ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/239938973

Chromosomal distribution of constitutive heterochromatin in eight species of mahseers (Family: Cyprinidae) from...

Article *in* Indian Journal of Biotechnology · June 2013

CITATIONS
0

READS
135

7 authors, including:

Indra Mani
Gargi College (University of Delhi)
95 PUBLICATIONS **138** CITATIONS
SEE PROFILE

Ravindra Kumar
National Bureau of Fish Genetic Resources
80 PUBLICATIONS **892** CITATIONS
SEE PROFILE

Basdeo Kushwaha
National Bureau of Fish Genetic Resources
84 PUBLICATIONS **728** CITATIONS
SEE PROFILE

Naresh Sahebrao Nagpure
National Bureau of Fish Genetic Resources
153 PUBLICATIONS **945** CITATIONS
SEE PROFILE

Some of the authors of this publication are also working on these related projects:

Whole genome sequencing of 2 commercially important fishes C. batrachus and L. rohita View project

Reservoir development through community participation, using enhancement techniques. View project

All content following this page was uploaded by Indra Mani on 03 June 2014.

The user has requested enhancement of the downloaded file. All in-text references underlined in blue are added to the original document and are linked to publications on ResearchGate, letting you access and read them immediately.

Indian Journal of Biotechnology
Vol 12, April 2013, pp 178-186

Chromosomal distribution of constitutive heterochromatin in eight species of mahseers (Family: Cyprinidae) from India

Indra Mani, Ravindra Kumar*, Mamta Singh, B Kushwaha, N S Nagpure, P K Srivastava[1] and W S Lakra

National Bureau of Fish Genetic Resources (NFBGR), Canal Ring Road, PO Dilkusha, Lucknow 226 002, India

[1]Department of Biochemistry, Faculty of Science, Banaras Hindu University, Varanasi 221 005, India

Received 22 March 2012 ; revised 16 May 2012; accepted 25 July 2012

The chromosomal distributions of constitutive heterochromatin were analyzed in 8 species of mahseer, namely, *Tor chelynoides* (McClelland), *T. khudree* (Sykes), *T. mosal mahanadicus* (David), *T. mussullah* (Sykes), *T. putitora* (Hamilton), *T. progeneius* (McClelland), *T. tor* (Hamilton) and *Neolissochilus hexagonolepis* (McClelland) using C-banding technique. The constitutive heterochromatin bands were observed on several pairs of chromosomes with maximum numbers in *T. progeneius* (11 pairs), followed by *T. putitora* (7 pairs), *T. mosal mahanadicus* (4), *T. khudree* (3), *T. mussullah* (3), *N. hexagonolepis* (3), *T. chelynoides* (3) and *T. tor* (2). In all species, the C-bands were observed mainly on p arm with few on centromeric region of chromosomes. This seems to be the characteristic feature for characterization of these species. The presence of similar chromosome numbers (2n=100) with diverse karyotypes in the mahseer species suggests evolution among the species through pericentric inversions and/or heterochromatin processes. The number and location of the heterochromatic bands were found species-specific and thus useful for the cytotaxonomy of these species. The findings of the present study can also help to understand the evolutionary relationships among the mahseer species.

Keywords: Constitutive heterochromatin, karyoevolution, mahseer, *Neolissochilus* sp., *Tor* spp.

Introduction

Cyprinidae is the most abundant and globally widespread family of the freshwater fishes, comprises of 220 genera and approx 2420 species[1]. Among the cyprinids, mahseer is the common name used for almost 50 species of freshwater fishes from genera *Tor*, *Neolissocheilus* and *Naziritor*. Currently, 46 mahseer species are recognized; of which 23 species belong to genus *Tor* Gray, 22 species to genus *Neolissochilus* Rainboth and 1 species to genus *Naziritor* Mirza[2]. Mahseers are widely distributed along the Himalayan region of India, Pakistan, Bhutan, Bangladesh and Indonesia. In India, difference of opinion prevails regarding the number of species existing in genus *Tor*.

In global perspective, the mahseer group is well known as commercially important game fish and highly esteemed food fish. The species of mahseer are large-scaled barbels that live in upstream, clear and running waters. The natural status of some mahseer species has been assigned as endangered[3-5]. Osteological and morphological characters, especially the proportion of head length to body depth, are the common criteria used to classify these species[6]. The plasticity in the morphological characters among mahseer species has been the source of taxonomic ambiguities leading to disagreement between researchers with respect to the validity of some species as well as subspecies. For example, taxonomic ambiguity prevails for *Tor mosal mahanadicus*[7] and its valid name has been reported to be *T. khudree* (www.fishbase.org, version 4/2009). Another mahseer species *Neolissochilus hexagonolepis*, commonly known as chocolate mahseer in India, has similar morphology like *Tor* species. Therefore, morphometric as well as meristic characters sometime fail to resolve the proper identification among *Tor* species.

