Karyotype characterization and gene mapping of 5S and 18S rDNA in three species of *Oligosarcus* (Teleostei: Characidae)

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Abstract — *Oligosarcus* is a monophyletic Characidae genus with 15 species described, distributed through the freshwater bodies of South and Southeastern parts of South America. Recently, a new classification of this genus has been proposed based on morphological characters and mtDNA sequence, but still now there are many uncertainties in relation to the taxonomic classification of this group. In this study, three *Oligosarcus* species were chromosomically analyzed using conventional and molecular techniques in order to characterize their karyotipic constitution. All the three species presented 2n = 50 chromosomes, distributed as follows: *O. jenynsii* — 2M/24SM/10ST/14A, *O. pintoi* — 2M/20SM/12ST/16A, and *O. hepsetus* — 2M/16SM/16ST/16A. A metacentric chromosome, larger than any other chromosome in the complement, was identified in all cases and probably corresponds to the marker chromosome of the Characidae family. The first submetacentric chromosome may also represent a marker chromosome, but at genus or subfamily level. The most frequent NOR location pattern was a large acrocentric pair bearing the NORs in the short arm and presenting size polymorphism. The presence of heterochromatin was more evident in *O. hepsetus* and *O. pintoi* than in *O. jenynsii*, with centromeric and telomeric bands on subtelocentric-acrocentric chromosomes. FISH with 18S rDNA identified three loci for *O. hepsetus* and *O. pintoi* and two for *O. jenynsii.* The 5S rDNA probe identified three *loci* for *O. hepsetus* and *O. pintoi* and two for *O. jenynsii*. A possible syntenic organization of these two ribosomal DNA sequences was identified for *O. hepsetus* and *O. pintoi* in the NOR-bearing chromosomes.

Keywords: Characidae, chromosomes, FISH, karyotype, *Oligosarcus*, rDNA.

INTRODUCTION

The genus *Oligosarcus* is formed by 15 species found only in South America, in the Paraná-Paraguay basin and in coastal streams of eastern and southern Brazil, Uruguay and north-eastern Argentina (Lima *et al.* 2003). The species of this genus are characterized as active predators, mainly of small fishes (Menezes 1988).

The phylogenetic position of this genus has undergone many modifications: initially, based on osteological characteristics, it was included with *Acestrorhynchus* in the subfamily Acestrorhynchinae, outside the family Characidae (Menezes 1969). However, in a revision analysis, Buckup (1991) proposed the separation of the genus *Oligosarcus* from Acestrorhynchinae and its inclusion in the subfamily Tetragonopterinae (Characidae), in a position probably more related to the genus *Astyanax*. More recently *Oligosarcus* was included in the genera Incertae Sedis in Characidae (Lima *et al.* 2003). Although the genus *Oligosarcus* is a well supported monophyletic group, its relationship with other groups of the family Characidae is so far undetermined.

Cytogenetic studies in this genus began with Falcão (1983), who characterized four *Oligosarcus* species karyotypically by Giemsa staining and NOR position. With the exception of some *O. pintoi* individuals from Pirassununga (State of São Paulo) that presented a supernumerary chromosome (2n = 51) (Falcão 1983), all the species had a diploid number of 2n = 50 chromosomes (see also Kavalco 2003).

The application of fluorescent *in situ* hybridization (FISH) with 5S and 18S rDNA probes has allowed their chromosomal localization in many Neotropical fishes, allowing the identification of other cytogenetic markers. In the Characidae

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family, 5 species of the genus *Astyanax* were analyzed by ALMEIDA-TOLEDO *et al*. (2002), disclosing the relationship that involves the localization of these genes.

In this study, we characterized the karyotype of three *Oligosarcus* species, using Giemsa staining, silver staining, C-banding, and FISH of 5S and 18S rDNA, with the aim of identifying possible cytogenetic markers as well as chromosome tendencies in the group.

