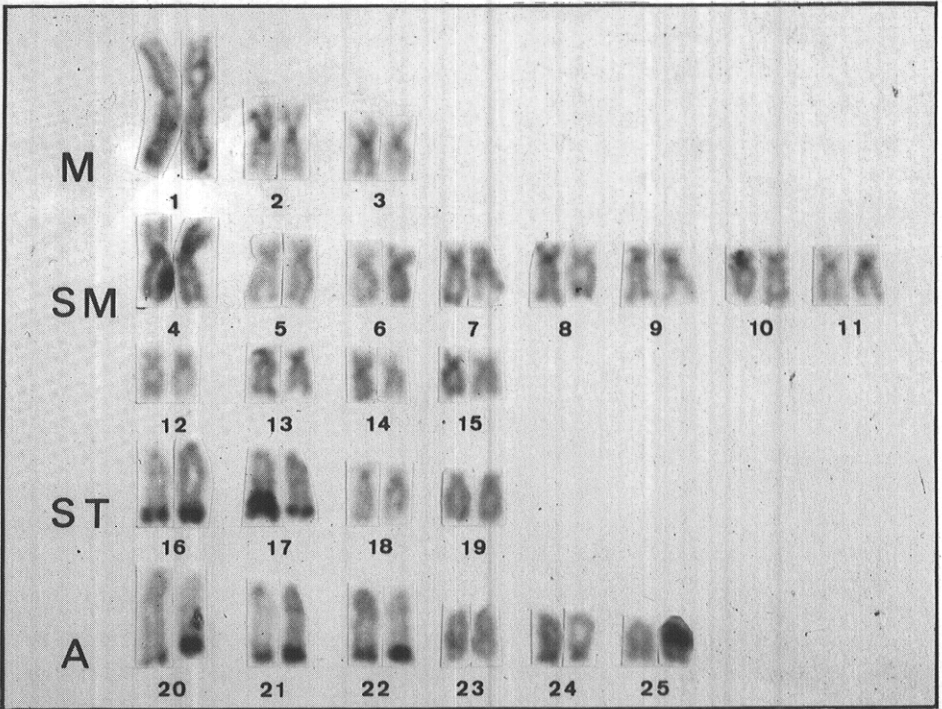


Figure 15 - C-banding pattern detected in specimens of the *scabripinnis* complex from the Monjolinho stream (São Carlos, SP).

Curral das Éguas (São Gonçalo do Abaeté, MG) (4), and Monjolinho (São Carlos, SP)/Rio Claro (Salesópolis, SP) (5) streams.

Morphological comparisons

The morphological diversity of the *scabripinnis* group has been a subject of study for many years. Eigenmann (1921) already referred to several subspecies for *Astyanax scabripinnis*, as later confirmed by Fowler (1948), who catalogued six subspecies in this group. Britski *et al.* (1986) also related two distinct forms (*A. scabripinnis rivularis* and *A. scabripinnis intermedius*) for the Três Marias region (São Francisco river). Studies by Caramaschi (1986) on 20 *A. scabripinnis* populations from the Botucatu (SP) region involving streams in the Pardo, Paranapanema and Tietê river basins, also pointed out morphological differentiations between populations, which, however, could not be attributed to isolation among the three large basins involved.