Earlier cytogenetic studies of mahseer species from India, undertaken using Giemsa and silver nitrate staining, have documented the diploid chromosome number (2n) as 100 and, in some studies, presence of species-specific variation in karyo-morphology has also been observed[8-11]. The C-banding studies in

*Author for correspondence:
Tel: +91-522-2442440/2442441; Fax: +91-522-2442403,
E-mail:Ravindra.Scientist@gmail.com;
ravindrakumar@scientist.com

teleost fishes have not only helped to correctly identify homologous chromosomes but also to visualize its role in evolution and speciation[12]. However, the reports on C-banding studies in the mahseer species are scanty. Therefore, the objectives of the present study were to: (a) identify chromosomes with C-banding patterns, and (b) discuss the inter-specific relationships based on C-banding patterns in mahseer species.

Materials and Methods

Collection of Specimens

Live specimens of eight undertaken mahseer species were collected from different water bodies (Table 1) and locations (Fig. 1) in India with the help of local fishermen. All the specimens were in juvenile stage and the sex was unidentifiable by visual examination.

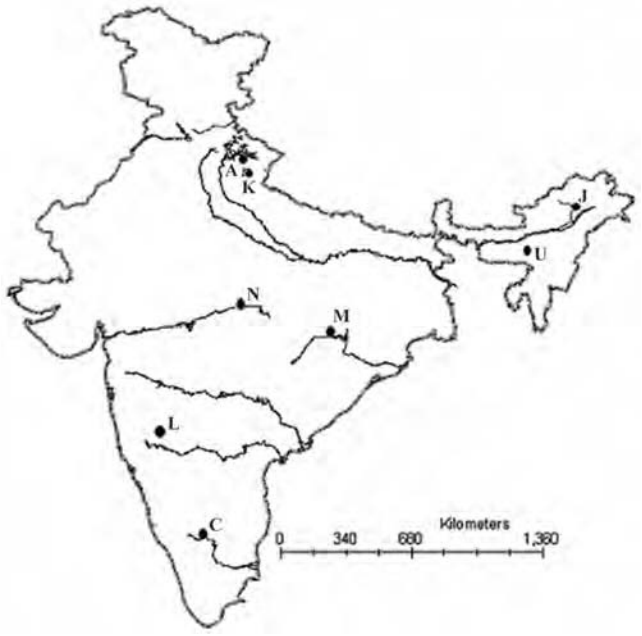


Fig. 1—Geographical locations of mahseer sampling from India: A. Alaknanda river, C. Cauvery river, M. Mahanadi river, L. Lonavala reservoir, K. Kosi river, J. Jia Bhoreli river, N. Narmada river & U. Tributaries of Umiam reservoir.

Chromosome Preparations

For chromosome preparations, the specimens were administered 0.05% colchicine (@ 1.0 mL/ 100 g body wt) intramuscularly and were sacrificed after 2 h for dissecting out the kidney tissue. The cells were processed for chromosome preparations using hypotonic treatment–methanol:acetic acid fixation-flame drying technique.

Fluorescence Staining

C-banding was done as per the technique described[13], followed by chromosome staining with propidium iodide (5 µg/ mL in DDW) instead of Giemsa. Constitutive heterochromatin (CH) band pattern for a species was determined by studying a minimum of 25 metaphase spreads per specimen. For karyo-morphology, chromosomes were grouped into metacentric (m), submetacentric (sm), subtelocentric (st) and telocentric (t) as per the classification proposed[14].

Results and Discussion

A marked chromosomal conservation of 100 diploid chromosomes has been observed in the eight undertaken mahseer species with different karyo-morphology. The C-banding patterns of these mahseer species are presented for comparison (Figs 2a-h). Differences in number and position of C-band on the chromosomes and location of C-heterochromatin chromosomes in the karyotype were observed among the species (Table 2). Enlarged view of the chromosomes bearing C-bands has been shown in Figs 3a-h.