MATERIAL AND METHODS

A total of 10 specimens of *O. jenynsii* (1), *O. hepsetus* (2), and *O. pintoi* (3), collected from the rivers Uruguay (Santa Catarina State), Paraíba do Sul and Mogi-Guaçu (both in São Paulo State), respectively, were analyzed.

Chromosome preparations of kidney cells were made using conventional air-drying technique, as described by ALMEIDA-TOLEDO (1993). The chromosome locations of the NORs (nucleolus organizer regions) were determined by silver staining, according to the protocol published by HOWELL and BLACK (1980), and C-banding was carried out by the method of SUMNER (1972). For hybridization, an 18S rDNA probe (HM456) from *Xenopus laevis* and a 5S rDNA from *Leporinus* were used, according to DANIEL-SILVA's method (2001). Metaphases with well-spread chromosomes and clear morphology were analyzed by pairing and ordering, according to LEVAN *et al.* (1964).

RESULTS AND DISCUSSION

Giemsa staining, silver staining and *C-banding* - All three species presented a diploid number of 2n = 50 chromosomes, but with different karyotypes (Table 1) (Fig. 1A to 3A). In none of them it was possible to distinguish the karyotypes of females from males. But interestingly, two pairs of chromosomes were clearly observed in all three karyotypes: a metacentric pair, the larger of the complement (or 1M), and a submetacentric pair, identified in all karyotypes as the first SM. The presence of a large M/SM pair is a characteristic that is very commonly observed among Characidae species studied so far. It was first described by SCHEEL (1973) and, since then, recognized by many other authors (for example, DANIEL-SILVA 2001). The presence of this marker chromosome in the three *Oligosarcus* here studied represents additional support for the inclusion of *Oligosarcus* genus within Characidae family, as proposed by BUCKUP (1991). However, we can not propose a more detailed relationship due to some limitations of our analysis. Phylogenetic analysis based on gene sequences coupled with comparative cytogenetic analysis may contribute to the positioning of this genus inside the Characidae group. In this context it would be interesting to compare cytogenetically *Oligosarcus* species with some members of genera Incertae Sedis in Characidae, in relation to the 1st SM and other possible marker chromosomes.

The silver staining evidenced NORs present in the larger pair of acrocentric chromosomes in all cases (Fig. 1A, 2A, and 3A), suggesting that it is well conserved in the genus In case of *O. hepsetus*, one specimen presented two NORs in one of the acrocentric pair, in the telomeric regions of the short arm and long arm (Fig. 1A). This situation can be explained by chromosomal rearrangements, such as inversions, incorrect pairing during cell division or even by the action of mobile elements associated to rDNA genes (ALMEIDA-TOLEDO *et al.* 2002). NOR size heteromorphism was also noticed in the three species and seems to be commonly observed in many fish species (FORESTI *et al.* 1981).

The C-banding patterns showed that, in these species, constitutive heterochromatin is distributed as small blocks, hardly occupying more than half of a chromosome. These blocks are present mainly in telomeric regions and secondarily in centromeric regions of subtelocentric-acrocentric chromo-

Table 1 — Karyotype characteristics of the three *Oligosarcus* species analyzed.

Species	*Chromosome morphology*				*2n*	*FN*	*Max. NORs.*
	M	SM	ST	A			
Oligosarcus hepsetus	2	16	16	16	50	68	3
Oligosarcus pintoi	2	20	12	16	50	72	2
Oligosarcus jenynsii	2	24	10	14	50	76	2

M = Metacentric, SM = Submetacentric, ST = Subtelocentric, and A = Acrocentric.
FN = Fundamental Number

