



Cytogenetic analysis of five species of the subfamily Corydoradinae (Teleostei: Siluriformes: Callichthyidae)

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Abstract

In the present study, five callichthyid species belonging to the subfamily Corydoradinae were karyotyped: three species of *Aspidoras* and two of *Corydoras*. The three species of *Aspidoras* had the same diploid number, $2n = 46$ chromosomes, similar karyotypic formulae, with most chromosomes metacentric or submetacentric, single interstitial Ag-NORs and C-band positive segments mainly found in the centromeric position. The comparative analysis of cytogenetic data available for the genus *Aspidoras* and other species of Corydoradinae suggest that several events of centric fusion occurred in the origin of the species of *Aspidoras*. The two analyzed species of *Corydoras* showed high diploid numbers, $2n = 74$ in *C. sodalis* and $2n = 90$ in *C. britskii*. While *C. sodalis* exhibited single Ag-NORs and terminal, interstitial and centromeric C-band positive segments in almost all chromosomes, *C. britskii* showed multiple Ag-NORs and a small number of C-band positive segments found in the terminal position in one acrocentric (A) pair and in the interstitial position in one subtelocentric (ST) pair. The occurrence of high diploid numbers and many ST and A chromosomes are uncommon among the Corydoradinae, suggesting the occurrence of a high number of chromosome rearrangements, mainly centric fissions, in the origin of the *Corydoras* species studied.

Key words: fish cytogenetic, karyotype, NOR, C-banding, karyotypic evolution.

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Introduction

The family Callichthyidae consists of two subfamilies: Corydoradinae and Callichthyinae (Reis, 2003). The subfamily Corydoradinae, with more than 90% of the species of the family Callichthyidae, presents 164 valid species divided in three genera: *Aspidoras*, *Brochis* and *Corydoras* (Reis, 1998; Reis, 2003). Phylogenetic studies, based on morphological data, showed that the subfamily Corydoradinae and the genera *Aspidoras* and *Brochis* were monophyletic groups while the genus *Corydoras* was paraphyletic or polyphyletic (Reis, 1998; Britto, 2003, respectively). With the main objective of identifying the monophyletic groups that compose the subfamily Corydoradinae, Britto (2003) proposed a new classification for the group, according to the tribe Aspidoradini, previously constituted only by the genus *Aspidoras*, is composed of *Aspidoras* and *Scleromystax*. The genus *Scleromystax* is composed of a monophyletic group of four species formerly belonging to the genus *Corydoras* (*C. barbatus*, *C. macropterus*, *C. prionotos*, and

C. lacerdai). The tribe Corydoradini, which included the genera *Brochis* and *Corydoras*, is now composed only of the genus *Corydoras*, and the genus *Brochis* was considered a junior synonymous with *Corydoras* (Britto, 2003).

The cytogenetic studies in the subfamily Corydoradinae are concentrated in *Corydoras*. Oliveira *et al.* (1992) reviewed the karyotypes and the nuclear DNA content of 29 species of the genus *Corydoras* and suggested the division of this genus into at least five groups of species based on their diploid number, karyotype and nuclear DNA content. A recent review of the cytogenetic data available for about 46 species of *Corydoras* (Shimabukuro-Dias *et al.*, 2004) corroborate the hypothesis of Oliveira *et al.* (1992) about the division of the genus *Corydoras* into five groups and revealed that the diploid number ranged from $2n = 40$ chromosomes in *Corydoras nattereri* (Oliveira *et al.*, 1990; Oliveira *et al.*, 1993b) to $2n = 134$ in *C. aeneus* (Turner *et al.*, 1992). This chromosome diversity found in *Corydoras* is one of the highest found among all fish genera (Scheel *et al.*, 1972; Oliveira *et al.*, 1992; Oliveira *et al.*, 1993a; Oliveira *et al.*, 1993b; Klinkhardt *et al.*, 1995). In the genus *Aspidoras*, only the species *A. fuscoguttatus*, with $2n = 44$ chromosomes, had its karyotype described

(Oliveira *et al.*, 1993a). Cytogenetic studies in the genus *Scleromystax* were conducted with the species *S. barbatus* ($2n = 64/2n = 66$), *S. macropterus* ($2n = 66$), and *S. prionotos* ($2n = 68/2n = 86$) (Oliveira *et al.*, 1993b).

