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Karyomorphology of Two Major Carps, *Cirrhinus Mrigala* (Hamilton) Buchanan (Mrigal) and *Hypophthalmichthys Molitrix Valenciennes* (Silver Carp)

Nandini. S
Department of Advanced Zoology and Biotechnology,
Quaid E Millath Govt. College for Women, Chennai, Tamil Nadu, India
Arockia Rita. J.J
Department of Advanced Zoology and Biotechnology,
Quaid E Millath Govt. College for Women, Chennai, Tamil Nadu, India
Sharmila. G
Department of Advanced Zoology and Biotechnology,
Quaid E Millath Govt. College for Women, Chennai, Tamil Nadu, India

Abstract:
A detailed study of chromosomes of two species of Indian inland fish revealed the chromosomal number and type. It describes the relation between the two species. Their peculiar morphological characters have been recorded. Karyological studies have provided basic information on the number, size and morphology of chromosomes (Tan et al., 2004). These karyological observations provide strong evidence to conclude that the two species are closely related and have phylogenetic links.

Keywords: *Cirrhinus mrigala, Hypophthalmichthys molitrix and Karyomorphology.*

1. Introduction

Cytogenetics is the study of chromosome morphology and the behaviour of chromosomes during mitosis and meiosis. The study of fish chromosome has become an active area of research in recent years (Mohammad Reza Kalbassi *et al*, 2008). The chromosome number and its morphology is specific for a particular species. Fishes are of particular interest to Ichthyologists as well as cytogenetists as they occupy a very important position in the systematic differentiation of vertebrates. The benefits of this Karyological study among fishes are great values as fishes are economically important.

Karyological methods of ascertaining the taxonomic position of different species of fish have been in wide use in recent years among Russian and other ichthyologists (E.A.Salmenkova *et al*, 2005). The available data in fishes show that almost all forms of chromosomal rearrangements have played a role in the evolution of the fish karyotypes (E.D.Vasil'eva 2011).

Among 23000 identified species, standard karyotype has been reported only for 2400 species (10.4%). While chromosomal study is very applicable for taxonomic, genetic, cytotoxicological, race improvement and biotechnological investigations, it also has application in chromosomes set manipulation and triploidy production as a tool to enhance of chromosomes alteration (Hosseini & Kalbassi, 2003; Gold *et al*., 1990; Al-Sabti, 1991).

2. Materials And Methods

2.1. Experimental Species

Two different species of freshwater fish namely *Cirrhinusmrigala* (Hamilton) Buchanan (Mrigal) and *Hypophthalmichthys molitrix valenciennes* (silver carp) were selected for the present study.

2.2. Maintainence in the Laboratory

The live animals were collected from Poondi fresh water aquaculture station and they were transported to the laboratory in oxygenated polythene bags and maintained in fiber glass tanks containing enough amount of water. The water was changed once a day and the fishes were given food *adlibitum*. The fishes were acclimatized for about one week before the experiments were conducted. Feeding was stopped two days prior to the experiment. On the third day, the experimental fishes were injected with colchicine (Hartwell *et* al., 2000) and introduced into the tank.

2.3. Chemicals Required

Colchicine 0.01% (0.1mg colchicine was dissolved in100ml of dis. water.
Potassium chloride:0.4%,400mg KCl was dissolved in 100ml of dis. water.
Sodium citrate:0.9% 900mg of sodium citrate was dissolved in 100ml of dis. water.
Carnoy's fixative: 3:1 ratio of methanol and Glacial acetic acid

2.3.1. Giemsa Stain

Prepared by dissolving 2ml of stock Giemsa solution and 2ml of 10% disodium hydrogen phosphate to 4.6ml of distilled water (PH 6.8)

2.3.2. Karyotyping

Procedure developed by Kligerman 1982 was followed with minor alterations.
Since blood samples could not be obtained in smaller fishes, chromosome preparation from the gill tissues were used for chromosome preparation.

