© Indian Academy of Sciences

RESEARCH ARTICLE

Population distribution of 45S and 5S rDNA in golden mahseer, *Tor putitora*: population-specific FISH marker

MAMTA SINGH[1], RAVINDRA KUMAR[1]*, N. S. NAGPURE[1], BASDEO KUSHWAHA[1], INDRA MANI[1], U. K. CHAUHAN[2] and W. S. LAKRA[1]

[1]*National Bureau of Fish Genetic Resources, Canal Ring Road, P.O. Dilkusha, Lucknow 226 002, India*
[2]*Faculty of Life Science, School of Environmental Biology, Awadhesh Pratap Singh University, Rewa 486 003, India*

Abstract

Chromosomal locations of major 45S and minor 5S ribosomal DNAs (rDNAs) and organization of 5S rRNA genes were analysed in five different populations of golden mahseers (*Tor putitora*) using fluorescence *in situ* hybridization (FISH) and Southern blot hybridization. All five populations of *T. putitora* ($2n = 100$) showed a similar type of macro-karyotype composed of 12 metacentric, 22 submetacentric, 14 subtelocentric and 52 telocentric chromosomes. Analysis of active nucleolar organizer regions (NORs) by silver staining did not show any differences in number and chromosomal position in different populations. But FISH data showed significant difference between the populations, four of the five populations showed six 18S (three pairs) and two 5S (one pair) signals with positional polymorphism, while one population showed eight 18S and four 5S signals, respectively. Southern blot data confirms that 5S rDNA clusters present on two different chromosome pairs in Kosi river population contain non-transcribed spacers (NTS) of same length. In the present study, simultaneous localization of 45S and 5S rDNA by *in situ* hybridization helped us to develop the discrete population-specific markers in different geographically isolated populations of *T. putitora*.

[Singh M., Kumar R., Nagpure N. S., Kushwaha B., Mani I., Chauhan U. K. and Lakra W. S. 2009 Population distribution of 45S and 5S rDNA in golden mahseer, *Tor Putitora*: population-specific FISH marker. *J. Genet.* **88**, 315–320]

Introduction

Repetitive DNA constitutes a considerable genome fraction in higher eukaryotes and represents the major component of heterochromatin (Miklos 1985; Charlesworth *et al*. 1994). These repetitive DNA sequences and specific associated proteins have been suggested to be involved in important processes such as stability of genome structure, position effect variegation, recombination, chromosome pairing and segregation, suprachromosomal organization as well as genetic differentiation and karyotypic evolution (Miklos 1985; Weiler and Wakimoto 1995; Ren *et al*. 1997). The existence of multiple copies of repetitive sequences, such as ribosomal DNA (rDNA), constitutes the basis for wide variation found among species, populations and individuals (Castro *et al*. 2001; Tigano *et al*. 2004; Santi-Rampazzo *et al*. 2008). Ribosomal RNA genes (rDNAs) are arranged in two different families including nucleolus forming major 45S rDNA family composed of multiple copies of a repeated unit that contain coding region of 18S, 5.8S and 28S rRNA (Long and David 1980; Pendas *et al*. 1993) and non-nucleolus forming tandemly arranged multiple copies of minor 5S rDNA family that comprise a highly conserved 120-bp long coding region and a variable non-transcribed spacer (NTS). As NTS is non-transcribed, it is expected to mutate freely. The 5S rDNA fits in a concerted evolution model (Drouin and Moniz 1995) and shows low intra-individual and intra-population heterogeneity due to homogenization of the repeated sequences (Dover 1982). For this reason, the NTS has been widely employed as a molecular marker for species identification and phylogenetic studies (Pasolini *et al*. 2006; Pinhal *et al*. 2009; Campo *et al*. 2009). The intense dynamisms of 5S rDNA repeats generate

*For correspondence. E-mail: ravindrakumar@scientist.com; rkumar@nbfgr.res.in.

