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Cytogenetic analysis of species of the genera *Acestrorhynchus*, *Oligosarcus* and *Rhaphiodon* (Teleostei: Characiformes)

EMANUEL RICARDO MONTEIRO MARTINEZ[1,2,*], CLAUDIO OLIVEIRA[1] and HORÁCIO F. JÚLIO-JUNIOR[2]

[1] Departamento de Morfologia, Instituto de Biociências, Universidade Estadual Paulista (UNESP), Botucatu, São Paulo, Brazil.
[2] Departamento de Biologia Celular e Genética, Universidade Estadual de Maringá, Maringá, Paraná, Brazil.

Abstract — In the present study the karyotypes and the number and position of nucleolus organizer regions (NORs) of four species of Characiformes are described. The analyses showed that *Acestrorhynchus lacustris* had 2n=50 chromosomes (8M+34SM+6ST+2A), *Oligosarcus longirostris* had 2n=50 chromosomes (2M+20SM+10ST+18A), *Oligosarcus* cf. *paranensis* from Keller and Mourão rivers had 2n=50 chromosomes (2M+26SM+8ST+14A), and *Rhaphiodon vulpinus* had 2n=54 chromosomes (32M+12SM+8ST+2A). The number of NOR-bearing chromosome pairs ranged from one to three among the species studied. The results available show that there is no variation in the diploid number within the genera analyzed. However, clear differences related to the karyotypic formulae and the number and position of Ag-NORs were found even in the comparison between populations. The possible relationships between the genera analyzed and other Characiformes are discussed.

Key words: Ag-NOR, Chromosomes, evolution, fish, Karyotype

INTRODUCTION

The fish species of the genera *Acestrorhynchus*, *Oligosarcus* and *Rhaphiodon* are predators that feed on small fishes. They present large mouths with many canine and conical teeth, and extended and compressed body (GÉRY 1977; MENEZES 1987; BRITSKI 1999). Recent studies, summarized in REIS *et al.* (2003), showed that the genus *Acestrorhynchus* is the only representative of the family Acestrorhynchidae; the genus *Rhaphiodon*, together with the genera *Cynodon, Gilbertolus, Hydrolycus* and *Roestes*, compose the family Cynodontidae; and the genus *Oligosarcus* belong to the family Characidae, but its relationship with other genera or subfamilies is still unresolved.

The karyotypes of three species of Acestrorhynchidae, two species of Cynodontidae and eleven species of *Oligosarcus* have already been described so far (Table 1). All species of Acestrorhynchidae and *Oligosarcus* karyotyped exhibited 2n=50 chromosomes and the species of Cynodontidae presented 2n=54 chromosomes (Table 1). In the present study the karyotypes and the number and position of Ag-NORs are described for one species of *Acestrorhynchus*, two species of *Oligosarcus* and one species of *Rhaphiodon* and the data are compared to other data available in literature.

MATERIALS AND METHODS

Four specimens (2 males and 2 females) of *Acestrorhynchus lacustris* from Paraná river (Porto Rico, Paraná, Brazil), three specimens (2 males and 1 female) of *Oligosarcus longirostris* from Iguaçu river (Três Barras, Paraná, Brazil), three specimens (2 males and 1 female) of *Oligosarcus* cf. *paranensis* from Keller river (Maringá, Paraná, Brazil), four specimens (2 males and 2 females) of *Oligosarcus* cf. *paranensis* from Mourão river (Maringá, Paraná, Brazil) and 16 specimens (13 males and 3 females) of *Rhaphiodon vulpinus* from Paraná river (Porto Rico, Paraná, Brazil) were analyzed. Fishes were identified and deposited in the fish collection of the Laboratório de Citogenética de Peixes, Departamento de Biologia Celular e Genética, Universidade Estadual de Maringá, Paraná, Brazil.

