© 2018 IJSRST | Volume 4 | Issue 2 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
Themed Section: Science and Technology

Chromosomal Analysis on A Few Species of Ornamental Fishes

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ABSTRACT

Karyology deals with the chromosome morphology and behaviour during cell cycle. Every organism is characterized by its own specific chromosomes both in number and morphology. Generally fish have been the subject of investigations for their systematic, mutagenesis and culture. The cytogenetic and karyomorphological studies would are aid in the improvement species including ornamental species. Currently, aquarium fish being ornamental gain new importance as a small scale industry product and the ability as in and established to be a balanced entry with great marketability. The present study has been carried out to provide some karyological information on the economically important ornamental fish species. The method of Kligerman and Bloom (1997) was standardized for the chromosome preparations. Chromosome spreads were made for gill, and kidney tissue. Well spread metaphase spreads were photographed from gill tissues. The diploid chromosome numbers were determined for the 8 ornamental fish species. Gymnocorymbus ternetzi 2n = 48; Hemigrammus armstrongi 2n =50; Lobeo bicolor 2n=54; Puntius gelius 2n =52; Betta aplendens 2n = 50; Colisa lalia 2n=68; Trichogaster leeri 2n =66 and Trichogaster trichopterus 2n=72. And it is provided the information on the variations in size, number, karyomorphological or structural variation and staining have been discussed in view of adaptive and evolutionary significance.

Keywords: Chromosome, Ornamental Fish, Karyology, Evolutionary, Significance.

I. INTRODUCTION

Karyology deals with the chromosome morphology and behaviour during cell cycle. Every organism is characterized by its own specific chromosomes both in number and morphology. Generally fish have been the subject of investigations for their systematic, mutagenesis and culture. Cytogenetic studies of fish include hybrid vigour, polyploidy, karyomorphological, chromosome banding and nature of sex chromosome.

The cytogenetic and karyomorphological studies would are aid in the genetic improvement of fish species including ornamental species. A cytogenetic of fishes is difficult compared to mammals and other groups, not much progress has been made in this field. Among approximately 20,000- 23,000 living species of fishes, the chromosome number is known for only about 650- 700 species and complete karyotyping has been made in about 500 species (Gold, 1979). Early fish cytologists were handicapped by numerous technical difficulties resulting in several reports of erroneous chromosome number and morphology now considered invalid (Cataudellas and Capana, 1973 and Denton 1973, Ohno 1974). Relatively recent developments of techniques have led to the current expansion of studies into the chromosomal basis of

successful crossing and selection in economically important and cultivable fishes.

The development of cytogenetic studies by current air dying method combined with colchicines treatment has made accurate delineation of chromosomes of somatic cells easier, (Kosswing 1973) has reviewed the place of fish in genetic research, and fish cytogenetic has been in detail by (Denton 1973, Gold 1979 and Blaxhal 1975).

1.1Aesthetic Value

In recent aquarium has been a decoration piece of homes. Now the scenario has changes. The barrier has been shattered down giving it the passage to schools colleges, research laboratories, hospitals, offices, shop and to lot more other public places. The reason for selecting ornamental fishes for breeding and marketing are as follows:-

They have wide temperature tolerance

They are easy to breed

They are hardy fish

They have good market demand and are easily disposed off.

1.2 Economic Importance

The disparity between rapidly growing human population and sluggish generation of job opportunities leave a big number of job searching youth. This problem can be improved if job- seekers being to resort the possibility of self employment. There are considerable numbers of people involved in the aquarium business. The countries that are pioneers in aquarium fish breeding technology, namely Japan and China both earn a sizable currency each year by exporting the fish food and various other aquarium appliances to other countries throughout the globe

Studies on fish cytogenetics began as early as the last decade of the nineteenth century. Some vague idea about the chromosomes could be possible from the investigations of Retziant (1890) on the agnatha Myxine glutinosa. Until the mid 1960's, all the analyses were based on the histologically sectioned gonadal material, particularly the testicular tissue. But for providing the chromosome numbers, the results obtained did not give an accurate description of the morphology of chromosomes. Subsequently, the application of hypnotic treatment coupled with squashing improved the spreading of chromosome at the metaphase stage though the chromosome morphology still remained obscure. Nevertheless, a realization persisted with the workers that the data obtained by these classical methods demanded a reappraisal.