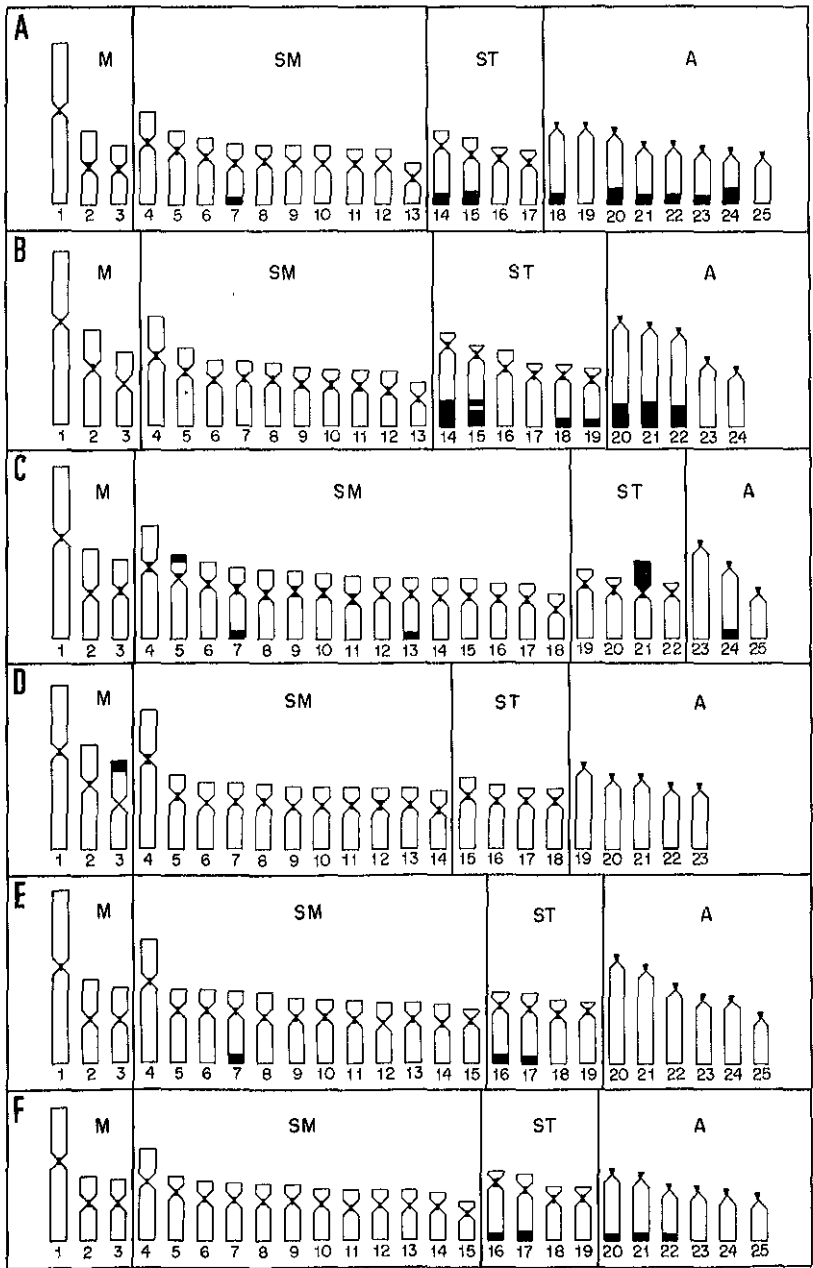


Figure 16 - Idiogram showing the chromosome types and the C-banding patterns (in black) of specimens of the *scabripinnis* complex from the different sites studied: A, Centenário stream; B, Marrecas stream; C, Viveiro de Mudás and Barreiro Grande streams; D, Curral das Éguas stream; E, Claro river; F, Monjolinho stream.