Keywords. 18S rDNA; 5S rDNA; FISH; rDNA; *Tor putitora*.

variant classes of 5S rDNA (on the basis of NTS length and their sequence variation), which have been reported in the genome of several fish species (Martins and Galetti 2001; Wasko *et al*. 2001). Campo *et al*. (2009) reported the presence of two types of 5S rDNA in 12 species of genus *Merluccius* that is differentiated by the absence or presence of a simple sequence repeat in the NTS. Pinhal *et al*. (2009) also reported presence of two types of 5S rDNA clusters in the sharks of the genus *Rhizoprionodon*, termed class I cluster, composed of approximately 185 bp; class II cluster composed of approximately 465 bp. In many species of fish, the chromosome locations of the two rDNA families are usually at different (Martinez *et al*. 1996; Fujiwara *et al*. 1998; Sajdak *et al*. 1998; Martins and Galetti 2000; Ferro *et al*. 2001), while in few others species they are colocalized on the same chromosome (Moran *et al*. 1996; Inafuku *et al*. 2000; Fontana *et al*. 2003; Tigano *et al*. 2004).

Tor putitora, commonly called golden mahseer, is a coldwater fish, belonging to the order Cypriniformes and family Cyprinidae, inhabits a wide range of river-systems ranging from tropical waters to sub-Himalayan regions, and has high demand as food and attraction for anglers as a sport fish. Genetic analyses of natural population of *T. putitora* from the rivers of the Indus, Ganges and Mahanadi river systems has been carried out using polymorphic microsatellite and allozyme markers (Mohindra *et al*. 2004) and some of the classical cytogenetic techniques like karyomorphology, chromosome distribution of heterochromatin and different banding/staining (C-, G-, NORs, CMA_3 etc.) have been utilized earlier for characterization of Indian fish species including *T. putitora* (Khuda-Bukhsh and Nayak 1982; Lakra 1996; Kushwaha *et al*. 2001). However, no molecular cytogenetic data are available on the population distribution of 45S and 5S rDNA in any of the *Tor* species including *T. putitora*. Within the framework of the research on macro-evolutionary processes in this species, that has not been characterized previously among different geographically isolated populations, we simultaneously localized the 45S and 5S rDNA by FISH and characterized 5S rRNA genes by Southern blot hybridization to better investigate that whether the discrete population chromosome markers are present in different populations of golden mahseer or not.

Materials and methods

Sample collection and chromosome preparation

A total of 37 samples of *T. putitora* were collected from five geographically isolated populations (figure 1) that include populations from rivers Kosi and Alaknanda (both from Uttarakhand), river Satluj near Bilaspur (Himachal Pradesh), river Jia-Bhoreli (Assam) and Lonavala reservoir (Maharastra), India. Metaphase chromosome spreads were prepared from the anterior kidney cells using standard hypotonic treatment, acetic acid-methanol fixation and flame-drying technique according to Bertollo *et al*. (1978).

DNA isolation, PCR amplification and probe labelling

The genomic DNA of *T. putitora* was extracted from whole blood using standard phenol : chloroform : isoamylalcohol procedure, described by Sambrook and Russell (2001). For amplification of partial 18S rDNA, primers were designed from sequence of *Cyprinus carpio* available in NCBI database (accession no. AF133089). The primers used were forward: 5′-TTGGTGACTCTCGATAACCTC-3′ and reverse: 5′-CCTTGTTACGACTTTTACTTCCTC-3′ and the sequence was submitted to NCBI database (accession no. EU597006). For 5S rDNA amplification, the primers, taken from Moran *et al*. (1996), were forward: 5′-TACGCCCGATCTCGTCCGATC-3′ and reverse: 5′-CAGGCTGGTATGGCCGTAAGC-3′ and the sequence was submitted to NCBI database (accession no. EU621853). The 18S rDNA probe was labelled with tetramethylrhodamine-5-dUTP (Roche, Basel, Switzerland) by nick translation while 5S rDNA probe was labelled with biotin, according to manufacturer's instructions (Vector Labs, Burlingame, USA).

