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Cytogenetic characterization of the sole *Solea senegalensis* (Teleostei: Pleuronectiformes: Soleidae): Ag-NOR, $(GATA)_n$, $(TTAGGG)_n$ and ribosomal genes by one-color and two-color FISH

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Abstract

A cytogenetic analysis of the sole *Solea senegalensis* was carried out using silver staining for the nucleolus organizer region (Ag-NOR) identification, one-color FISH for chromosomal mapping of 45S and 5S ribosomal DNAs (rDNAs), $(GATA)_n$, and $(TTAGGG)_n$, and two-color FISH for co-localization of both rDNAs. The Ag-NORs and the 45S rDNA were mapped to a medium-sized submetacentric chromosomal pair. Hybridization with the 5S rDNA showed a major signal on the short arm of a medium-sized submetacentric chromosome pair and a minor signal on a centromeric site of a small acrocentric chromosome pair. Differences in the Ag-NOR and 45S and 5S rDNAs FISH signal sizes were observed between homologous chromosomes and among individuals. A two-color FISH co-localized 45S and 5S rDNAs to a medium-sized submetacentric chromosomal pair. The hybridization with the telomeric $(TTAGGG)_n$ repeat displayed small signals at all chromosomal telomeres. Finally, the $(GATA)_n$ probe produced dispersed and small hybridization signals on all chromosome spreads, showing its ubiquitous existence in the genome. These results were compared with those from other Pleuronectiformes and discussed in terms of karyotype evolution.

Introduction

The sole *Solea senagalensis* (Teleostei: Pleuronectiformes: Soleidae) (Kaup, 1858) is a common flatfish, which occurs throughout the north-eastern Atlantic Ocean, from the north of Senegal to France, and from the Strait of Gibraltar to the coast of Tunisia in the Mediterranean Sea. In the Iberian Peninsula, this sole is mainly found in the Gulf of Cádiz (Spain) whereas is absent or rare in the Cantabrian and Mediterranean waters. The reduction in sole landings and its high commercial value have motivated an increasing interest for the aquaculture of this species in both the south of Spain and Portugal (Dinis et al., 1999). Earlier studies have mainly focused on physiology, reproduction and nutrition (Dinis et al., 1999; Ribeiro, Sarasquete & Dinis, 1999). Nevertheless, little knowledge exists on the genetic aspects of this flatfish and other soleids in general.

Flatfish chromosomes have been studied so far by means of Giemsa staining, chromosome

banding and silver staining for the nucleolus organizer regions (Ag-NOR) (Vitturi, Catalano & Colombera, 1993; Bouza, Sanchez & Martinez, 1994). To date, Ag-NORs have been characterized in only five flatfish species: *Scophthalmus maximus*, *Scophthalmus rhombus*, *Platichthys flesus*, *Solea solea* and *Solea lascaris* (Pardo et al., 2001). These Ag-NORs have demonstrated their utility as species-specific chromosome markers due to their number, location and size polymorphisms (Cross, Vega & Rebordinos, 2003).

Ribosomal RNA (rRNA) genes have proven to be useful in karyotype characterization studies (de Azevedo et al., 2005). In higher eukaryotes, tandem arrays of rRNA genes are organized in two distinct multigene families. One class is represented by the 45S rDNA that contains for the 18S, 5.8S and 28S rDNA and internal transcribed spacers (ITS). The second class codes for the 5S rRNA that consist of a coding region of 120 nucleotides and an intergenic non-transcribed spacer (NTS) of variable length and sequence (Suzuki et al., 1996).