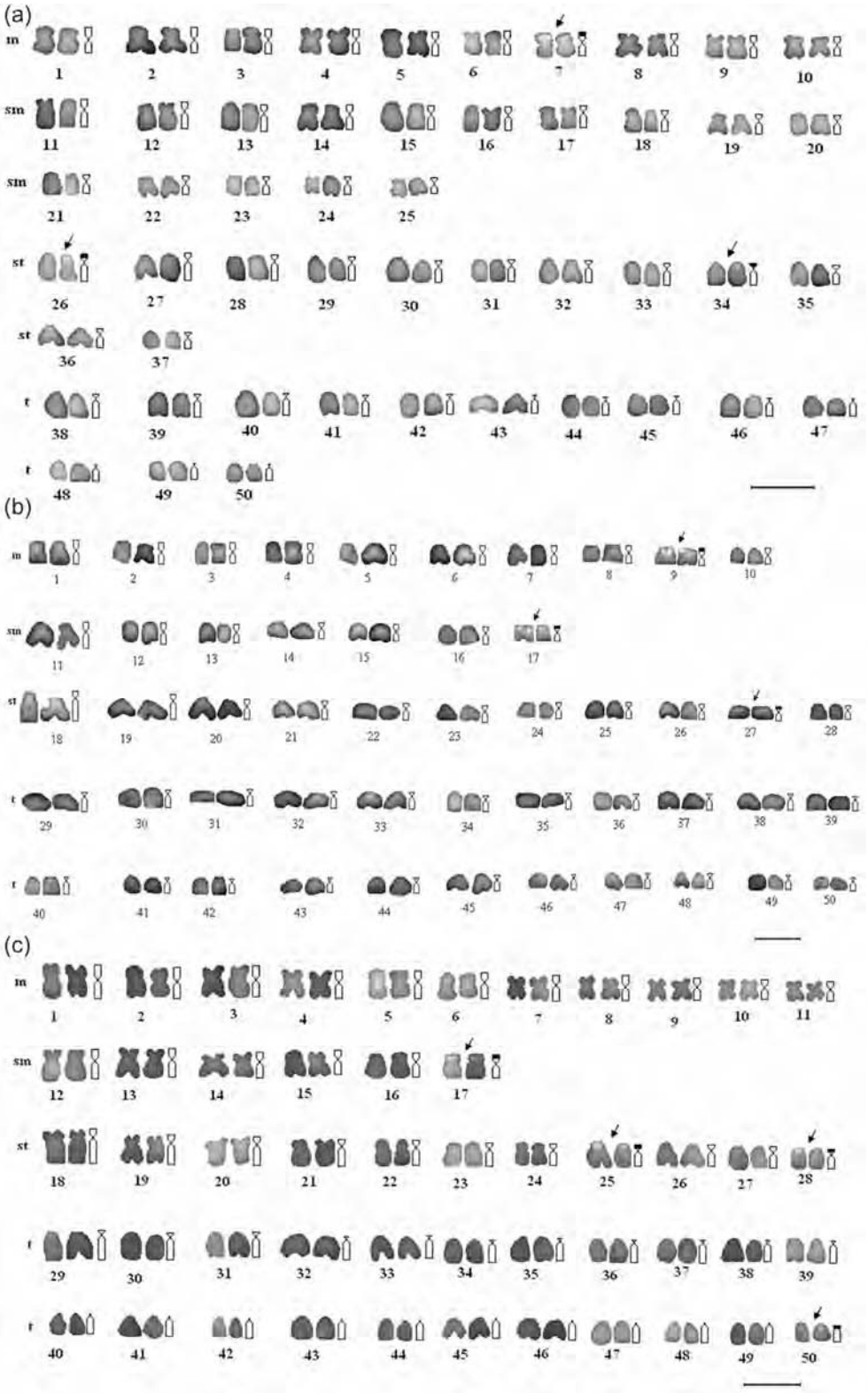
To date, the cytogenetic investigations in family Cyprinidae have been undertaken in approx 416

Table 1—Collection sites of live specimens of different mahseer species

No.	Name of the species	n*	Site of collection
1	*Tor chelynoides* (McClelland)	8	Tributaries of river Alaknanda, Garhwal, Uttarakhand
2	*T. khudree* (Sykes)	12	River Cauvery, Karnataka
3	*T. mosal mahanadicus* (David)	23	River Mahanadi, Sonepur, Orissa
4	*T. mussullah* (Sykes)	6	TATA Electric Company Reservoir, Lonavala, Maharastra
5	*T. progeneius* (McClelland)	6	River Jia Bhoreli, near Bhalukpong, Assom
6	*T. putitora* (Hamilton)	7	River Kosi, Manan, Almora, Uttarakhand
7	*T. tor* (Hamilton)	6	River Narmada near Hosangabad, Madhya Pradesh
8	*Neolissochilus hexagonolepis* (McClelland)	8	Tributaries of Umiam Reservoir, Shillong, Maghalaya