2.3.3. Staining

The air dried slides were then stained with freshly prepared Giemsa staining solution (4%) for 15-18mts. The slides were then destained with distilled water and air dried. The slides were then screened for chromosomal spreads under the light microscope.
A karyotype was prepared by high-contrast chromosome photographs and the individual chromosomes were cut of the photographs. Classification and karyotype of the chromosomes were performed according to the techniques described by Levan *et al*.,(1964) and Ergene *et al*. (1998a,b). The final karyogram was scanned and printed.

2.4. Chromosomal Complement in Cirrhinus Mrigala

The total diploid number was found to be 50(2n=50). This was confirmed by observing 157 metaphase Figures which showed the diploid number 50.
Based on the idogram individual karyomorphology of the diploid set was analysed and the chromosome length was measured. The length ranges from $8.5X10^{-3}\mu$ to $3.0\ X10^{-3}\mu$ of the largest to the smallest chromosomes. In the diploid set four pairs are metacentric (4,9,15 and 20), Thirteen pairs are submetacentric (1,2,3,5,6,8,10,11,12,14,16,19 and 21) and remaining eight pairs are acrocentric (7,13,17,18,22,23,24 and 25). Relative length percent (RL%) ranges from 5.64 to 1.99. Nucleolar organiser region (NOR) and heterochromatin region (HCR) were also observed. (Figure 4, Table 1 and Figure 1 & 2)

Figure 1: Cirrhinus mrigala

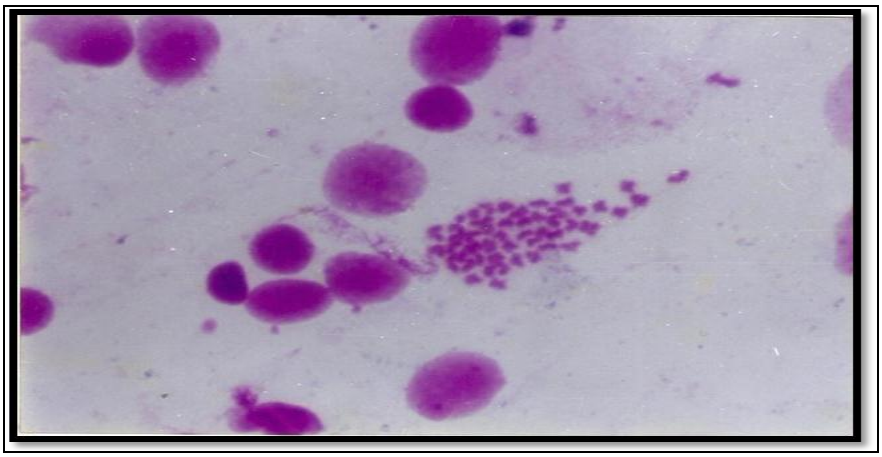

Figure 2: Metaphase Figure showing chromosomal complement 2n = 50 of Cirrhinus mrigala

