

CYTOGENETIC AND DNA CONTENT STUDIES OF ARMoured CATFISHES OF THE GENUS *Corydoras* (PISCES, SILURIFORMES, CALLICHTHYIDAE) FROM THE SOUTHEAST COAST OF BRAZIL

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ABSTRACT

Cytogenetic and DNA content studies were done on six nominal species of *Corydoras* from the southeast coast of Brazil. The data show that several nominal species present local populations with differences in karyotype or DNA content. There are at least two groups of *Corydoras* species with similar karyotypic structure in this region: the first composed by *C. ehrhardi*, *C. nattereri* and *C. paleatus* and the second composed of *C. barbatus*, *C. macropterus* and *C. prionotos*. These two groups of species are probably not derived directly from the same ancestral line. The speciation process of *Corydoras* species from the southeastern coast of Brazil is discussed.

INTRODUCTION

The genus *Corydoras* comprises about 115 species and subspecies distributed throughout South America (Nijssen and Isbrucker, 1986). Of these, about ten nominal species occur in the coastal streams of southern and eastern Brazil (Nijssen and Isbrucker, 1980). *Corydoras* species are primary freshwater fishes, without the ability to disperse through saltwater, and the marine environment presents an ecological barrier to their dispersal along coastal waters.

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According to some references cited by Weitzman *et al.* (1988), during the periods of lower sea levels, some streams along the Brazilian coast were presumably united as they flowed into extended coastal river valleys. Therefore, speciation in the Glandulocaudini and in other freshwater fishes restricted to these coastal streams was influenced by complicated vicariance and dispersal events associated with large fluctuations in sea level during alternative glacial and interglacial periods of the Pleistocene.

The present paper presents the cytogenetic analysis of *Corydoras* species from the coastal streams of southeastern Brazil with the objective of evaluating the nature and extension of karyotypical changes which occurred during the speciation process.

MATERIAL AND METHODS

Six nominal species of fishes belonging to the genus *Corydoras* from five Brazilian States (Rio de Janeiro (RJ), São Paulo (SP), Paraná (PR), Santa Catarina (SC) and Rio Grande do Sul (RS)) were studied. Figure 1 shows the localities sampled and Table I lists the samples analysed, the collection sites and the number and sex of the specimens.

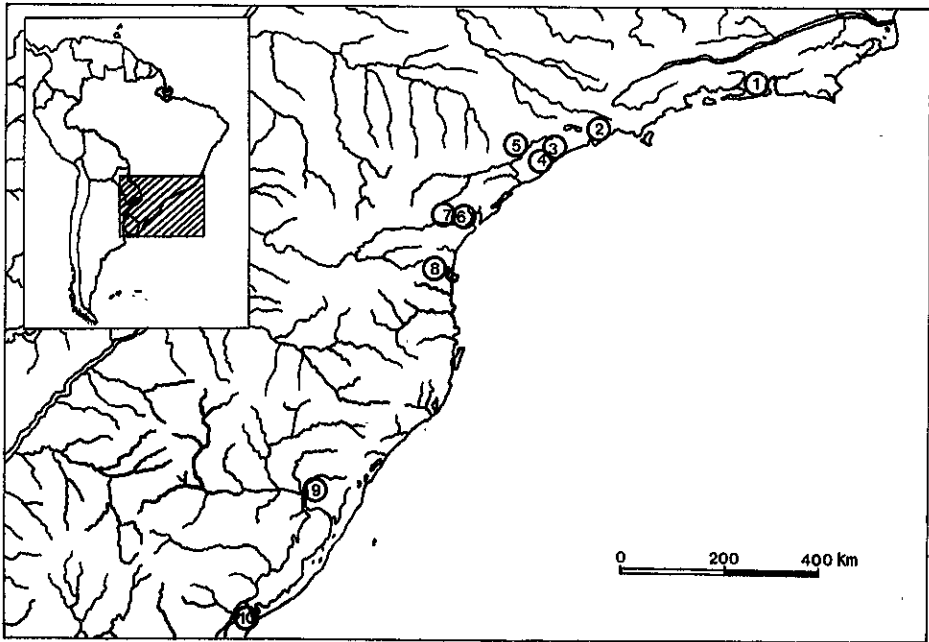


Figure 1 - Map showing the collecting sites: 1 - Nova Iguaçu, RJ; 2 - Bertioga, SP; 3 - Itanhaém, SP; 4 - Peruíbe, SP; 5 - Juquiá, SP; 6 - Morretes, PR; 7 - Curitiba, PR; 8 - Jaraguá do Sul, SC; 9 - São Leopoldo, RS; 10 - Rio Grande, RS.

