Analysis of Karyotypic Evolution in Natural Populations of *Cynolebias* (Pisces: Cyprinodontiformes, Rivulidae) Using Banding Techniques

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Many genera belonging to the family Rivulidae (Pisces, Cyprinodontiformes) show evolutionary tendency to a decrease in their chromosome numbers, considering that the basal chromosomal number found in Teleosts is $2n=48$. This process is accompanied by an increase in the number of banded chromosomes. Studying the karyotypes of 127 genera Rivulinae, Scheel (1972) postulated two types of chromosomal rearrangements alternating the evolution of these karyotypes: pericentric inversions and centric fusions. This author suggested that centric fusions can occur between either subtelocentric or acrocentric chromosomes (ST-A), giving rise to metacentrics. Pericentric inversions would then transform the metacentric chromosomes into larger telocentrics. These pericentric inversions would then be followed by new centric fusions, thus generating especially large banded chromosomes. Under this apparently simple reorganization system, parallel evolution would have occurred in many genera. Scheel (op. cit.) particularly described the chromosomal differences found in species of the genus *Aphyosemion*.

The genus *Cynolebias* Steindachner 1876 is considered to be biologically related to genera *Aphyosemion* and *Notobranchius* because all their species have annual life cycles. The species of *Cynolebias* originally proved to be chromosomally uniform, having 48 acrocentric chromosomes (Sola *et al.* 1981). However, extensive chromosomal variation was found in specimens belonging to populations collected in Uruguay (Máspoli and García 1988, García *et al.* 1988). These studies demonstrated that: 1) Species with low chromosome number have more banded chromosomes. 2) The "Número fundamental" (N.F.s) of some species is the same as the chromosome number of others. 3) Species with the same diploid number have different N.F.s. This evidence led us to consider the possibility that the alternating fusions and pericentric inversions proposed by Scheel (1972) for all Rivulinae were actually occurring during the karyotypic evolution of *Cynolebias*.

The main objectives of this work were: first, to analyze the karyotypic dynamics in natural populations of *Cynolebias* using C and NOR chromosome banding techniques; and second to further examine Scheel's hypothesis of karyotypic evolution using these chromosome banding techniques.

Material and methods

The specimens used in this study came from natural populations found in temporary pools belonging to 8 species of *Cynolebias*. These species are separated in two groups by an apparent geographic barrier: "Cuchilla Grande" and "Cuchilla Grande del Este". Specimens coming from 10 different localities in Uruguay and one in Argentina (Fig. 1) were analyzed.

Fig. 1. Location of the 11 collection sites of the genus *Cynolebias*: 10 from Uruguay, one from Argentina. The species collected are: (1) *C. bellottii*, (2) *C. nigripinnis*, (3) *C. viarius*, (4) *C. sp.*, (5) *C. melanotaenia*, (6) *C. wolterstorffi*, (7) *C. prognatus*, (8) *C. luteoflammulatus*.

Table 1. Sex, number of specimens and localities (numbers are in Fig. 1)

Species	Localities	No ♂♂	No ♀♀	Total N°
1. <i>C. bellottii</i>	Salto*	3 (161,155,192)	—	7
	R. 12-km 145**	4 (248,261,262,260)	—	
2. <i>C. nigripinnis</i>	Salto*	2 (152,157)	1 (154)	5
	Artigas*	1 (50)	—	
	R. 12-km 145**	1 (263)	—	
3. <i>C. viarius</i>	"La Cruz"	4 (101,118,225,226)	—	
	19 de Abril*	1 (102)	—	6
		1 (6)	—	
4. <i>C. sp.</i>	R. 19*	4 (227,228,229,68)	1 (95)	
	"Kambara"	1 (92)	—	7
	"Camino Indio"	—	1 (99)	
5. <i>C. melanotaenia</i>	"Camino Indio"	1 (223)	—	
	R. 19*	—	1 (204)	4
	R. 10*	1 (163)	1 (187)	
6. <i>C. wolterstorffi</i>	"La Salina"	2 (104,130)	—	2
7. <i>C. prognatus</i>	R. 19	1 (120)	1 (121)	3
	"La Salina"	1 (105)	—	
8. <i>C. luteoflammulatus</i>	"Pto. de los Botes"	2 (56,57)	—	4
	R. 19	—	1 (184)	
	"Camino Indio"	1 (39)	—	