Double-target FISH

Double-target FISH was performed to determine the colocalization of 18S major and 5S minor rDNA clusters. Two-to-three days aged chromosome preparations were baked at 90°C for 1 h followed by the FISH protocol described by Winterfeld and Roser (2007), with minor modifications in post-hybridization washing at 45°C instead of 42°C. Before detection of biotin-labelled probe, slides were incubated in 1× blocking solution (Vector Labs, Burlingame, USA) at 37°C for 30 min and detection reagents were diluted in 1× blocking solution approximately 30 min before use to further reduce any non-specific binding. Detection of biotin-labelled probe was carried out with fluorescein avidin DCS 5 μg/mL (Vector Labs, Burlingame, USA) followed by two rounds of signal amplification. After each step of amplification, slides were washed in 1× blocking solution while 18S rDNA probe detection did not require any antibody conjugate. The preparations were then rinsed twice each for 5 min with 4× SSC / 0.1% Tween 20 and counterstained with DAPI and mounted on Vectashield mounting medium (Vector Labs, Burlingame, USA). Slides were examined under fluorescence microscope (Leica, Illinois, USA) with triple band filter for simultaneous visualization of the three colours, i.e. DAPI (blue), rhodamine (red), and fluorescein (green).

Southern blot hybridization

The genomic organization of 5S rDNA was determined by Southern blot hybridization. Genomic DNA (10 μg) from all the populations was partially digested with *Hin*dIII at 37°C for 30 min and complete digestion was performed only for Kosi river samples. Partially to completely digested DNA was subjected to gel electrophoresis in 1% agarose gel and transferred to positively charged nylon membrane, according to Sambrook and Russell (2001). Probe of 5S rRNA gene of

T. putitora, collected from river Satluj, was labelled with biotin, according to manufacturer's instructions (Vector Labs, Burlingame, USA). Hybridization of filter immobilized DNA and post-hybridization washes were performed according to Sambrook and Russell (2001). Hybridized DNA was detected by BCIP/NBT substrate kit (Vector Labs, Burlingame, USA).

Results

In the present investigation 37 specimens from five geographically isolated populations showed a monomorphic macrokaryotype constitution with $2n = 100$ chromosome (12 metacentric, 22 submetacentric, 14 subtelocentric and 52 telocentric chromosomes). There was no visible differentiation among the populations from the five collection sites and between the sexes. Analysis of active NORs by silver staining did not show any difference in number and chromosomal position in different populations and were present on two pairs of chromosomes (data not shown). In examined polulations double-target FISH revealed an interesting chromosomal pattern in major and minor rDNA clusters. In Alaknanda, Satluj and Lonavala populations, major rDNA positive sites were present on telomeric position of three pairs of chromosomes (metacentric, submetacentric and telocentric) (figures 1 and 2a) while specimens of *T. putitora* from Jia-Bhoreli river showed six positive signals (three pairs) of major rDNA with positional polymorphism (one pair metacentric and two pair telocentric) (figure 2d). On the other hand, Kosi population showed one extra pair of major rDNA site, resulting in eight telomeric fluorescent signals (one pair metacentric, one pair submetacentic and two pairs telocentric) (figure 2b). Most of the metaphase spreads of *T. putitora* from the populations showed heterogeneity in signal intensity. Comparisons between FISH data using 18S rDNA probe and Ag-NOR in different populations strongly suggested the possible inactivation of NORs localized at the telomeres of a telocentric chromosome pair. On the other, 5S rDNA loci were present in only one submetacentric chromosome pair and the position was near the centromere in all the populations, except in Kosi river (figure 2a–e). Samples collected from Kosi river showed minor rDNA clusters on two pairs of chromosomes (one pair on submetacentirc chromosome and an extra pair on telocentric chromosome).