1.3 Current Techniques

Almost all the techniques currently in vague for fish chromosome analysis make use of pretreatment with colchicine and a hypotonic solution, particularly potassium chloride (0.56%) or sodium citrate (1.0%) these techniques are mentioned below:

1.4Squashing

Although an old method, it still carries an advantage since preparations are possible from small bits of tissues removable without much injury to the animals. For example, slides can be made from scale epithelium, gill epithelium, marginal snips of fins and barbells and corneal tissues (Denton, 1973). Sometimes it is possible to enhance the mitotic activity by making a local injury and allowing to heal. Colchicine may be injected into the specimens or the tissues may be treated with colchicine. Squashing is generally done in the stain fixatives like, Aceto-orcein or Aceto-carmine after pretreatment with a hypotonic solution. Whereas the hypotonic media mentioned above give the best result, use can be made of distilled water, tap water. The ideal treatment time is 20 to 30 minutes. If facilities for air-drying do not exist, squashing may be resorted to even for such tissues as kidney, spleen, liver and gonads (Roberts 1967). Egg squashing before gastrulation also gives satisfactory results. Even young larvae can give good metaphase plates.

1.5 Air/flame-drying Method

This method is based on making cell suspensions of the mitotically active tissues of pre colchicinised specimens in a hypotonic solution. The cells are fixed after pretreatment for an optimal period and centrifugation. The Cells are gain suspended several times in the fixative (usually 1:3 acetic methanol) and are then spread on clean slides. These are then left for drying in the air or are ignited to dry. Slides are stained in diluted giemsa. This simple method has given extremely satisfactory results (Manna & Prasad 1968, 1977).

In addition to kidney, several other tissues can also yield good chromosomes by air-drying, as for example, liver spleen, gill epithelium. Analysis of both mitotic and meiotic chromosomes is also possible from the testicular tissue.

1.6 Tissue Culture

Significant progress has been made in developing tissue-culture techniques for the fish material. Both short-term and long-term culture methods have been standardized. Tissue – culture gives much better pictures of Chromosomes. A number of tissues such as embryo, fins, testes, ovary, kidney, spleen, liver, swim bladder and blood have been exploited. The period of growth of cultures varies from 6 days to 2 months, depending on the size and age of specimens.

It is expected that closely related fishes which reveal similar karyotypes may actually be differing in several chromosomal regions and their evolution may have been due to paracentric changes.

The present study investigated the diploid number of chromosome characteristic of selected species such as Gymnocorymbus ternetzi (Black tetra). Hemigrammus armstrongi (Golden tetra), Labeo bicolor (Red tailed black shark), Puntius gelius (Golden dwarf barb), Betta aplendans (Siamese fighter) Colisa lalia (Dwarf gourami) Trichogaster leeri (Pearl gourami) and Trichogaster trichopterus (Blue gourami) and it is providing the information on the karyomorphological or structural variation.

II. METHODOLOGY

2.1Collection of Experimental Animal

The experimental fishes were procured form aquarium keepers in Chennai and reared in laboratory condition in well aerated aquarium tanks using well water. The fishes were fed with pelletized food (Hitachi) adlibitum and the renewed once in two days

Feeding was stopped two days before the experiment. On the third day the experimental fishes were injected with 0.01% to 0.02% of 0.05ml/gm body weight colchicines for the know weight of the fishes, and placed in an aerated aquarium tanks. Care was taken to maintain the fishes actively during the period (Plate-1).

2.2 Experimental Species

Eight different species of aquarium fishes namely Gymnocorymbus ternetzi (Black tetra), Hemigrammus armstrongi (Golden tetra), Labeo bicolor (Red tailed black shark), Puntius gelius (Golden dwarf barb), Betta aplendans (Siamese fighter), Colisa lalia (Dwarf gourami), Trichogaster leeri (Pearl gourami) and Trichogaster trichopterus (Blue gouranmi).