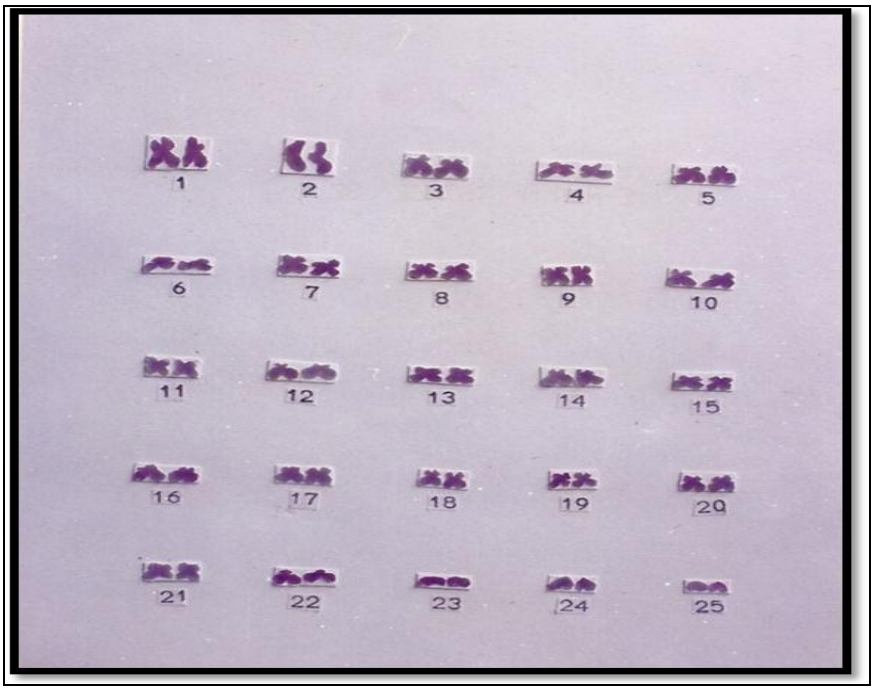

Figure 3: Karyotype of Cirrhinus mrigala

Pair No	Pair mean p(μ)	Pair mean q(μ)	Pair mean TL(μ)	RL%	Ic	TYPE OF CHROMOSOME
1	$3.2X10^{-3}$	$3.6X10^{-3}$	$7.3X10^{-3}$	6.80	0.44	Metacentric
2	$3.0x10^{-3}$	$3.3x10^{-3}$	$6.82x10^{-3}$	6.35	0.44	Metacentric
3	$2.5x10^{-3}$	$3.5x10^{-3}$	$6.5x10^{-3}$	6.05	0.38	Submetacentric
4	$1.5x10^{-3}$	$3.3x10^{-3}$	$5.3x10^{-3}$	4.93	0.28	Submetacentric
5	$1.5x10^{-3}$	$3.0x10^{-3}$	$5.0x10^{-3}$	4.65	0.30	Submetacentric
6	$1.2x10^{-3}$	$3.0x10^{-3}$	$4.7x10^{-3}$	4.38	0.26	Submetacentric
7	$1.7x10^{-3}$	$2.5x10^{-3}$	$4.7x10^{-3}$	4.38	0.36	Submetacentric
8	$1.6x10^{-3}$	$2.5x10^{-3}$	$4.6x10^{-3}$	4.28	0.35	Submetacentric
9	$2.0x10^{-3}$	$2.0x10^{-3}$	$4.5x10^{-3}$	4.19	0.44	Metacentric
10	$1.5x10^{-3}$	$2.5x10^{-3}$	$4.5x10^{-3}$	4.19	0.33	Submetacentric
11	$2.0x10^{-3}$	$2.0x10^{-3}$	$4.5x10^{-3}$	4.19	0.44	Metacentric
12	$1.0x10^{-3}$	$2.16x10^{-3}$	$4.1x10^{-3}$	3.82	0.38	Acrocentric
13	$1.5x10^{-3}$	$2.0x10^{-3}$	$4.0x10^{-3}$	3.72	0.38	Metacentric
14	$1.0x10^{-3}$	$2.5x10^{-3}$	$4.0x10^{-3}$	3.72	0.25	Acrocentric
15	$1.75x10^{-3}$	$1.75x10^{-3}$	$4.0x10^{-3}$	3.72	0.44	Metacentric
16	$1.0x10^{-3}$	$2.5x10^{-3}$	$4.0x10^{-3}$	3.72	0.25	Submetacentric
17	$1.2x10^{-3}$	$2.0x10^{-3}$	$3.7x10^{-3}$	3.44	0.32	Submetacentric
18	$1.2x10^{-3}$	$2.0x10^{-3}$	$3.7x10^{-3}$	3.44	0.32	Submetacentric
19	$1.6x10^{-3}$	$1.6x10^{-3}$	$3.7x10^{-3}$	3.44	0.43	Metacentric
20	$1.5x10^{-3}$	$1.5x10^{-3}$	$3.5x10^{-3}$	3.26	0.43	Metacentric
21	$1.2x10^{-3}$	$1.7x10^{-3}$	$3.4x10^{-3}$	3.17	0.35	Submetacentric
22	$0.5x10^{-3}$	$2.25x10^{-3}$	$3.25x10^{-3}$	3.03	0.15	Acrocentric
23	$1.25x10^{-3}$	$1.5x10^{-3}$	$3.25x10^{-3}$	3.03	0.38	Metacentric
24	$0.5x10^{-3}$	$1.2x10^{-3}$	$2.2x10^{-3}$	2.05	0.16	Submetacentric
25	$0.5x10^{-3}$	$1.2x10^{-3}$	$2.2x10^{-3}$	2.05	0.23	Submetacentric