The main objective of the present study was to gather new cytogenetic data for the genera *Aspidoras* and *Corydoras* and thus construct a more robust hypothesis regarding the pattern of relationships between species of the Corydoradinae subfamily. Thus, the karyotype of three species of *Aspidoras* and two species of *Corydoras* is described, through the Giemsa staining, staining of nucleolus organizer regions with Silver Nitrate (Ag-NORs) and C-banding.

Material and Methods

Cytogenetic studies were conducted with the following species: *Aspidoras taurus*, three males and one female from the Itiquira River, 17°02'36.3" S 53°28'43.3" W, Alto Garça, Mato Grosso, Brazil; *Aspidoras cf. fuscoguttatus*, two males and two females from aquarium; *Aspidoras poecilus*, four females from the Araguaia River, 17°33'42.4" S 53°18'29.7" W, Alto Araguaia, Mato Grosso, Brazil; *Corydoras sodalis*, two males and one female from aquarium; and *Corydoras britskii*, one male and one female from a tributary of the Pirai River, 16°25.280' S 56°25.143' W, Poconé, Mato Grosso, Brazil. After processing, fishes were identified and stored as vouchers at the fish collection center of Laboratório de Biologia de Peixes (LBP), Instituto de Biociências, Universidade Estadual Paulista, Botucatu, São Paulo, Brazil.

Chromosome preparations were carried out according to Foresti *et al.* (1993). Silver staining of the nucleolus organizer regions was performed according to the technique proposed by Howell and Black (1981), the C-banding technique was performed as described by Sumner (1972). Chromosome morphology was determined on the basis of arm ratio as proposed by Levan *et al.* (1964) and chromosomes were classified as metacentric (M), submetacentric (SM), subtelocentric (ST) and acrocentric (A).

Results

The three studied species of the genus *Aspidoras* showed $2n = 46$ chromosomes. The species *A. taurus* (Figure 1a) and *A. poecilus* (Figure 1c) exhibited karyotypic formulae composed of 15 pairs of M chromosomes, 5 pairs of SM and 3 pairs of ST. The karyotype of the species *Aspidoras cf. fuscoguttatus* had 16 pairs of M chromosomes, 5 pairs of SM and 2 pairs of ST (Figure 1b). *Corydoras sodalis* displayed $2n = 74$ chromosomes, divided into 8 pairs of M chromosomes, 9 pairs of SM, 5 pairs of ST and 15 pairs of A (Figure 2a). *Corydoras britskii* showed $2n = 90$ chromosomes and karyotypic formulae constituted by 2 pairs of M chromosomes, 5 pairs of SM, 11 pairs of ST and 27 pairs of A (Figure 2b).

The chromosome analysis with the Ag-NOR technique showed the presence of single marks, interstitially located at the short arms of the fifth M pair in all species of *Aspidoras* (Figure 1). Only one of the NOR-bearing chromosomes was marked in *A. taurus*. *C. sodalis* showed single Ag-NORs, in terminal position, at the long arms of the fifth pair of A chromosomes (Figure 2a). *C. britskii* exhibited terminal Ag-NORs at the short arms of the ST chromosome pairs number 14 and 16 (Figure 2b).

The analysis of the C-banding pattern showed that the species *A. cf. fuscoguttatus* (Figure 3b) and *A. poecilus* (Figure 3c) presented large C-band positive segments located at the centromeric position in most chromosomes. *A. taurus* (Figure 3a) exhibited C-band positive segments in the centromeric position in almost all chromosomes and some terminal segments in a few chromosomes. *C. sodalis* showed C-band positive segments at terminal, interstitial and centromeric positions in many chromosomes (Figure 3d). The species *C. britskii* presented a small amount of C-band positive segments restricted to the terminal position in one A pair and another at the interstitial position in one pair of ST chromosomes (Figure 3e).

Discussion

Chromosome evolution in the genus *Aspidoras*

The three studied species of the genus *Aspidoras* showed the same diploid number, $2n = 46$, and similar, but not identical, karyotypes. Although the species *A. taurus* and *A. poecilus* presented the same karyotypic macrostructure, their karyotypes were different due to the presence of a higher amount of C-band positive segments in *A. taurus*. The species *A. cf. fuscoguttatus* exhibited a different karyotypic formulae from those observed in the other species due to its different number of M and SM chromosomes.