2.2.1Method: Kligerman and Bloom (1977)

The fish were allowed to reside in a well aerated tank after an intramuscular injection of 0.001% Colchicine (in Cl/100 mg body weight of the fish).After 21/2 hrs the fish were sacrificed by pithing and the kidney and gills dissected out.

The individual tissues were transferred to 10 times their volume of 1% sodium citrate or 0.4% KCl hypotonic solution for 30 minutes.The blood vessels, mucosa and other impurities were removed. The tissues were then fixed in methanol; glacial acetic acid (3:1) by slowly adding the fixative drop by drop. The fixative was poured off and fresh fixative added. The

tissues were kept in a refrigerator. After about 1 hr, the fixative was again changed. For preparing slides, a few pieces of the tissue were removed from the fixative and touched to a piece of filter paper to remove excess of fixative. The tissue was placed in an embryo cup and 5-8 drops of 50% acetic acid was added to it. The tissues were rinsed gently for about 2 minutes to form a cell suspension. This was sucked by a clean pasture pipette and dropped on free slides heated between 40 to 50°C.This suspension was dropped from a height of about 8-15 cm and immediately after dropping it was withdrawn back in to the pipette leaving a ring of cell approximately 1 cm on the slide.

Two or rings were made on one slide. The slides were air dried and stained in 2 % Giemsa stain (98ml of Sorenson's buffer at pH 6.8) for 25-30 minutes.

The slides could be observed with or without mounting.

III. RESULTS

3.1 FAMILY: CHARACIDAE

3.1.1Gymnocorymbus ternetzi : (Plate .2)

The total diploid Gymnocorymbus ternetzi is found to be 48 and is based on 130 well spread metaphase plate observed from 10 experimental species, of which 80 metaphase plate represents model diploid numbers 48 chromosomes. However varying frequency of diploid numbers and the number of metaphase counted are shown in (Table 1, Figure 1).

Metaphase plates of G. ternetzi (Plate. 3) reveals condensed, darkly stained karyomorphology with the distinct centromeric position in the chromosome. The entire chromosomes are in diad stage and metacentric in nature. A distinct feature of this existence of a ring chromosome. Heterochromatic regions are observed distinctly in 20 pairs remaining pairs indistinct. Secondary constriction was noted in a few chromosomes.

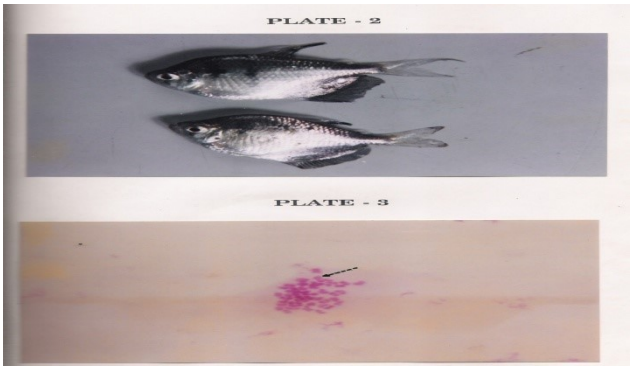

Figure 1. Illustrates the varying frequency of diploid numbers and the number of metaphase counted in Gymnocorymbus ternetzi

Table 1. Varying frequency of diploid number being obtained from the metaphase of 10 specimen of Gymnocorymbus ternetzi (Black tetra)

S.NO	DIPLOID NUMBER	NO.OF METAPHASE OBSERVED
1.	42	5
2.	43	7
3.	44	8
4.	45	14
5.	46	3
6.	47	8
7.	48	80
8.	49	3
9.	50	0
10.	51	2
2n =48		130

3.1.2 Hemigrammus armstrongi (Plate .4)

The diploid number of chromosome in H.armstrongi is found to be 50 (2n =50). It is observed from total

number of 107 metaphase plates from 10 specimens of the same species. The model diploid number is observed from 70 metaphase plates and the other model diploidies are also observed (Table 5 & Figure 2).