During the past few years, chromosomal localization of DNA sequences by means of fluorescence *in situ* hybridization (FISH) has been applied to fish species (Hatanaka & Galetti Jr., 2004; Tigano et al., 2004; Kavalco et al., 2005; Rossi & Gornung, 2005). Particularly in flatfish, this technique has only been employed for the mapping of some subunits of the 45S (Pardo et al., 2001; de Azevedo et al., 2005) and 5S (Manchado et al., 2005) rRNA genes. Two different classes of 5S rDNA have been obtained in *S. senegalensis*. They were designated as one major and one minor 5S rDNA loci which were located to different chromosomal pairs (Manchado et al., 2005). These two NTS clones, NTS-I and NTS-II, represent paralogous copies that have evolved independently. The existence of several 5S loci has been correlated with genome polyploidization in salmonids (Morán et al., 1996) or duplication events in *Coris julis* (Mandrioli, Colomba & Vitturi, 2000). The 5S rDNA genes can be found clustered in a single chromosomes pair or in different chromosomes loci in fish (Pendás et al., 1994). Moreover, the 5S rDNA appear usually not to be syntenic to the 45 S with regards to different polymerases involved in transcribing these genes (Martins & Galetti Jr., 2001). Other repetitive DNA sequences, such as the telomeric $(TTAGGG)_n$ sequences in vertebrates and the GATA repeats associated sometimes with sex chromosomes in humans and other vertebrates (Singh & Jones, 1982; Jones & Singh, 1985; Subramanian, Mishra & Singh, 2003), have not been described previously in flatfish.

In the present study, both advanced and conventional cytogenetic techniques were applied in order to gain more genetic knowledge in *S. senegalensis*. In addition to the Ag-NOR identification, chromosomal mapping of the two rDNA families (45S and 5S) and the repetitive DNA sequences $(TTAGGG)_n$ and $(GATA)_n$, were carried out using the FISH technique. Moreover, the major and minor rDNAs were localized by means of a simultaneous two-color FISH technique.

Material and methods

Chromosome preparations and silver staining

Chromosome preparations started with 2–4 day-old *Solea senegalensis* larvae. They were pretreated with 0.02% colchicine for 3 h, subjected to hypotonic shock and finally fixed in a freshly prepared solution of absolute ethanol-acetic acid (3:1). Larvae were homogenized in 45% acetic acid and several drops were splashed onto a slide heated at 43 °C.

The nucleolus organizer regions (Ag-NORs) were detected by silver nitrate staining according to Howell and Black (1980).

Probe amplifications and FISH

Telomeric $(TTAGGG)_n$ and $(GATA)_n$ probes were generated by PCR in the absence of templates (Ijdo et al., 1991), using $(TTAGGG)_5$ and $(CCCTAA)_5$, and $(GATA)_7$ and $(TATC)_7$, as primers, respectively. The $(TTAGGG)_n$ and $(GATA)_n$ probes were labelled with digoxigenin by nick translation according to the manufacturer's instructions (Roche Molecular Biochemicals). FISH conditions were performed according to Cross et al. (2005).

A probe pDm238 (Roiha et al., 1981), containing 18-5.8-28S rDNA genes and spacers of *Drosophila melanogaster* cloned into the plasmid pBR322, was used to detect the rDNA clusters on chromosomes (Cross, Vega & Rebordinos, 2003). The whole plasmid was labelled with

digoxigenin-11–dUTP according to the manufacturer's instructions (Boehringer Mannheim). 5S rDNA probes were obtained from a recombinant vector TOPO® by PCR using primers T3 and T7 according to Manchado et al. (2005). Labelling was obtained with the following dNTPs concentration: 200 μM dATP, 200 μM dCTP, 200 μM dGTP, 150 μM dTTP and 50 μM dig−11-dUTP for one-color FISH. For two-color FISH 200 μM dATP, 200 μM dCTP, 200 μM dGTP, 130 μM dTTP and 70 μM biotin-dUTP (Roche Molecular Biochemicals) were used. The amplification profile was 94 °C/5 min, 35 cycles of 94 °C/45 sec, 59 °C/45 sec, 72 °C/1 min, followed by a final extension step of 72 °C/10 min.

