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KARYOMORPHOLOGY OF THREE INDIAN MAJOR CARPS FROM HARYANA, INDIA

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Abstract: The purpose of the present study was to investigate and compare the karyotypes of three Indian major carps, *Catla catla* (Hamilton, 1822), *Labeo rohita* (Hamilton, 1822), *Cirrhinus mrigla* (Hamilton, 1822) in terms of chromosomal architechture, karyotype formula and number of chromosomes from aquatic ecosystems of Haryana, India. Karyotypes of these carps were investigated by examining metaphase chromosomes. The results indicated that the diploid (2n) chromosome number of all the three major carps was 50. *Catla catla* consisted of 22 acrocentric, 2 subtelocentric, 20 submetacentric and 6 metacentric chromosomes. *Labeo rohita* consisted of 32 acrocentric, 4 subtelocentric, 6 submetacentric, 8 metacentric while *Cirrhinus mrigla* consisted of 30 acrocentric, 8 subtelocentric, 6 submetacentric, 6 metacentric chromosomes. Centromeric Index, arm ratio and fundamental number was also determined. No heteromorphic sex chromosomes were cytologically detected. The variability in size, shape and arm number (NF) of chromosomes among these three species suggest that diversification in these fish species of same family is related to structural changes in chromosomes. Variations in karyotype formulae are also observed with respect to earlier studies which may be due to variations in habitat conditions as a result of anthropogenic activities.

Keywords: *Catla catla*, Chromosome, *Cirrhinus mrigla*, Karyotype, *Labeo rohita*

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Öz: **Hindistan, Haryana bölgesinden üç ana sazan türünün karyomorfolojisi**

Çalışmanın amacı, Hindistanın Haryana su ekosistemlerinde bulunan başlıca üç sazan türü olan, Catla catla (Hamilton, 1822), Labeo rohita (Hamilton 1822) ve Cirrhinus mrigla (Hamilton, 1822) türlerinin karyotiplerinin kromozom yapıları, karyotip formülleri ve kromozom sayıları açısından incelenmesidir. Bu türlerin karyotipleri metafaz kromozomlarına bakılarak incelenmiştir. Sonuçlar üç türünde diploid (2n) kromozom sayısının 50 olduğunu göstermiştir. Catla catla, 22 akrosentrik, 2 subtelosentrik, 20 submetasentrik ve 6 metasentrik kromozom içerirken; Labeo rohita 32 akrosentrik, 4 subtelosentrik, 6 submetasentrik,8 metasentrik; Cirrhinus mrigla ise 30 akrosentrik, 8 subtelosentrik, 6 sub metasentrik ve 6 metasentrik kromozom içermektedir. Sentromerik index, (arm ratio) ve temel sayıda belirlenmiştir. Sitolojik olarak heteromorfik cinsiyete rastlanmamıştır. Bu üç türün kromozomlarının boyut, şekil ve (arm number) (NF) çeşitliliği aynı familya içerisindeki yapısal kromozom farklılıklarından ileri gelmektedir. Daha önceki çeşitli çalışmalar ile karşılaştırıldığında karyotip formüllerinde de varyasyonlara rastlanmıştır. Bunun nedeni olarak habitat özelliklerinde meydana gelen değişimlerin antropojenik aktivitelere etkisi düşünülebilir.

Anahtar Kelimeler: *Catla catla*, Chromosome, *Cirrhinus mrigla*, Karyotype, *Labeo rohita*

Introduction

Cyprinidae is a large family of freshwater fishes that is commonly called the carp family. The vast majority of bony fish belongs to this family and these are widely distributed in freshwater resources (Abdoli, 1999). Family Cyprinidae is comprised of 220 genera and 2420 species (Nelson, 2006). In fishes, cytogenetic data is important because in this group the usual morphotaxonomical characters are not so clear and phylogenetic understanding is beset with many hurdles. The increasing knowledge of chromosomes can provide reliable information on the phyletic relationship in the Cyprinidae to a certain extent (Kalbassi et al., 2006).

Systematically, *Catla catla*, *Labeo rohita* and *Cirrhinus mrigla* belongs to class Teleostei, order Cypriniformes, family Cyprinidae (Jayaram, 1999). The study of fish chromosomes was initiated in India from 1960s by using basically the methodologies available for mammals. Karyological studies on Indian major carps have been carried out by some workers (Rishi, 1973; Khuda-Bukhsh and Manna, 1974; Majumdar and Ray-Chaudhari, 1976; Zhang and Reddy, 1991). The different variety of karyotypes found in different species was evidence that the process of evolution was associated with karyotypic changes (Fredga, 1977).