The test species chromosomes are characterized by short condensed and darkly stained karyomorphology (Plate.5). Heterochromatin region are shown in all the chromosomes. In addition to this 2 pairs of chromosomes are shown to have Nucleolar organizers region (NOR).

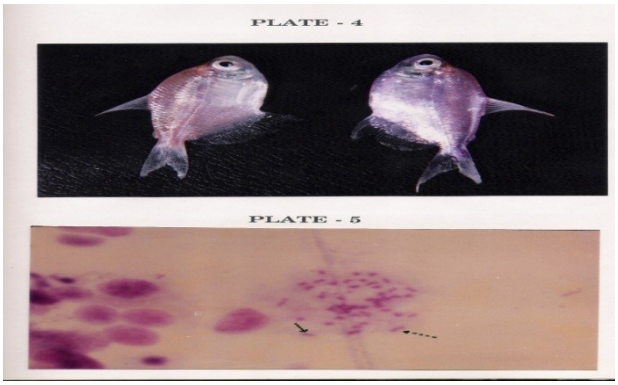

Figure:2 Illustrates the observed model diploid number from 70 metaphase plates and the other model diploidies of Hemigrammus armstrongi

Table 2. Varying frequency of diploid number being obtained from the metaphase of 10 specimen of Hemigrammus armstrongi (Golden tetra)

S.NO	DIPLOID NUMBER	NO.OF METAPHASE OBSERVED
1.	46	1
2.	47	0
3.	48	6
4.	49	8
5.	50	70
6.	51	10
7.	52	8
8.	53	2
9.	54	1
10.	55	0
2n =50		107

3.2 FAMILY: CYPRINIDAE

3.2.1 Labeo bicolor (Plate .6)

About 125 metaphase plates are observed form 10 specimens and the total diploid number is found to be 54 (2n = 54) and the other varying diploid numbers observed (Table .6 & Figure 3).

The metaphase plate of L.bicolor (Plate. 7) shoes darkly stained chromosomes with short, condensed and round karyomorphology. The chromosomes exhibit heterochromatin region and one of the long chromosome and a short chromosome show secondary constriction of Nuclear organizer region (NOR). All the chromosomes are metacentric in nature. One pair of chromosomes shows tetrad and remaining pairs diad condition.

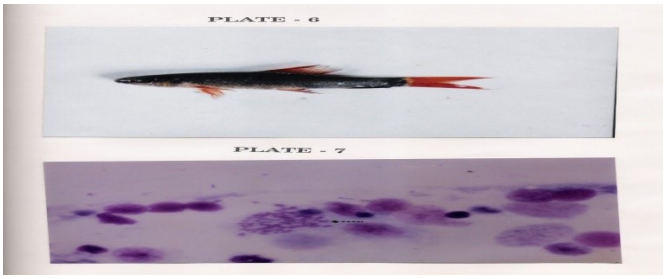

Figure 3. Illustrates the observed 125 metaphase plates form 10 specimens and the total diploid number is found to be 54 (2n = 54) and the other varying diploid numbers of Labeo bicolor

Table 3. Varying frequency of diploid number being obtained from the metaphase of 10 specimen of Labeo bicolor (Red tailed black shark)

S.NO	DIPLOID NUMBER	NO.OF METAPHASE OBSERVED
1.	51	2
2.	52	6
3.	53	4
4.	54	73
5.	55	8
6.	56	5
7.	57	9
8.	58	5
9.	59	10

10.	60	3
2n =54		125

3.2.2 Puntius gelius (Plate.8) In this species the total number is found to be 52 (2n = 52). It is confirmed on the basis of 144 metaphase plates observed in all 10 specimens. The frequency of the diploid number 52 is found in 94 metaphase plates out of 144 as shown in (Table 4 & Figure 4)

The metaphase plates of this species shows three pairs of chromosomes (Plate.9) with elongated morphology and remaining pairs shows condensed karyomorphology. HCR was found in two pairs of chromosomes. Chromosomes are moderately stained in this species. In addition to this one pair of chromosome shows nuclear organizer region and all the pairs exhibit metacentric nature. Unique of this species is presence of a ring chromosome.