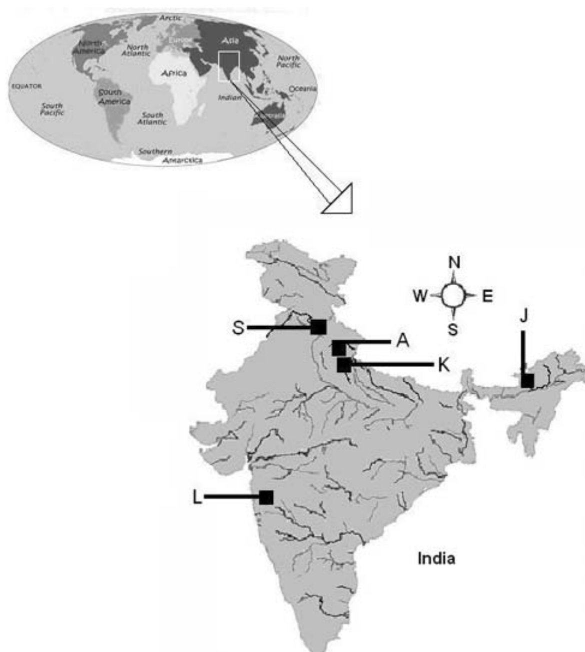

Figure 1. Collection sites of *T. putitora* specimens from India. S, Satluj river, belongs to Indus river system; A & K, Alaknanda and Kosi rivers, both belong to Ganges river system; J, Jia Bhoreli river, belongs to Brahmaputra river system; L, Lonavala reservoir.

PCR amplification of partial 18S rDNA generated a band of 1560 bp in *T. putitora* (NCBI accession no. EU597006). This sequence showed about 96% average similarity to the sequences of other fish species deposited in the NCBI database. After sequencing, the length of single repeat of the minor rDNA family was found to be 201-bp long (NCBI accession no. EU621853). The nucleotide sequences of the 5S rDNA coding region in *T. putitora* species shared a highly conserved gene (100% identity) of 120-bp length which contained three elements; namely Box A, Box C and IE, of the internal control regions that functions as a promoter for the gene (Hallenberg *et al.* 1994). Non-transcribed spacer (NTS) region of the minor family was reported to be 81-bp long. The NTS region contained three TATA box-like sequences in all the populations of *T. putitora* studied by us. The coding sequence of minor rDNA family in *T. putitora* after searches using BLASTn program (Altschul *et al.* 1997) showed an average sequence similarity of 95.74% with other fishes belonging to order Cypriniformes, Characiformes, Salmoniformes, Gadiformes, Perciformes and Tetradontiformes. In all the populations of *T. putitora*, the genomic DNA was partially digested with *Hin*dIII, which only cleaves once in the 5S rRNA gene of the species studied and then hybridized with 5S rRNA gene probe to examine the organization of the 5S rDNA. Comparative analysis of band pattern (ladder of exact integers of 201) after Southern blot hybridization in all the populations showed identical results (figure 3). Complete digestion with *Hin*dIII in the sample collected from Kosi river showed one 201-bp long band after Southern blot detection (figure 4).

Discussion

Tor putitora has shown to be an excellent material for evolutionary studies due to its wide distribution as well as its morphological and karyotypic diversities. Comparative analysis of five populations of *T. putitora* demonstrated differentiation at chromosomal level with fixed variation in number as well as position of major and minor rDNA clusters. In all the populations, Ag-NOR positive signal was observed on two pairs of chromosomes but major rDNA FISH showed positive signals on three to four pairs of chromosomes. The