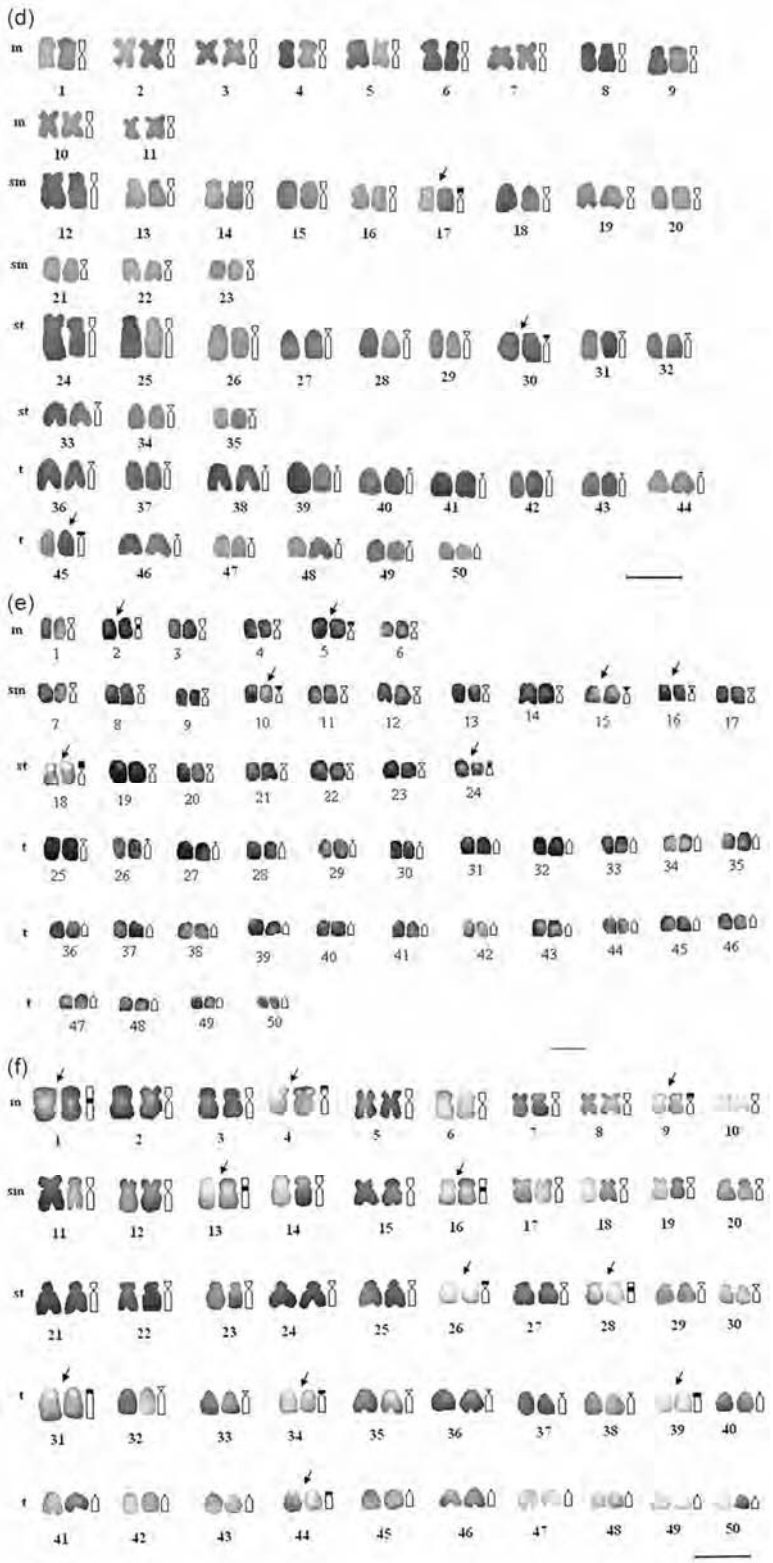
*n= number of live specimens collected.