p : Short arm
q : Long arm
TL : Total length
RL % : Relative length percent
Ic : Centromere index

Table 1: Measurement of chromosomal complement of Cirrhinus mrigala

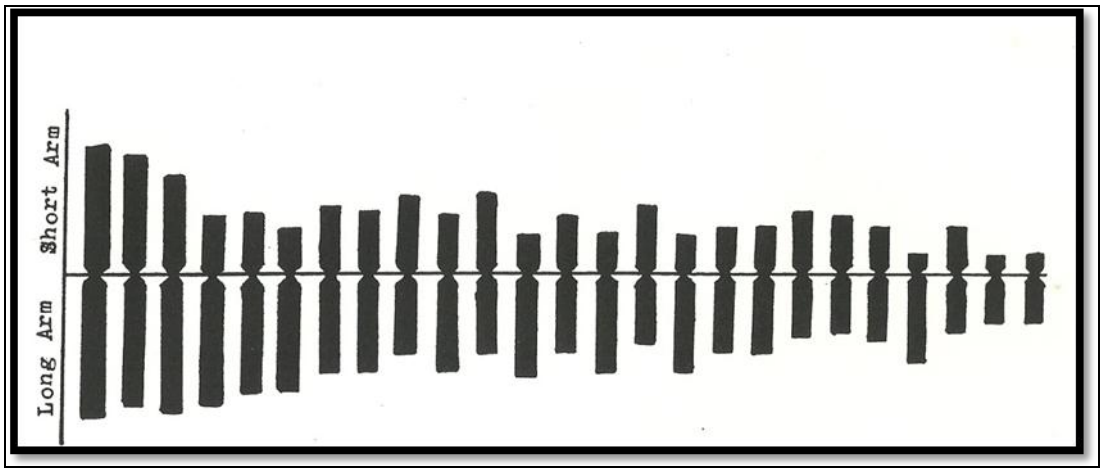

Figure 4: An Idiogram of Cirrhinus mrigala

2.5. Chromosomal complement in Hypopthalmichthys molitrix.

The diploid number was found to be 48 (2n=48). This was confirmed by observing 147 metaphase Figures which showed the diploid number 48.

The chromosomes are condensed in nature and darkly stained with distinct karyomorphology. Based on the idogram individual karyomorphology of the diploid set was analysed and the chromosome length was measured. The length ranges from $7.0X10^{-3}\mu$ to $3.5\ X10^{-3}\mu$ of the largest to the smallest chromosomes. In the diploid set eleven pairs exhibit metacentric (2,3,7,9,11,14,15,18,19,20 and 24). Eleven pairs are submetacentric (1,4,5,8,12,13,16,17,21,22 and 23) and remaining two pairs are acrocentric (6 and 10). Relative length percent (RL%) ranges from 5.79 to 2.90. Nucleolar organiser region (NOR) was distinctly observed in the first pair and heterochromatin region (HCR) were observed in all the pairs.

From the observations it is found that the three species of Indian major carps namely *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* have the same diploid number 2n=50 while that of the exotic species *Hypopthalmichthys molitrix* is 48.