Mitotic chromosome preparations were performed according to the technique described by BERTOLLO *et al.* (1978). The silver staining of the nucleolar organizer regions (Ag-NORs) was performed according to the technique proposed by HOWELL and BLACK (1980). The chromosome morphology was determined on the

* Corresponding author: Departamento de Morfologia, Instituto de Biociências, Universidade Estadual Paulista, 18618-000 Botucatu, SP, Brazil. phone/fax: 55 14 3811-6264. e-mail: emanuel@ibb.unesp.br.

Table 1 — Summary of the cytogenetic data available for the families Acestrorhynchidae, Cynodontidae and the genus *Oligosarcus*. 2n= diploid number; M = metacentrics; SM = submetacentrics; ST = subtelocentrics; A= acrocentrics; NORs= nucleolus organizer regions.

Species	2n	Karyotype	Pairs with NORs	References
Acestrorhynchidae				
Acestrorhynchus altus	50	8M+22SM+14ST+6A		Falcão and Bertollo (1985)
Acestrorhynchus falcatus	50		1	Pastori and Fenocchio (1996)
Acestrorhynchus lacustris	50	12M+32SM+4ST+2A		Falcão and Bertollo (1985)
Acestrorhynchus lacustris	50	8M+34SM+6ST+2A	1	Present study
Acestrorhynchus pantaneiro	50	22M+14SM+8ST+6A	2	Miyazawa (1997)
Cynodontidae				
Cynodon gibbus	54	16M+30SM+8ST	1	Nascimento and Vênere (2000)
Hydrolycus scomberoides	54	24M+30SM	1	Nascimento and Vênere (2000)
Rhaphiodon vulpinus	54	32M+12SM+8ST+2A	1	Present study
Oligosarcus				
Oligosarcus hepsetus	50	2M+26SM+4ST+18A		Falcão and Bertollo (1985)
Oligosarcus hepsetus	50		1-4	Hattori *et al.* (2002)
Oligosarcus jenynsii	50	6M+22SM+6ST+16A		Falcão and Bertollo (1985)
Oligosarcus jenynsii	50	28M,SM+22ST,A	1	Veiga *et al.* (1998)
Oligosarcus jenynsii	50		1-4	Hattori *et al.* (2002)
Oligosarcus longirostris	50	2M+20SM+10ST+18A	2	Present study
Oligosarcus longirostris	50	6M+16SM+12ST+16A	1	Cunha *et al.* (2001)
Oligosarcus macrolepis	50	8M+20SM+6ST+16A		Falcão and Bertollo (1985)
Oligosarcus menezesis	50	2M+24SM+8ST+16A	3	Mortati and Dias (2001)
Oligosarcus paranensis	50			Carvalho *et al.* (1998)
Oligosarcus paranensis	50	2M+26SM+8ST+14A	1	Mortati and Dias (2002)
Oligosarcus paranensis	50	2M+24SM+8ST+16A	3	Mortati and Dias (2002)
Oligosarcus cf. *paranensis* Keller river	50	2M+26SM+8ST+14A	1	Present study
Oligosarcus cf. *paranensis* Mourão river	50	2M+26SM+8ST+14A	3	Present study
Oligosarcus pintoi	50	4M+20SM+10ST+16A		Falcão and Bertollo (1985)
Oligosarcus pintoi	50		1-4	Hattori *et al.* (2002)
Oligosarcus sp.	50	10M+12SM+12ST+16A	3	Salvador and Margarido (1999)
Oligosarcus sp.	50	6M+16SM+12ST+16A	3	Cunha *et al.* (2001)
Oligosarcus sp.	50	2M+16SM+8ST+14A	1	Mortati and Dias (2001)

basis of arm ratios as proposed by LEVAN *et al.* (1964), and the chromosomes were classified according to the morphology as metacentrics (M), submetacentrics (SM), subtelocentrics (ST), and acrocentrics (A).

RESULTS

The specimens of *Acestrorhynchus lacustris* had 2n=50 chromosomes (8M+34SM+6ST+2A) and one ST pair with Ag-NORs in the terminal position on the short arm (Table 1, Figure 1a).