One-color FISH and two-colour FISH hybridizations were carried out using ribosomal probes according to Cross, Vega and Rebordinas (2003) with some modifications. For digoxigenin-labelled probes, hybridization sites were detected using mouse anti-digoxigenin, rabbit anti-mouse-FITC (fluorescein isothiocyanate) and goat anti-rabbit-FITC immunological reagents (Roche Diagnostics). Streptavidin Texas Red (Amersham Biosciences) and biotinylated anti-streptavidin (Vector) were used for biotin-labelled probes. The chromosomes were counterstained with 500 ng/ml propidium iodide (Vector Labs) for one-color FISH and 0.4 μg/ml DAPI (4′−6-Diamidino−2-phenylindole) for two-color FISH, both of them in Vectashield antifade. Images were recorded using a Zeiss Axioskop 2 plus fluorescence microscope equipped with a digital camera.

Results

The Ag-NORs staining showed that NORs were located on the short arm of a medium-sized submetacentric chromosome pair in *S. senegalensis* (Figure 1a). Positive stains were not observed on any other chromosome regions, although differences in the NOR sizes were observed both in homologous chromosomes and in different individuals. FISH using the 45S rDNA probe revealed strong signals on the short arm of a medium-sized submetacentric chromosomal pair, apparently identical to the NOR chromosome pair (Figure 1b). In addition, some metaphase spreads showing additional hybridization signals and variable signal sizes were observed both between chromosome chromatids and between homologous chromosome pairs. Hybridization with 5S rDNA showed one major signal occupying the short arm of a medium-sized submetacentric chromosome pair, similar to the 45S chromosome, and another minor signal on centromeric sites of a small acrocentric pair (Figure 1c). These results are coincident with the recently published by Manchado et al. (2005). Different signal sizes between homologous chromosomes and individuals were observed frequently. In order to determine if the major and minor ribosomal genes were mapped to the same chromosome, a two-color FISH with both ribosomal probes was performed. Two color signals representing the 5S and the 45S rDNAs were visualized on a medium-sized submetacentric chromosomal pair. It indicated the co-localization on the major 5S and 45 S rDNAs and hence, the synteny of both rDNAs arrays (Figure 1d). The telomeric $(TTAGGG)_n$ repeat hybridized to all chromosomal telomeres but not to the NORs (Figure 2b). The intensity of the telomeric signals varied on different chromosomes. Finally, the $(GATA)_n$ probe produced dispersed and weak hybridization signals throughout all the chromosomes, indicating the ubiquitous presence of the repeat throughout all the genome (Figure 2a).

Discussion

The number and location of the NORs have been used as a cytotaxonomic parameter. The *S. senegalensis* karyotype contains a diploid number of 42 chromosomes, including 6 metacentrics, 4 submeta-subtelocentrics, 8 subtelocentrics and 24 acrocentrics, presenting a variety of chromosomes (Vega et al., 2002). Among soleid karyotypes, only those of *S. solea* (Barker, 1972) and *S. lascaris* (Vasil'ev, 1978) are known having $2n = 42$ chromosomes. Only one NOR site was found in *S. senegalensis*. It was located on the short arm of a medium-sized submetacentric chromosome pair. This finding appear to agree with those reported for another two previously identified soleid species (Pardo et al., 2001). The existence of only one NOR chromosome pair should be considered as a plesiomorphic character in fish and other organisms (Hartley, 1987). In addition, the Ag-NORs have been observed on two other locations in the

