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Short Communication

Cytotaxonomy and karyoevolution of the genus *Crenicichla* (Perciformes, Cichlidae)

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Abstract

Karyotypes of six cichlid species of the genus *Crenicichla* were investigated. The species *C. cincta, C. inpa, C. reticulata*, *C. lugubris*, and *C.* cf. *johanna* were collected from Amazon basin, and *C. britskii* was collected from the Paraná-Paraguai basin. All of the analysed species showed 2n = 48 chromosomes; however, *C. cincta*, *C. lugubris, C.* cf. *johanna*, and *C. britskii* had a karyotype formula of 8M-SM+40ST-A, FN = 56, while *C. inpa* and *C. reticulata* exhibited the formula 6M-SM+42ST-A, FN = 54. Analysis of active Ag-NORs revealed two NOR-bearing chromosomes in all species; however, theses cistrons were located on different chromosome pairs and/or in different chromosome locations in each species. This condition bears evolutionary significance, since it is the main chromosome marker of the process of karyotypic evolution among the species of the genus *Crenicichla*. In general, C-banding revealed a similar constitutive heterochromatin pattern in all species, although it was possible to detect some features that led us to infer that *Crenicichla* also presents a species-specific heterochromatin pattern.

Key words: karyotype, pike cichlid, chromosome marker, Amazon.

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In the Perciformes group, cichlids are the most species-rich non-Ostariophysan freshwater fish family in the world, and one of the largest vertebrate families, with at least 1,300 species and estimates to approach 1,900 species (Kullander, 1998). The pike cichlids of the genus *Crenicichla* Heckel, 1840 figure among the most species-rich genera in the family and the genus includes about 80 species. Its geographical distribution comprises most of cis-Andean South America, from Trinidad to Argentina, including the Orinoco, Amazon, and Paraguay-Paraná basins and most coastal rivers between Guianas and the La Plata River (Kullander, 1982; Ploeg, 1991; Lucena and Kullander, 1992; Kullander, 1998, 2003). Based on characteristics such as body shape, the number of scale series below lateral lines, and presence or absence of a humeral blotch, the *Crenicichla* species are included into nine groups (*lugubris*, *acutirostris*, *lacustris, missioneira, reticulata*, *saxatilis*, *scotti*, *wallacii,* and *macrophtalma*) or into two more inclusive groups: large-scaled and small-scaled species (Ploeg, 1991; Lucena and Kullander, 1992; Kullander and Lucena, 2006).

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The family Cichlidae is considered a group with a conservative chromosome macrostructure, where 60% of the analysed species have a diploid number of 48 chromosomes (Feldberg *et al.*, 2003). This characteristic has been proposed by some authors as a conserved character for this family, suggesting that the ancestral karyotype consisted of 48 acrocentric chromosomes (Thompson, 1979; Kornfield *et al.*, 1979; Martins *et al.*, 1995; Feldberg *et al.*, 2003).

The aim of this paper is to update the karyotypic information on *Crenicichla* species and to present additional cytogenetic data for six species, and to evaluate the usefulness of cytotaxonomic information for the understanding of the evolution of pike cichlids.

Karyotype analyses were performed on *Crenicichla cincta* (eight males, seven females), *C. inpa* (five males, seven females), *C. reticulata* (two males, one female), *C. lugubris* (five males, three females), *C.* cf. *johanna* (one male, one female), and *C. britskii* (one male). The five former species were collected in the area near the confluence of the Negro and Solimões Rivers and the surrounding area (between 59° -60° W and 2° -3° S), and *C. britskii* was collected in the Paraná River near the Jupiá Hydroelectric Dam in the Paraná-Paraguai basin (51° 35' 35" W and 20° 45' 55" S). The specimens were deposited in the collection of Laboratório de Genética de Peixes (INPA/CPBA).

Chromosome preparations were obtained from a kidney cell suspension using the standard air-drying technique of Bertollo *et al.* (1978). Fish were anesthetized in chilled water before being sacrificed. Mitotic induction with biological yeast (Oliveira *et al.*, 1988) was performed in order to obtain a higher number of dividing cells. Nucleolar organizer regions (NORs) were identified by silver nitrate staining according to Howell and Black (1980), and C-banding to locate constitutive heterochromatin was performed using barium hydroxide according to Sumner (1972). When possible, slides were stained sequentially with Giemsa, C-banding, and silver nitrate solution (Ag-NOR) according to Centofante *et al.* (2002). Chromosomes were measured and arranged in decreasing order of size into two groups: metacentric-submetacentric (M-SM) and subtelocentric-acrocentric (ST-A) according to Levan *et al.* (1964) and Thompson (1979). The fundamental number (FN) or chromosome arm number was determined by counting M-SM chromosomes with two arms and ST-A with only one.