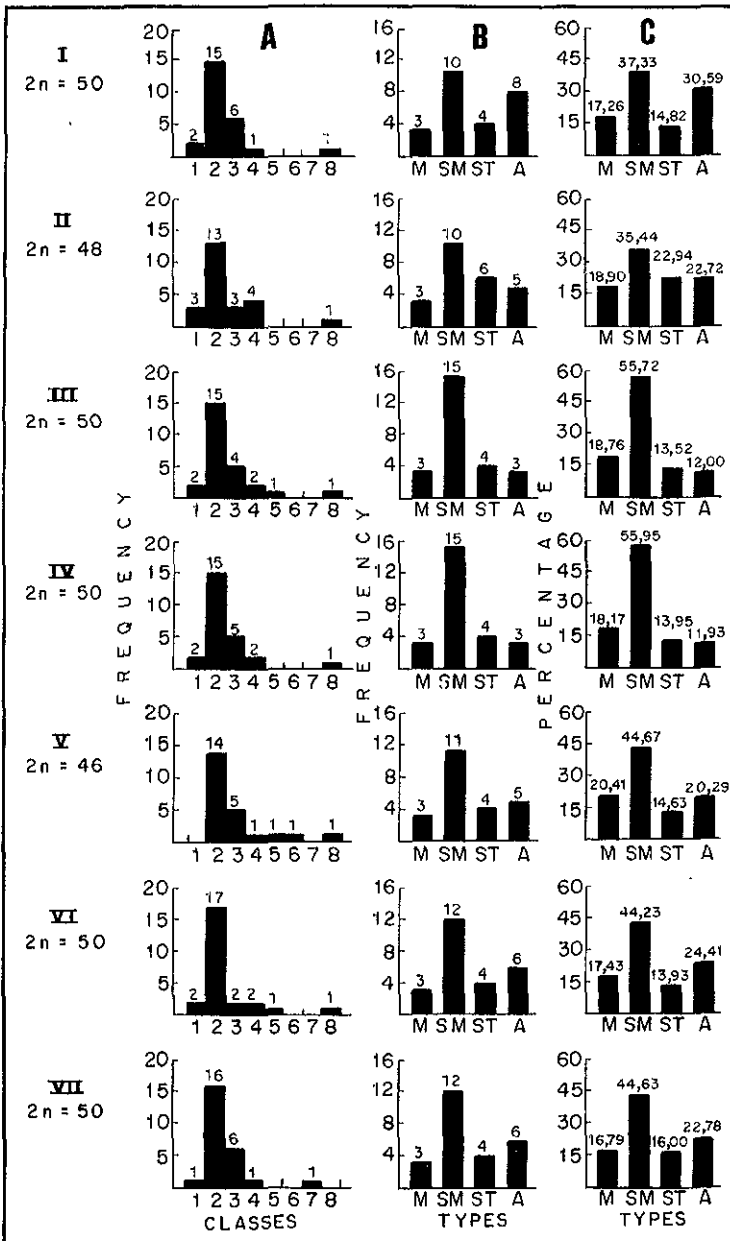


Figure 17 - Comparison of karyotype data on the basis of the haploid complement of specimens of the *scabripinnis* complex from the Centenário stream (I), Marrecas stream (II), Viveiro de Mudanças stream (III), Barreiro Grande stream (IV), Curral das Águas stream (V), Claro river (VI), and Monjolinho stream (VII). A, Chromosome frequency by size class; B, chromosome type frequency; C, participation (%) of chromosome types in relation to the size of the haploid lot.

Table III - Grouping of the *scabripinnis* complex specimens according to canonical correlation, by collection site Marrecas stream (1), Curral das Éguas (2), Monjolinho stream (3), Centenário stream (4), Barreiro Grande stream (5) and Viveiro de Mudás stream (6).

	1	2	3	4	5	6	Total no. of individuals /sample
1	28	0	0	0	0	2	30
2	0	29	0	0	0	1	30
3	0	0	27	0	0	3	30
4	1	0	0	24	0	5	30
5	0	0	1	0	19	0	20
6	2	0	3	0	0	24	29
Total no. of individuals	31	29	31	24	19	35	169

Table IV - Canonical variable coefficients for the two axes VCI and VCII, considering the parameters studied in the *scabripinnis* complex.

Morphometric parameters	Axes	
	VCI	VCII
Standard length	-4.275*	-2.128
Rostrordorsal distance	-0.096	5.156*
Rostro-anal distance	0.567	0.699
Body height	-3.357*	-2.856*
Head length	1.897	0.020
Snout length	2.450*	-4.297*
Eye diameter	1.385	-0.144
Interorbital width	1.620	1.661
3rd intraorbital width	-0.140	1.835

* Coefficients with the highest values, i.e., indicating the most important traits for discrimination among populations.