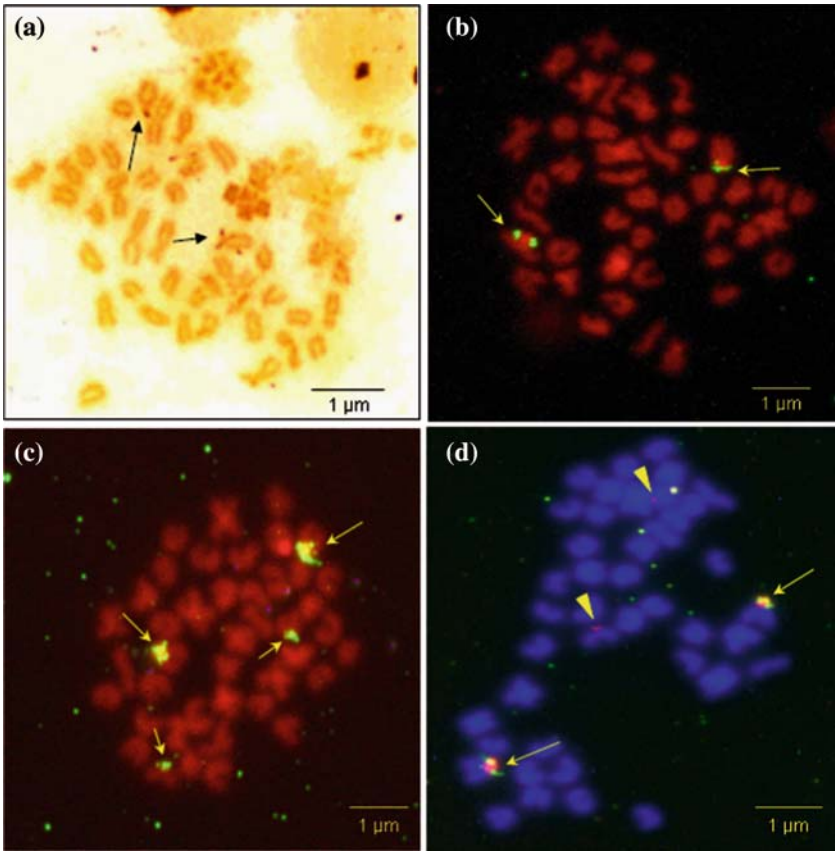

Figure 1. *S. senegalensis* metaphases showing Ag-NORs (arrows) (a), one-color FISH using the 45S (b) and 5S (c) rDNAs probes (yellow) and two-colour FISH of 45S and 5S ribosomal genes (d). Pink FISH signals (arrowheads) correspond to the minor 5S rDNA gene whereas the pink-yellow signals the 45S and the major 5S rDNAs co-localization (arrows).

order Pleuronectiformes. The marker was seen in telomeres in the family Soleidae, Scophtalmidae and Pleuronectidae (Pardo et al., 2001), and in centromeric or subcentromeric sites in the family Paralichthyidae (Kikuno, Ojima & Yamashita, 1986) and Bothidae (Vitturi, Catalano & Colombera, 1993). The family Pleuronectidae has been considered as the most ancestral group among the order Pleuronectiformes based on some cytogenetic data (Le Grande, 1975); nevertheless, other molecular and morphologic studies suggest the Soleidae family being much more ancestral (Verneau et al., 1993). The centromeric/subcentromeric NOR in the family Bothidae could represent a more derived condition within this order. If we take into account that species in a higher degree of evolution carry lower number of chromosomes with respect to the ancestral karyotype, the family Pleuronectidae (*P. flesus*, $2n = 48$) should be considered the oldest lineage within this order as hypothesized by Le Grande (1975). Nevertheless, additional NORs have been detected by means of rDNA-FISH in *S. maximus* and *P. flesus* (Pardo et al., 2001), but not in *S. senegalensis*. The silver staining technique only allows to visualize the actively transcribed NORs during the previous interphase, while some inactive ribosomal sequences or pseudogenes in other regions can only be detected by means of FISH (Martins & Galetti Jr., 2001). In addition, variability in the size of both NOR and rDNAs that we observed in *S. senegalensis* has been described in different organisms and attributed to duplications and/or deletions, which habitually take place in long arrays of repeats (Garrido-Ramos et al., 1995; Zurita et al., 1998; Cross, Vega & Rebordinos, 2003).