Fig. 2


(a)
m
1 2 3 4 5 6 7 8 9 10
sm
11 12 13 14 15 16 17 18 19 20
sm
21 22 23 24 25
st
26 27 28 29 30 31 32 33 34 35
st
36 37
t
38 39 40 41 42 43 44 45 46 47
t
48 49 50
(b)
m
1 2 3 4 5 6 7 8 9 10
sm
11 12 13 14 15 16 17
st
18 19 20 21 22 23 24 25 26 27 28
t
29 30 31 32 33 34 35 36 37 38 39
t
40 41 42 43 44 45 46 47 48 49 50
(c)
m
1 2 3 4 5 6 7 8 9 10 11
sm
12 13 14 15 16 17
st
18 19 20 21 22 23 24 25 26 27 28
t
29 30 31 32 33 34 35 36 37 38 39
t
40 41 42 43 44 45 46 47 48 49 50

(*Contd.*)

Fig. 2– *Contd.*



(*Contd.*)

Fig. 2– *Contd.*

Fig. 2 (a-h)—The karyotype of C-banded chromosomes and ideogram in: a. *Tor chelynoides*, b. *T. khudree*, c. *T. mosal mahanadicus*, d. *T. mussullah*, e. *T. putitora*, f. *T. progeneius*, g. *T. tor* & h. *Neolissochilus hexagonolepis*. (Arrows indicates C-bands positive chromosomes; m, metacentric; sm, submetacentric; st, subtelocentric; t, telocentric; Bar=5 µm)

species belonging to 152 genera (www.fishbase.org, version 2/2012). In the preliminary karyotypic analyses, the mahseer group can be considered conservative in maintaining the same 100 diploid chromosome number with different karyotype formulae/ karyo-morphology. In fish, several studies have shown that CH (identified by C-banding technique) is an important element in chromosome

Table 2—Comparative chromosomal distributions of C-bands in different mahseer species

No.	Name of species	Karyo-morphology	No. of C-band pairs	Position of C-band on chromosome number/in karyotype
1	*Tor chelynoides*	20 m+30 sm+24 st+26 t	3	p arm of 7th (7 m), 26th (1 st) and 34th (9 st)
2	*T. khudree*	20 m+14 sm+22 st+44 t	3	telomeric regions of 9th (9 m), 17th (7 sm) and 27th (10 st)
3	*T. mosal mahanadicus*	22 m+12 sm+22 st+44 t	4	telomeric regions of 17th (6 sm), 25th (8 st) and 28th (11 st) and centromeric region of 50th (22 t)
4	*T. mussullah*	22 m+24 sm+24 st+30 t	3	telomeric positions of 17th (6 sm) and 30th (7 st), and centromeric region of 45th (10 t).
5	*T. progeneius*	20 m+20 sm+20 st+40 t	11	telomeric regions of 4th (4 m), 9th (9 m) and 26th (6 st), and centromeric regions of 1st (1 m), 13th (3 sm), 16th (6 sm), 28th (8 st), 31st (1 t), 34th (4 t), 39th (9 t) and 44th (14 t)
6	*T. putitora*	12 m+22 sm+14 st+52 t	7	telomeric positions of 5th (5 m), 10th (4 sm), 15th (9 sm) and 18th (1 st), and centromeric positions of 2nd (2 m), 16th (10 sm) and 24th (7 st)
7	*T. tor*	20 m+24 sm+24 st+32 t	2	telomeric regions of 20th (10 sm) and 27th (5 st).
8	*Neolissochilus hexagonolepis*	20 m+18 sm+14 st+48 t	3	telomeric regions of 15th (5 sm) and 25th (6 st), and centromeric region of 39th (13 t)

Fig. 3 (a-h)—Enlarge view of C-banded chromosomes in: a. *Tor chelynoides*, b. *T. khudree*, c. *T. mosal mahanadicus*, d. *T. mussullah*, e. *T. putitora*, f. *T. progeneius*, g. *T. tor* & h. *Neolissochilus hexagonolepis*.

differentiation[12]. In the present study, CH patterns in eight mahseer species were analyzed.