The specimens of *Oligosarcus longirostris* presented 2n=50 chromosomes (2M+20SM+10ST+18A) and Ag-NORs in the terminal position on the long arm of two A pairs (Figure 1b, Table 1).

The specimens of *Oligosarcus* cf. *paranensis* from Keller river exhibited 2n=50 chromosomes (2M+26SM+8ST+14A) and notably polymorphic Ag-NORs in the terminal position on the short arm of the largest ST pair (Figure 2a, Table 1). Specimens of *Oligosarcus* cf. *paranensis* from Mourão river presented 2n=50 chromosomes (2M+26SM+8ST+14A) and Ag-NORs in the terminal position on the short arm and on the long arm of two pairs of a ST and in the terminal position on the long arm of an A pair (Figure 2b, Table 1).

The specimens of *Rhaphiodon vulpinus* exhibited 2n=54 chromosomes (32M+12SM+8ST+2A) and Ag-NORs in the terminal position on the long arm of a ST pair (Figure 3, Table 1).

Chromosome differences between sexes were not observed in the species analyzed.

DISCUSSION

The species *A. lacustris* and the two species of *Oligosarcus* analyzed in the present study exhibited 2n=50 chromosomes, as other species of those genera previously karyotyped (Table 1), which characterize the occurrence of a marked conserved diploid number in those genera. With regard to the karyo-