The location of the 5S rRNA genes has been described in approximately 50 fish species pertaining to different groups (Hatanaka & Galetti

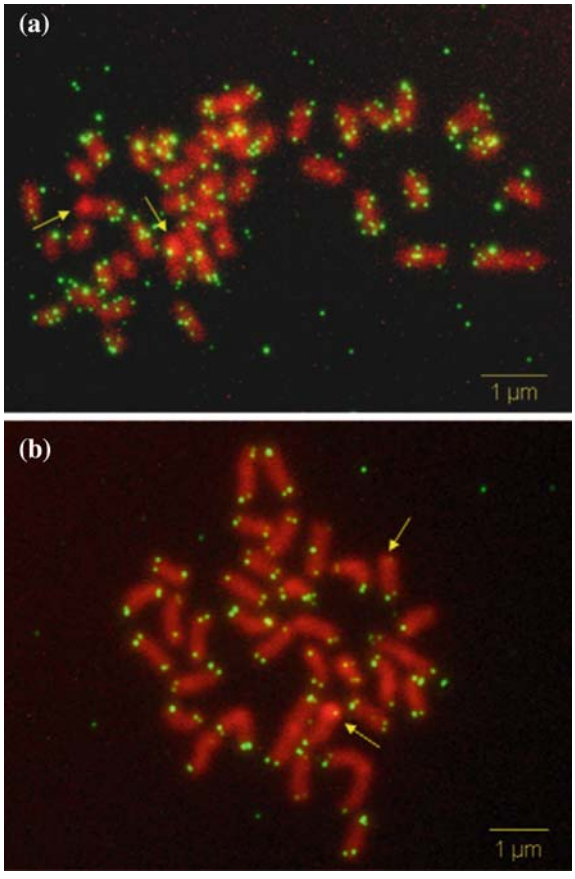

Figure 2. FISH signals of $(GATA)_n$ (a) and $(TTAGGG)_n$ probes in a partial metaphase of *S. senegalensis*.

Jr., 2004). In most fish it appears in an interstitial position of the chromosomes (Martins & Galetti Jr., 2001). In the present study the 5S rDNA gene was observed on two different chromosomic pairs. Nevertheless, the co-localization of both the major 45S and minor 5S ribosomal genes on the same chromosomal pair in *S. senegalensis* appears to be quite rare. Location of 5S rDNA in fish has been described in synteny with the 45S in few studies (Fujiwara et al., 1998; Almeida-Toledo et al., 2002) but on different chromosomes in most studies (Martins & Galetti Jr., 2001). Some surveys showed both genes quite separated on a chromosome pair (Hatanaka & Galetti Jr., 2004). In addition, mechanisms, such as the gene conversion and the unequal crossingover, that act in the evolutionary processes of the multiple tandem arrays, could produce undesirable translocations of 5S to the 45S cluster, possibly providing an advantage to those species having rRNA genes on different chromosomes (Martins & Galetti Jr., 2001).

The telomeric sequence $(TTAGGG)_n$ present at all the chromosomal termini in *S. senegalensis* has been widely described in vertebrates (Gornung, Gabrielli & Sola, 1998). Moreover, the finding of $(TTAGGG)_n$ repeat in marine invertebrate telomeres points to its wider distribution among eukaryotic organisms and suggests an ancestry older than originally presumed from its vertebrate distinctiveness (Plohl et al., 2002). In *S. senegalensis*, the occurrence of these sequences at the telomeres, but not interstitially, exclusively suggests that the repeated sequence is not dispersed within the genome of this species, at least to a significant extent for being detected by FISH. The comparison between the karyotype of *S. senegalensis* ($2n = 42$ and 60 chromosome arms (NF)) and the karyotype of *S. rhombus* and *S. maximus*, other Pleuronectiformes species ($2n = 44$ chromosomes and NF = 48)(Pardo et al., 2001) suggests that certain chromosome rearrangements may cause the chromosome number reduction and the NF increasing. The detection of interstitial (TTAGGG)*n* signals support the hypothesis of chromosome number reduction in some species of the family Cichlidae (Chew et al., 2002). However, chromosome rearrangements have occurred in some species of ants without the presence of telomeric interstitial signals (Meyne, Hirai & Imani, 1995). Hence, chromosome fusion in *S. senegalensis* could have involved the elimination of telomeric interstitial sequences by deletion or mutation (Martins et al., 2004).