Cytogenetic studies conducted with the species *A. fuscoguttatus*, showed that it presented $2n = 44$ chromosomes ($28M+12SM+4ST$), interstitial Ag-NORs in the long arms of the biggest SM chromosome pair and C-band positive segments distributed in the pericentromeric position in almost all chromosomes (Oliveira *et al.*, 1993a). These characteristics are similar to those found in the three species of *Aspidoras* analyzed in the present study, which suggest that few chromosome rearrangements - fusions or translocations - occurred during the chromosome evolution of the species of this genus.

The presence of diploid numbers close to $2n = 44/46$ was also observed among some species of *Corydoras* (*C. nattereri*, *C. ehrhardti*, *C. paleatus*, *C. arcuatus*, *C. schwartzi*, *C. trilineatus*, and *C. panda*) (Oliveira *et al.*, 1990; Oliveira *et al.*, 1992; Oliveira *et al.*, 1993a; Oliveira *et al.*, 1993b). However, while those species of *Corydoras* presented large chromosomes (with size variation from 3 μm to 8 μm) and about 4.4 pg of DNA per diploid nuclei

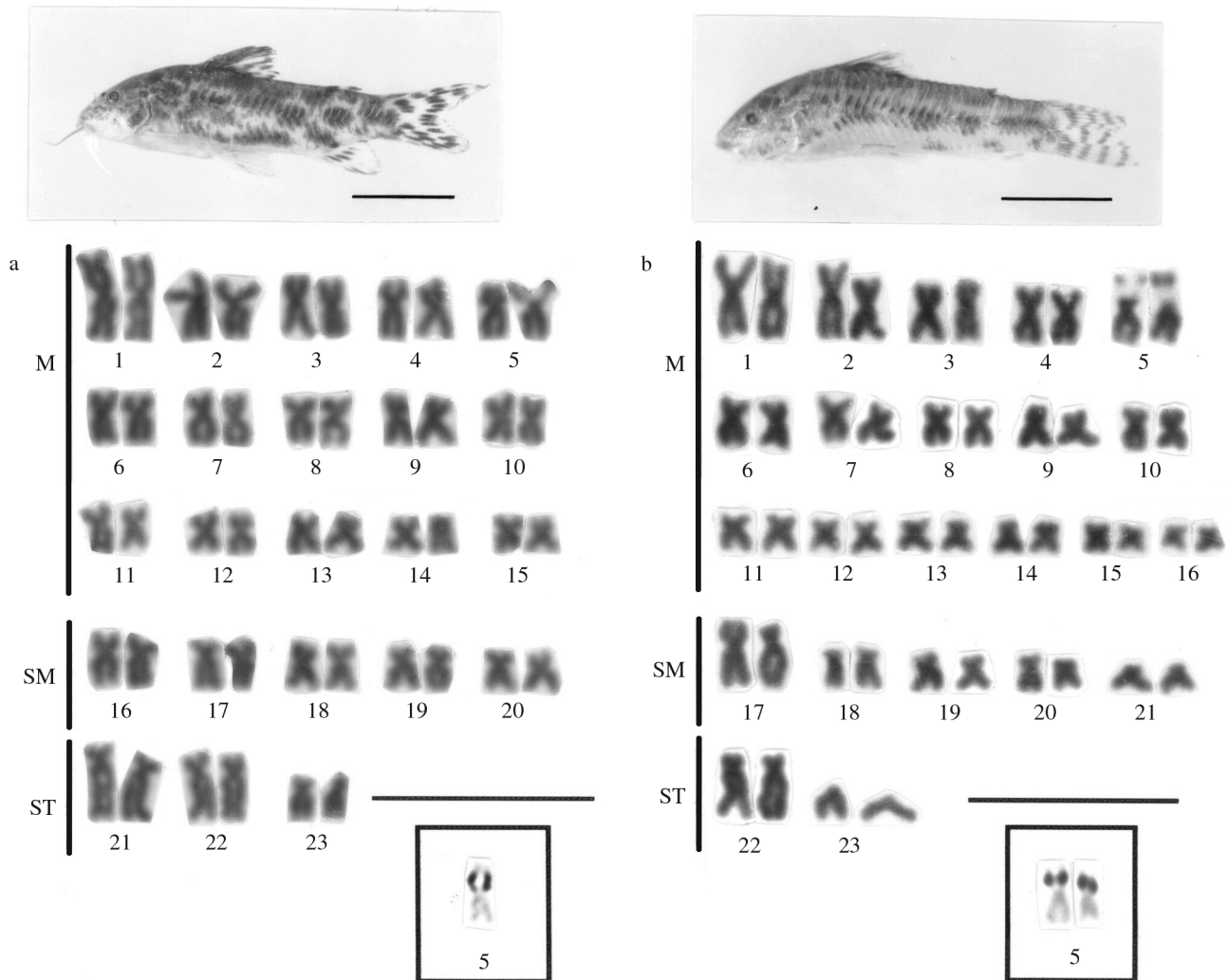


Figure 1 - Karyotypes, Ag-NORs and specimens of: a) *Aspidoras taurus*, $2n = 46$ and b) *A. cf. fuscoguttatus*, $2n = 46$.

(Oliveira *et al.*, 1992), the species of *Aspidoras* presented very small chromosomes (with size variation from 1 μm to 4 μm) and about 1.5 pg of DNA per diploid nuclei in *A. fuscoguttatus* (Oliveira *et al.*, 1993a; present study).