Table 1 summarizes the chromosome characteristics of the *Crenicichla* species that were analyzed in the present study and those available in the literature. These data are hierarchically organized according to the species group division proposed by Ploeg (1991) and Lucena and Kullander

Table 1 - Chromosome characteristics in the genus *Crenicichla* (2n = diploid number; KF = karyotypic formula; FN = fundamental number; NOR = Nucleolar organizer region, p = short arm, q = long arm, t = terminal, i = interstitial; * = updated species names as shown in the Fish Database http://www.fishbase.org/search.cfm.

Group	Species	Locality	2n	KF	FN	NOR(Pair)	Reference
saxatilis	*Crenicichla "sexatilis"*	Uruguai	48	4m, sm+44st, a	52	-	Oyhenart-Perera *et al.*, 1975
	Crenicichla lepidota		48	-		-	Schell, 1973
	Crenicichla lepidota	Miranda, MS, Brazil	48	6m, sm+42st, a	54	1°, q, i	Feldberg and Bertollo, 1985a, b
	Crenicichla lepidota	Commercial source	48	6m, sm+42st, t	54	-	Thompson, 1979
	Crenicichla lepidota	Paraná River, PR, Brazil	48	6m, sm+42st, a	54	1°, p, t 5°, q, t	Martins *et al.*, 1995
	Crenicichla lepidota	Misiones, Argentina	48	6m, sm+42st, a	54	1°, -, i	Fenocchio *et al.*, 2003
	Crenicichla lucius	Commercial source	48	-		-	Thompson, 1979
	Crenicichla inpa	Br 174, km 14, AM, Brazil	48	6m, sm+42st, a	54	1°, q, i	Present paper
	Crenicichla britskii	Jupiá River, PR, Brazil	48	8m, sm+40st, a	56	4°, q, t	Present paper
lacustris	*Crenicichla iguassuensis*	Iguaçu River, PR, Brazil	48	8m, sm+40st, a	56	1°, -, i	Mizoguchi and Martins-Santos, 2000
	Crenicichla iguassuensis	Iguaçu River, PR, Brazil	48	6m, sm+42st, a	54	1°, p, i	Lorscheider and Margarido, 2004
	Crenicichla lacustris	Registro, SP, Brazil	48	6m, sm+42st, a	54	1°, p, i	Feldberg and Bertollo, 1985a, b
	Crenicichla niederleinii	Paraná River, PR, Brazil	48	14m, sm+34st, a	62	1°, p, i	Martins *et al.*, 1995
	Crenicichla niederleinii	Tibagí River, PR, Brazil	48	10m, sm+40st, a	58	2 cr.	Loureiro *et al.*, 2000
	Crenicichla niederleinii	Misiones, Argentina	48	6m, sm+42st, a	54	1°, p, i	Fenocchio *et al.*, 2003
wallacii	*Crenicichla notophthalmus*	Commercial source	48	6m, sm+42st, a	54	-	Thompson, 1979
reticulata	*Crenicichla reticulata*	Uatumã River, AM, Brazil	48	6m, sm+42st, a	54	1°, p, i	Feldberg *et al.*, 2004
	Crenicichla reticulata	Careiro, AM, Brazil	48	6m, sm+42st, a	54	1°, p, i	Present paper
	Crenicichla semifasciata	Misiones, Argentina	48	6m, sm+42st, a	54	1°, -, i	Fenocchio *et al.*, 2003
	*Crenicichla semifasciata**	Miranda, MS, Brazil	48	6m, sm+42st, a	54	1°, p, i	Feldberg and Bertollo, 1985a, b
lugubris	*Crenicichla strigata*	Commercial source	48	6m, sm+42st, a	54	-	Thompson, 1979
	Crenicichla vittata	Miranda, MS, Brazil	48	6m, sm+42st, a	54	1°, p, i	Feldberg and Bertollo, 1985a, b
	Crenicichla cincta	Catalão, AM, Brazil	48	8m, sm+40st, a	56	1°, p, t	Present paper
	Crenicichla lugubris	Catalão, AM, Brazil	48	8m, sm+40st, a	56	3°, q, t	Present paper
	Crenicichla cf. *johanna*	Catalão, AM, Brazil	48	8m, sm+40st, a	56	24°, p, t	Present paper
Unidentified	*Crenicichla* sp.	São Benedito River, Itajaí, SC, Brazil	48	8m, sm+40 st, a	56	2 cr.	Loureiro *et al.*, 2000