* localities from Uruguay

** localities from Argentina

Fig. 2. Conventional karyograms of four species of *Cynolebias*. a. *Cynolebias nigripinnis*. Conventional karyogram of male specimen 157 (Salto, Uruguay) obtained from Giemsa stained somatic, mitotic chromosomes. $2n=48, m (M+SM)=8$ and $a (ST+A)=40$. b. *Cynolebias viarius*. Conventional karyogram of male specimen 6 (Valizas, Rocha, Uruguay) obtained from lacto-acetic orcein stained somatic, mitotic chromosomes. $2n=46 m (M+SM)=2$ and $a (ST+A)=44$. c. *Cynolebias prognatus*. Conventional karyogram of male specimen 120 (San Miguel, Rocha, Uruguay) obtained from lacto-acetic orcein stained spermatogonial, mitotic chromosomes. $2n=36+1 m (M+SM)=12$ and $a (ST+A)=24+1$. d. *Cynolebias luteoflammulatus*. Conventional karyogram of male specimen 39 (Cno. del Indio, Rocha, Uruguay) obtained from lacto-acetic orcein stained somatic, mitotic chromosomes. $2n=34 m (M+SM)=16$ and $a (ST+A)=18$. The horizontal bars represent 10 μm.

Table 1. gives the detailed number, sex and localities of all specimens.

All individuals were processed using conventional techniques for the study of fish chromosomes (Mc Phail and Jones 1966, Bertollo 1988). Somatic tissues, (branchia, kidney and intestines) and in some cases gonadal mitosis were studied. Chromosomes were classified according to Levan *et al.* (1964) adopting the modifications by Denton (1973) for fish studies. They were grouped in two categories: biarmed *m* (M-SM) and uniarmed *a* (ST-A). When calculating the N.F.s, evident small arms were considered.

Giemsa and Lacto—Acetic Orcein were used as conventional stains. C-banding was carried out according to Sumner (1972) and modified for this material. Howell and Black's (1980) silver staining technique for NOR was used. This silver NOR technique was applied to 17 specimens (one or two per species), and ten metaphases per individual were examined.

Table 2. Chromosomal constitutions of species analysed

Species	2n	NF	<i>m</i>	<i>a</i>	C-bands position	N° NORs	Positions NORs
<i>C. bellottii</i>	48	52	4	44	t	5-6	t
	48	54	6	42			
<i>C. nigripinnis</i>	48	76	6	42	c-t	5-6	t
	48	80	6	42			
	48	72	8	40			
	48	62	6	42			
<i>C. viarius</i>	48	50	2	46	c	6	t
	46	50	4	42			
	46	50	2	44			
<i>C. sp.</i>	48	52	4	44	c-t	6	t
	46	52	6	40			
	48	56	8	48			
<i>C. melanotaenia</i>	44	58	14	30	t	5	Subm/peric peric/t
<i>C. wolterstorffi</i>	46	50	4	42	c	5	t
<i>C. prognatus*</i>	36	48	12	24	c-t	3	Subm/t
	36	52	10	26			
	36+1	55	12	24+1			
<i>C. luteoflammulatus**</i>	34	56	16	18	c-t	4	t
	34	48	14	20			

m=M+SM, *a*=ST+A; t=terminal, c=centromeric, i=interstitial, Subm=submedian, peric=pericentromeric.

*3 to 5 pairs of large biarmed chromosomes.

**6 pairs of large biarmed chromosomes.

Results

Chromosome numbers and N.F.s

Among the 37 specimens, belonging to 8 species of the genus *Cynolebias*, diploid numbers varied from the basic 48 chromosomes to 2n=34. The "Nombre fundamental" had more extensive variation, from 48 to 80 (Table 2). The specific chromosomal constitutions (*m*=M+SM, *a*=ST+A) are presented in Table 2.