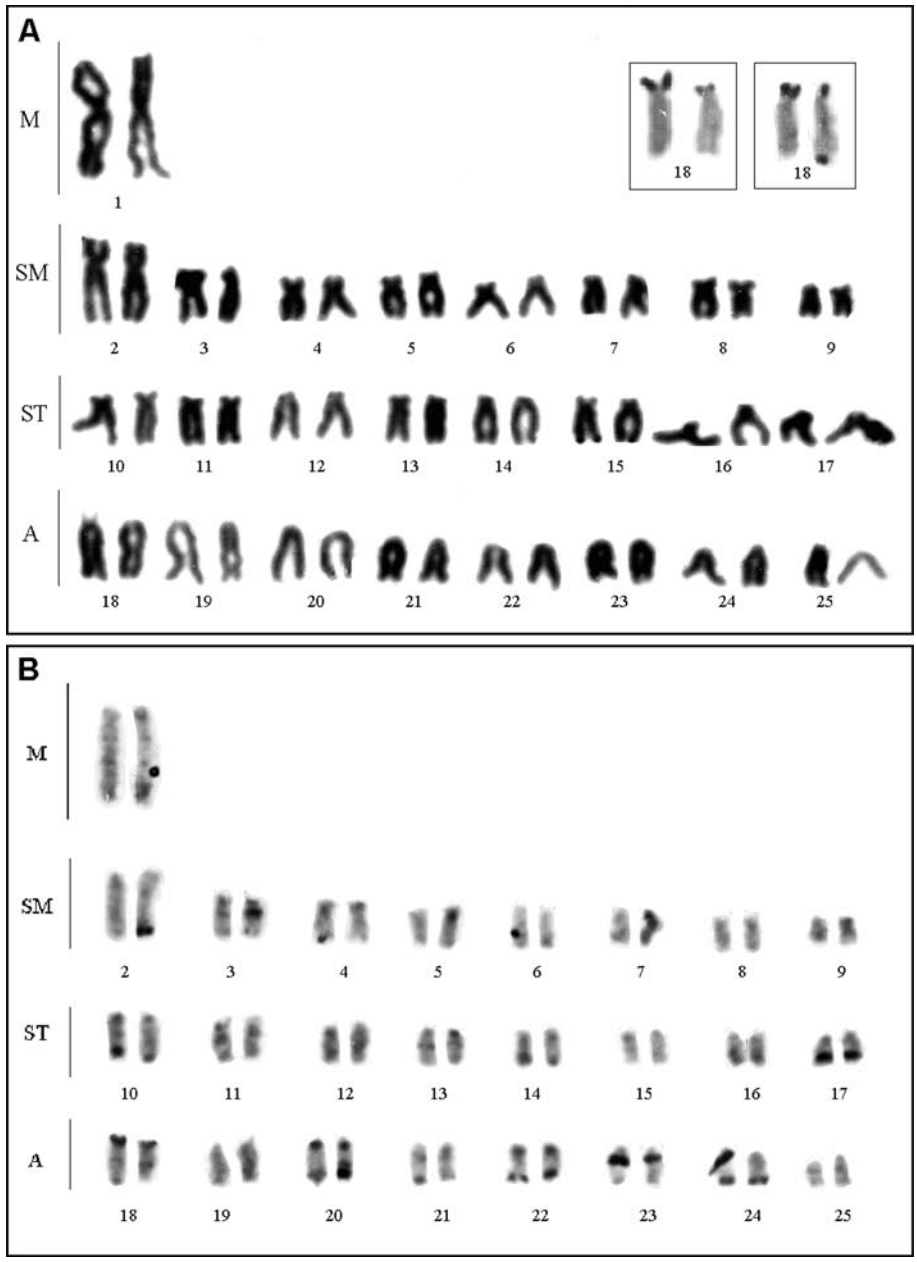

Fig. 1 — *Oligosarcus hepsetus*. **A** Giemsa-stained karyotype; in the inset, the two patterns of NOR-bearing chromosomes; **B** C-banded karyotype.

somes. The association between heterochromatin blocks and NORs was more evident in *O. hepsetus* and *O. pintoi*. Considering the three species focused here, *O. jenynsii* (Fig. 2B) is apparently the species with less heterochromatin, followed by *O. pintoi* (Fig. 3B) and *O. hepsetus* (Fig. 1B).