Figure 5: Hypopthalmichthys molitrix

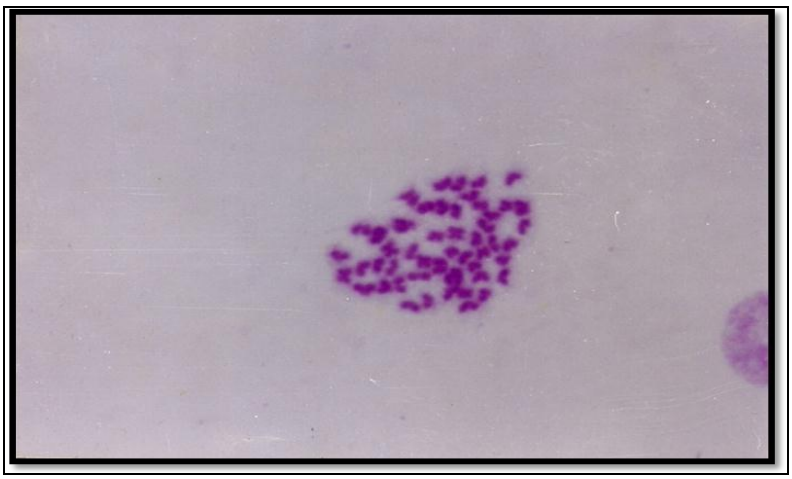

Figure 6: Metaphase Figure showing chromosomal complement 2n = 48 of Hypopthalmichthys molitrix