The positive C-bands identify the regions of heterochromatin consisting of repetitive DNA sequences. The C-bands were observed on several pairs of the chromosomes with maximum number in *T. progeneius* (11 pairs), followed by *T. putitora* (7 pairs), *T. mosal mahanadicus* (4), *T. khudree* (3), *T. mussullah* (3), *N. hexagonolepis* (3), *T. chelynoides* (3) and *T. tor* (2). It is interesting to note that the C-bands were localized only on telomeric positions in *T. chelynoides, T. khudree* and *T. tor*; whereas in *T. mosal mahanadicus, T. mussullah*, *T. putitora, T. progeneius* and *N. hexagonolepis*, the bands were localized on both telomeric and centromeric regions. The C-bands were also reported at telomeric and centromeric positions in six species of flatfishes[15] and in *Labeo rohita* and *Cirrhinus mrigala*[16]. The centromeric C-bands were commonly observed in a number of fish species[17,18]. In *Danio rerio*, the CH was located at the centromeric position in all chromosome pairs[19]. Similarly, in Brazilian marine fishes belonging to families Sciaenidae and Sparidae, the heterochromatin blocks were detected at the centromeric positions in most of chromosome pairs[20].

In the studies conducted in two cyprinids, *Acanthobrama marmid* and *Cyprinion macrostomus* from Turkey, CH bands were seen in the pericentromeric regions of all chromosomes[21]. However, inter-specific variation was observed in cyprinid genera *Rutilus* and *Scardinius* for the distribution of CH. In both genera, the heterochromatin differentiation appears to be directed to a centromere-telomere direction, particularly evident along the metacentric elements of their

karyotypes[22]. In the present study, no C-band was present on interstitial and/or q arm of the chromosomes in the undertaken species. On the other hand, in another study, a pair of C-band was found on long arm of a metacentric choromosome of three species of genus *Serrasalmus*[23].

A prominent heterochromatic block was observed on the largest st chromosome in *T. putitora*, which could have been formed by tandem duplication of heterochromatic DNA[24]. Since similar type of prominent heterochromatic block was not observed in other species, it may be considered as a marker chromosome for identification of *T. putitora*. Earlier, the chromosomal distributions of CH in other fish species[25-26] have been utilized in identification of marker chromosomes in the species. Moreover, an association may also be established between the C-positive heterochromatin, observed in the present study, and the NOR (nucleolar organizer region) reported on the terminal region of the short arms of 4th sm chromosome in *T. putitora*. Flanking of C-bands with NORs is a common feature as reported in other fish species[27-28]. Further, in *Brycon* sp.[29] and *Hypostomus* sp. B[30], the rDNA were found to be interspersed adjacent to heterochromatin segments.

In general, the distributions of C-bands on metacentric (7) and telocentric (7) chromosomes were less as compared to the submetacentric (10) and subtelocentric (12) chromosomes that allowed a more accurate, homologous pairing. The maximum (4) presence of C-bands on telocentric chromosomes was found in *T. progeneius*. It may be possible that these heterochromatic regions are involved in the non-homologous pairing of chromosomes during karyo-evolution of these mahseer groups. In the present study, the C-bands mostly exhibited telomeric distribution (22) rather than centromeric (14) as the chromosomes of *T. chelynoides, T. khudree* and *T. tor* exhibited C-bands only on telomeric regions.

The extensive variations in C-bands, in terms of number, chromosomal localization and position in the karyotype, could be used as species-specific marker for identification in these fishes. These variations in the heterochromatic blocks can be attributed to various types of chromosomal rearrangements, *viz.* addition of heterochromatin, robertsonian/tandem fusion, pericentric/paracentric inversions and reciprocal translocation[31] during karyo-evolution of these mahseer species. These variations in CH may also be utilized at initial stage to establish cytotaxonomic relationships among the species. Based on the number and position of C-bands on the chromosomes, the *T. khudree*, *T. mussullah* and *T. mosal mahanadicus* can be considered in one cytotype.

T. putitora and *T. progeneius* have primitive character due to the presence of maximum number of C-bands as compared to other species in the study. The higher CH diversity is an ancestral condition[32]. Further, the loss of heterochromatic blocks may also be responsible for a smaller number of C-bands during the course of evolution. The elimination of CH accompanies the phyletic evolution of fishes[33]. The C-banding pattern were analyzed in 10 species of Chinese cyprinid fish and found extensive variation in the CH among the fishes. Some species possess considerable quantities of heterochromatin and some possess little heterochromatin[34]. The mechanisms, such as, multiple replications, unequal exchanges, amplifications, accumulations and deletions, can lead to the quantitative variation of heterochromatin within and between species[35]. These heterochromatin differentiations often suggest an evolutionary role in the process of species divergence[36].