Table I - Cytogenetic data obtained for *Corydoras* species from southeastern Brazil.

Species	Locality	Number of specimens ♂♂/♀♀	2n DNA content (in picograms) mean ± S.E.	2n	Karyotype*			NF**	Pairs with NORS***	
					M	SM	ST A			
<i>C. nattereri</i>	Niterói, RJ	4/6	3.57 ± 0.29	40	20	20		80	1 ^a	
	Nova Iguaçu, RJ	2/5	ND	44	20	24		88	1 ^a	
	Juquiá, SP	3/3	ND	42	18	24		84	2 ^a	
	Morretes, PR	4/6	ND	44	18	26		88	1	
<i>C. ehrhardti</i>	Jaraguá do Sul, SC	7/3	ND	44	18	26		88	2	
<i>C. paleatus</i>	Curitiba, PR	4/2	ND	44	20	24		88	3	
	São Leopoldo, RS	3/6	ND	44	20	24		88	2	
	Rio Grande, RS	3/3	ND	44	22	22		88	2	
<i>C. barbatus</i>	Bertioga, SP	4/2	1.87 ± 0.15	64	38	20	4	2	122	4
	Itanhaém, SP	5/2	1.73 ± 0.20	64	38	20	4	2	122	4
	Morretes, PR	4/0	ND	66	38	22	4	2	126	4
	Jaraguá do Sul, SC	0/2	ND	66	38	22	4	2	126	3
<i>C. macropterus</i>	Itanhaém, SP	3/1	1.63 ± 0.10	66	28	14	16	8	108	3
	Perube, SP	3/0	1.35 ± 0.02	66	28	14	16	8	108	3
<i>C. prionotos</i>	Juquiá, SP	4/2	1.19 ± 0.13	68	14	12	14	28	94	2
	Nova Iguaçu, RJ	0/4	1.64 ± 0.00	86	20	28	20	18	134	2

*M = metacentrics; SM = submetacentrics, ST = subtelocentrics and A = acrocentrics. **Chromosome arm number. ***Nucleolus Organizer Regions. ND = Not determined. a = data from Oliveira *et al.*, 1988.

Chromosome spreads, silver staining of Nucleolus Organizer Regions (NORs) and C-banding were done as described by Oliveira *et al.* (1988). Chromosome preparations were obtained from gill and kidney tissues and about 30 metaphases were examined 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 as metacentrics (M), submetacentrics (SM), subtelocentrics (ST) and acrocentrics (A). NF (chromosome arm number) was determined considering M/SM chromosomes to have two arms and ST/A chromosomes to have 1 arm. Nuclear DNA content was determined using erythrocytes of *C. nattereri* with $2n=40$ (eight specimens from Niterói, RJ) whose karyotype was described by Oliveira *et al.* (1990), of *C. barbatus* (three specimens from Bertioga and four specimens from Itanhaém), *C. macropterus* (two specimens from Itanhaém and three specimens from Peruíbe), and *C. prionotos* (three specimens from Juquiá and one specimen from Nova Iguaçu), as described by Oliveira *et al.* (1992).

Specimens were identified and deposited in the fish collection of Museu de Zoologia, Universidade de São Paulo, São Paulo, Brazil (MZUSP) (*C. nattereri*, *C. paleatus* from São Leopoldo (RS) and Curitiba (PR), *C. barbatus* from Itanhaém (SP) and Bertioga (SP), *C. macropterus*, and *C. prionotos*), and Museu de Ciências, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, Brazil (MCP) (*C. ehrhardti*, and *C. barbatus* from Morretes (PR) and Jaraguá do Sul (SC)).

RESULTS

Figures 2, 3, 5 and 6 show the karyotypes of the six species investigated. The karyotypes were similar for males and females in the species where both sexes were analyzed. Diploid numbers and chromosome formulae of each population are summarized in Table I.

The number and position of NORs, visualized by the silver-staining technique, were highly variable. However, in all samples analyzed the NORs were located in the terminal position. *C. nattereri* from Morretes was the only sample with only one chromosome pair with NORs (Figure 2a, Table I). The other species had multiple NORs: *C. ehrhardti*, *C. paleatus* from São Leopoldo and Rio Grande, and *C. prionotos* had two chromosome pairs with NORs (Figures 2b, 3b, 3c, 6a and 6b, Table I), *C. paleatus* from Curitiba and *C. macropterus* had three chromosome pairs with NORs (Figures 3a and 5c, Table I), and *C. barbatus* from Bertioga, Itanhaém and Morretes had four chromosome pairs with NORs (Figures 5a and 5b, Table I) and two fish analysed from Jaraguá do Sul presented three chromosome pairs with NORs (Table I).