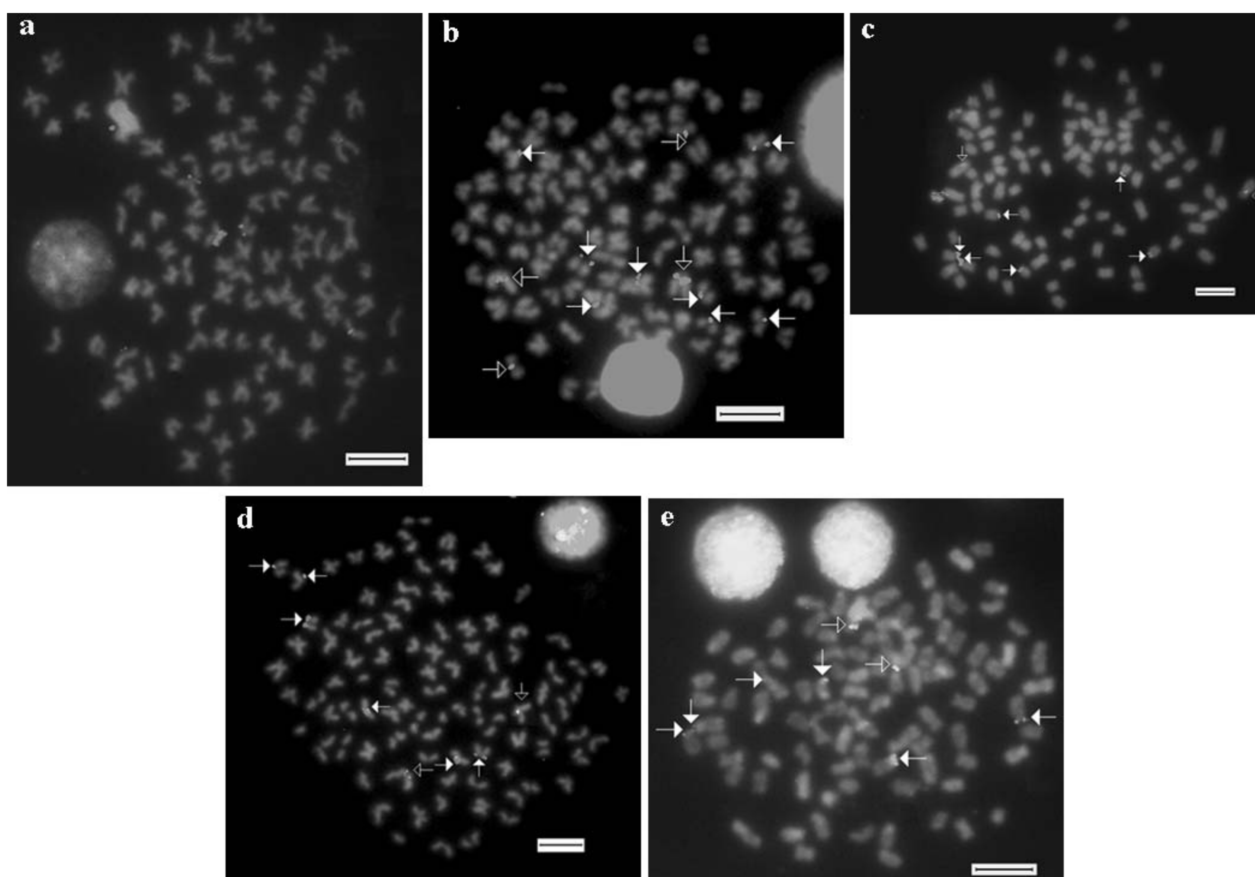

Figure 2. Dual colour FISH on metaphase spread in different populations of *T. putitora* (a) Alaknanda river population. (b) Kosi river population. (c) Satluj river population. (d) Jia-Bhoreli river population. (e) Lonavala reservoir population. The micrographs were taken with triple band filter allowing the simultaneous visualization of DAPI stained chromosomes, the hybridization sites of 18S (white arrows) and the 5S (hollow arrows) rDNA probe. Bar = 5 μm.

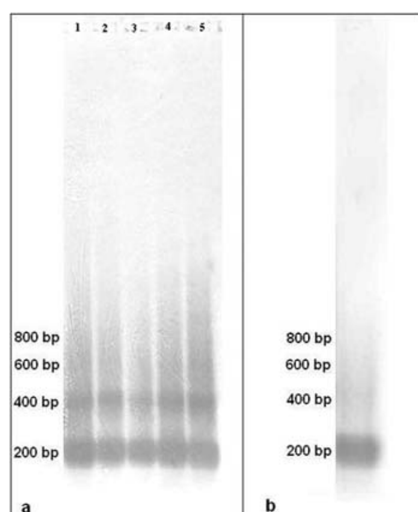

Figure 3. Southern blot of 5S rRNA gene in geographically isolated populations of *T. putitiora*: (a) partially digested (*Hin*dIII) genomic DNA in specimen of lane 1, Satlej river; lane 2, Lonavalla reservoir; lane 3, Jia-Bhoreli river; lane 4, Alaknanda river; lane 5, Kosi river, and (b) completely digested (*Hin*dIII) genomic DNA in specimen collected from Kosi river.