Chromosomal analysis is important for fish breeding from the viewpoint of genetic control, the rapid production of inbred lines, cytotaxonomy and evolutionary studies (Kirpichnikov, 1981). Karyological studies provide basic information on the number, size and morphology of chromosomes (Tan et al., 2004) that is important to undertake chromosome manipulation in fish (Khan et al., 2000). However, the studies dealing with karyotype of fishes are few because of large number and small size of chromosomes. Hence the present study was designed to determine the karyotype, chromosomal architecture, the proportion of acrocentric, submetacentric and metacentric chromosomes and number of chromosomes in Indian major carps, *C. catla, L. rohita, C. mrigla* from the geographic area of Haryana.

Materials and Methods

Fifteen fishes were obtained from the local fish farm and transported live to the Fish and Fisheries laboratory of Department of Zoology, Kurukshetra University, and Kurukshetra. Kidney and gill epithelium tissue were used for karyotype analysis. Three living specimens each of *C. catla*, *L. rohita* and *C. mrigla* from Haryana were analysed. The preparation of chromosomes was performed according to air drying technique given by Tjio and Whang (1965). Each specimen was injected intraperitoneally with a colchicine solution (0.05%; 1 mL/100g body weight). The fishes were maintained in a well aerated aquarium and after 2 hr., Kidney and gill epithelium were extracted and placed in hypotonic solution of 0.56% KCl. After 30 minutes in the hypotonic solution, the cellular suspension was centrifuged at 1000 rpm for 10 minutes. The hypotonic solution was discarded and the pellet was suspended and washed three times in methanol: glacial acetic acid (3: 1). After centrifugation at 1000 rpm

for 10 minutes, the drops of cellular suspension was put on a clean grease free microscopic slide, previously chilled in a freezer from a height of 2 feet. The slides were allowed to air dry.

For the conventional karyotype, the preparations were stained for 15 minutes with 5% Giemsa in phosphate buffer (pH 6.88). Mitotic metaphases spreads were scanned to determine the modal chromosome number. Mitotic metaphase were photographed by using Olympus C-7070 wide zoom camera at magnification of 1000X and used for preparation of karyotype. The arm ratio and centromeric indices of metaphase chromosomes were determined following Levan *et al*. (1964) to assign the morphological types and the chromosomal formulae.

Results and Discussion

Catla catla 2n = 50, NF^a = 76

The diploid chromosome number of all three major carps was found to be 50. In case of *C. catla*, the somatic metaphase showed the presence of 50 chromosomes (Figure 1A) and fundamental arm number 76. The somatic karyotype (Figure 1B) was prepared according to decreasing chromosome length. Somatic karyotype showed 22 acrocentric (pair nos. 4, 9, 11, 15-19, 22-23 and 25), 2 subtelocentric (pair no. 3), 20 submetacentric (pair nos. 1-2, 5-7, 12, 14, 20-21 and 24) and 6 metacentric (pair nos. 8, 10 and 13) chromosomes. Sex chromosomes could not be distinguished. The size of the chromosome ranged from as low as 0.251µm of 25th pair of chromosome to as high as 5.911µm of 1st pair of chromosomes (Table 1). Relative % length of the smallest chromosome was 0.640 while largest chromosome was 15.070. The total haploid mean length was calculated to be 39.218 µm.

Labeo rohita 2n = 50, NF^a = 64

The somatic metaphase in the kidney cells of the *L. rohita* showed diploid chromosome number 50 i. e. 2n=50 (Fig. 2A) and fundamental arm number 64. The somatic karyotype (Fig.2B) showed 32 acrocentric (pair nos. 2, 6-8, 10-13, 15, 18-21, 23-24), 4 subtelocentric (pair no. 4 and 14), 6 submetacentric (pair nos. 1, 9 and 16) and 8 metacentric (pair nos. 3, 5, 17 and 22) chromosomes. Sex chromosomes could not be distinguished. The size of the chromosomes in this case ranged from as low as 1.087 µm of 25th pair of chromosome to as high as 1.736 µm of 1st pair of chromosomes (Table 2). Relative % length of the smallest chromosome was 1.126 while largest chromosome was 9.528. The total haploid mean length was calculated to be 32.724 µm.