The low nuclear DNA content observed in *A. fuscoguttatus* (Oliveira *et al.*, 1993a) was also found in the species of the genus *Scleromystax* (cited as *Corydoras*), including *S. barbatus*, *S. prionotos*, and *S. macropterus* (Oliveira *et al.*, 1993b), which allow Oliveira *et al.* (1993a) to propose that these species of *Scleromystax* could be related to the species of *Aspidoras*. This hypothesis was corroborated by the phylogenetic study conducted by Britto (2003), that showed that the genera *Aspidoras* and *Scleromystax* compose a monophyletic group (the tribe Aspidoradini). The presence of low diploid numbers in *Aspidoras* ($2n = 44-46$) and high diploid numbers in *Scleromystax* ($2n = 64-86$) and other genera of Callichthyidae suggest that several terminal fusions may have occurred in the ancestor which gave origin to the ge-

nus *Aspidoras* as initially proposed by Oliveira *et al.* (1993a).

In Corydoradinae, interstitial Ag-NORs, as those observed in *Aspidoras*, were found only in a few species of *Corydoras* (Oliveira *et al.*, 1990; Oliveira *et al.*, 1992). In other groups of Siluriformes, the presence of interstitial Ag-NORs is uncommon, though it has been described for some species of the families Trichomycteridae (Torres *et al.*, 1998) and Loricariidae (Alves *et al.*, 2003).

The occurrence of large C-band positive segments located at the centromeric position in most chromosomes of the species of *Aspidoras* is also found in the *Corydoras* species with $2n = 44/46$ chromosomes as with *C. nattereri*, *C. ehrhardti*, *C. paleatus*, *C. arcuatus*, *C. schwartzi*, *C. trilineatus*, and *C. panda* (Oliveira *et al.*, 1990; Oliveira *et al.*, 1992; Oliveira *et al.*, 1993a; Oliveira *et al.*, 1993b). On the other hand, this kind of distribution of C-band positive segments is very different from that observed in the callichthyid species of the genus *Scleromystax* which are more closely related to *Aspidoras*, since in the species of