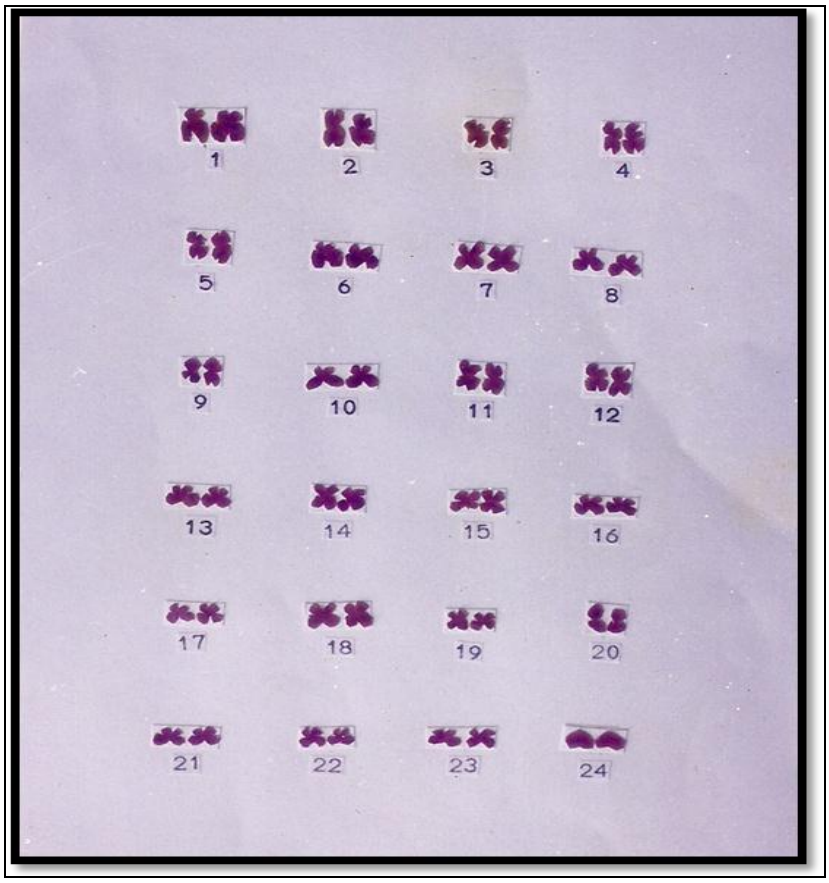

Figure 7: Karyotype of Hypopthalmichthys molitrix

Pair No	Pair mean p(μ)	Pair mean q(μ)	Pair mean TL(μ)	RL%	Ic	TYPE OF CHROMOSOME
1	$1.5X10^{-3}$	$5.0X10^{-3}$	$7.0X10^{-3}$	5.79	0.21	Submetacentric
2	$3.2x10^{-3}$	$3.2x10^{-3}$	$6.9x10^{-3}$	5.71	0.46	Metacentric
3	$3.0x10^{-3}$	$3.10x10^{-3}$	$6.5x10^{-3}$	5.38	0.46	Metacentric
4	$2.0x10^{-3}$	$3.8x10^{-3}$	$6.3x10^{-3}$	5.21	0.32	Submetacentric
5	$2.0x10^{-3}$	$3.5x10^{-3}$	$6.0x10^{-3}$	4.96	0.38	Submetacentric
6	$1.0x10^{-3}$	$4.2x10^{-3}$	$5.7x10^{-3}$	4.74	0.18	Acrocentric
7	$2.6x10^{-3}$	$2.6x10^{-3}$	$5.7x10^{-3}$	4.72	0.46	Metacentric
8	$2.0x10^{-3}$	$3.0x10^{-3}$	$5.5x10^{-3}$	4.55	0.36	Submetacentric
9	$2.5x10^{-3}$	$2.5x10^{-3}$	$5.5x10^{-3}$	4.55	0.45	Metacentric
10	$1.0x10^{-3}$	$3.5x10^{-3}$	$5.0x10^{-3}$	4.14	0.20	Acrocentric
11	$2.0x10^{-3}$	$2.5x10^{-3}$	$5.5x10^{-3}$	4.14	0.40	Metacentric
12	$1.5x10^{-3}$	$3.0x10^{-3}$	$5.0x10^{-3}$	4.14	0.30	Submetacentric
13	$1.0x10^{-3}$	$3.5x10^{-3}$	$5.0x10^{-3}$	4.14	0.20	Submetacentric
14	$2.2x10^{-3}$	$2.2x10^{-3}$	$4.9x10^{-3}$	4.05	0.45	Metacentric
15	$2.1x10^{-3}$	$2.1x10^{-3}$	$4.7x10^{-3}$	3.89	0.45	Metacentric
16	$1.5x10^{-3}$	$2.5x10^{-3}$	$4.5x10^{-3}$	3.72	0.33	Submetacentric
17	$1.5x10^{-3}$	$2.5x10^{-3}$	$4.5x10^{-3}$	3.72	0.33	Submetacentric
18	$1.8x10^{-3}$	$2.0x10^{-3}$	$4.3x10^{-3}$	3.56	0.44	Metacentric
19	$1.8x10^{-3}$	$1.8x10^{-3}$	$4.1x10^{-3}$	3.39	0.44	Metacentric
20	$1.75x10^{-3}$	$1.75x10^{-3}$	$4.0x10^{-3}$	3.31	0.44	Metacentric
21	$1.5x10^{-3}$	$2.0x10^{-3}$	$4.0x10^{-3}$	3.31	0.375	Submetacentric
22	$1.0x10^{-3}$	$2.25x10^{-3}$	$3.75x10^{-3}$	3.10	0.27	Submetacentric
23	$1.0x10^{-3}$	$2.0x10^{-3}$	$3.5x10^{-3}$	2.90	0.29	Submetacentric
24	$1.5x10^{-3}$	$1.5x10^{-3}$	$3.5x10^{-3}$	2.90	0.43	Metacentric

p : Short arm
q : Long arm
TL : Total length
RL % : Relative length percent
Ic : Centromere index