Cirrhinus mrigla 2n = 50, NF^a = 62

The somatic metaphase in *C. mrigla* also showed the presence of 50 chromosome number (Figure 3A) and fundamental arm number 62. The somatic karyotype (Figure 3B) comprised of 30 acrocentric (pair nos. 3, 5, 7, 9, 11-16, 19, 21-23 and 25), 8 subtelocentric (pair no. 2, 4, 6 and 20), 6 submetacentric (pair nos. 1, 10 and 24) and 6 metacentric (pair nos. 8, 17 and 18) chromosomes. Sex chromosomes could not be distinguished. The size of the chromosome ranged from 0.251 µm in case of 25th pair of chromosome and 1.400 µm of 1st pair of chromosomes (Table 3). Relative % length of the smallest chromosome was 3.357 while largest chromosome was 4.333. The total haploid mean length was calculated to be 29.575 µm.

In Cyprinidae 2n ranges from 44 to 100 (Arai, 1982). The high diploid chromosome number 2n=98-100 are thought to have resulted by polyploidisation of 2n=48 or 50. Chromosomal analysis in the present study revealed that these three Indian major carps from Haryana shared the same diploid number i. e. 2n=50. The karyological study of *C. catla* and *L. rohita* done by Khuda-Bukhsh and Manna (1974), Manna (1977), Majumdar and Ray Chaudhari (1976), Zhang and Reddy (1991), Jana (1993), Manna and Prasad (1971), Gui et al (1986) reported the similar results i. e. 2n=50. Karyotype studies on *C. mrigla* have been performed by Manna and Prasad (1971), Majumdar and Ray Chaudhari, (1976) and Zhang and Reddy (1991). All these studies have shown the diploid number as 50, confirming the present results. According to Manna (1984) and Rishi (1989) the most commonly occuring diploid number in family Cyprinidae is 50, considered to be the modal number of this species. Presence of same modal number in the present studies reinforces the hypothesis that Indian major carps are karyologically very conserved and represent plesiomorphic condition.

The primitive teleost karyotype is thought to have consisted of 46 to 48 chromosomes (Fitzsimons, 1972; LeGrande, 1975) all acrocentrics. Karyotypes with biarmed chromosomes are generally regarded to represent a derived condition (Fredga, 1977). Therefore, cyprinids investigated

in the present study showed a derived karyotype configuration. No heteromorphic sex chromosomes were found.

For comparative purpose, the arm number (NF) of karyotyped fishes is calculated assigning a value 2 to biarmed chromosomes (metacentric and submetacentric) and value of 1 to uniarmed chromosomes (acrocentric and subtelocentric) and is regarded as karyotype formulae. Despite the similarity of diploid numbers in all the three selected cyprinid species, there are differences in their karyotypic formulae. Comparision of the karyotypic formulae revealed deviations from the earlier reports for these species.

In case of *C. catla*, Khuda- Bukhsh and Manna (1976) reported 22 acrocentric, 24 submetacentric and 4 metacentric chromosomes. Manna (1977) reported 26 acrocentric, 16 submetacentric and 8 metacentric chromosomes. Zhang and Reddy (1991) and Jana (1993) showed similar results i. e. 22 acrocentric, 16 submetacentric and 12 metacentric chromosomes. But the present results showed a slight variation in chromosome morphology indicating 22 acrocentric, 2 subtelocentric, 20 submetacentric and 6 metacentric chromosomes.

Karyological study of *L. rohita* done by Manna and Prasad (1971), Majumdar and Ray Chaudhari (1976), Gui et al (1986), Zhang and Reddy (1991), Jana (1993) showed the same diploid number as found in present studies i.e. 2n=50. Manna and Prasad (1971) observed 24 subtelocentric, 8 submetacentric and 18 metacentric chromosomes. Gui et al (1986) observed 24 subtelocentric, 16 submetacentric and 10 metacentric chromosomes. Zhang and Reddy (1991) and Jana (1993) reported similar results in *L. rohita* i. e. 22 subtelocentric, 18 submetacentric and 10 metacentric chromosomes. Present results showed 32 acrocentric, 4 subtelocentric, 6 submetacentric and 8 metacentric chromosomes.