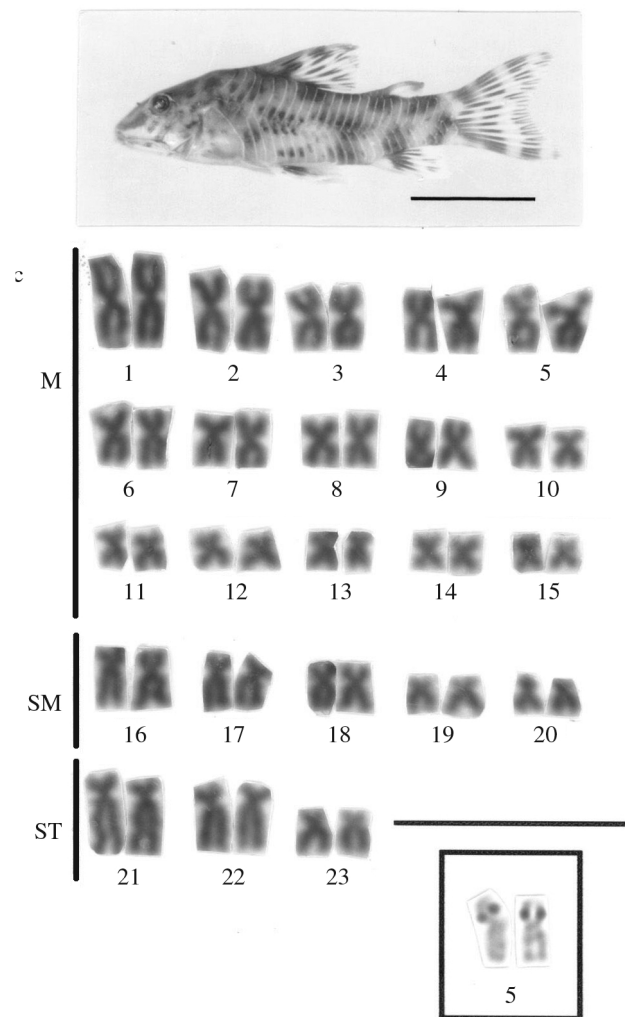


Figure 1 (cont.) - Karyotypes, Ag-NORs and specimens of: c) *A. poecilus*, $2n = 46$. Scale bars: 10 μm (karyotype) and 10 mm (specimen).

Scleromystax the C-band positive segments were mainly distributed in the short arms of several chromosome pairs (Oliveira *et al.*, 1993b).

Although the cytogenetic studies in the genus *Aspidoras* have been conducted in about one fifth of the described species, it is possible to observe the remarkable homogeneity in the karyotypes of the species of the genus. Moreover, the set of cytogenetic characteristics found in *Aspidoras* is not found in any other genera of Callichthyidae, which reinforces the hypothesis that this genus is monophyletic.

Chromosome evolution in the genus *Corydoras*

The cytogenetic analysis of the species *C. sodalis* revealed that it presented a high diploid number ($2n = 74$), a high number of ST and A chromosomes, a single Ag-NORs-bearing chromosome pair, and small C-band positive segments distributed in all chromosomes. These characteristics are very similar to those found in other species of

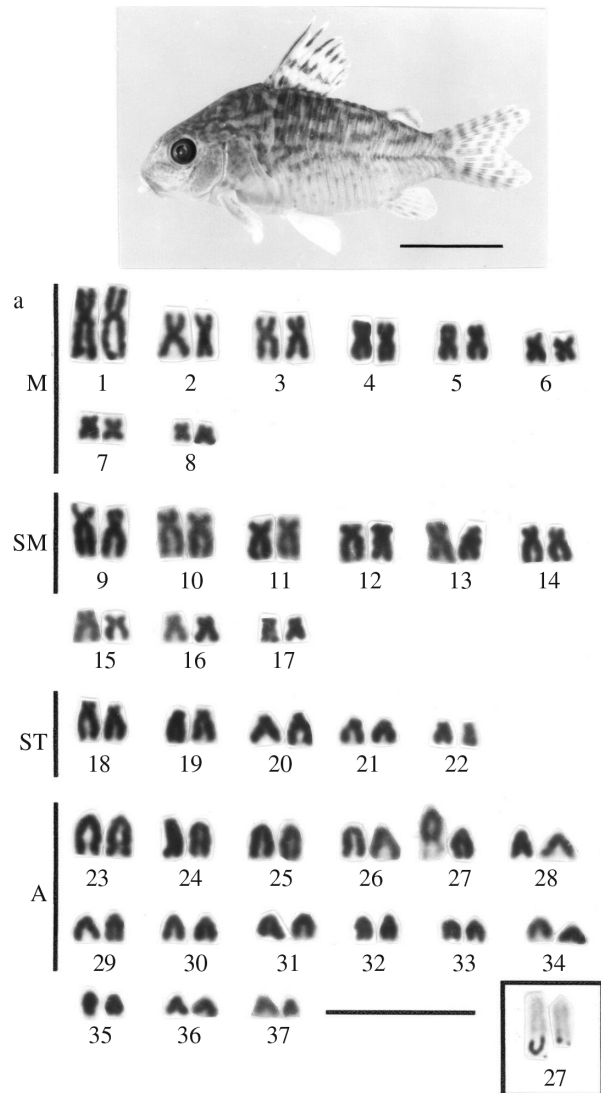


Figure 2 - Karyotypes, Ag-NORs and specimens of a) *Corydoras sodalis*, $2n = 74$.