variation in number of signals could be attributed to the fact that the Ag-NORs are visualized only in transcriptionally-active state. The silver nitrate gives positive signals only to those NORs that were expressed during the last interphase, since silver binds to a complex of acidic protein associated with the nucleolus and nascent pre-RNA (Jordan 1987). On the other hand, FISH allows specific identification of major rDNA clusters irrespective of their transcriptional state. The size heteromorphism of major rDNA clusters, reflected in the signal strength at different loci, indicates high rates of non-homologous crossing over in these chromosome regions, which was considered to be an alternative mechanism of NOR inactivation in mammals (Guillen *et al*. 2004).

The minor rDNA clusters showed invariability in their number and position (interstitial), except for the samples from Kosi population. Martins and Wasko (2004) suggested that in fishes, the 5S rDNA clusters are most commonly located at interstitial chromosome site and this interstitial position is optimal for its organization in fish, since it has been found in most species of several orders. The length of single repeat of 5S rDNA was found to be 201 bp and the

nucleotide sequences of 5S rDNA have highly conserved 120-bp long coding gene region and 81-bp long variable NTS region. Organization of 5S rRNA in all the populations, examined by Southern blot hybridization, showed the presence of single type of 5S rDNA repeat in *T. putitora*. Detection of a ladder of exact integers of 201 in partially digested DNA provides the evidence that these 5S rDNA units were organized in repeats of tandem array. Two different genomic locations of 5S rDNA unit were also characterized by same type of non-transcribed spacers in Kosi river population because complete digestion of DNA showed only 201-bp band after Southern blot detection.

Simultaneous localization of the major and minor rDNAs in the examined populations reveal interesting results that lead to a different hybridization pattern in Kosi river population as compared to other four populations. Kosi population showed eight fluorescent signals (four pairs) of major rDNA and four fluorescent signals (two pairs) for minor rDNA while remaining four populations showed six signals (three pairs) for major rDNA and two signals (one pair) for minor rDNA. Observed extensive inter-population variation in chromosomal localization of these two rDNA clusters over the genome does not show a geographical pattern. This could be due to: (i) the existence of effective barriers to gene flow, (ii) the occurrence of random expansion of the repetitive DNAs yielding different patterns in each population, (iii) adaptation to an unknown environmental factor not related to geographical distance between populations, or (iv) a combination of these factors.

The active process of genomic changes at different levels of chromosomal organization leads to a strong interspecific and intra-specific as well as inter-population and intra-population variability. This points to the need for more extensive research; especially on the species with wide geographical distribution, possibly through integrated and complementary approaches to evaluate degrees of genetic exchange among populations. In this respect, intra-specific and intra-population cytogenetic polymorphism can have implications for stock identity.

To date, no population-specific FISH marker, employed in *T. putitora*, is recorded. Indeed, no effort has been made for application of FISH tools in genomic investigation of Indian fish species. In the present study, we have characterized various populations of *T. putitora*, albeit with a rather limited sample size, on the basis of localization and distribution of major and minor repetitive rDNA clusters and made an attempt towards the development of population-specific FISH marker for Kosi river population. In light of widespread concerns about ecological health of exploited *T. putitora* populations, we tried to focus on the development of population identification method that will help in effective planning and management, and to develop conservation strategies for severely declining populations. In conclusion, the present study describes the population distribution of major and minor rDNA and by this analysis we were able to develop a population-specific FISH marker for the Kosi river population of *T. putitora*.

Acknowledgements

The authors are thankful to the Department of Biotechnology, Ministry of Science and Technology, Government of India for financial support.

References

Altschul S. F., Madden T. L., Schäffer A. A., Zhang J., Zhang Z., Miller W. *et al.* 1997 Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* **25**, 3389–3402.

Bertollo L. A. C., Takahashi C. S. and Moreira-Filho O. 1978 Cytotaxonomic consideration on *Hoplias lacrdae* (Pisces, Erythrinidae). *Braz. J. Genet.* **1**, 103–120.