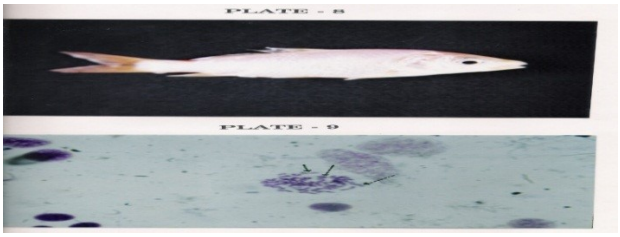

Figure 4. Illustrates the frequency of the diploid number 52 is found in 94 metaphase plates out of 144 in Puntius gelius

Table 4. Varying frequency of diploid number being obtained from the metaphase of 10 specimens of Puntius gelius (Golden dwarf barb).

S.NO	DIPLOID NUMBER	NO.OF METAPHASE OBSERVED
1.	45	2
2.	46	3
3.	47	5
4.	48	4
5.	49	6
6.	50	8
7.	51	12
8.	52	94
9.	53	7
10.	54	3
2n =52		144

3.3 FAMILY: ANABANTIDAE

3.3.1 Betta aplendens

The diploid number of chromosomes in B. aplendens is found to be 50 (2n=50) and it is derived form 86 metaphase plates out of 146 number of total metaphase plates observed from specimens of the same species as shown in (Table 5 & Figure 5).

The metaphase plate of B. aplendens shows distinct karyomorphology (Plate.11). All the chromosomes are medium size darkly stained with distinct heterochromatin region. One pair of chromosome show secondary constriction. Two pairs of chromosomes exhibit tetrad condition while the remaining show diad condition. The metaphase plate is also plate is also characterized by the existence of distinct centromere position.

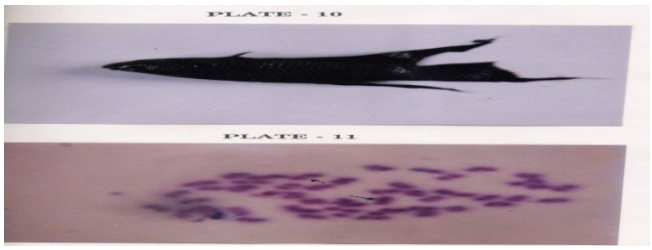

Figure 5. Illustrates the observed 86 metaphase plates out of 146 number of total metaphase plates from Betta aplendens

Table 5. Varying frequency of diploid number being obtained from the metaphase of 10 specimen of Betta aplendens (Siamese fighter)

S.NO	DIPLOID NUMBER	NO.OF METAPHASE OBSERVED
1.	44	5

2.	45	3
3.	46	6
4.	47	4
5.	48	8
6.	49	15
7.	50	86
8.	51	12
9.	52	5
10.	53	2
2n =50		146

3.3.2 Colisa Lalia (Plate .12)

About 110 metaphase plates are observed from 10 specimen of C. Lalia of which 68 metaphase plates show the highest modal diploid number, and it is found to be 62 (2n =62). The other modal diploid numbers observed (Table.9 & Figure 6).

Metaphase plate of Colisa lalia show all the chromosomes are characterized by is more or less uniform contour (Plate.13) with distinct heterochromatin region except two pairs, which show indistinct HCR. The location of centromere was clearly noted as a distinct constriction in the chromosome and in addition to this one pair show secondary constriction. Further the chromosomes are metacentric in nature.