The proposal for karyotypic evolution of mahseer species seems to reveal a general trend regarding the distribution of CH and each species showed a specific C-banding pattern useful in species identification. Similar species- and population-specific differentiations have also been reported in other studies[37,38]. The data on distribution of CH are also helpful in understanding the genomic organization and differentiation in the cyprinidae, evidently showing structural variability of the chromosomes with a karyotypic diversity, since the variations in the amount and distribution of CH throughout the karyotype were most probably caused by heterochromatin-mediated chromosome rearrangements[39].

Acknowledgments

The authors are thankful to the Department of Biotechnology (DBT), Ministry of Science and Technology, New Delhi, India for the financial support. They are also thankful to A K Pathak, Scientist, NBFGR for necessary help in preparation of the manuscript.

References

1 Nelson J S, *Fishes of the world*, 4th edn (John Wiley & Sons, Inc. Hoboken, New Jersey, USA) 2006, pp 601.

2 Escmeyer W N, Ferraris C J Jr, Hong M D & Long D J, The catalog of fishes, on line species of fishes, 2004. http://www.calacademy.org/research/ichthyology/catalog/intro.html.

3 Sharma R C, Fish diversity and their ecological status in protected areas of Uttaranchal, in *Protected habitats and biodiversity*, *Natl Conserv Publ,* 8 (2003) 617-638.

4 Raghavan R, Anvar Ali P H & Prasad G, Need for a comprehensive re-assessment of the conservation status of critically endangered (?) freshwater fishes of Kerala, *Curr Sci*, 92 (2007) 721-723.

5 Menon A G K, Checklist of freshwater fishes of India, *Rec Zool Surv India*, 175 (1999) 56-57.

6 Talwar P K & Jhingran A G, *Inland fishes of India and adjacent countries*, vol 1 & 2 (Oxford and IBH Publishing Company Pvt. Ltd., New Delhi, India) 1991, 1158 p.

7 David A, On some new records of fish from the Damodar and the Mahanadi River systems, *J Zool Soc India*, 5 (1953) 243-254.

8 Khuda-Bukhsh A R, Karyomorphology of two species of *Tor* (Pisces, Cyprinidae) with a high number of chromosomes, *Experientia*, 38 (1982) 82-83.

9 Lakra W S, Cytogenetic studies on endangered fish species: 1. Karyotype of three species of Mahseers: *Tor khudree, T. tor* and *T. putitora* (Cyprinidae, Pisces), *Cytobios*, 85 (1996) 205-218.

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

11 Mani I, Kumar R, Singh M, Kushwaha B, Nagpure N S *et al*, Karyotypic diversity and evolution of seven mahseer species (Cyprinidae) from India, *J Fish Biol*, 75 (2009) 1079-1091.

12 Sharma O P, Tripathi N K & Sharma K K, A review of chromosome banding in fishes, in *Some aspects of chromosome structure and functions*, edited by R C Sobti, G Obe & R S Athwal (Narosa Publishing House, New Delhi, India) 2002, 109-115.

13 Sumner A T, A simple technique for demonstrating centromeric heterochromatin, *Exp Cell Res*, 75 (1972) 304-306.

14 Levan A, Fredga K & Sandberg A A, Nomenclature for centromeric position on chromosomes, *Hereditas*, 52 (1964) 201-220.

15 Azevedo M F C, Oliveria C, Pardo B G, Martinez P & Foresti F, Cytogenetic characterization of six species of flatfishes with comments to karyotype differentiation patterns in Pleuronectiformes (Teleostei), *J Fish Biol*, 70 (2007) 1-15.

16 Khuda-Bukhsh A R & Chakrabarty C, Localization of C-band heterochromatin in metaphase chromosomes of two species in Indian major carp, *J Inland Fish Soc India*, 26 (1994) 44-46.

17 Gold J R, Li Y C, Shipley N S & Powers P K, Improved methods for working with fish chromosomes with a review of metaphase chromosome banding, *J Fish Biol*, 37 (1990) 563-575.

18 Rishi K K & Mandhan R P, Analysis of C-banded heterochromatin in chromosomes of *Labeo rohita* (Ham) (Cyprinidae), *Chromosome Inform Serv*, 48 (1990) 3-4.

19 Daga R R, Thode G & Amores A, Chromosome complement, C-banding, Ag-NOR and replication banding in the zebrafish *Danio rerio*, *Chromosome Res*, 4 (1996) 29-32.

20 Accioly I V & Molina W F, Cytogenetic studies in Brazilian marine Sciaenidae and Sparidae fishes (Perciformes), *Genet Mol Res*, 7 (2008) 358-370.