FISH with 5S and 18S rDNA - O. hepsetus - In this species the two probes detected two different patterns of hybridization. In case of the 18S rDNA probe (Fig. 4c), one pattern corresponded to the one observed with silver-staining method, in which one of the acrocentric presented two NORs (Fig. 1A). In the second pattern (Fig. 4a), besides the first acrocentric pair, another short acrocentric chromosome also presented a hybridization signal in the short arm. Since this second pattern was not observed by silver-staining, we supposed that this 18S rDNA site is hardly active during cell cycle of kidney cells. The 5S rDNA subunit was

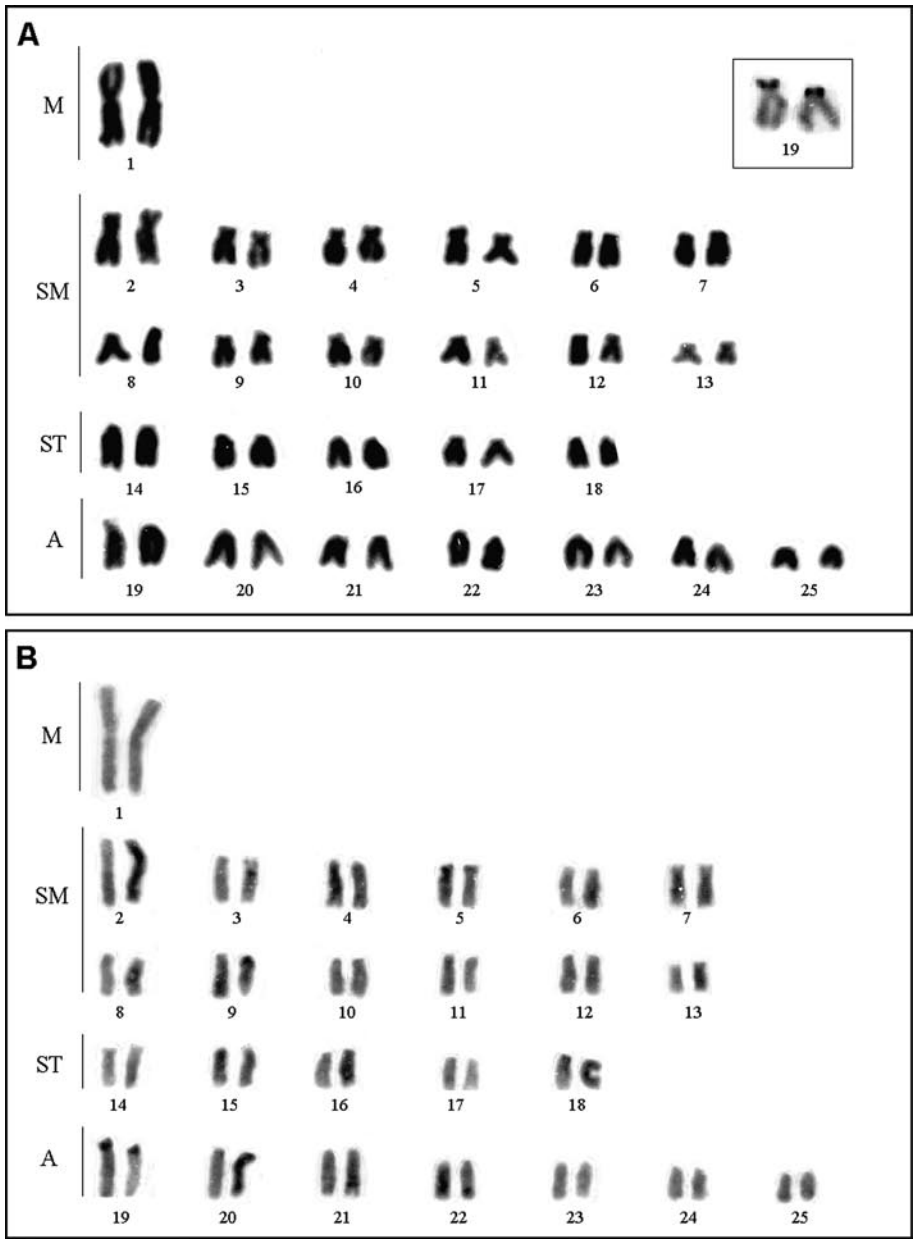

Fig. 2 — *Oligosarcus jenynsii*. **A** Giemsa-stained karyotype; in the inset, pattern of NOR-bearing chromosomes; **B** C-banded karyotype.