Telomeric signals were undetected in the NOR-bearing chromosomes. Although they have associated with the NORs in salmonids (Reed & Phillips, 1995) they were not seen in other species such as the Nile tilapia (Chew et al., 2002). It would be interesting to carry out some FISH studies of the telomeric repeats in the Pleuronectiformes where there are big differences in both the chromosome number and the NF (*S. senegalensis* $2n = 42$ and NF = 60, *Solea solea* and *S. lascaris* $2n = 42$ and NF = 56–58, *Scophthalmus rhombus* and *S. maximus* $2n = 44$ NF = 48 and *Platichthys flesus* $2n = 48$ NF = 48) (Pardo et al., 2001), in

order to further elucidate the chromosomal evolution in this fish group.

Results with the $(GATA)_n$ probe, one member of satellite DNA families, revealed the ubiquitous presence of the $(GATA)_n$ repeat throughout all the genome limiting its utility as a chromosome marker. The $(GATA)_n$ has been associated with the sex in some species (Demas & Wachtel, 1991; Subramanian, Mishra & Singh, 2003). In spite of being a potential marker associated with the sex and evolution, the localization of the $(GATA)_n$ by means of FISH has been used rarely (Cross et al., 2005; Gallardo-Escárate et al., 2005). However, the recent finding that GATA repeats have been gradually accumulated in highly evolved organisms (Subramanian, Mishra & Singh, 2003), has converted this sequence into a valuable tool to be used in studies of cytogenetic characterization.This paper contains a first approach to the molecular characterization of the Sole (*Solea senegalensis*) and it provides some valuable cytogenetic markers in the study of phylogenetic relationships in Pleuronectiformes.

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References

Almeida-Toledo, L.F., C. Ozouf-Costaz, F. Foresti, C. Bonillo, F. Porto-Foresti & M. Daniel-Silva, 2002. Conservation of the 5S-bearing chromosome pair and co-localization with major rDNA clusters in five species of Astyanax (Pisces, Characidae). Cytogenet. Genome Res. 97: 229–233.

Barker, C.J., 1972. A new method for the display of chromosomes plaice, *Pleuronectes platessa*, and other marine fishes. Copeia 2: 365–368.

Bouza, C., L. Sanchez & P. Martinez, 1994. Karotypic characterization of turbot (*Scophthalmus maximus*) with conventional, fluorochrome and restriction endonuclease-banding techniques. Mar. Biol. 120: 609–613.

Cross, I., L. Vega & L. Rebordinos, 2003. Nucleolar organizing regions in *Crassostrea angulata*: chomosomal location and polymorphism. Genetica 119: 65–74.

Cross, I., E. Díaz, I. Sánchez & L. Rebordinos, 2005. Molecular and cytogenetic characterization of *Crassostrea angulata* chromosomes. Aquaculture 247: 135–144.

de Azevedo, M.F., C. Oliveira, B.G. Pardo, P. Martínez & F. Foresti, 2005. Chromosome banding and 18S rDNA *in situ* hybridization analysis of seven species of the family Achiridae (Teleostei: Pleuronectiformes). Genetica 125: 125–132.

Demas, S. & S. Wachtel, 1991. DNA fingerprinting in reptiles: Bkm hybridization patterns in Crocodilia and Chelonia. Genome 34: 472–476.

Dinis, M.T., L. Ribeiro, F. Soares & C. Sarasquete, 1999. A review on the cultivation potential of *Solea senegalensis* in Spain and Portugal. Aquaculture 176: 27–38.

Fujiwara, A., S. Abe, E. Yamaha, F. Yamazaki & C. Yoshida, 1998. Chromosomal localization and heterochromatin association of ribosomal RNA gene loci and silver-stained nucleolar organizer regions in salmonid fishes. Chromosome Res. 6: 463–471.