In case of *C. mrigla*, different chromosome morphology was given by different workers but basic diploid number was same i.e. 2n=50. Manna and Prasad (1971) reported 36 acrocentric, 8 submetacentric and 6 metacentric chromosomes which showed little similarity with the present results. In the present study 30 acrocentric, 6 submetacentric, 6 metacentric and 8 subtelocentric chromosomes were found. Majumdar and Ray Chaudhari (1976) observed 18 acrocentric, 26 submetacentric and 6 metacentric chromosomes. Zhang and Reddy (1991) showed 20 acrocentric, 18 submetacentric and 12 metacentric chromosomes. Many authors considered that diploid chromosomes are all acrocentric as the ancestral karyotype in fishes (Nogusa, 1960; Post, 1965; Denton, 1973). It may be pointed out that all acrocentric karyotype happens to be absent in a number of primitive group like chondrichthyes. Therefore it may be assumed that acrocentricity is certainily a more primitive condition that the biarmed condition.

The acrocentric chromosomes have a tendency to stick to each other by their centromere and in this way they form metacentric chromosomes (Dogramci et al. 1994). Denton (1973), Gold (1979) also stated that karyotypes with biarmed chromosomes are regarded as derived condition confirming that karyomorphology of all the three species could be derived mainly by envisaging per centric inversion at various regions with respect to time, geographical condition and ecological characteristics.

A comparision of karyotypic formulae of these three Indian major carps species revealed that larger numbers of acrocentric chromosome are observed in *L. rohita* followed by *C. mrigla* and *C. catla.* 6 metacentric chromosomes were observed in both *C. mrigla* and *C. catla* whereas 8 metacentric chromosomes were observed in *L. rohita*. According to Le Grande (1981), differences in the NF among close species can be the result of pericentric inversions. The karyotype formulae of Indian major carps in the present studies can be interpreted as the result of structural chromosomal rearrangements as well as a series of pericentric inversions, generating biarmed chromosomes and so increase the NF to 74 in *C. catla*, 64 in *L. rohita* and 62 in *C. mrigla.* The karyotypes of these species have been compared with the related ones and it has been suggested that large number of acrocentric chromosomes have been observed during present investigation in comparision to earlier studies. It may be due to mechanism of centric fission. Centric fission seemed to have played a significant role in evolution of teleost fishes (Manna and Khuda-Bukhsh, 1978). Different groups of fishes exhibit different processes of karyotype evolution.

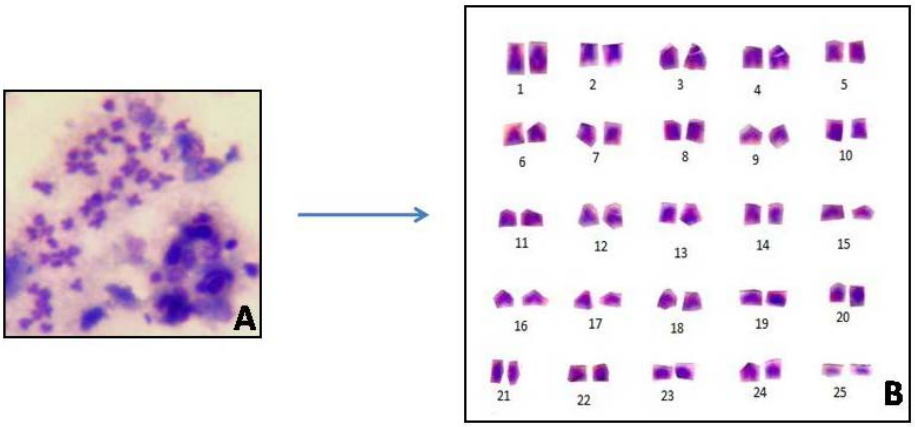

Fig. 1

Figure 1A. Somatic metaphase of *C. catla*
Figure 1B. Karyotype of *C. catla*

Table 1. Morphometric data of somatic karyotype of *C. catla* (2n= 50)