Analysis of heterochromatin distribution by C-banding showed that *C. ehrhardti*, *C. nattereri*, and *C. paleatus* have large heterochromatic blocks in the pericentromeric position in almost all chromosome pairs (Figure 4). The species *C.*

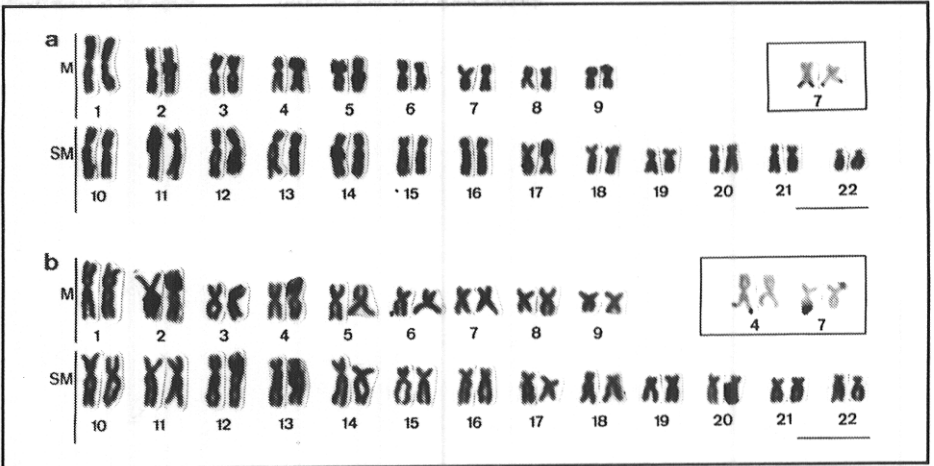


Figure 2 - Karyotype of a *C. nattereri* female from Morretes with $2n=44$ (a), and, in the inset, the chromosome pair with NORs; and karyotype of a *C. ehrhardii* female from Jaraguá do Sul with $2n=44$ (b), and, in the inset, the chromosome pairs with NORs. Bars - 10 μ m.

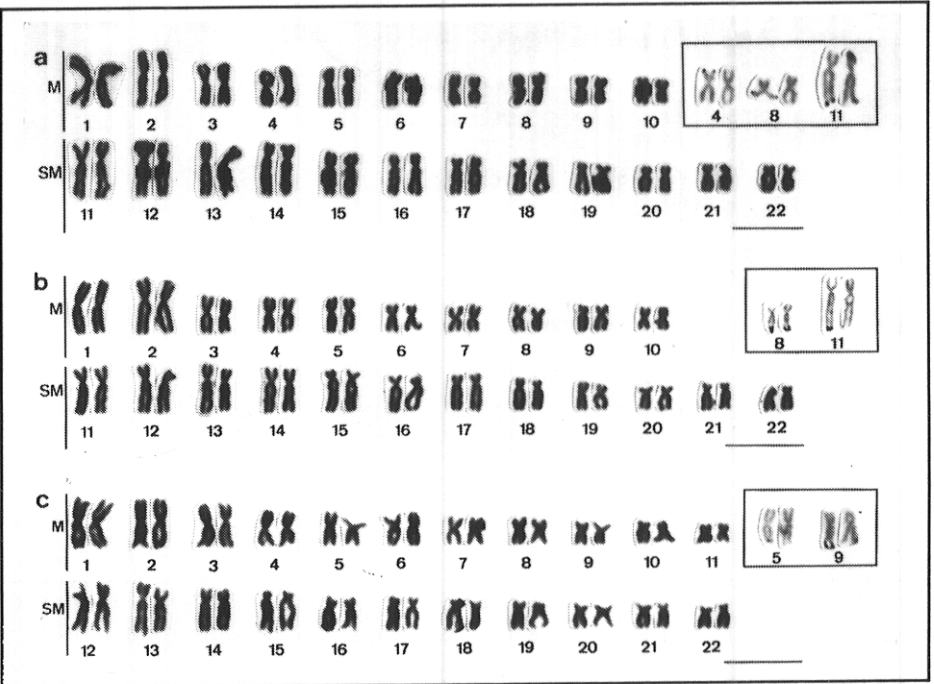


Figure 3 - Karyotype of a *C. paleatus* male from Curitiba with $2n=44$ (a), and, in the inset, the chromosome pairs with NORs; karyotype of a *C. paleatus* male from São Leopoldo with $2n=44$ (b), and, in the inset, the chromosome pairs with NORs; and karyotype of a *C. paleatus* male from Rio Grande with $2n=44$ (c), and, in the inset, the chromosome pairs with NORs. Bars - 10 μ m.