Six specimens of *C. bellottii* had 2n=48 and NF=52 while one specimen from Argentina had 2n=48 and NF=54 (Table 2). Three specimens of *C. nigripinnis* had 2n=48 and NF=76, while one specimen from Argentina had 2n=48 and NF=80 (Table 2). Specimen in Fig. 2a belonging to the same species, had 2n=48 and NF=72 and the individual of Fig. 3a had a 2n=48 and NF=62. Three specimens of *C. viarius* had 2n=48 and NF=50 and two other

Fig. 3. C-banded karyogram of two species of *Cynolebias*. The corresponding Ag-NOR bearing chromosomes are located under homologous C-pair.

a. *Cynolebias nigripinnis*. C-banded karyogram of somatic mitotic chromosomes obtained from male specimen 50 (Artigas, Uruguay). $2n=48m$ ($M+SM$)=6 and a ($ST+A$)=42. Ag-NOR bearing chromosomes of male specimen 152 (Salto, Uruguay) were obtained from a somatic, mitotic metaphase and set under the corresponding C-pair. Pairs 1, 6 and 17 carry Ag-NORs. Pair 1 of the C-banded karyotype has a heterochromatic small arm corresponding to the NOR position. Members of C-pair 6 have subterminal secondary constrictions and terminal C-bands on the long arm. Members of pair 17 have terminal C-bands corresponding to the NOR position. b. *Cynolebias prognatus*. C-banded karyogram obtained from somatic mitotic chromosomes of male specimen 120 (San Miguel, Rocha, Uruguay). $2n=36m$ ($M+SM$)=10, a ($ST+A$)=26. The position of C-bands on the 5 $M+SM$ pairs has been indicated (c=centromeric, t=terminal, i=interstitial). Ag-NOR bearing chromosomes were obtained from somatic, mitotic chromosomes of the same specimen (120). Both members of pair 8 have terminal C-bands on the same position as the Ag-NOR. The smallest pair, 17 also has terminal C-bands in the same position of the Ag-NOR. The horizontal bars represent 10 μm .

specimens showed $2n=46$ and $NF=50$ Table 2 (Fig. 2b). Five specimens of *C. sp.* had $2n=48$ and $NF=52$, but others had $2n=46$ and $NF=52$, while still others showed $2n=48$ and $NF=56$ (Table 2). Four specimens of *C. melanotaenia* had $2n=44$ and $NF=58$ (Table 2). Two specimens of *C. wolterstorffi* had $2n=46$ and $NF=50$. Three specimens of *C. prognatus* had $2n=36$ and $NF=48$ (Table 2). A specimen of the same species had $2n=36$ on somatic tissues and NF of 52 (Fig. 3b). In the spermatogonial mitotic metaphase diagrammed in Fig. 2c, of the same individual $2n=36+1$ and $NF=55$. Three specimens of *C. luteoflammulatus* showed $2n=34$ and $NF=48$ (Table 2), and one specimen $2n=34$ and $NF=55$ (Fig. 2d).

Fig. 4. Ag-NOR bearing chromosomes in three species of *Cynolebias*.

a. *Cynolebias sp.* A mitotic nucleus treated with Ag-NOR technique from male specimen 228 (Rta. 19, Rocha, Uruguay) $2n=46$. The NOR bearing chromosomes are indicated (N). b. *Cynolebias sp.* Under NOR specification chromosomes bearing the Ag-NORs (four pairs) were cut out from a nucleus as in Fig. 3a. The conventionally stained (CS) mitotic chromosomes corresponding to those carrying Ag-NORs were obtained from somatic, mitotic chromosomes of female specimen 22 (Chuy, Rocha, Uruguay) stained with lacto-acetic orcein. $2n=46$. c. *Cynolebias viarius*. Mitotic chromosomes carrying Ag-NORs of male specimen 226 (Rta. 10, 16, "La Cruz", Rocha, Uruguay) are found under the NOR specification (three pairs). $2n=46$. Conventionally stained (CS) chromosomes homologous to those coming from the same somatic, mitotic nucleus in Fig. 1, b of male specimen 6 (Valizas, Rocha, Uruguay) $2n=46$. d. *Cynolebias luteoflammulatus*. Under NOR, the two somatic mitotic chromosome pairs bearing Ag-NOR of female specimen 184 (Chuy, Rocha, Uruguay). $2n=34$. The homologous pairs (CS) come from the same somatic, mitotic nucleus in Fig. 1, d, male specimen 39 (Cno. del Indio, Rocha, Uruguay).