(1992). All species analyzed in the present study exhibited 2n = 48 chromosomes. Although the overall karyotypes are uniform (Figure 1A-F), they could be separated into two groups, based on their chromosomal formulae: *C. cincta, C. lugubris, C.* cf. *johanna*, and *C. britskii* exhibit 8M-SM+40ST-A and FN = 56, and *C. inpa* and *C. reticulata* exhibit 6M-SM+42ST-A and FN = 54. No sex heteromorphism was detected in species where it was possible to analyze males and females.

A single pair of NORs was observed in all six species; however, these cistrons were located on different chromosome pairs and/or different locations. In *C. cincta* the Ag-NORs were located on the terminal region of the short arms of the first pair (M-SM); in *C. inpa* they were located on the interstitial region of the long arms of the first pair (M-SM); in *C. reticulata* the Ag-NORs were located on the interstitial region of the short arms of the first pair (M-SM); in *C. lugubris* the Ag-NORs were located on the terminal region of the long arms of the third pair (M-SM); in *C.* cf. *johanna* the Ag-NORs were located on the terminal region of the short arms of the 24th pair (ST-A); and in *C. britskii* the Ag-NORs were located on the terminal region of the long arms of the fourth pair (M-SM). Heteromorphism in the size of the NORs was detected in all species, more markedly in *C. britskii* and *C.* cf. *johanna* (Figure 1A-F).

All the six analyzed species were characterized by several C-band positive heterochromatin blocks in the pericentromeric region of all chromosomes, although some of theses blocks were more conspicuous than others. In *C. cincta, C. inpa* and *C. reticulata,* the Ag-NORs were not coincident with heterochromatic blocks, although these ribosomal cistrons were closely adjacent to clearly evident C-bands. In contrast, in *C lugubris*, *C.* cf. *Johanna*, and *C. britskii,* Ag-NORs were coincident with C-bands. Moreover, some other conspicuous heterochromatic blocks could also be observed in the six species. *C. reticulata* presented an interstitial heterochromatic block on the long arms of the 10th chromosome pair of its karyotype complement. *C. lugubris* showed an interstitial C-band on the long arms of the second chromosome pair, and *C.* cf. *johanna* has a terminal heterochromatic block on the long arms of the 19th chromosome pair. In *C britskii*, it was possible to detect a heterochromatic block that occupies the whole short arms of the first chromosome pair (Figure 1 G-L). These unique heterochromatic blocks are chromosome markers that could also be useful in future comparisons among several *Crenicichla* species.

The relationships among Neotropical cichlids have been the object of evolutionary studies using morphological (Kullander, 1998), molecular (Farias *et al.*, 2000), and karyological data (Feldberg *et al.*, 2003). All these data agree that Neotropical cichlids form a monophyletic group.

According to Kullander (1998), Neotropical cichlids are organized in five major lineages, where the subfamily Cichlinae occupies a basal position and encompass only two genera: *Cichla* and *Crenicichla*. In a recent review, Feldberg *et al.* (2003) showed that *Crenicichla* and *Cichla* appear to fall into different karyoevolution directions. *Cichla* species exhibit only acrocentric chromosomes and seem to be the most primitive (Thompson, 1979; Alves-Brinn *et al.*, 2004), while *Crenicichla* exhibits chromosomal variability in characteristics like karyotypic formula and fundamental chromosome number (Thompson, 1979; Feldberg and Bertollo, 1985a,b; Martins *et al.*, 1995; Loureiro *et al.*, 2000; present paper). According to Thompson (1979), the presence of a greater number of meta-submetacentric chromosomes characterizes a derived state, since the karyotype considered ancestral for this group has only acrocentric chromosomes.