Corydoras, as *C. reticulatus* ($2n = 74$) (Oliveira *et al.*, 1992) and *C. difluviatilis* ($2n = 78$) (our unpublished data), suggesting that these species could belong to a natural group, identified as group 2 by Oliveira *et al.* (1992).

The species *C. britskii* showed the same diploid number and karyotypic formulae described for some specimens studied and identified as *Brochis britskii* by Oliveira *et al.* (1993a). However, while in the present study the occurrence of multiple Ag-NORs and C-band positive segments restricted to two chromosome pairs was observed, the sample of *C. britskii* studied by Oliveira *et al.* (1993a) showed single Ag-NORs and large C-band positive segments in almost all chromosome pairs. These differences could be attributed to the occurrence of technical problems or to the existence of different cytotypes in this species. Another species, earlier identified as *Brochis splendens*, and now identified as *Corydoras splendens*, exhibited $2n = 100$

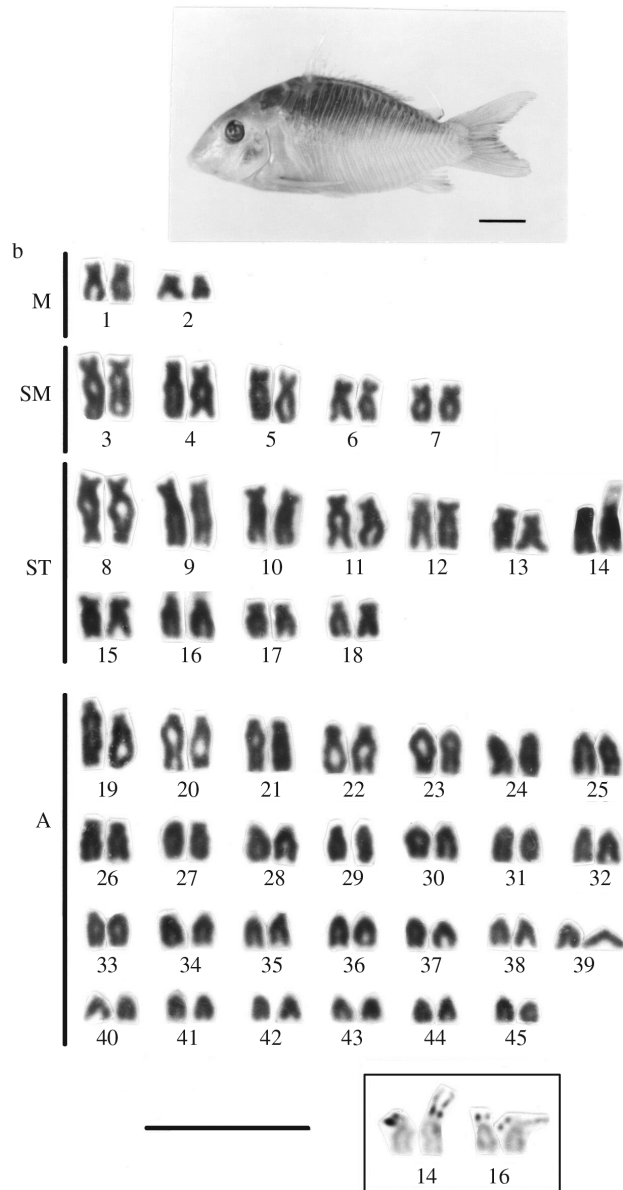


Figure 2 (cont.) - Karyotypes, Ag-NORs and specimens of b) *C. britskii*, $2n = 90$. Scale bars: 10 μm (karyotype) and 10 mm (specimen).

chromosomes (18M+18SM+20ST+44A), multiple Ag-NORs and centromeric C-band positive segments (Oliveira *et al.*, 1993a). These results show that these two species, formerly placed in the genus *Brochis*, have many cytogenetic characteristics in common, reinforcing the hypotheses that both belong to a monophyletic group (Reis, 1998; Britto, 2003).