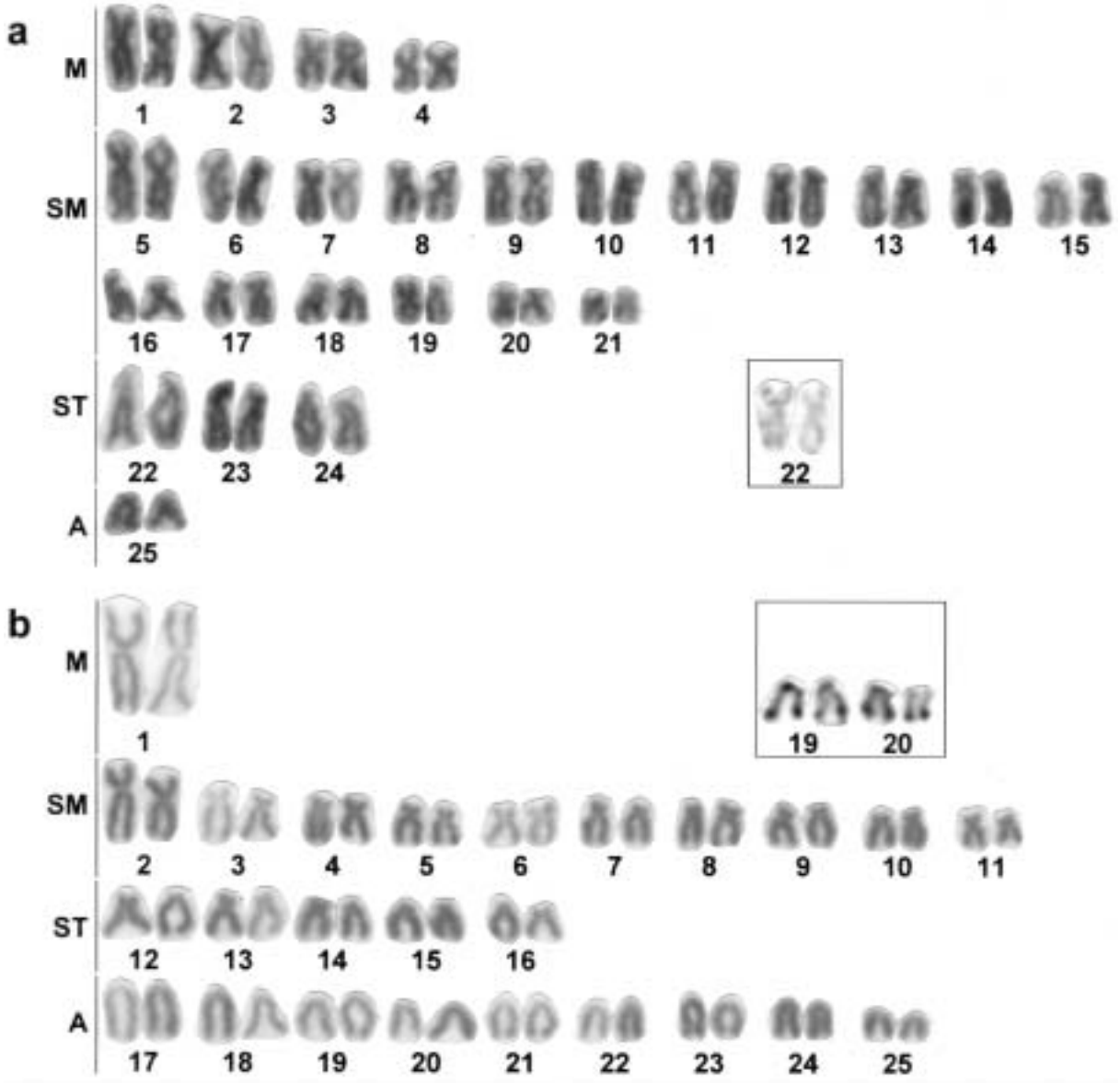

Fig. 1 — Giemsa stained karyotypes of: (a) *Acestrorhynchus lacustris* with 2n=50 chromosomes; and (b) *Oligosarcus longirostris* with 2n=50 chromosomes. In the insets, silver stained chromosomes with the nucleolar organizer regions.

typic structure, chromosomes of types M, SM, ST and A were found in all species analyzed. However, the species of *Acestrorhynchus* have a high number of M chromosomes and a small number of A chromosomes when compared to the species of *Oligosarcus* (Table 1). The presence of 2n=50 chromosomes, as observed in the genera *Acestrorhynchus* and *Oligosarcus*, is common in some groups of the order Characiformes, as in the family Crenuchidae, in the subfamilies Bryconinae, Salmininae, and some Tetragonopterinae (Oliveira *et al.* 1988).

The species of Cynodontidae analyzed, *R. vulpinus*, as the other two previously karyotyped, has 2n=54 chromosomes (Table 1), also suggesting the occurrence of a conserved diploid number in this family. With regard to the karyotypic structure, there is a predominance of chromosomes of types M and SM in all species. Only a few ST chromosomes were observed in *C. gibbus* and *R. vulpinus*, and A chromosomes were only observed in *R. vulpinus* (Table 1). The presence of 2n=54 chromosomes, as observed in the family Cynodontidae, is very common in several groups of Characiformes, as in the families Anostomidae, Chilodontidae, Curimatidae, Hemiodontidae, Parodontidae and Prochilodontidae (Oliveira *et al.* 1988). Although these similarities regarding the chromosome number might suggest the occurrence of some kind of relationship between the