Whereas Farias *et al.* (2000) proposed that *Crenicichla* might be related with *Apistogramma* forming the subfamily Geophaginae, Feldberg *et al.* (2003) showed that *Crenicichla* and *Apistogramma* fall into different karyoevolution directions with *Apistogramma* presenting 46 chromosomes. However, a closer look on the chromosomal data presented by the authors reveal that the gross karyotype macrostructure of *Crenicichla* and the remaining geophagines is quite similar.

In fact, *Crenicichla* species exhibit a diploid number of 48 chromosomes, except for *Crenicichla* sp. from the Paraná-Paraguay basin which supposedly possess 2n = 46 chromosomes (nome dos autores, unpublished data). Often, chromosomal variability can be attributed to problems in analysis, as well as to the interpretation of chromosome measurements. Special care must be taken when these comparisons are made among cichlids, since there are cases where cytogenetic differences indicated the existence of different species (Feldberg *et al.*, 2003; Alves-Brinn *et al.*, 2004).

Data regarding the prevalence of chromosomal rearrangements in Neotropical cichlids have been obtained mainly through conventional cytogenetic studies (Feldberg *et al.*, 2003). Apparently, non-Robertsonian rearrangements, such as paracentric/pericentric inversions and translocation, might be postulated as the source of differentiation among *Crenicichla* species, taking into account the NORs carriers as a hallmark. Thus, the phenetic NOR character seems to be a good tool for differentiating *Crenicichla* species (Figure 1, Table 1). Of the 17 nominal *Crenicichla* species that have already been studied (Table 1), 13 had their NOR pattern determined. All 13 species exhibit a single NOR pattern, with the exception of a population of *C. lepidota* from the Paraná River. This population exhibits four NOR-bearing chromosome markers, characterizing a multiple NOR system (Martins *et al.*, 1995).

According to Feldberg *et al.* (2003), the general trend in the family Cichlidae is to exhibit a single NOR system, with only one pair of NORs located on the largest chromosome of the complement. This chromosomal character seems to be plesiomorphic for cichlids. This is particularly