Campo D., Machado-Schiaffino G., Horreo J. L. and Garcia-Vazquez E. 2009 Molecular organization and evolution of 5S rDNA in the genus *Merluccius* and their phylogenetic implications. *J. Mol. Evol.* **68**, 208–216.

Castro J., Santiago R., Padro B. G., Sanchez L. and Martinez P. 2001 Population analysis of an unusual NOR-site polymorphism in Brown trout (*Salmo trutta* L.). *Heredity* **86**, 291–302.

Charlesworth B., Sniegowski P. and Stephan W. 1994 The evolutionary dynamics of repetitive DNA in eukaryotes. *Nature* **371**, 215–220.

Dover G. A. 1982 Molecular drive: a cohesive model of species evolution. *Nature* **299**, 111–117.

Drouin G. and Moniz de Sa M. 1995 The concerted evolution of 5S ribosomal genes linked to the repeat units of other multigene families. *Mol. Biol. Evol.* **12**, 481–493.

Ferro D. A. M., Neo D. M. , Moreira-Filho O. and Bertollo L. A. C. 2001 Nucleolar organizing regions, 18S and 5S rDNA in *Astyanax scabripinnis* (Pisces, Characidae): population distribution and functional diversity. *Genetica* **110**, 55–62.

Fontana F., Lanfredi M., Congiu L., Leis M., Chicca M. and Rossi R. 2003 Chromosomal mapping of 18S-28S and 5S rRNA genes by two-colour fluorescent in situ hybridization in six sturgeon species. *Genome* **46**, 473–477.

Fujiwara A., Abe S., Yamaha E., Yamazaki F. and Yoshida M. C. 1998 Chromosomal localization and hetrochromatin association of ribosomal RNA genes loci and silver stained nucleolar organizer regions in salmonid fishes. *Chromosome* Res. **6**, 463–471.

Guillen A. K., Hirai Y., Tanoue T. and Hirai H. 2004 Transcriptional repression mechanisms of nucleolus organizer regions (NORs) in humans and chimpanzees. *Chromosome Res.* **12**, 225–237.

Hallenberg C., Nederby-Nielson J., Frederiksen S. 1994 Characterization of 5S rRNA genes from mouse. *Gene* **142**, 291–295.

Inafuku J., Nabeyama M., Kikuma Y., Saitoh J., Kubota S. and Kohno S. 2000 Chromosomal location and nucleotide sequences of 5S ribosomal DNA of two cyprinid species (Osteichthyes, Pisces). *Chromosome Res.* **8**, 193–199.

Jordan G. 1987 At the heart of the nucleolus. *Nature* **329**, 489–499.

Khuda-Bukhsh A. R. and Nayak K. 1982 Karyomorpological studies in two species of hill stream fishes from Kashmir, India: occurrence of a high number of chromosomes. *Chromosome Inf. Sci.* **33**, 12–14.

Kushwaha B., Srivastava S. K., Nagpure N. S., Ogale S. N. and Ponniah A. G. 2001 Cytogenetic studies in two species of mahseer, *Tor khudree* and *Tor mussullah* (Cyprinidae, Pisces) from India. *Chromosome Sci.* **5**, 47–50.

Lakra W. S. 1996 Cytogenetic studies on endangered fish species: karyotypes of three species of mahseers, *Tor putitora, T. tor* and *T. khudree* (Cyprinidae Pisces). *Cytobios* **85**, 205–218.

Long E. O. and David I. D. 1980 Repeated genes in eukaryotes. *Annu. Rev. Biochem*. **49**, 727–764.

Martinez J. L., Moran P., Garcia-Vazquez S. and Pendas A. M. 1996 Chromosomal localization of major and 5S rRNA genes in the European eel (*Anguilla anguilla*). *Cytogenet. Cell Genet*. **73**, 149–152.

Martins C. and Galetti P. M. 1999 Chromosomal localization of 5S rRNA genes in Leporinus fish (Anostomidae, Characiformes). *Chromosome Res*. **7**, 363–367.