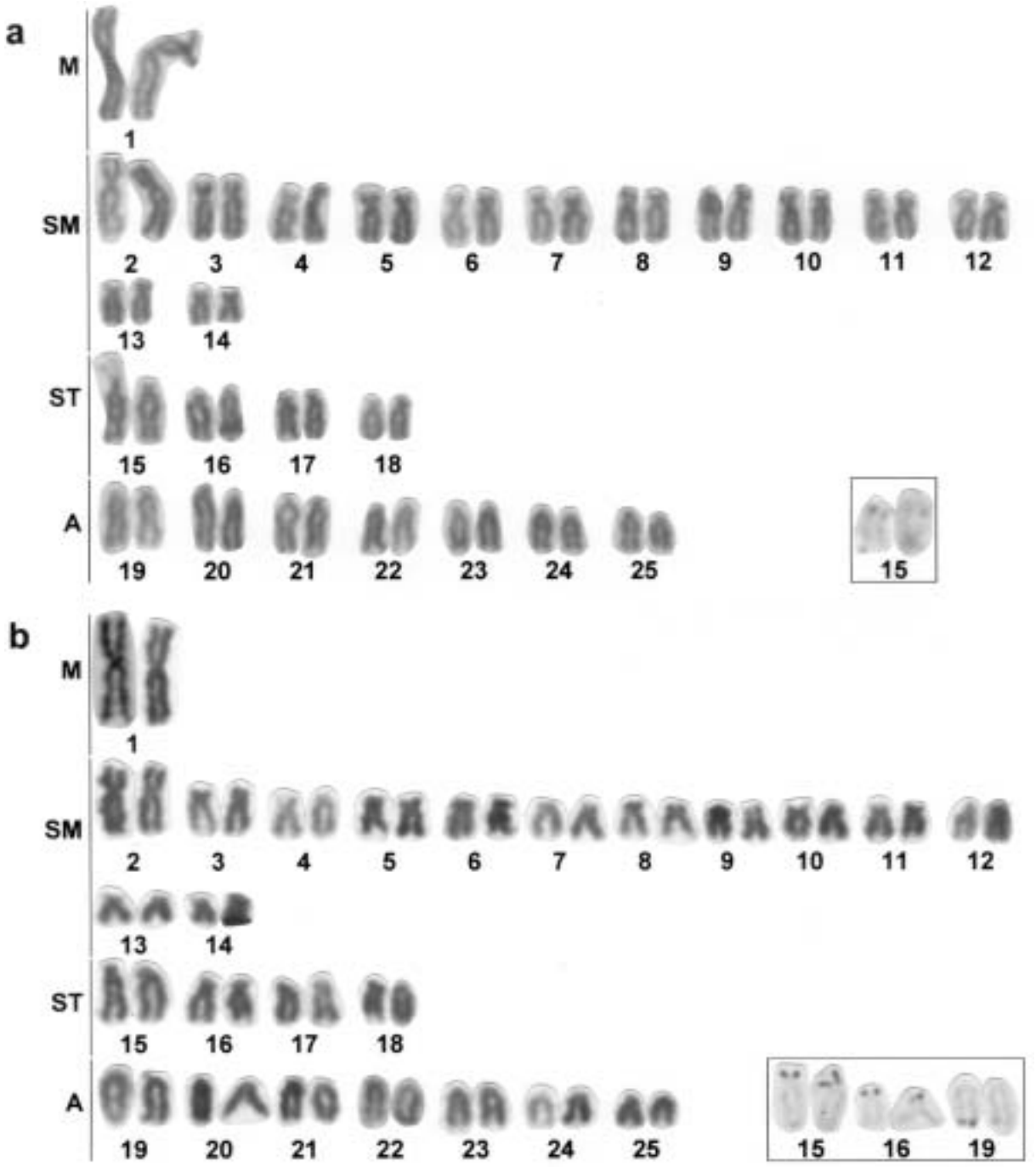

Fig. 2 — Giemsa stained karyotypes of: (a) *Oligosarcus* cf. *paranensis* from Keller river with 2n=50 chromosomes; and (b) *Oligosarcus* cf. *paranensis* from Mourão river with 2n=50 chromosomes. In the insets, silver stained chromosomes with the nucleolar organizer regions.

groups cited above, further systematic studies should be conduced to test this hypothesis.

The analysis of the karyotypic structure of *Oligosarcus* showed that all species of this genus have the first M pair considerably larger than all other M pairs, as previously observed by Falcao and Bertollo (1985). This chromosomic characteristic has been observed for several species of the family Characidae, since its initial description by Scheel (1973). On the other hand, this characteristic was not identified in any species of Acestrorhynchidae or Cynodontidae.