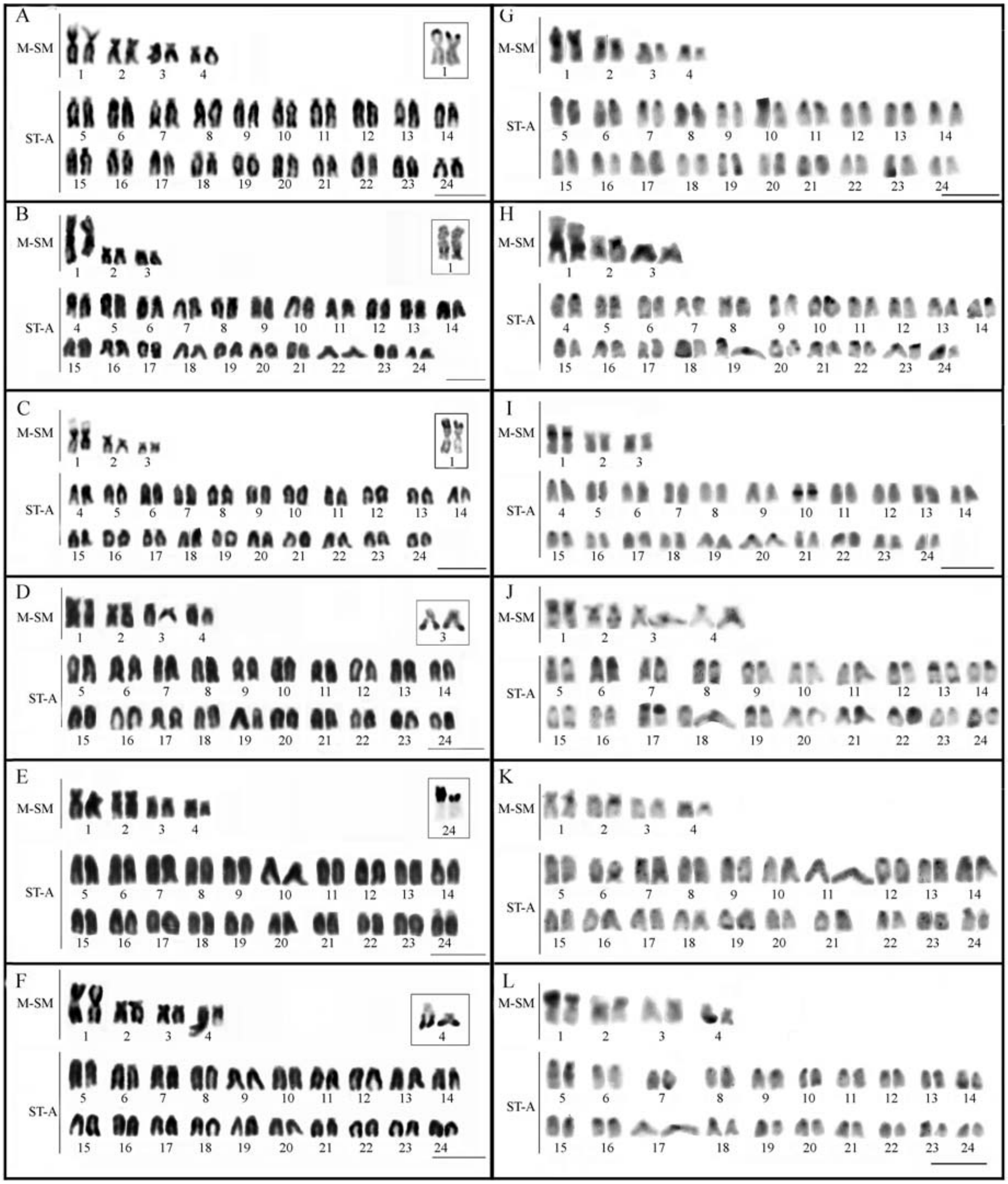

Figure 1 - Conventional Giemsa-stained and C-banding karyotypes of: (A and G) *C. cincta*; (B and H) *C. inpa*; (C and I) *C. reticulata*; (D and J) *C. lugubris*; (E and K) *C.* cf. *johanna*; and (F and L) *C. britskii.* In evidence, Ag-NORs pairs. Bar represents 10 µm.

true for the majority of the *Crenicichla* species. Every time that a large M-SM chromosome pair is observed, it usually bears Ag-NORs (except for *Crenicichla britskii*). When such a large M-SM chromosome pair is not so evident, then the Ag-NORs position is not on the first chromosome pair. In this way, *C. reticulata*, *C. inpa*, and *C. cincta* might be considered more primitive than *C.* cf. *joahna*, *C. lugubris*, and *C. britskii*, although the last species also presents a large M-SM chromosome pair in the complement.

Feldberg *et al.* (2003) also reported that constitutive heterochromatin, in the few Neotropical cichlids analyzed so far, is present in the pericentromeric region of all chromosomes or in most of them. However, interspecific differences are clearly observed in *Crenicichla*, where interstitial and/or telomeric blocks, as well as regions at or near the NORs, reflect part of the chromosomal differentiation of the species.

The heterochromatin patterns of the species analyzed here are very similar; however, each species exhibits at least one species-specific block (Figure 1G-L). In addition, sequential Ag-NOR staining/C-banding in the same metaphase led us to observe the correspondence of C-bands and NORs in *C lugubris*, *C*. cf *Johanna*, and *C. britskii*, indicating the presence of heterochromatin associated with ribosomal cistrons. Yet, in *C. cincta, C. inpa*, and *C. reticulata* the Ag-NORs were C-band negative, but adjacent to a heterochromatic block.

Several studies in fish shave shown that the association of heterochromatin and NORs is an important element in chromosome differentiation. For example, Galetti *et al.* (1991) postulated for anostomids that heterochromatin associated with NORs may have favored breaks and consequent rearrangements during the chromosomal evolution. Furthermore, Ruiz *et al.* (1981) and Clark and Wall (1996) claimed that constitutive heterochromatin is often associated with ribosomal cistrons in the NORs of eukaryotic chromosomes. Thus, we are assuming for *Crenicichla* species that the association of heterochromatin and NORs would have played an important role in their chromosomal differentiation.

Our comparisons of cytogenetic patterns of the genera *Cichla* and *Crenicichla* make it clear that *Crenicichla* presents a more derived condition. It is interesting to note that *Crenicichla* possesses a much wider natural distribution than *Cichla*. Species of the *C. lacustris* group, which are restricted to the southern portion of the genus distribution, exhibit less-conserved characteristics than other members of this taxon. This may indicate that the dispersion and speciation of the genus *Crenicichla* involved non-Robertsonian rearrangements, principally inversions, and that these have occurred from North to South on the South American continent.

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Internet Resources

Fish Database, http://www.fishbase.org/search.cfm (access on july 19th, 2006).

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