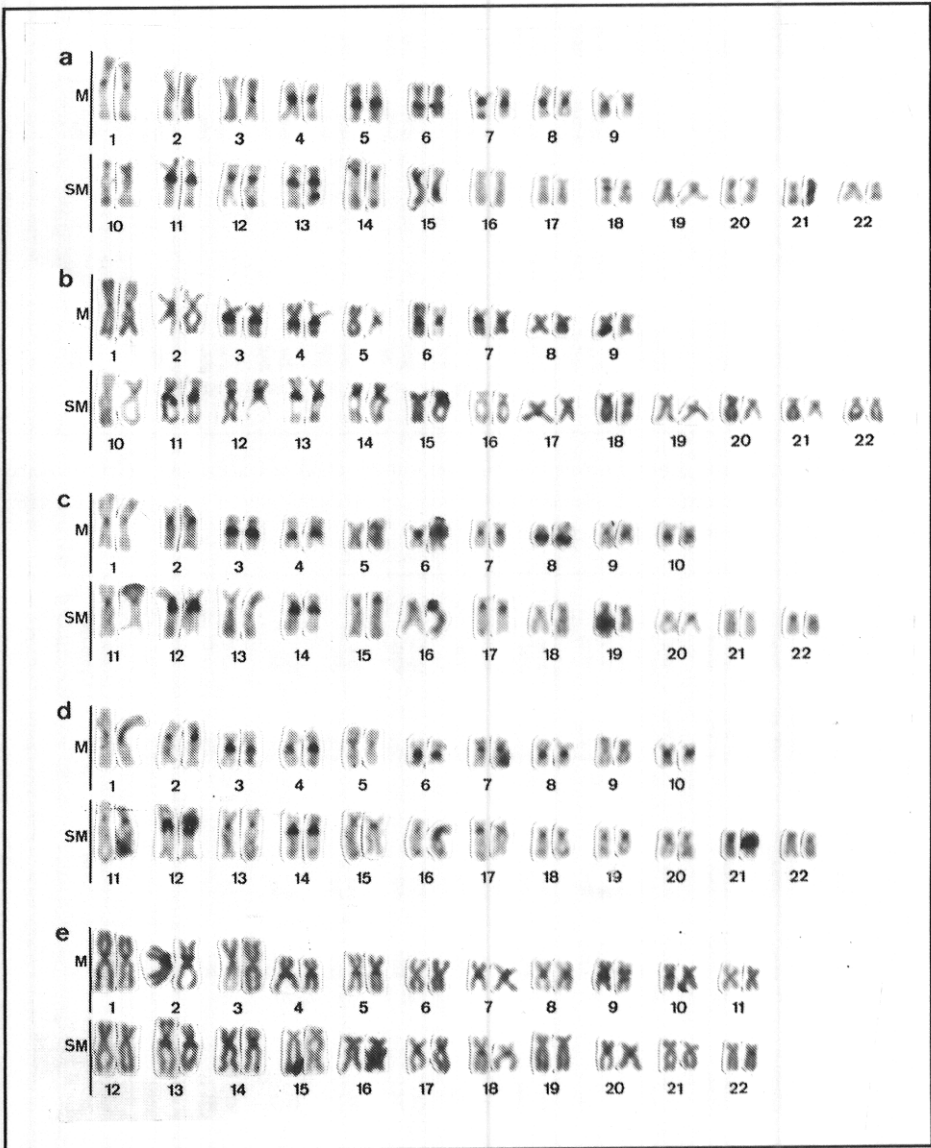


Figure 4 - C-banded karyotypes of *C. nattereri* from Morretes (a), *C. ehrhardti* from Jaraguá do Sul (b), *C. paleatus* from Curitiba (c), *C. paleatus* from São Leopoldo (d) and *C. paleatus* from Rio Grande (e).

barbatus, *C. macropterus*, and *C. prionotos* have several chromosome pairs with heterochromatic short arms and some chromosomes with heterochromatic blocks in the pericentromeric or in the terminal position (Figures 7 and 8).