C-banding

All species were analyzed with the C-banding technique (28 individuals). C-bands were observed in three chromosomal regions: centromeric, interstitial and telomeric.

The distribution of these C-bands followed a definite pattern in the different species (Table 2). *C. nigripinnis* had centromeric and telomeric bands (Fig. 3a, Table 2). *C. wolterstorffi* had predominantly centromeric C-bands (Table 2). In *C. bellottii*, *C. viarius* and *C. melanotaenia* C-bands have a telomeric position (Table 2). *C. sp.* has telomeric, centromeric and few interstitial C-bands (Table 2). *C. prognatus* and *C. luteoflammulatus* showed three types of C-bands (Fig. 3b, Table 2).

NOR technique

The NOR regions were identified on metaphase chromosomes with silver stain. They varied in number, 2 to 4 homologous pairs bearing Ag-NORs according to each species (Fig. 3a, b, 4a, b, c, d, Table 2). They were localized mainly at terminal positions showing no specificity to chromosome types.

In *C. bellottii* two specimens had 5 chromosomes with NORs while one individual from the same population had 6. All NORs in this species were found at terminal positions. The pairs having NORs at the terminal regions of the corresponding chromosomes, were found in the only specimen belonging to *C. nigripinnis* (Fig. 3a) from Salto. However, pair 1 bears an Ag-NOR on the short arm which is observed as C-heterochromatic (Fig. 3a). The other terminal pairs involved in NORs have them in terminal position on the long arm. In *C. viarius* (Fig. 4c) three pairs having Ag-NORs at terminal positions were observed. *C. sp.* (Figs. 4a, b) presents 4 chromosome pairs with 8 Ag-NORs. In pair 3 which appears positively heteropycnotic with orcein stain had a submedian to terminal position; pair 5 has a pericentromeric Ag-NOR. In *C. melanotaenia* (Table 2) 5 NORs were observed. They had submedian-pericentromeric position on pairs 5 and 12, while one Ag-NOR was found in pericentromeric-terminal position on chromosome 16. *C. wolterstorffi* (Table 2) had 5 NORs at terminal positions in one specimen. In *C. prognatus* (Fig. 3b) 3 Ag-NORs were observed at submedian to terminal positions. In one specimen of *C. luteoflammulatus* (Fig. 4d) only 2 pairs of chromosomes were found to bear NORs at terminal positions. Heteromorphism for Ag-NORs was observed in all species.

Heteromorphic pairs

Chromosome pairs varying in size and morphology were observed in a few cases. (Fig. 2b, pair 1, 2d, pairs 1, 8, 9, 3a, pair 1, 3b, pair 1).

Discussion

Variation in chromosome numbers observed here in species of the genus *Cynolebias* from Uruguay confirm our previous findings of high levels of karyotypic variation (Maspoli and Garcia 1988, Garcia *et al.* 1988). In these papers standard karyotypes of 7 species were compared. Intra population variation of chromosome numbers and NF were also observed. The present data are consistent with the hypothesis that pericentric inversions and centric fusions have occurred in the karyotypic reorganization of this group, just like those postulated by Scheel (1972) for all the Rivulinae.