Chrm. Pair no.	Short arm length (µm) (p)	Long arm length (µm) (q)	Total length (µm)	% Relative length (RL%)	Arm ratio	Centromeric Index (%)	Chromosome morphology
1	1.960	3.951	5.911	15.072	2.015	33.158	Submetacentric
2	1.981	3.661	5.642	14.386	1.848	35.111	Submetacentric
3	1.020	3.540	4.560	11.627	3.470	22.368	Subtelocentric
4		3.520	3.520	8.975			Acrocentric
5	0.998	1.603	2.601	6.632	1.606	38.369	Submetacentric
6	0.781	1.413	2.194	5.594	1.809	35.597	Submetacentric
7	0.615	1.054	1.669	4.255	1.713	36.848	Submetacentric
8	0.584	0.852	1.436	3.661	1.458	40.668	Metacentric
9		1.210	1.210	3.085			Acrocentric
10	0.480	0.715	1.195	3.047	1.489	67.132	Metacentric
11		1.091	1.091	2.781			Acrocentric
12	0.385	0.684	1.069	2.725	1.776	56.286	Submetacentric
13	0.384	0.551	0.935	2.384	1.434	69.691	Metacentric
14	0.354	0.541	0.895	2.282	1.528	65.434	Submetacentric
15		0.715	0.715	1.823			Acrocentric
16		0.691	0.691	1.761			Acrocentric
17		0.598	0.598	1.524			Acrocentric
18		0.544	0.544	1.387			Acrocentric
19		0.523	0.523	1.333			Acrocentric
20	0.158	0.301	0.459	1.170	1.898	34.422	Submetacentric
21	0.145	0.285	0.430	1.096	1.965	33.720	Submetacentric
22		0.398	0.398	1.014			Acrocentric
23		0.377	0.377	0.961			Acrocentric
24	0.115	0.189	0.304	0.775	1.643	37.828	Submetacentric
25		0.251	0.251	0.640			Acrocentric

Total mean haploid length = 39.218 µm

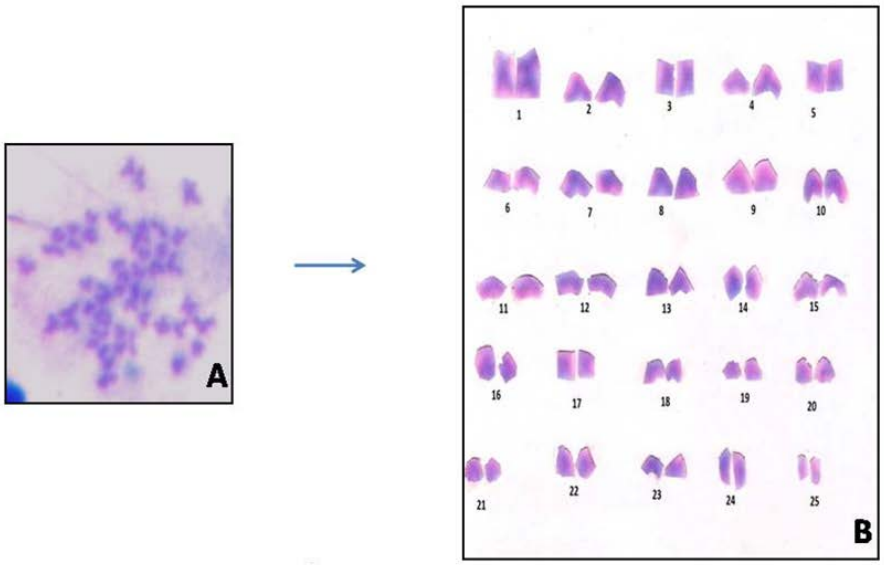

Fig. 2

Figure 2A. Somatic metaphase of *L. rohita*
Figure 2B. Karyotype of *L. rohita*

Table 2. Morphometric data of somatic karyotype of *L. rohita* (2n= 50)

Chrm. Pair no.	Short arm length (µm) (p)	Long arm length (µm) (q)	Total length (µm)	% Relative length (RL%)	Arm ratio	Centromeric Index (%)	Chromosome morphology
1	0.756	0.980	1.736	9.528	1.875	34.782	Submetacentric
2		1.543	1.543	7.030			Acrocentric
3	0.681	0.784	1.465	6.020	1.569	38.924	Metacentric
4	0.587	0.874	1.461	5.968	4.298	23.262	Subtelocentric
5	0.719	0.734	1.453	5.864	1.068	48.344	Metacentric
6		1.379	1.379	4.906			Acrocentric
7		1.373	1.373	4.829			Acrocentric
8		1.361	1.361	4.673			Acrocentric
9	0.601	0.759	1.360	4.660	2.564	28.055	Submetacentric
10		1.343	1.343	4.440			Acrocentric
11		1.341	1.341	4.418			Acrocentric
12		1.337	1.337	4.363			Acrocentric
13		1.329	1.329	4.259			Acrocentric
14	0.591	0.701	1.292	3.780	3.197	31.164	Subtelocentric
15		1.281	1.281	3.638			Acrocentric
16	0.587	0.692	1.279	3.612	2.206	31.182	Submetacentric
17	0.598	0.601	1.199	2.576	1.030	49.246	Metacentric
18		1.187	1.187	2.421			Acrocentric
19		1.178	1.178	2.304			Acrocentric
20		1.174	1.174	2.252			Acrocentric
21		1.168	1.168	2.175			Acrocentric
22	0.568	0.580	1.159	2.058	0.176	42.767	Metacentric
23		1.140	1.140	1.812			Acrocentric
24		1.099	1.099	1.281			Acrocentric
25		1.087	1.087	1.126			Acrocentric