21 Gaffaroglu G & Yuksel E, Constitutive heterochromatin in *Acanthobrama marmid* and *Cyprinion macrostomus* (Osteichthyes, Cyprinidae), *Kafkas Univ Vet Fak Derg*, 15 (2009) 169-172.

22 Bianco P G, Aprea G, Balletto E, Capriglione T, Fulgione D *et al*, The karyology of the cyprinid genera *Scardinius*and *Rutilus* in southern Europe, *Ichthyol Res*, 51 (2004) 274-278.

23 Nakayama C M, Feldberg E & Bertollo L A C, Mapping of ribosomal genes and chromosomal markers in three species of the genus *Serrasalmus* (Characidae, Serrasalminae) from the Amazon basin, *Genet Mol Biol*, 31 (2008) 868-873.

24 Almeida-Toledo L F, Foresti F & Toledo S A, Constitutive heterochromatin and nucleolus organizer regions in the knifefish *Apteronotus albifrons* (Pisces, Apteronotidae), *Experientia*, 37 (1981) 953-954.

25 Nagpure N S, Distribution of C-band heterochromatin in *Labeo rohita* Ham (Cyprinidae), *Chromosome Sci*, 1 (1997) 45-46.

26 Sharma R & Lakra W S, Karyotype and localisation of C-band heterochromatin in an Indian major carp, *Catla catla* (Ham), *Indian J Anim Sci*, 69 (1999) 996-998.

27 Pendas A M, Moran P & Garcia-Vázquez E, Ribosomal RNA genes are interspersed throughout a heterochromatic chromosome arm in Atlantic salmon, *Cytogenet Cell Genet*, 63 (1993) 128-130.

28 Wasko A P & Galetti P M Jr, Mapping 18S ribosomal genes in fish of the genus *Brycon* (Characidae) by fluorescence *in situ* hybridization (FISH), *Genet Mol Biol*, 23 (2000) 135-138.

29 Galetti P M Jr, Mestriner C A, Monaco P J & Rasch E M, Post-zygotic modifications and intra- and inter-individual nucleolar organizing region variations in fish: report of a case involving *Leporinus fridereci*, *Chromosome Res*, 3 (1995) 285-290.

30 Artoni R F & Bertollo L A C, Nature and distribution of constitutive heterochromatin in fishes, genus *Hypostomus* (Loricariidae), *Genetica*, 106 (1999) 209-214.

31 Sen S & Sharma T, Role of constitutive heterochromatin in evolutionary divergent: Results of chromosome banding and condensation in inhibition studies in *Mus musculus*, *Mus booduga* and *Mus dunni*, *Evolution*, 37 (1983) 928-636.

32 Elder J F Jr & Turner B J, Concerted evolution of repetitive DNA sequences in eukaryotes, *Q Rev Biol*, 70 (1995) 297-320.

33 Kornfield I L, Rittem U, Richler C & Wahrman J, Biochemical and cytological differentiation among cichlid fishes of the Sea of Galilee, *Evolution*, 33 (1979) 1-14.

34 Ren X, Cui J & Yu Q, Chromosomal heterochromatin differentiation of cyprinid fishes, *Genetica*, 87 (1992) 47-51.

35 John B, The biology of heterochromatin, in *Heterochromatin*, edited by R S Verma (Cambridge University Press, Cambridge, UK) 1988, 1-147.

36 Baimai V, Constitutive heterochromatin differentiation and evolutionary divergence of karyotype in Oriental *Anopheles* (*Cellia*), *Pac Sci*, 42 (1988) 13-27.

37 Andreata A A, Oliveira C & Foresti F, Karyological characterization of four Neotropical fish species of the genus *Hisonotus* (Teleostei, Loricariidae, Hypoptopomatinae) from distinct Brazilian river basins, *Genet Mol Biol*, 29 (2006) 62-66.

38 Mantovani M, Abel L D S, Mestriner C A & Moreira-Filho O, Evidence of the differentiated structural arrangement of constitutive heterochromatin between two populations of *Astyanax scabripinnis* (Pisces, Characidae), *Genet Mol Biol*, 27 (2004) 536-542.

39 Koehler M R, Dehm D, Guttenbach M, Nanda I, Haaf T *et al*, Cytogenetics of the genus *Leporinus* (Pisces, Anostomidae) 1. Karyotype analysis, heterochromatin distribution and sex chromosomes, *Chromosome Res*, 5 (1997) 12-22.

View publication stats