The C-banding results found in the 8 species are different to those observed in other Cyprinodontiformes. Members of this order studied so far show pericentromeric C-bands and very few interstitial and telomeric ones. These typical positions were documented by Kornfield (1981) in many species of *Fundulus*; Uwa and Ojima (1981) in *Oryzias latipes* and *O. celebensis*; and by Turner (1985) in *Ilyodon furcidens*. This situation is not the same in other species of Pisces. Foresti *et al.* (1981) found interstitial blocks of C-heterochromatin along with pericentromeric C-bands in *Apteronotus albifrons*. Oliveira *et al.* (1990) report

in *Corydoras natteri* many chromosome pairs with small pericentromeric C-band positive blocks and other pairs which showed pericentromeric blocks extending to the long arms. Phillips and Hartley (1988) described three species of the genus *Salmo* with C-bands in three positions: telomeric, centromeric and interstitial.

The observations found for the different species of *Cynolebias* are in agreement with these statements. In addition, several papers have revealed a considerable degree of polymorphism in the amount and position of C-bands (John and King 1977, John and Micklos 1979, King and John 1980).

How do the C-banding patterns found in *Cynolebias* relate to Scheel's hypothesis for karyotypic evolution in the Rivulinae? The existence of a hypothetical ancestral karyotype with 48 ST-A chromosomes, as proposed by Ebeling and Chen (1970) for Cyprinodontiformes, would presumably imply the occurrence of centromeric C-bands. These were observed in *C. wolterstorffi* and in *C. nigripinnis* (Fig. 3a). Centric fusions, and the formation of two-armed chromosomes would maintain the centromeric positions of C-bands. This is observed in most biarmed elements in *C. luteoflammulatus* and *C. prognatus* (Fig. 4b, pairs 2, 3, 4). Through pericentric inversions, the biarmed chromosomes can be transformed into ST-A elements so that small C-heterochromatic blocks should be observed at interstitial or telomeric positions. Pericentric inversions of acrocentric chromosomes with predominantly telomeric blocks can give rise to biarmed chromosomes with C-heterochromatin in three positions: telomeric, centromeric and interstitial. (Fig. 3b, pairs 1, 2). Both types of rearrangements occurring in alternation would finally originate large biarmed chromosomes like those proposed by Scheel (1972) with the three types of C-bands. This last situation was observed in *C. prognatus* (Fig. 3b) and *C. luteoflammulatus*.

The present data for 17 specimens of the genus *Cynolebias* are preliminary with respect to number and localization of NORs (Table 2). Intraspecific variation was observed for *C. bellottii* indicating that one obvious focal point for future research is to undergo further studies on the patterns and numbers of rDNA cistrons.

Terminal localization of NORs was frequently observed in most species (Figs. 3a, b, 4a, b, c, d) but in some cases pericentromeric and submedian NORs occurred (Figs. 3b, 4a, b). This variation in number and localization of NORs has been considered by several authors. The present data are consistent with the findings of a high number and variable position for rDNA. Galetti Jr. *et al.* (1985) working with *Serrasalmus spilopleura* (Serrasalminae, Characidae) found multiple NORs, involving up to 10 chromosomes ($2n=60$). In the genus *Astyanax* (Characidae) multiple NORs were also identified, with two to six chromosomes participating in this system (Morelli 1981). Many species in the subfamily Tetragonopterinae (Characidae) have differences in the number and behavior of NORs (Portela *et al.* 1988). One of these species, *Piabina argentea*, has a maximum of 4 NORs per cell. In this case two NORs were found on homologous chromosomes so that only three pairs were involved in rRNA transcription. Bertollo (1988) analyzed three genera of the Erythrinidae and stated that multiple NORs are the most common condition in this family.

There are several interpretations to explain this great variability in numbers and patterns of rDNA. Some include chromosomal rearrangements like deletions and translocations, events that could control the expression and copy number of ribosomal genes, according to their localization. Portela *et al.* (1988) propose that the multiplicity of NORs and their interspecific differences suggests that karyotypic reorganizations could have included the regions that contain the ribosomal genes. Galetti and Foresti (1987) stated that the variability found in NORs of different species of *Leporinus* shows the degree of differentiation present in their karyotypes, including the occurrence of inversions or other types of rearrangements accompanying the process of speciation in this genus.

