Cytogenetic Studies and Evolutive Considerations on Fishes of the Family Cichlidae (Perciformes) from Parana River (Argentina)

Héctor Alberto Roncati, María Cristina Pastori
and Alberto Sergio Fenocchio

Universidad Nacional de Misiones, Facultad de Ciencias Exactas, Químicas y Naturales,
Departamento de Genética, Cátedra de Citogenética General, Félix de Azara 1552 3300 Posadas,
Misiones, Argentina

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Summary Among the Perciformes, Cichlidae represent the more specious group, geographically distributed in Africa, Asia and America. In the present work, 7 species of 5 genera of cichlids from the Paraná River (Misiones, Argentina) were cytogenetically characterized and discussed some aspects of the chromosome evolution. The following chromosome numbers and formulae: were found for each analyzed species: *Crenicichla niederleini* $2n=48$ (6 M-SM; 42 ST-A), *Crenicichla lepidota* $2n=48$ (6 M-SM; 42 ST-A), *Gymnogeophagus* sp. n. $2n=48$ (2 M-SM; 46 ST-A), *Gymnogeophagus balzanii* $2n=48$ (2 M-SM; 46 ST-A), *Apistogramma trifasciata* $2n=46$ (16 M-SM; 30 ST-A), *Cichlasoma dimerus* $2n=48$ (8 M-SM; 40 ST-A) and *Bujurquina vittata* $2n=44$ (22 M-SM; 8 ST-A; 14 microchromosomes). The C-positive heterochromatin was observed preferentially in pericentromeric regions of some chromosomes and NORs at the secondary constriction on the first chromosome pair. In *B. vittata* the microchromosomes are not heterochromatic and the 5th pair shows short arms entirely stained and, in coincidence, the AgNORs appears located at the same region. The results obtained evidence a wide chromosomal variation, either in number and structure that could be integrated with morphological and molecular data in phylogenetic studies.

Key words Fishes, Cichlidae, Cytogenetics.

Among the Perciformes, the Cichlidae family shows the highest species number in freshwater and are distributed in Africa, Asia and America, along the Neotropical Region. At now have been described about 450 species (Kullander 1998) and new species are continuously being described (Kullander 2004, López-Fernández and Taphorn 2004, Ready and Kullander 2004, Schelly and Stiasny 2004, Staack and Schindler 2005).

With the background of discoveries on Cichlidae biodiversity in the great lakes of Eastern Africa, new hypothesis and theories are built to explain the adaptive radiations of the group (Nagl *et al.* 1998, Albertson *et al.* 1999, Kocher 2004, Salzburger *et al.* 2005). The first cytotaxonomic studies (Thompson 1979) showed that cichlids maintain the karyotype macrostructure with $2n=48$ chromosomes, varying slightly by means of pericentric inversions and deletions. However, in some further reports including other techniques (C-bands, AgNORs) were studied species from Brazil, showing variation in chromosome number and morphology (Feldberg and Bertollo 1985a, b, Martins-Santos *et al.* 1995, 2005, Salgado *et al.* 1994, Brum *et al.* 1998, Loureiro *et al.* 2000). In the present paper were cytogenetically characterized species belonging to 5 genera of cichlids from the Paraná River (Misiones, Argentina), discussing some aspects about the chromosome evolution of the group.

* Corresponding author, e-mail: hroncati@gwail.com

Materials and methods

Were cytogenetically analyzed 120 individuals of the Cichlidae family, belonging to the following subfamilies: Cichlinae: *Crenicichla niederleinii*, *Crenicichla lepidota*; Geophaginae: *Gymnogeophagus* sp. n., *Gymnogeophagus balzanii*, *Apistogramma trifasciata* and Cichlastominae: *Cichlasoma dimerus*, *Bujurquina vittata* (Kullander 1998). Samples were collected at the Paraná River (Posadas, Misiones, Argentina).

Mitotic preparations were obtained from kidney cells according the techniques of Bertollo *et al.* (1978) and Fenocchio *et al.* (1991). C-banding and nucleolus organizer regions were evidenced as described by Sumner (1972) and Howell and Black (1980), respectively. Chromosomes were arranged in karyotypes and classified as metacentric (M), submetacentric (SM), subtelocentric (ST) and acrocentric (A) (Guerra 1986).

Results and discussion

Were found for each analyzed species the following diploid numbers and chromosome formulae: *Crenicichla niederleinii* $2n=48$ (6 M-SM; 42 ST-A) (Fig. 1a), *Crenicichla lepidota* $2n=48$ (6 M-SM; 42 ST-A) (Fig. 2a), *Gymnogeophagus* sp. n. $2n=48$ (2 M-SM; 46 ST-A) (Fig. 3a), *Gymnogeophagus balzanii* $2n=48$ (2 M-SM; 46 ST-A) (Fig. 4a), *Apistogramma trifasciata* $2n=46$ (16 M-SM; 30 ST-A) (Fig. 5a), *Cichlasoma dimerus* $2n=48$ (8 M-SM; 40 ST-A) (Fig. 6a) and *Bujurquina vittata* $2n=44$ (22 M-SM; 8 ST-A; microchromosomes) (Fig. 7a).