also observed in a large pair of acrocentric chromosomes (Fig. 4b), that probably corresponds to the first acrocentric pair bearing the NORs. The syntenic organization of 18S and 5S rDNA can be explained by the figure 4c and 4d, in which both probes produced an identical pattern of hybridization (signals in the telomeric regions of the short and long arm of an acrocentric chromosome).

O. pintoi - The 18S rDNA probe revealed three acrocentric chromosomes (Fig. 4e), including the two NOR-bearing acrocentrics, similar to one of the patterns found in *O. hepsetus* (Fig. 4a). The 5S rDNA probe also hybridized with three acrocentric chromosomes (Fig. 4f), that we supposed to be the same bearing the 18S rDNA genes and therefore presenting syntenic organization as suggested to *O. hepsetus*.

O. jenynsii - Hybridization with the 18S rDNA probe confirmed the two NORs detected by silver staining on the first pair of acrocentric chromosomes

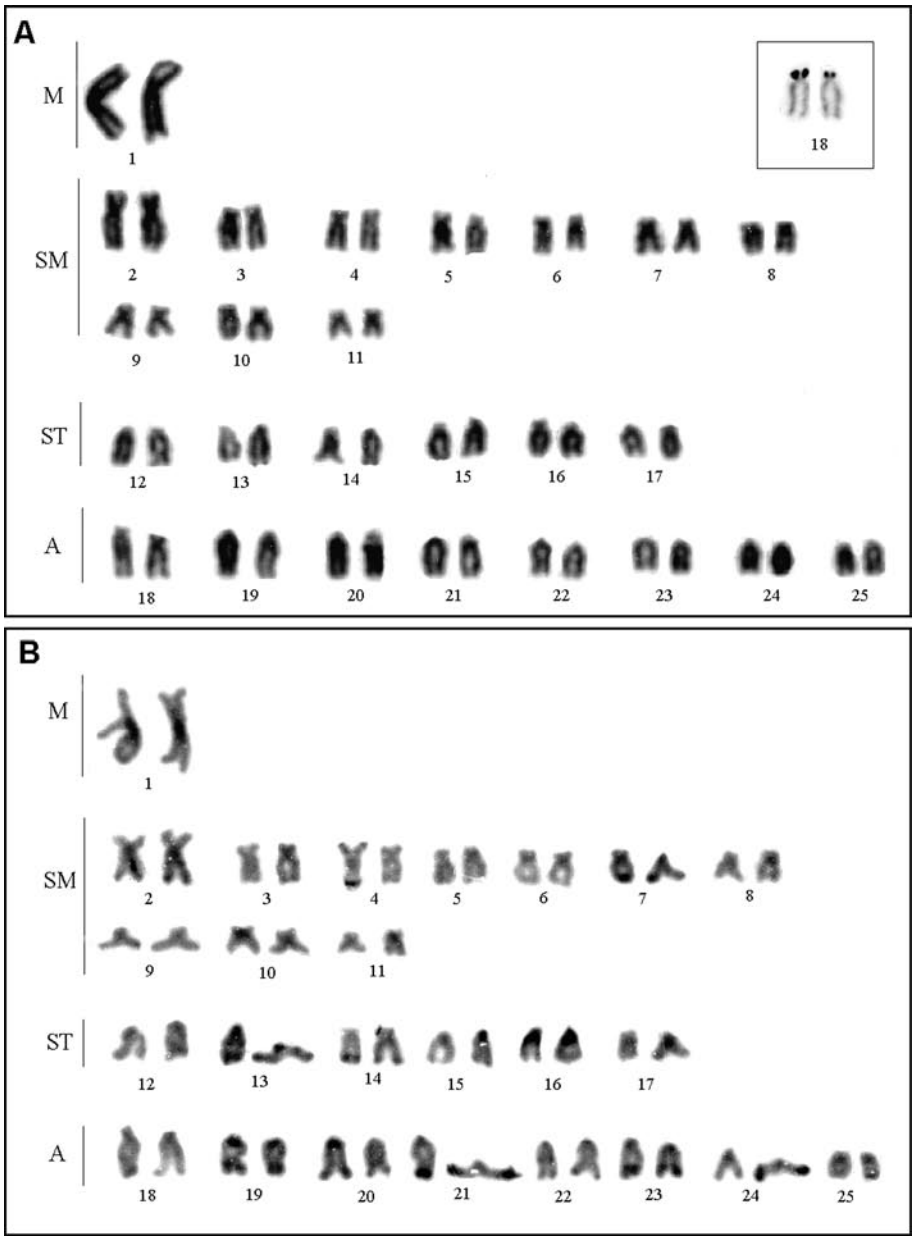

Fig. 3 — *Oligosarcus pintoi.* **A** Giemsa-stained karyotype; in the inset, pattern of NOR-bearing chromosomes; **B** C-banded karyotype.

(Fig. 4g). The 5S rDNA probe also hybridized with one pair of acrocentric chromosomes, which may be the same pair bearing the NORs (Fig. 4h).

The syntenic organization of the 5S and 18S subunits is not a very common situation, not only in fishes but also in eukaryotes in general (MARTINS 2000). However, the syntenic organization of these subunits has been reported, for instance in the genus *Oncorhynchus* (MORÁN *et al.* 1996; FUJIWARA *et al.* 1998) and *Salmo* (PENDÁS *et al.* 1994). More recently, ALMEIDA-TOLEDO *et al.* (2002) reported the same situation in some Neotropical species of the genus *Astyanax*.

In 1999 and 2000, MARTINS and GALETTI Jr. reported the existence of two subtypes of 5S rDNA in the genus *Leporinus* and *Schizodon*, characterized as major and minor. VICENTE *et al.* (2001) also observed this situation in three *Parodon* species. In the present study the only species that presented more than two chromosomes bearing 5S rDNA subunits was *O. pintoi.* The two larger acrocentrics present a stronger signal compared to the