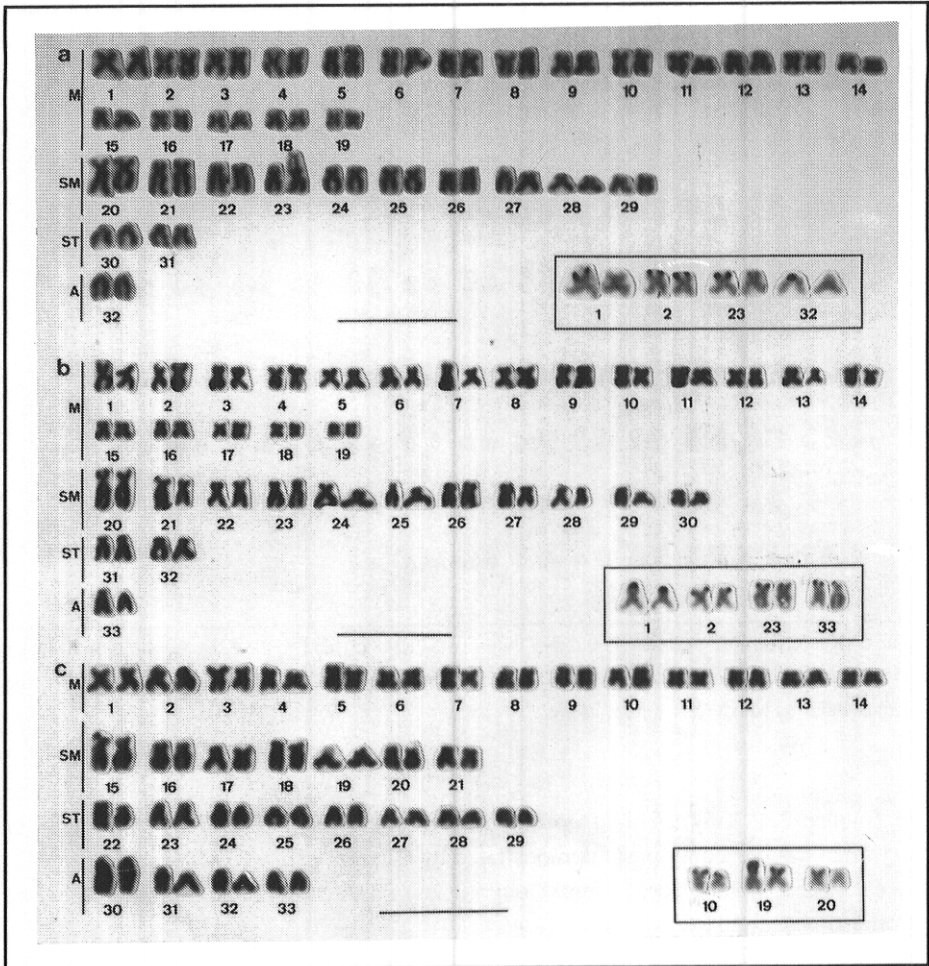


Figure 5 - Karyotype of *C. barbatus* male from Bertioga with $2n=64$ (a), and, in the inset, the chromosome pairs with NORs; karyotype of *C. barbatus* male from Morretes with $2n=66$ (b), and, in the inset, the chromosome pairs with NORs; karyotype of *C. macropterus* male from Itanhaém with $2n=66$ (c), and, in the inset, the chromosome pairs with NORs. Bars - 10 μ m.

The nuclear DNA content ranged from 1.19 ± 0.13 pg/nucleus for *C. prionotos* from Juquiá ($2n=68$) to 3.57 ± 0.29 pg/nucleus for *C. nattereri* from Niterói ($2n=40$). The species *C. barbatus*, *C. macropterus* and *C. prionotos*, with reduced DNA content, had small chromosomes ranging in size from 1 to 4 μ m (Figures 5 and 6) and the species *C. nattereri*, with a large DNA content, had large chromosomes ranging in size from 5 to 10