Gallardo-Escárate, C., J. Álvarez-Borrego, M.A. Del Río-Portilla, I. Cross, M.A. Merlo & L. Rebordinos, 2005. Fluorescence *in situ* hybridization of rDNA, telomeric (TAAGGG)n and (GATA)*n* repeats in the red abalone *Haliotis rufescens* (Archaeogastropoda: Haliotidae). Hereditas 142: 73–79.

Garrido-Ramos, M.A., M. Jamilena, R. Lozano, S. Cárdenas, C. Ruiz-Rejón & M. Ruiz-Rejón, 1995. Cytogenetic analysis of gilthead seabream *Sparus aurata* (Pisces, Perciformes), a deletion affecting the NOR in a hatchery stock. Cytogenet. Cell Genet. 68: 3–7.

Gornung, E., I. Gabrielli & L. Sola, 1998. Localization of the (TTAGGG)n telomeric sequence in zebrafish chromosomes. Genome 41: 136–138.

Hartley, S.E., 1987. The chromosomes of salmonid fishes. Biol. Rev. 62: 197–214.

Hatanaka, T. & P.M. Galetti Jr., 2004. Mapping of the 18S and 5S ribosomal RNA genes in the fish *Prochilodus argenteus* Agassiz, 1829 (Characiformes, Prochilodontidae). Genetica 122: 239–244.

Howell, W.M. & D.A. Black, 1980. Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. Experientia 36: 1014–1015.

Ijdo, J.W., R.A. Wells, A. Baldini & S.T. Reeders, 1991. Improved telomere detection using a telomere repeat probe (TTAGGG)*n* generated by PCR. Nucleic Acids Res. 19: 4780.

Jones, K.W. & L. Singh, 1985. Snakes and evolution of sex chromosomes. Trends Genet. 1: 55–61.

Kavalco, K.F., R. Pazza, A.C. Bertollo & O. Moreira-Filho, 2005. Molecular cytogenetics of *Oligosarcus hepsetus* (Teleostei, Characiformes) from two Brazilian locations. Genetica 124: 85–91.

Kikuno, T., Y. Ojima & N. Yamashita, 1986. Chromosomes of flounders, *Paralichthys olivaceus*. Proc. Jap. Acad. 62B: 194–196.

Le Grande, W.H., 1975. Karyology of six species of Louisiana flatfishes (Pleuronectiformes: Osteichthyes). Copeia 3: 516–522.

Manchado M., E. Zuasti, I. Cross, M.A. Merlo, C. Infante & L. Rebordinos, 2005. Molecular characterization and chromosomal mapping of the 5S rRNA gene in *Solea*

senegalensis: a new linkage to the U1, U2 and U5 small nuclear RNA genes. Genome (in press).

Mandrioli, M., M.S. Colomba & R. Vitturi, 2000. Chromosomal analysis of repeated DNAs in the rainbow wrasse *Coris julis* (Pisces, Labridae). Genetica 108: 191–195.

Martins, C. & P.M. Galetti Jr., 2001. Two 5S arrays in Neotropical fish species: is it a general rule for fishes? Genetica 111: 439–446.

Morán, P., J.L. Martinez, E. Garcia-Vazquez & A.M. Pendás, 1996. Sex chromosome linkage of 5S rDNA in rainbow trout (*Oncorhynchus mykiss*). Cytogenet. Cell Genet. 75: 145–150.

Pardo, B.G., C. Bouza, J. Castro, P. Martinez & L. Sánchez, 2001. Localization of ribosomal genes in Pleuronectiformes using Ag- CMA_3-banding and in situ hybridization. Heredity 86: 531–536.

Plohl, M., E. Prats, A. Martínez-Lage, A. González-Tizón, J. Méndez & L. Cornudella, 2002. Telomeric localization of the vertebrate-type hexamer repeat, (TTAGGG)n in the wedgeshell clam *Donax trunculus* and other marine invertebrate genomes. J. Biol. Chem. 277: 19839–19846.