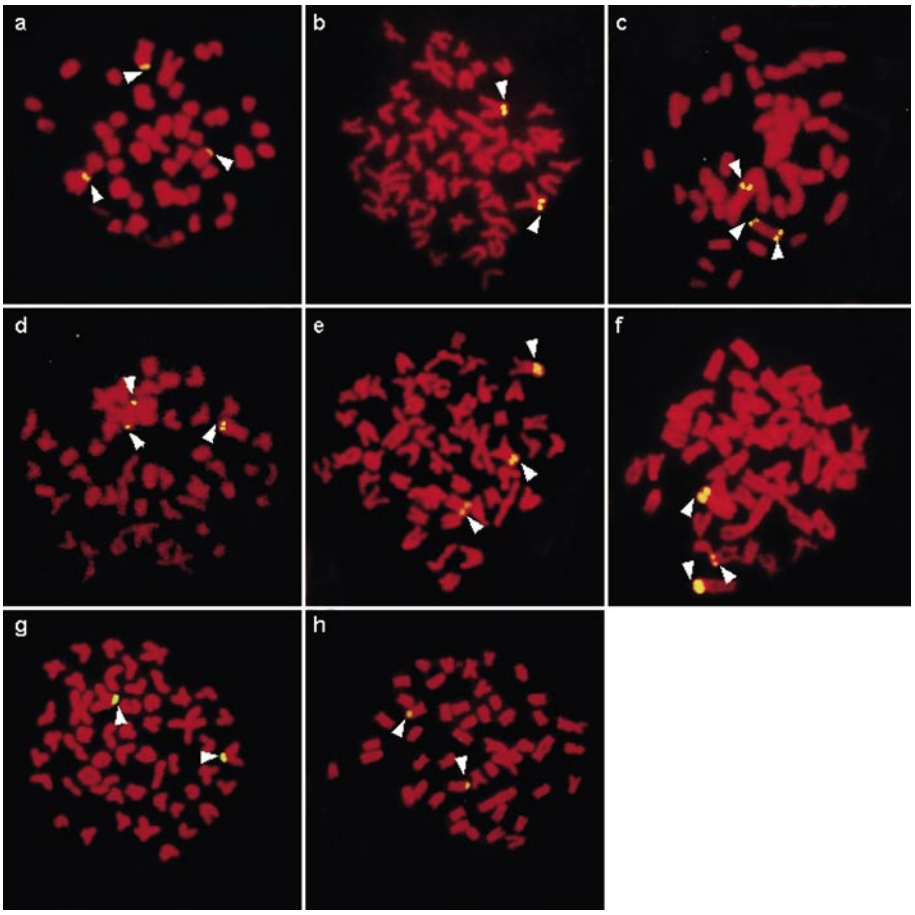

Fig. 4 — Fluorescent in situ hybridization (FISH) with 18S (**a**, **c**, **e** and **g**) and 5S (**b**, **d**, **f** and **h**) rDNA probes. **a** to **d**: *Oligosarcus hepsetus*; **e** and **f**: *Oligosarcus pintoi*; **g** and **h**: *Oligosarcus jenynsii*. The arrows indicate rDNA location.

short acrocentric, what may correspond the major and minor 5S rDNA. The increase of sensitivity in FISH may reveal other small sites of rDNA genes probably undetected by our protocol.

In summary, the clear presence of the 1st M/SM characteristic of Characidae family supports the inclusion of *Oligosarcus* within Characidae family, but further analysis are necessary to give a clearer phylogenetic position. Karyotype comparison of the 1st M and the 1st SM chromosome pairs of *Oligosarcus* and *Astyanax* using not only conventional but also molecular techniques (R-banding or chromosome hybridization, for instance) are suggested in order to verify the old classification proposed by BUCKUP (1991) and the more recent one, proposed by LIMA *et al.* (2003). Although some variations do occur, the sites of rDNA as well as NORs sites seem to be well conserved in the first pair of acrocentric chromosomes.

The cytogenetic markers considered here, with different levels of variability, may comprise useful tools for characterizing and distinguishing populations and species of the genus *Oligosarcus*. Some tendencies observed in the repetitive genes or sequences, as in the case of NORs, constitutive heterochromatin and rDNA genes, can be reinforced by studying other species and related genera.

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REFERENCES

ALMEIDA-TOLEDO L.F., FORESTI F, DANIEL M.F.Z. and TOLEDO-FILHO S.A. 1993 — *Nucleolar chromosome variants in Sternopygus macrurus (Pisces, Sternopy-*

gidae) from three Brazilian river basins. Caryologia, 46: 53-61.

ALMEIDA-TOLEDO L.F., OZOUF-COSTAZ C., FORESTI F., BONILLO C., PORTO-FORESTI, F. and DANIEL-SILVA M.F.Z. 2002 — *Conservation of the 5S-bearing chromosome pair and co-localization with major rDNA clusters in five species of Astyanax (Pisces, Characidae)*. Cytogenetic and Genome Research, 97: 229-233.

BUCKUP P.A. 1991 — *The Characidiidae: A phylogenetic study of the South American Darters and their relationship with other characiform fishes.* PhD. Thesis Ann Arbor, University of Michigan.

DANIEL-SILVA M.F.Z., 2001 — *Análises citogenéticas comparativas em Characidae (Pisces, Characiformes)*. PhD. Thesis. Universidade de São Paulo.