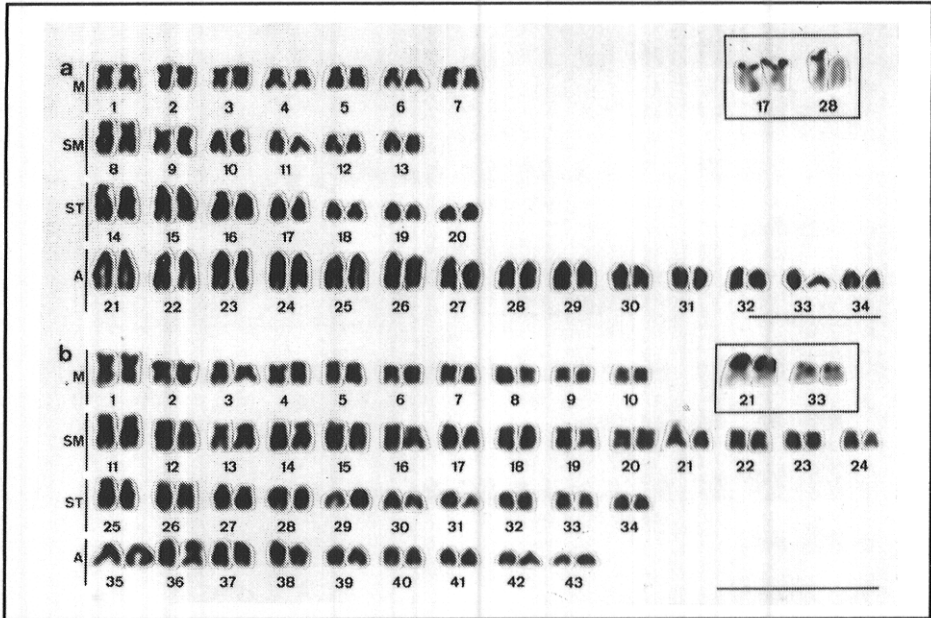


Figure 6 - Karyotype of *C. prionotos* male from Jussiaé with $2n=68$ (a), and, in the inset, the chromosome pairs with NORs; karyotype of *C. prionotos* female from Nova Iguaçu with $2n=86$ (b), and, in the inset, the chromosome pairs with NORs. Bars - 10 μm .

μm (Figure 2a). This relationship between chromosome size and DNA content in *Corydorax* has been previously demonstrated by Oliveira *et al.* (1992).

There was no correlation among nuclear DNA content and diploid number or chromosome formulae (Table I).

DISCUSSION

To date, karyotypic information about *Corydorax* species from the southeastern coast of Brazil was restricted to the species *C. paleatus* (Scheel *et al.*, 1972; Calcagnoto *et al.*, 1986), *C. undulatus* (Calcagnoto *et al.*, 1986) and *C. nattereri* (Oliveira *et al.*, 1990).

The present analysis of ten samples from different coastal streams revealed the occurrence of several differences in diploid number for different geographic populations of the same nominal species. Thus, *C. nattereri* had $2n=44$ chromosomes in Morretes which is located at the southern limit of distribution of this species (Figure 2a), $2n=44$ in Nova Iguaçu, $2n=42$ in Jussiaé and $2n=40$ in Niterói (Oliveira *et al.*, 1990); *C. barbatus*