Table 2: Measurement of chromosomal complement of Hypopthalmichthys molitrix

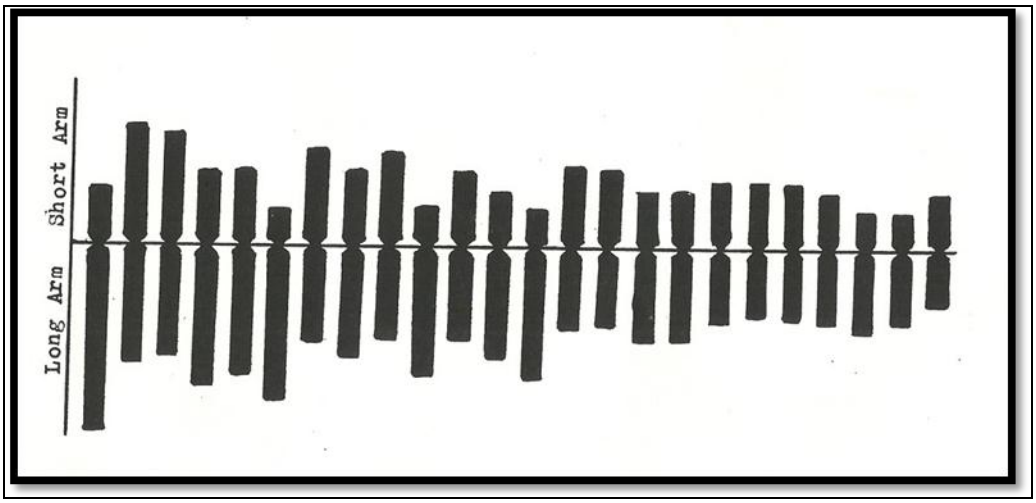

Figure 8: An Idiogram of Hypopthalmichthys molitrix

3. Discussion

The chromosomal study was conducted in several steps. The first step in the procedure was treatment of the cells with colchicine, which arrests cell division at the metaphase (Baksi SM, Means JC 1988) . In chromosome studies, improvement of colchicine treatment has a basic role in obtaining suitable metaphase spreads. In this study, the optimum colchicines concentration was determined to be 0.01ml/mg body weight for 3 ½ hours exposure time which is similar to that of Nahavandi *et al*., (2001) and Nowruzfashkhami *et al*., (2002)

In the recent study the slides were first warmed and then the suspension fell on to them which is similar to the studies carried out by Baksi and Means, (1988), Nahavandi *et al*., (2001), Varasteh *et al*., (2002), Nowruzfashkhami *et al*., (2002) and Hosseini and Kalbasii *et al*., (2003).

Air drying technique which was originally developed for the study of mammalians chromosomes (Serap and Tolga 2004) can now be applied to chromosome studies in other species.

Diversity is a land mark of evolution. Each species is characterized by a specific chromosome complement commonly referred to as karyotype. Karyodiversity is mainly due to the variation in the position of centromere. Centromere is the special region of chromosome which gets firmly attached to mitotic spindle at the time of metaphase during cell cycle. These are usually observed during the early stage of cell division as non-staining gaps. The chromatids, both attached to the kinetochore part of centromere and to the spindle during metaphase which can be observed by the influence of a drug, colchicines. Besides the centromere, non-stained gaps in the form of secondary constriction is also a common feature of chromosomes.

During the early and late metaphase, the chromosomes reach maximum degree of condensation and constriction, to take part in the on-going chromosomal division. Hence, metaphase holds the secret of cell cycle and expresses the full complement of chromosomes in the somatic cells of a species is referred to as somatic number and designated as 2n. In the present study gill cells, conventional karyological technique of Kligerman and Bloom (1977) was adopted with due modification to suit the experimental fish. In the present study the diploid complement 2n was 50 in both the species of carps.

In 70% of the examined Cyprinids, the chromosome number was 2n=50 and it is considered as a model and base number for Cyprinids (Khuda-Bukhsh *et al*., 1986).

The number of chromosomes per cell is rather conservation characteristic and may be used as an indicator of closeness of species, within families. The number and position of the arms of the chromosomes is even more conservative than chromosome number and is often equally useful in taxonomic studies.

4. Conclusion

In conclusion it is emphasized that the order Cypriniformes include fish groups which is not much variable from karyological point of view. The major carp *Cirrhinus mrigala* had the diploid number 50 (2n=50), while that of Exotic carp *Hypopthalmichthys molitrix* had the diploid number 48 (2n=48). But to analyse in detail the chromosome evolution processes and taxonomical relationship, it is necessary to collect more cytological banding methods.

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