The C-positive heterochromatin was located in pericentromeric regions of some chromosomes in *Crenicichla niederleinii*, *Gymnogeophagus* sp. n. and *Gymnogeophagus balzanii* (Figs. 1b, 3b and 4b). In *Crenicichla lepidota* C-banding evidenced great blocks and some chromosomes totally heterochromatic (Fig. 2b). In some of these species the NORs were located on the secondary constriction in the first chromosome pair (Figs. 1c and 4c).

In *Cichlasoma dimerus* the greather part heterochromatin are in the pericentromeric region, being observed conspicuous bands on some small chromosomes (Fig. 6b). *Apistogramma trifasciata* and *Bujurquina vittata* also show some pericentromeric weak C-bands, with size and stain variations (Figs. 5c and 7c). In *B. vittata* the microchromosomes are not heterochromatic and the pair 5th shows their short arms entirely stained (Fig. 7b) and, in coincidence, the AgNORs are appears located on these same regions (Fig. 7c).

Here are confirmed the cytogenetic characteristic of some species and reported for the first time the karyotypes of other, and could be observed, in agreement with the available literature that several of the studied species showed $2n=48$ indicating the conservatism of the karyotypic macrostructure, characteristic trait of the Order and Family (Thompson 1979). Among the Cichlinae, *Crenicichla* presents $2n=48$ with 2 to 8 biarmed chromosomes (Martins *et al.* 1995, Loureiro *et al.* 2000) and due to these karyotype characteristics these group could be located with Chaetobranchinae in a basal phylogenetic level in relation to the rest of the Neotropical Cichlidae (Brum *et al.* 1998, Kullander 1998).

Among the Geophaginae, *Apistogramma trifasciata* ($2n=46$) have shown a reduction in the chromosome number related with an increase in the number of biarmed ones, suggesting that centric fusions could be important in the evolution of the group (Oliveira *et al.* 1988). *Gymnogeophagus* ($2n=48$; NF=50–52) and *Guianacara* ($2n=48$; NF=52) have nearly basal karyotypes among the Perciformes, however, *Apistogramma* that is considered a more derivate genus, show diploid numbers ranged from 38 to 46 and 48 with NF=60 and 70 (Brum *et al.* 1998).

Bujurquina vittata (Cichlasomatinae) have the lower chromosome number among the studied species, almost all biarmed, few acrocentric and 14 microchromosomes, features that clearly evidence the complex rearrangements occurred along the evolution of this specie. In Neotropical cich-

Fig. 1. *Crenicichla niederleinii*. a. Karyotype $2n=48$, b. C banding, c. NOR banding the arrows indicate NORs regions.

Fig. 2. *Crenicichla lepidota*. a. Karyotype $2n=48$, b. C banding.

Fig. 3. *Gymnogeophagus* sp. n. a. Karyotype $2n=48$.

Fig. 4. *Gymnogeophagus balzanii*. a. Karyotype $2n=48$, b. C banding, c. NOR banding.

Fig. 5. *Apistogramma trifasciata*. a. Karyotype $2n=46$, b. C banding arrows indicate NORs regions.

Fig. 6. *Cichlasoma dimerus*. a. Karyotype $2n=48$, b. C banding.

Fig. 7. *Bujurquina vittata*. a. Karyotype $2n=44$, b. C banding, c. NOR banding, the arrows indicate NORs regions.

Table 1. Chromosome numbers and morphology of the studied species (M-SM, ST-A and Microchromosomes)

Species	Diploid number	Chromosome types			
		NF	M-SM	ST-A	Microchromosomes
<i>Crenicichla lepidota</i>	48	54	6	42	
<i>Crenicichla niederleinii</i>	48	54	6	42	
<i>Gymnogeophagus balzanii</i>	48	50	2	46	
<i>Gymnogeophagus</i> sp. n.	48	50	2	46	
<i>Cichlasoma dimerus</i>	48	56	8	40	
<i>Apistogramma trifasciata</i>	46	62	16	30	
<i>Bujurquina vittata</i>	44	66	22	8	14

lids have been reported only another case of microchromosomes in *Symphysodon discus* (Salgado *et al.* 1994). On the other hand, in *Laetacara dorsigera*, from the Paraná River (Brazil), belonging to the same subfamily, were found 4 cytotypes, with variation in number and chromosome morphology (Martins *et al.* 2005), evidencing a particular evolution pathway. Supporting these observations, the molecular data suggest that this subfamily represent the more derivative lineage of the Neotropical cichlids (Farias *et al.* 1998). All the karyotype features, chromosome numbers and formulae are summarized in Table 1.

C-banding revealed pericentromeric heterochromatic blocks in some chromosomes of the species with $2n=48$, except *Crenicichla lepidota* and *Cichlasoma dimerus*, and a not-regular pericentromeric, interstitial and telomeric distribution in *Apistogramma trifasciata* ($2n=46$) and *Bujurquina vittata* ($2n=44$). Variation in amount and localization of C-bands reveals that events as duplication and rearrangement of heterochromatin were important in the evolution of these species.

Present data, obtained from species belonging to several subfamilies evidence, contrarily to the general view of the Cichlidae as a highly conservative group, a broad chromosomal variation, either in number and structure, that could be integrated with morphological and molecular ones in phylogenetic studies.

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