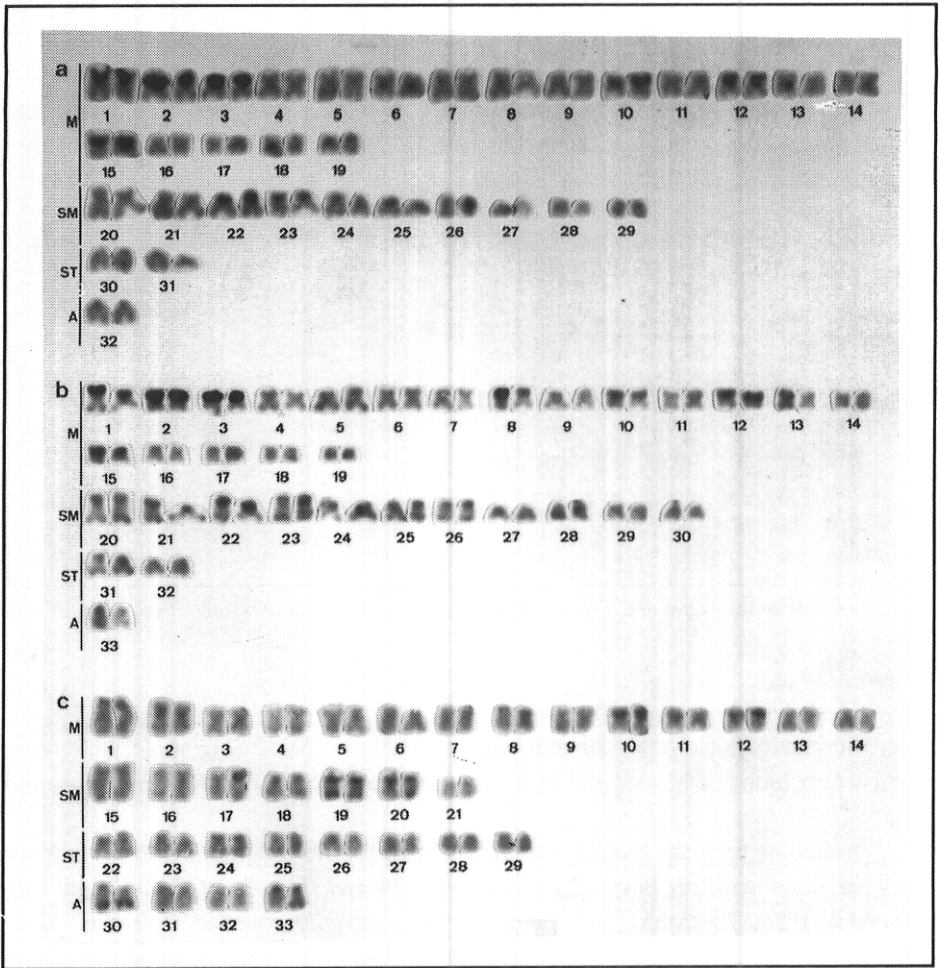


Figure 7 - C-banded karyotypes of *C. barbatus* from Bertioiga (a), *C. barbatus* from Morretes (b), and *C. macropterus* from Itanhaém (c).

had $2n=64$ in Bertioiga and Itanhaém and $2n=66$ in Morretes and Jaraguá do Sul; and *C. prionotos* had $2n=68$ in Juquiá and $2n=86$ in Nova Iguaçú.

Some other samples presented no variation in diploid numbers, but they differed in karyotypic structure. *C. paleatus* had $2n=44$ chromosomes and three chromosome pairs with NORs in specimens from Curitiba (Figure 3a) and $2n=44$ and two chromosome pairs with NORs in specimens from São Leopoldo and Rio Grande (Figures 3b and 3c). There were also differences in C-banding pattern, i.e., *C. paleatus* from Rio Grande had a large

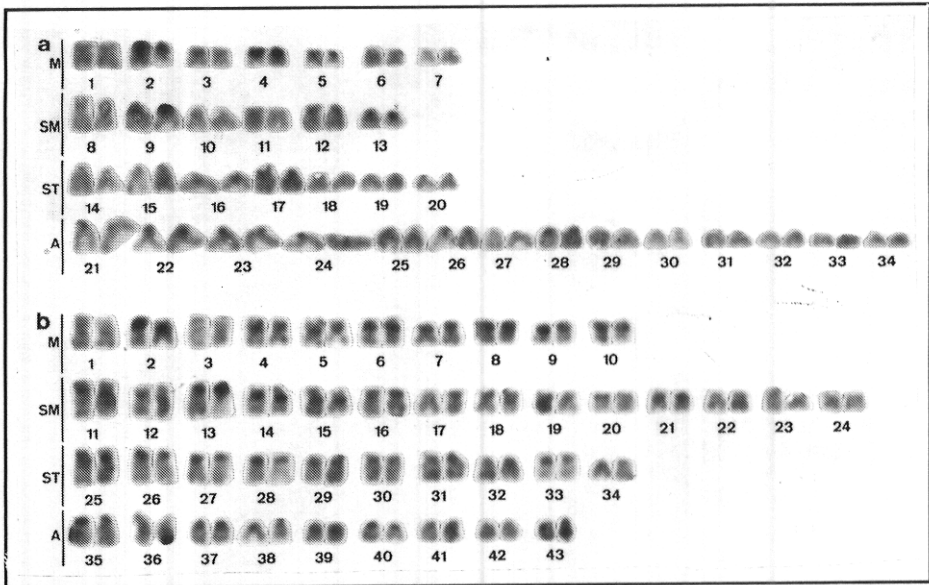


Figure 8 - C-banded karyotypes of *C. prionotos* from Juquiá (a), and *C. prionotos* from Nova Iguaçu (b).

heterochromatic block in the pericentromeric position of pair 2 (Figure 4e) but only a small heterochromatic block in samples from Curitiba and São Leopoldo (Figures 4c and 4d).

Among the fish, several examples of intraspecific variation in diploid number or in karyotype structure have been reported (Black and Howell, 1979; LeGrande and Cavender, 1980; Thorgaard, 1983; Foresti *et al.*, 1984; Dergam and Bertollo, 1990; Moreira-Filho and Bertollo, 1991; among others). This variation has been interpreted to be due to the occurrence of subspecies, cryptic species or unidentified species.