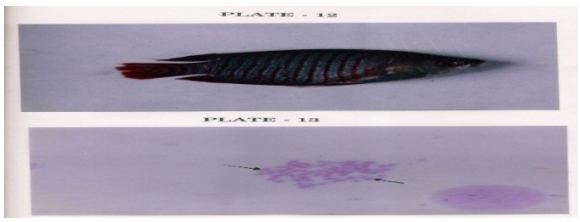

Figure:6 Illustrates the observed modal diploid numbers in Colisa Lalia

Table 6. Varying frequency of diploid number being obtained from the metaphase of 10 specimen of Colisa lalia (Dwarf gourami)

S.NO	DIPLOID NUMBER	NO.OF METAPHASE OBSERVED
1.	64	5
2.	65	2
3.	66	10
4.	67	1
5.	68	62
6.	69	8
7.	70	6
8.	71	2
9.	72	9
10.	73	5
2n = 68		110

3.3.3 Trichogaster leeri (Plate .14)

About 116 metaphase plates are observed form 10 specimens of T.leeri which indicates the diploid number should necessarily be 66 and is based on 75 metaphase plates. The other probable modal diploid numbers observed from this species are tabulated and plotted as in (Table 7 & Figure 7)

The metaphase plate of T. leeri show chromosomes having small, short, rod like karyomarphology and are moderately stained 5 pairs of chromosomes show metacentric of which 2 pair shown tetrads, while the remaining are acrocentric (Plate15).Heterochromatic region was noted as indistinct region since most of the

chromosomes are characterized by condensed karyomorphology. It is difficult to locate the exact position of nucleolar organizer region in this species.

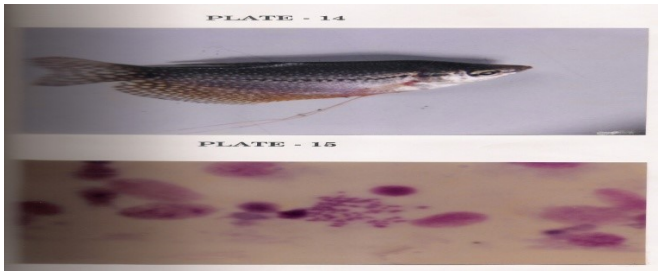

Figure 7. Illustrates the 116 metaphase plates are observed from 10 specimens of T.leeri

Table 7. Varying frequency of diploid number being obtained from the metaphase of 10 specimen of Trichogaster leeri (Pearl gourami).

S.NO	DIPLOID NUMBER	NO.OF METAPHASE OBSERVED
1.	63	5
2.	64	2
3.	65	9
4.	66	75
5.	67	7
6.	68	0
7.	69	8
8.	70	3
9.	71	1
10.	72	6
2n = 66		116

3.3.4 Trichogaster trichogasterus

The diploid number of this species T. trichogasterus was found to be 72 (2n =72). It is arrived form highest of thes number from 137 metaphase plates out of 10 specimens of the same test species. Other modes of diploid number and frequencies of metaphase plates observed are tabulated in (Table 8 & Figure 8).

The metaphase plate of T. trichogatsreus show the following features: the chromosomes are in short, slender, and darkly stained with metacentric dyad nature of chromosomes; and it also includes 3 pairs of acrocentric chromosomes (Plate 17). Besides the nucleolar organizing region is distinctly observed in a pair of chromosome.

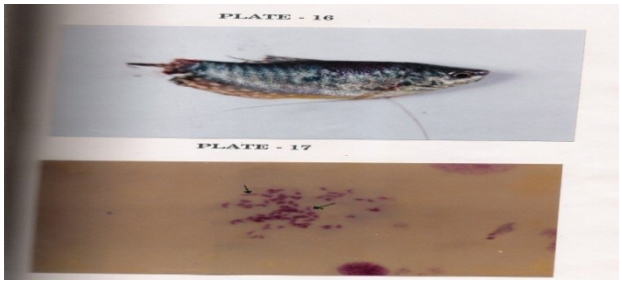

Figure 8. Illustrates the 137 metaphase plates out of 10 specimens of T.trichogasterus

Table 8. Varying frequency of diploid number being obtained from the metaphase of 10 specimens of Trichogaster Trichopterus (Blue gourami).