FALCÃO J.N., 1983 — *Estudos Citogenéticos em Acestrorhynchinae e Cynopotaminae (Pisces, Characidae)*. Master`s Thesis. Universidade de São Paulo.

FORESTI F., ALMEIDA-TOLEDO L.F. and TOLEDO-FILHO S.A., 1981 — *Polymorphic nature of nucleolus organizer regions in fishes.* Cytogenetic and Cell Genetics, 31: 137-144.

FUJIWARA A, ABE S., YAMAHA E., YAMAZAKI F. and YOSHIDA M.C., 1998 — *Chromosomal localization and heterochromatin association of ribosomal RNA genes loci and silver stained nucleolus organizer regions in salmonid fishes*. Chromosome Research, 6: 463-471.

HOWELL W.M. and BLACK D.A., 1980 — *Controlled silver staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method.* Experientia, 36: 1014-1015.

KAVALCO K.F., 2003 — *Contribuição citogenética à análise da biodiversidade das nascentes da ictiofauna das nascentes do rio Paraitinga.* Master`s Thesis. Universidade Federal de São Carlos.

LEVAN A., FREDGA K. and SANDBERG A.A., 1964 — *Nomenclature for centromeric position on chromosomes.* Hereditas, 52: 201-220.

LIMA F.C.T., MALABARBA L.R., BUCKUP P.A., SILVA J.F.P., VARI R.P., HAROLD A., BENINE R., OYAKAWA R., PAVANELLI C.S., MENEZES N.A., LUCENA C.A.S., MALABARBA M.C.S.L., LUCENA Z.M.S., REIS R.E., LANGEANI F., CASSATI L., BERTACCO V.A., MOREIRA C. and LUCINDA P.H.F., 2003 — *Genera incertae Sedis in Characidae.* In: REIS R.E., KULLANDER C.J. and FERRARIS C.J. (Eds.) Check list of the freshwater fishes of South and Central America, p. 106-169*,* EDIPUCRS, Porto Alegre.

MARTINS C., 2000 — *Organização do DNA ribossômico 5S no genoma de peixes, com ênfase em Leporinus.* PhD Thesis. Universidade Federal de São Carlos.

MARTINS C. and GALETTI JR. P.M., 1999 — *Chromosomal localization of 5S rDNA genes in Leporinus fish (Anostomidae, Characiformes)*. Chromosome Research, 7: 363-367.

MENEZES, N.A. 1969 — *Systematics and evolution of the tribe Acestrorhynchini (Pisces, Characidae).* Arquives of Zoology*,* SP, 18(1-2): 1-150.

MENEZES, N.A. 1988 — *Implications of the distribution patterns of the species of Oligosarcus (Teleostei, Characidae) from Central and Southern America*. In: Workshop on Neotropical Distribution Patterns. Proceedings. Rio de Janeiro: Academia Brasileira de Ciências, 295-304.

MORÁN P., MARTINEZ J.L., GARCIA-VÁSQUEZ E. and PENDÁS A.M., 1996 — *Sex linkage of 5S rDNA in rainbow trout (Oncorhynchus mykiss).* Cytogenetics and Cell Genetics, 75: 145-150.

PENDÁS A.M., MORÁN P., FREIJE J.P. and GARCIA-VAZQUEZ E., 1994 — *Chromosomal mapping and nucleotide sequence of two tandem repeats of Atlantic salmon 5S rDNA*. Cytogenetics and Cell Genetics, 67: 31-36.

SCHEEL J.J., 1973 — *Fish chromosomes and their evolution.* Internal Report of Danmarks Akuatium, Charllottenlund, Denmark.

SUMNER A.T., 1972 — *A simple technique demonstrating centromeric heterochromatin.* Experimental Cell Research, 75: 304-306.

VICENTE V.E., JESUS C.M. and MOREIRA-FILHO O., 2001 — *Chromosomal localization of 5S and 18S rRNA genes in three Parodon species (Pisces, Parodontidae).* Caryologia, 54: 365-369.

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