Species in the genus *Cynolebias* (Table 2) have a maximum of 5 to 6 chromosomes with NORs, having diploid numbers near 48. Those species with low chromosome number (34 and 36) only have 3 to 4 NORs (Figs. 3b, 4d). This reduction in the number or expression of the rDNA cistrons could be a consequence of rearrangements. We could also consider the possibility of NORs dominance (Nicoloff *et al.* 1979). The fusion of two elements having terminal NORs (Fig. 3b) could have functionally inactivated one of them or actually reduced the copy number of the rDNA cistrons. It is possible that during karyotypic reorganization, small segments of rDNA could have been lost, especially those with an initial pericentromeric position as that observed in *C. melanotaenia*. This way we could associate fusions events with a decrease in active NORs.

Otherwise, considering the ubiquity of NORs at terminal regions in the specimens of *Cynolebias* analyzed here, we can suggest that through the occurrence of inversions they could be transferred to a submedian or pericentromeric position, just as was observed in some individuals (Fig. 4b, Table 2).

It is known that Ag-NOR techniques detect only those that were active in the previous interphase. Not all of them are active and heteromorphic chromosome pairs appear as those observed in Figs. 3a, b, 4b, c, d.

In conclusion, we have shown that variation in diploid and fundamental numbers, in amounts and distribution of heterochromatin, and number of NORs, is ubiquitous in the genus *Cynolebias*. The types of variation observed here are consistent with Scheel's (1972) hypothesis about chromosomal evolution in the Rivulinae, by Robertsonian fusions and pericentric inversions. Thus, *Cynolebias* offers us with one ideal system for the study of speciation and chromosomal evolution. Further studies are needed to extend our findings of intraspecific polymorphisms and of somatic variation.

Summary

High levels of variation in chromosome numbers and N.F.s from 48–80. C-banding analysis allowed us to detect C-positive regions in three positions: centromeric, telomeric and interstitial. A differential C-banding pattern was found in each species. These banding patterns can be interpreted as a result of namely pericentric inversions and centric fusions occurring alternatively during the karyotypic reorganization of these species.

The karyotypic analysis of 17 specimens of *Cynolebias* using silver stain NOR technique showed 3–6 rDNA active regions among these species. A reduction in the number or expression of NORs occurred in the species with lower chromosome number as a result of centric fusions. Terminal active NORs seem to be the most common situation among these species. Other positions of the active rDNA cistrons can be explained through chromosomal rearrangements.

All present data are consistent with Scheel's hypothesis that pericentric inversions and centric fusions occurred in the karyotypic reorganization in the Rivulinae.