Ribeiro, L., C. Sarasquete & M.T. Dinis, 1999. Histological and histochemical development of the digestive system of *Solea senegalensis* (Kaup. 1858) larvae. Aquaculture 171: 293–308.

Roiha, H., J.R. Miller, L.C. Woods & D.M. Glover, 1981. Arrangements and rearrangements of sequences flanking the two types of rDNA insertion in *Drosophila melanogaster*. Nature 290: 749–753.

Rossi, A.R. & E. Gornung, 2005. Cytogenetic analysis of three italian populations of *Coregonus lavaretus* (Pisces, Salmoniformes) with chromosomal localization of major and minor ribosomal genes, and telomeric repeats. Hereditas 142: 15–21.

Singh, L. & K.W. Jones, 1982. Sex reversal in the mouse (*Mus musculus*) is caused by a recurrent nonreciprocal crossover involving the X and an aberrant Y-chromosome. Cell 28: 205–216.

Sola, L., E. Gornung, H. Naoi, R. Gunji, C. Sato, K. Kawamura, R. Arai & T. Ueda, 2003. FISH-mapping of 18S ribosomal RNA genes and telomeric sequences in the Japanese biterlings *Rhodeus ocellatus kurumeus* and *Tanakia limbata* (Pisces, Cyprinidae) reveals significant cytogenetic differences in morphologically similar karyotypes. Genetica 119: 99–106.

Subramanian, S., R.K. Mishra & L. Singh, 2003. Genome-wide analysis of Bkm sequences (GATA repeats): predominant association with sex chromosomes and potential role in higher order chromatin organization and function. Bioinformatics 19: 681–685.

Suzuki, H., S. Sakurai & Y. Matsuda, 1996. Rat rDNA spacer sequences and chromosomal assigment of the genes to the extreme terminal region of chromosome 19. Cytogenet. Cell Genet. 72: 1–4.

Tiersch, T.R., B.A. Simco, K.B. Davis & S.S. Wachtel, 1992. Molecular genetics of sex determination in channel catfish: studies on SRY, ZFY, Bkm and human telomeric repeats. Biol. Reprod. 47: 185–192.

Tigano, C., L. Rocco, V. Ferrito, D. Costagliola, A.M. Pappalardo & V. Stingo, 2004. Chromosomal and molecular characterization of ribosomal RNA genes in *Lebias fasciata* (Telostei, Cyprinodontidae). Genetica 131: 95–100.

Traut, W., 1987. Hypervariable Bkm DNA loci in a moth *Ephesia kuehniellla*: does transposition cause restriction fragment length polymorphism? Genetics 115: 112–118.

Verneau, O., C. Moreau, F.M. Catzeflis & F. Renaud, 1993. Phylogeny of flatfishes (Pleuronectiformes): comparisons and contradictions of molecular and morpho-anatomical data. J. Fish Biol. 45: 685–696.

Vasil'ev, V.P., 1978. Karyotypes of 5 species of fishes (Pisces) from the Black Sea. Tsitologiya 20: 1092–1094.

Vega, L., E. Díaz, I. Cross & L. Rebordinos, 2002. Caracterización citogenética e isoenzimática del lenguado *Solea senegalensis* Kaup, 1858. Bol. Inst. Esp. Oceanogr. 18: 245–250.

Vitturi, R., R. Catalano & D. Colombera, 1993. Chromosome analysis of *Bothus podas* (Pisces, Pleuronectiformes) from the Mediterranean Sea. J. Fish Biol. 43: 221–227.

Zurita, F., R. Jiménez, M. Burgos, R. Díaz & de Guardia la, 1998. Sequential silver staining and *in situ* hybridization reveal a direct association between rDNA levels and the expression of homologous nucleolar organizing regions: a hypothesis for NOR structure and function. J. Cell Sci. 111: 1433–1439.