Systematic studies conducted by Lucena and Menezes (1998), based on morphological character-

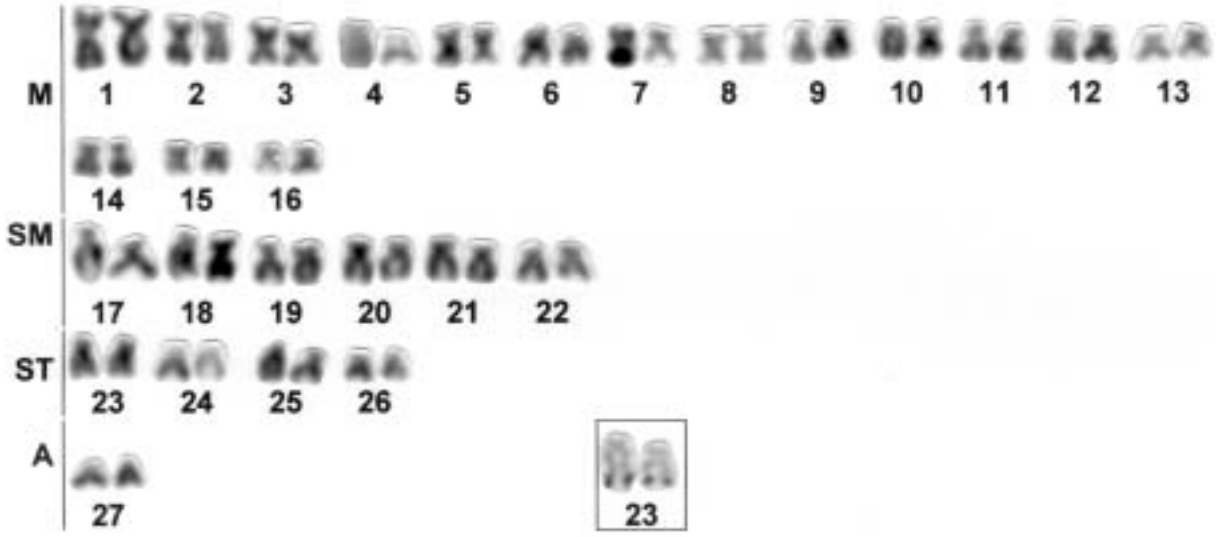

Fig. 3 — Giemsa stained karyotypes of: *Rhaphiodon vulpinus* with 2n=54 chromosomes. In the insets, silver stained chromosomes with the nucleolar organizer regions.

istics, suggested that the genus *Acestrorhynchus* could be the primitive sister-group of the family Cynodontidae. Although the diploid number of these groups are different (2n=50 and 2n=54, respectively) the occurrence in both groups of a first M pair without great difference in size when compared to other M pairs, as discussed above, corroborate the hypotheses that *Acestrorhynchus* and Cynodontidae could belong to a natural group. Considering that the diploid number 2n=54 is found in several representatives of Characiformes and may constitute an apomorphy for the order (OLIVEIRA *et al.* 1988) the presence of 2n=54 chromosomes in the species of Cynodontidae may represent a primitive characteristic for this family. On the other hand, the presence of a very large first M pair in the species of *Oligosarcus* is also in accord with the hypotheses of LUCENA and MENEZES (1998) that these genera must not belong to the family Acestrorhynchidae, as previously suggested (MENEZES 1987).

The number of chromosome pairs with Ag-NORs varies extensively in the families Acestrorhynchidae and Cynodontidae and in the genus *Oligosarcus* (Table 1). Ten species/populations presented single Ag-NORs and ten exhibited multiple Ag-NORs. With regard to the species here identified as *O.* cf. *paranensis*, single Ag-NORs were observed in the sample from Keller river and multiple Ag-NORs were observed in the sample from Mourão river (Table 1). In the species *O. longirostris*, samples with single and multiple Ag-NORs were also observed (Table 1). The species *Acestrorhynchus lacustris* displayed single Ag-NORs, as observed in *A. falcatus*, and differing from *A. pantaneiro*, which has multiple Ag-NORs (Table 1). The differences in the number of chromosomes with Ag-NORs may suggest that these samples are reproductively isolated or that different mechanism of NOR activation are operating in these samples. The data obtained reinforce the hypotheses that the Ag-NORs may constitute good chromosome markers at population or species levels as reported in several studies (VŸNERE and GALETTI JR. 1989; CARVALHO *et al.* 2002).

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