Martins C. and Galetti P. M. 2000 Conservative distribution of 5s rDNA loci in Schizodon (Pisa, Anastomidae) chromosomes. *Chromosome Res.* **85**, 353–355.

Martins C. and Galetti P. M. 2001 Organization of 5S rDNA in species of the fish *Leporinus*: two different genomic locations are characterized by distinct nontranscribed spacers. *Genome* **44**, 903–910.

Martins C. and Wasko A. P. 2004 Organization and evolution of 5S ribosomal DNA in the fish genome. In *Focus on genome research* (ed. C. R. Williams), pp. 289–319. Nova Science Publishers, New York, USA.

Miklos G. L. G. 1985 Localized highly repetitive DNA sequences in vertebrates and invertebrates genomes. In *Molecular evolutionary genetics* (ed. R. J. MacIntyre), pp. 241–321. Plenum Press, New York, USA.

Mohindra V., Khulbe R. L., Ponniah A. G. and Lal K. K. 2004 Microsatellite loci to assess genetic variation in *Tor putitora. J. Appl. Ichthyol*. **20**, 466–469.

Moran P., Martinez J. L., Garcia-Vazquez S. and Pendas A. M. 1996 Sex-linkage of 5S r DNA in rainbow trout (*Oncorhynchus mykiss*). *Cytogenet. Cell Genet*. **75**, 145–150.

Pasolini P., Costagliola D., Rocco L. and Tinti F. 2006 Molecular organization of 5s rDNA in Rajidae (Chondrichthyes): structural features and evolution of piscine 5S rRNA genes and nontranscribed intergenic spacers. *J. Mol. Evol*. **62**, 564–574.

Pendas A. M. , Moran P. and Garcia-Vazquez E. 1993 Multi chromosomal location of ribosomal RNA genes and heterochromatin association in brown trout. *Chromosome Res*. **1**, 63–67.

Pinhal D., Araki C., Gadig O. B. F. and Martins C. 2009 molecular organization of 5S rDNA in sharks of the genus *Rhizoprionodon*: insights into the evolutionary dynamics of 5S rDNA in vertebrate genomes. *Genet. Res*. **91**, 61–72.

Ren X., Eisenhour L., Hong C., Lee Y. and McKee B. D. 1997 Roles of rDNA spacer and transcription unit-sequences in X-Y meiotic chromosome pairing in *Drosophila melanogaster* males. *Chromosoma* **106**, 29–36.

Sajdak S. L., Reed K. M. and Phillips R. B. 1998 Intra individual and interspecies variation in the 5S r DNA of coregonid fish. *J. Mol. Evol*. **46**, 680–688.

Sambrook J. and Russell I. 2001 *Molecular cloning: a laboratory manual*, 3rd edition. Cold Spring Harbor Laboratory Press, Plainsveiw, New York, USA.

Santi-Rampazzo A. P., Nishiyama P. B., Ferreira P. E. B. and Martins-Santos I. C. 2008 Intrapopulational polymorphism of nucleolus organizer regions in Serrapinnus notomelas (Characidae, Cheirodontinae) from the Parana River. *J. Fish Biol*. **72**, 1236–1243.

Tigano C., Rocco L., Ferrito V., Costagliola D., Pappalardo A. M. and Stinso V. 2004 Chromosomal mapping and molecular characterization of ribosomal RNA genes in *Lebias fasciata* (Teleostei, Cyprinodontidae). *Genetica* **121**, 95–100.

Wasko A. P., Martins C., Wright J. M. and Galetti P. M. 2001 Molecular organization of 5S rDNA in fishes of genus *Brycon. Genome* **44**, 893–902.

Weiler K. S. and Wakimoto B. T. 1995 Heterochromatin and gene expression in *Drosophila. Annu. Rev. Genet*. **29**, 577–605.

Winterfeld G. and Roser M. 2007 Deposition of ribosomal DNAs in the chromosome of perennial oats (Poaceae: Aveneae). *Bot. J. Linn. Soc*. **155**, 193–210.

Received 26 May 2009, in revised form 18 July 2009; accepted 20 August 2009

Published on the Web: 18 November 2009