C. macropterus from Itanhaém and Peruíbe had the same number of chromosomes, two chromosome pairs with NORs and a similar C-banding pattern, but differed in nuclear DNA content (Table I). Differences in DNA content may be due to intraspecific polymorphism as described in cyprinids (Gold and Amemiya, 1987 and Gold *et al.*, 1990), in *Lepomis* (Ragland and Gold, 1989) and in *Corydoras flaveolus* (Oliveira *et al.*, 1992).

The occurrence of samples with different cytogenetic characteristics in the same nominal species of *Corydoras* points to the necessity for a taxonomic revision of this genus as well as of other groups of the South American freshwater fish fauna (Bohlke *et al.*, 1978) using other techniques in addition to anatomy for species identification.

A preliminary cytogenetic analysis of eleven *Corydoras* species showed that the genus is composed of at least five groups of species sharing particular karyotypes and nuclear DNA content (Oliveira *et al.*, 1992). Our data show that at least two of these five groups of *Corydoras* species occur in the southeastern coastal region of Brazil.

One is composed of *C. barbatus*, *C. macropterus*, and *C. prionotos* which present $2n=64-86$ chromosomes, two to four chromosome pairs with NORs, several chromosomes with heterochromatic arms or with heterochromatic blocks in the pericentromeric or in the terminal position, small chromosomes (from 1 to 5 μm , Figures 5 and 6) and low nuclear DNA content (from 1.19 ± 0.13 to 1.87 ± 0.15 pg/nucleus, Table I). Similar characteristics were found in *Corydoras* species from the group 1 proposed by Oliveira *et al.* (1992).

The second group is composed of *C. nattereri*, *C. ehrhardti* and *C. paleatus*, which present $2n=40-44$, one to three chromosome pairs with NORs, many chromosomes with large heterochromatic blocks in the pericentromeric position, large chromosomes (from 5 to 10 μm , Figures 2 and 3) and high nuclear DNA content, as determined for *C. nattereri* from Niterói (RJ) (Table I). Similar characteristics were found in *Corydoras* species from the group 4 proposed by Oliveira *et al.* (1992).

Another species of *Corydoras* found in the southeastern coastal region, *C. undulatus*, has $2n=52$ chromosomes and a karyotypic formula of $24M+14SM+12ST+2A$ (Calcagnoto *et al.*, 1986). The lack of information about DNA content, number and position of NORs, and C-banding pattern makes it impossible to define whether this species belongs to one of the two groups cited above or to a third group of species.

The differences between the two groups of species suggest that they did not derive from the same ancestral stock, and probably originated from two ancestral lines that colonized this region independently. A fact that may reinforce this hypothesis is the frequent occurrence of species of both proposed groups in sympatry as in the case of *C. nattereri* and *C. prionotos* in the Tinguá and Biguá rivers and *C. ehrhardti* and *C. barbatus* in the Figueirinha river.

The present data suggest that events of vicariance and dispersion may have occurred with fishes of the genus *Corydoras* in the southeastern coastal rivers, associated with large fluctuations in sea level during alternating glacial and interglacial periods. The karyotypic differences found have probably arisen after local isolation. This hypothesis follows the proposition of Weitzman *et al.* (1988) that considerable fish evolution has taken place on the southeastern coast of Brazil.

Complementation of the present study by other cytogenetic techniques (as R-banding) would permit the construction of cladograms for the *Corydoras* species, which may provide new insights for the understanding of the evolution of this genus and the biogeographic history of this region.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Heraldo A. Britski and Mr. Roberto E. Reis for taxonomic identification. Funds supporting this study were provided by CNPq, FAPESP, FINEP and CAPES.

Publication supported by FAPESP.

RESUMO

Estudos citogenéticos e de conteúdo de ADN nuclear foram feitos com seis espécies nominais de *Corydoras* provenientes da região costeira do Sudeste do Brasil. Os dados mostram que várias das espécies nominais apresentam populações locais com diferenças cariotípicas ou de conteúdo de ADN. Há pelo menos dois grupos de espécies de *Corydoras* com estruturas cariotípicas similares nessa região: o primeiro composto por *C. ehrhardti*, *C. nattereri* e *C. paleatus* e o segundo composto por *C. barbatus*, *C. macropterus* e *C. prionotos*. Esses dois grupos de espécies provavelmente não são diretamente derivados de uma mesma linhagem ancestral. O processo de especiação das espécies de *Corydoras* da região costeira do Sudeste do Brasil é discutido.

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(Received June 4, 1992)