Total mean haploid length = 32.724 µm

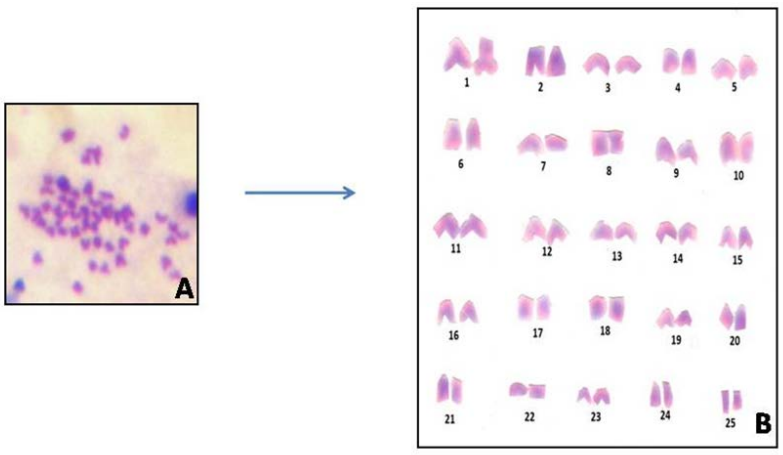

Fig. 3

Figure 3A. Somatic metaphase of *C. mrigla*

Figure 3B. Karyotype of *C. mrigla*

Table 3. Morphometric data of somatic karyotype of *C. mrigla* (2n= 50)

Chrm. Pair no.	Short arm length (µm) (p)	Long arm length (µm) (q)	Total length (µm)	% Relative length (RL%)	Arm ratio	Centromeric Index (%)	Chromosome morphology
1	0.499	0.901	1.400	4.733	1.805	35.642	Submetacentric
2	0.300	1.099	1.399	4.730	3.651	21.443	Subtelocentric
3		1.395	1.395	4.716			Acrocentric
4	0.291	1.096	1.387	4.689	3.766	20.980	Subtelocentric
5		1.381	1.381	4.669			Acrocentric
6	0.288	1.089	1.377	4.655	3.781	20.915	Subtelocentric
7		1.375	1.375	4.649			Acrocentric
8	0.683	0.691	1.374	4.645	1.011	49.708	Metacentric
9		1.374	1.374	4.645			Acrocentric
10	0.299	1.073	1.372	4.639	3.588	21.793	Submetacentric
11		1.370	1.370	4.632			Acrocentric
12		1.369	1.369	4.628			Acrocentric
13		1.368	1.368	4.625			Acrocentric
14		1.365	1.365	4.615			Acrocentric
15		1.361	1.361	4.601			Acrocentric
16		1.358	1.358	4.591			Acrocentric
17	0.651	0.705	1.356	4.581	1.082	48.008	Metacentric
18	0.651	0.702	1.353	4.574	1.078	48.115	Metacentric
19		1.347	1.347	4.554			Acrocentric
20	0.277	1.067	1.344	4.554	3.851	20.610	Subtelocentric
21		1.399	1.399	4.527			Acrocentric
22		1.398	1.398	4.527			Acrocentric
23		1.374	1.374	4.645			Acrocentric
24	0.275	0.925	0.304	4.057	3.363	20.916	Submetacentric
25		0.251	0.251	3.357			Acrocentric

Total mean haploid length = 29.575 µm

Conclusions

In conclusion, the chromosome analysis of three Indian major carp species *C. catla* (Hamilton, 1822), *L. rohita* (Hamilton, 1822), *C. mrigla* (Hamilton,1822) using conventional staining procedure revealed the same diploid number (2n=50) with variability in size, shape and arm number (NF) of chromosomes suggesting that diversification in these fishes of the same family is related to structural changes in chromosomes. The variations in karyotype formulae in comparison to earlier studies may be because of pericentric inversions or centric fission and appears to be due to variations in habitat conditions as a result of anthropogenic activities.

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