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References

- Bertollo, L. A. C. 1978. Estudos citogeneticos no genero *Hoplias* Gill, 1903 (Pisces, Erythrinidae). Ph. D. thesis, Universidade de São Paulo, Faculdade de Medicina de Ribeirão Preto, São Paulo, Brasil.
- 1988. As regioes organizadoras de Nucleolos na familia Erythrinidae (Teleostei, Characiformes). Res. II Simp. Citogenetica Evolutiva e Aplicada de Peixes Neotropicais. Maringa, RP, Brazil. pp. 20.
- Denton, T. E. 1973. Fish Chromosome Methodology. C. C. Thomas Ed: Illinois.
- Ebeling, A. W. and Chen, T. R. 1970. Heterogamety in teleostean fishes. Trans. Amer. Fish Soc. **99**: 131–136.
- Foresti, F., Almeida Toledo, L. F. and Toledo Filho, S. A. 1981. Polymorphic nature of nucleolus organizer regions in fishes. Cytogenet. Cell Genet. **31**: 137–144.
- Galetti Jr., P. M., Barbosa da Silva, E. and Cerminaro, R. T. 1985. A multiple NOR System in the *Serrasalmus spilopleura* (Serrasalminae, Characidae). Brazil. J. Genetics **VIII** (3): 479–484.
- and Foresti, F. 1987. Two new cases of ZZ/ZW heterogamety in *Leporinus* (Anastomidae, Characiformes) and their relationship in the phylogeny of the group. Brazil. J. Genetics **x** (1): 135–140.
- García, G., Máspoli, M. C. and Scvortzoff, E. 1988. Estudio citogenético en especies del género *Cynolebias* Steindachner, 1876 (Cyprinodontiformes, Rivulidae). Resúmenes V Reunión Iberoamericana de Conservación y Zoología de Vertebrados. Montevideo, Uruguay. pp. 22.
- Howell, W. M. and Black, D. A. 1989. Controlled silver staining nucleolus organizer regions with protective colloidal developer: a 1-step method. Experientia **36**: 1014–1015.
- John, B. and King, M. 1977. Heterochromatin variation in *Cryptobothrus chrysophorus*. I. Chromosome differentiation in natural populations. Chromosoma **64**: 219–239.
- and Miklos, G. 1979. Functional aspects of satellite DNA and heterochromatin. Int. Rev. Cytol. **58**: 1–114.
- King, M. and John, B. 1980. Regularities and restrictions governing C-band variation in Acridoid grasshopper. Chromosoma **76**: 123–150.
- Kornfield, I. 1981. Distribution of constitutive heterochromatin and the evolution of the sex chromosomes in *Fundulus*. Copeia **4**: 914–918.
- Levan, A., Fredga, K. and Sandberg, A. A. 1964. Nomenclature for centromeric position on chromosomes. Hereditas **52**: 201–220.
- Máspoli, M. C. and García, G. 1988. Estudio comparativo del cariotipo de especies del género *Cynolebias* Steindachner, 1876 (Cyprinodontiformes, Rivulidae) del Uruguay. Bol. Soc. Zool. Uruguay, 2a. epoca **4**: 27–33.
- Mc. Phail, J. D. and Jones, R. L. 1966. A simple technique for obtaining chromosomes from teleost fishes. Journal of the Fisheries Research Board of Canada **23**: 767–769.
- Morelli, S. 1981. Aspectos citogeneticos do genero *Astyanax* (pisces, Characidae). M. S. thesis. Universidade Federal de Sao Carlos, Sao Paulo, Brasil.
- Nicoloff, H., Anastosova-Kristeva, M., Rieger, R. and Kunzel, G. 1979. Nucleolar dominance as observed in barley translocation with specifically reconstructed SAT-chromosomes. Theor. Appl. Genet. **55**: 47–251.
- Oliveira, C., Foresti de Almeida Toledo, L. and de Almeida Toledo Filho, S. 1990. Comparative cytogenetic analysis of three cytotypes of *Corydoras natterii* (Pisces, Siluriformes, Callichthyidae). Cytologia **55**: 21–26.
- Phillips, R. L. and Hartley, S. E. 1988. Fluorescent banding patterns of the chromosomes of the genus *Salmo*. Genome **30**: 193–197.
- Portela, A. L., Galetti Jr., P. M. and Bertollo, L. A. C. 1988. Considerations on the chromosome evolution of Tetragonopterinae (Pisces, Characidae). Brazil. J. Genetics **XI** (2): 307–316.
- Scheel, J. 1972. Rivulinae karyotypes and their evolution (Rivulinae, Cyprinodontidae, Pisces). Sonderdruck aus Z. F. Zool. Systematik u. Evolutionsforschung **10**: 108–209.
- Sola, L., Cataudella, S. and Capanna, E. 1981. New development in vertebrate cytotaxonomy. III. Karyology of bony fishes: review. Genetica **54**: 285–328.
- Sumner, A. T. 1972. A simple technique for demonstrating centromeric heterochromatin. Exp. Cell Res. **75**: 304–306.
- Turner, B. J., Grudizen, T. A., Adkisson, K. P. and Worrell, R. A. 1985. Extensive chromosomal divergence within a single River Basin in the Goodeid Fish, *Ilyodon furcidens*. Evolution **39**(1): 122–134.
- Uwa, H. and Ojima, Y. 1981. Detailed and banding karyotype analyses of the Medaka, *Oryzias latipes* in cultured cells. Proc. Japan Acad., **57**(B): 39–43.