S.NO	DIPLOID NUMBER	NO.OF METAPHASE OBSERVED
1.	67	8
2.	68	4
3.	69	1
4.	70	10
5.	71	7
6.	72	82
7.	73	11
8.	74	6
9.	75	2
10.	76	0
2n =72		137

IV. DISCUSSION

Information about the karyomorphology of fish is available from approximately 1700 or 50 species,

belonging to about 600 families and 36 or 50 orders. The most recent list is that of Vasilev (1985) who recorded 1415 families and 36 orders. Earlier, Manna (1984) mentioned 1400 species belonging to 593 genera, 146 families and 36 orders. These estimates are quite complete but data form more and more forms are regularly accurating in the fish literature. Recent emphasis of analyses has now shifted form working out new species to deciphering details or karyotypes through the views of various type of banding techniques. However, some broad generalizations are possible from the so far data available, which are still fragment and uneven in many groups of fishes.

The diploid numbers in fish ranged form 12 or 16 Calton and Denton (1974) to 239 ± 7 Fontana and Colombo (1974). In the present study each species show distinct diploid number and Gymnocorymbus ternetzi 2n = 48; Hemigrammus armstrongi 2n =50 ; Lobeo bicolor 2n=54; Puntius gelius 2n =52; Betta aplendens 2n = 50; Colisa lalia 2n=68; Trichogaster leeri 2n =66 and Trichogaster trichopterus 2n=72.

Another important generalization is about the model diploid number in fish. Many workers have mentioned 2n-48 is common in many species and are acrocentric deemed ancestral karyotype in fish general Noguse, (1960); Post (1965); Robert's (1967); Ohno et al., (1968) and Foresti (1993). The above hypothesis seems to have some justification as 2n = 48 as the model diploid number of fish species by virtue to its sheer prepondence. However, 2n = 48 is the acrocentric karyotype being ancestral to all fishes is a highly debatable point. Inspite of the fact that 48 is the most frequent number in fishes and acrocentric is certainly a more primitive feature than the biarmed condition.

An interesting feature of fish cytogenetics has been the data on DNA values in various species, where, the DNA values are correlated with the chromosomal data and it becomes a better method of determining the relationships have been beautifully presented on the basis of both these parameters Ohno (1960) et al., (1969). The present observation on C. lalia, T. leeri and T. trichopterus confirms the above finding. But in several groups of fishes the diploid numbers and DNA content values are useful in finding out weather the karyotypes changes are on gene duplication mechanism or not.

Fish species exhibit variation in chromosomes form population to population or within a population of the same species. They even show chromosomal variabilities in different individuals Ohno et al., (1965) and Juhnxiu (1983). Different individuals within a population show chromosomal variabilities due to centricfusion as observed in the case of rainbow trout. But in the present study on 8 different species shows difference in chromosomal number and contour. Therefore, the possibility of intraspecific variation in chromosome number may be attributed.

V. CONCLUSION

The present study has been carried out to provide some karyological information on the economically important ornamental fish species.
Selected methods were adopted.

The methods of Kligerman and Bloom (1997) was standardized for the chromosome preparations of Gymnocorymbus ternetzi (Balck tetra), Hemigrammus armstrongi (Golden tetra), Labeo bicolor (Red tailed black shark), Puntius gelius (Golden dwarf barb), Betta aplendans (Siamese fuighter) Colisa lalia (Dwarf gourami) Trichogaster leeri (Pearl gourami) and Trichogaster trichopterus (Blue gourami).

Chromosome spreads were made for gill, and kidney tissue. Well spread metaphase spreads were photographed from gill tissues. The diploid chromosome numbers were determined for the 8 ornamental fish species. Gymnocorymbus ternetzi 2n

= 48; Hemigrammus armstrongi 2n =50 ; Lobeo bicolor 2n=54; Puntius gelius 2n =52; Betta aplendens 2n = 50; Colisa lalia 2n=68; Trichogaster leeri 2n =66 and Trichogaster trichopterus 2n=72.

Variations in size, number, karyomorphology and staining have been discussed in view of adaptive and evolutionary significance.

Further study will throw more light on the karyodiversity of the in view of karyotaxonomy.

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