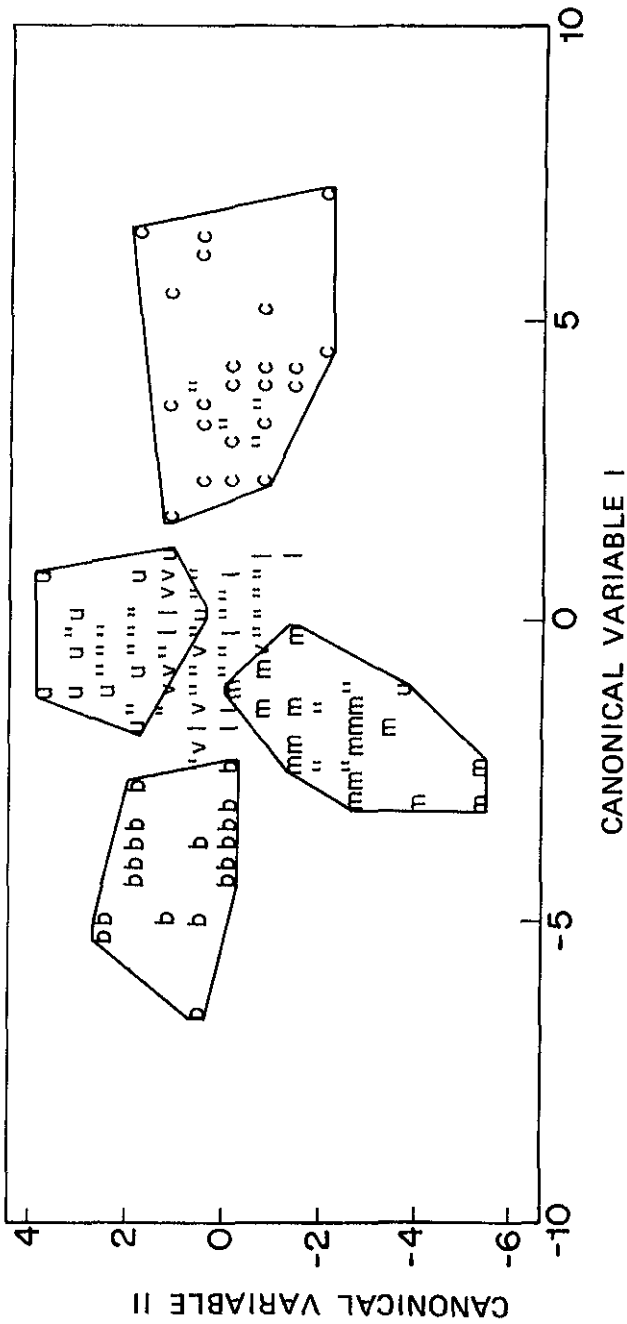


Figure 18 - Discrimination of six populations of the *scabripinnis* complex on the basis of the first canonical variable (x). b, Barreiro Grande stream; c, Curral das Éguas stream; i, Marrecas stream; m, Monjolinho stream; v, Viveiro de Mudas stream; x, overlapping points.

In general, these differentiations in the *scabripinnis* group are based on small morphological differences, a fact that impairs analysis when forms from different hydrographic basins are compared. However, with the development of new analytical methods for systematics, advances have recently been made in this field of study.

The present analyses specifically permitted us to determine that four of the six populations studied (from Curral das Éguas - São Gonçalo do Abaeté, from the Monjolinho - São Carlos stream, from the Centenário - Maringá stream and from the Barreiro Grande - Três Marias streams) can be perfectly differentiated from one another, mainly in terms of standard length, body height and snout length, as shown by canonical variable analysis (Figure 18). The other two populations, from the Viveiro de Mudas (Três Marias) and the Marrecas (Londrina) streams, were very similar with respect to these traits, which could not be used to differentiate them. For the Claro river (Salesópolis, SP) population, the impossibility of performing a satisfactory sampling analysis has not permitted thus far comparisons with the other populations.