The presence of high diploid numbers (greater than $2n = 70$) and a high number of ST and A chromosomes, found in several species of *Corydoras* (Oliveira *et al.*, 1992), and also observed in *C. britskii* and *C. sodalis* analyzed in the present study, reinforce the hypotheses that these two species belong to a natural group (Oliveira *et al.*, 1993a). In the only phylogenetic study based on morpho-

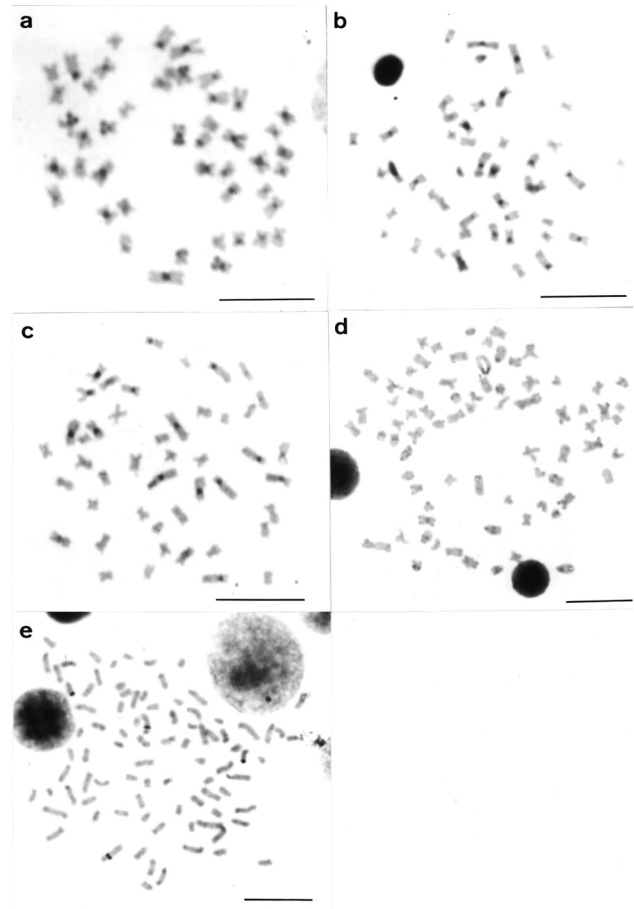


Figure 3 - Somatic metaphases treated with C-banding technique. a) *Aspidoras taurus*; b) *A. cf. fuscoguttatus*; c) *A. poecilus*; d) *Corydoras sodalis*; and e) *C. britskii*. Bars = 10 μm .

logical characters of a high number of species of Corydoradini, conducted by Britto (2003), a close relationship among the species *C. difluviatilis* (cited as *Corydoras* sp. by Britto, 2003), *C. reticulatus*, and *C. britskii* (cited as *Brochis britskii* by Britto, 2003) was not observed. The species *C. sodalis* was not included in the analysis of Britto (2003).

The presence of single Ag-NORs in *C. sodalis* and multiple Ag-NORs in *C. britskii*, as observed in the present study, were also described for other species of *Corydoras*, reinforcing the hypothesis that these species belong to a natural group, group 2 from Oliveira *et al.* (1992). The Ag-NORs, in the analyzed species of *Corydoras*, exhibited a large size polymorphism. This characteristic has been found in many species of Neotropical fish, and it may be due to the occurrence of duplication in the ribosomal DNA sequences, caused by unequal *crossing-over* rearrangements during meiosis (Almeida-Toledo *et al.*, 2000). The distribution patterns of C-band positive segments in *C. sodalis* and *C. britskii* have also been observed in other species of *Corydoras* of group 2 from Oliveira *et al.* (1992).

The presence of high diploid numbers and a high number of ST and A chromosomes, found in some species of the genus *Corydoras*, are uncommon characteristics in the family Callichthyidae (Oliveira *et al.*, 1993a) and even in other families of Siluriformes as well (Oliveira and Gosztanyi, 2000). Thus, it seems that in the origin of this group of species of *Corydoras* a high number of chromosome rearrangements - such as centric fissions - occurred, contrary to what occurred in the genus *Aspidoras*, in which a high number of chromosome fusions could have occurred. Moreover, the differences in the number and position of Ag-NORs and in the amount and distribution of C-band positive segments observed among species of Corydoradinae suggest that, besides the large morphological changes, which occurred in the karyotype, several small changes in the chromosomes also occurred in the evolutionary history of the group.

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