Integration of karyotypic and morphological studies

The cytogenetic and morphological analyses were coincident in terms of the characterization of four of the populations studied as distinct entities within the "scabripinnis complex", i.e., those from the Curral das Éguas (São Gonçalo do Abaeté, MG), Barreiro Grande (Três Marias, MG), Centenário (Maringá, PR) and Monjolinho (São Carlos, SP) streams.

It is interesting to note that the specimens from the Marrecas (Londrina, PR) and Viveiro de Mudas (Três Marias, MG) streams belonging to geographically isolated hydrographic complexes (Paranapanema and São Francisco basins) were not differentiated by morphological analysis but were grouped in the same set of sampling points (Figure 18). However, they were perfectly distinguished by karyotypic analysis, not only in terms of diploid number, but also in terms of chromosome types and C-band distribution in the karyotypes (Figure 16). In contrast, the specimens from the Barreiro Grande and Viveiro de Mudas streams, geographically very close (both in the municipality of Três Marias, MG) and showing the same karyotypic pattern, could be perfectly separated by morphological analysis.

On this basis, the association of karyotypic and morphological data permitted the *a priori* differentiation of six distinct forms which we believe to be representative of different species, with only the Claro river population (Salesópolis, SP) needing further definition. *A. scabripinnis* can therefore be better characterized as a species complex. The present results demonstrate the validity of including these analyses in systematics and taxonomy, thus obtaining more conclusive information.

Forms that are not chromosomally differentiated can be separated by morphological analysis and, conversely, identical morphological forms can be separated chromosomally. The two approaches proved to be quite effective and to complement each other as means of identification of distinct forms within this group.

The reason for the wide diversity observed in *scabripinnis* may perhaps reside in the biological characteristics of the group itself. Gomes and Azevedo (1960), Britski (1972) and Caramaschi (1986) pointed out that *A. scabripinnis* is a typical headwaters species and as such may be restricted to these environments. Thus, large rivers may represent barriers to the free transit of the species, at least during present times (Caramaschi, 1986). Lowe McConnell (1969) emphasized that the absolute size of certain water systems is an important factor in the evolutionary process of fish since it may permit many species to evolve within the system itself by developing in isolation in the headwaters of the tributaries where interpopulation barriers may be physical, chemical or even biotic. According to Caramaschi (1986), the tolerance shown by *A. scabripinnis* to variation in abiotic factors may be a preponderant factor in its process of occupation of headwaters environments and in its presence in small rivers.

The present results fully support the above propositions. Forms that are chromosomally or morphologically distinct are found in small waterways belonging to the same basin (two distinct forms in the Paranapanema river and three in the São Francisco river). Furthermore, no different forms were found to coexist at a given site, nor was any evidence of intermediate forms observed.

Thus, in the study of species of the *scabripinnis* complex, as well as of other species with similar characteristics, it is important to analyze and interpret the data obtained not only as a function of the isolation caused by the dividers of the major hydrographic systems, but mainly as a function of the different environments represented by the microbasins in each drainage system, where the populations are located.

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RESUMO

Foram realizados estudos cariotípicos em 7 populações de *A. scabripinnis*, provenientes de algumas bacias hidrográficas distintas. Em 6 dessas populações foram efetuados também estudos morfológicos, aplicando-se a Análise das Variáveis Canônicas. Através da associação das análises

cariotípicas e morfológicas foi possível uma melhor compreensão da diversidade apresentada por esse grupo de peixes possibilitando caracterizá-lo com um complexo de espécies, isoladas nas cabeceiras dos pequenos tributários dos grandes sistemas hidrográficos.

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