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# Karyomorphometric analysis of fresh water fish species of India, with special reference to cold water fishes of Kashmir Himalayas. A Mini Review

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**Abstract.** Cytogenetics is the diagnostic study of chromosomal structure and properties, as well as cell division, using a variety of methods, one of which is "karyotyping." It refers to a method of photographing a stained preparation in which the chromosomes are organised in a uniform pattern. The advent of modern techniques such as "karyotyping" has made it feasible to visualize undetected chromosomal abnormalities such as short chromosome segments and chromosome translocations. Because such techniques enabled each pair of chromosomes to be identified separately, they have further aided our understanding of the chromosomal basis of a certain significant genetic diseases. Every organism has its own unique karyotype, which is defined by its number and shape. Karyotypic variation, on the other hand, occurs in different individuals of the same species, as well as between different species. Monitoring cytogenetic data of economically significant fishes as well as threatened fishes can hold importance of the succeeding generations. This review article highlights the variation in the chromosomal number & classification, methods of chromosome preparation and karyotypic analysis of various fish species of India with a special reference to fishes of Kashmir Himalayas.

**Keywords:** fish chromosomes, fish karyology, freshwater fish, Kashmir, Himalaya.

## INTRODUCTION

The study of chromosome number, morphology and size at the metaphase stage is the basis of cytogenetics, which involves karyotype analysis. A species' chromosomes can be arranged in size order. The karyotype is the full set of chromosomes grouped according to their number Shao et al. (2010). Karyotyping is the method of pairing and ordering all of an organism's chromosomes, resulting in a genome-wide snapshot of the chromosomes of an individual. Karyotypes are produced using standardised stain-

ing procedures that expose each chromosome's specific structural features. Changes in chromosome number associated with aneuploid conditions may be revealed through karyotypes. More subtle structural shifts, such as chromosomal deletions, duplications, translocations, or inversions, can be identified by carefully studying karyotypes. Indeed, karyotypes are increasingly being used to diagnose birth defects, genetic disorders, and even cancers O'Connor (2008). DNA, which is packed into chromosomes, provides the blueprint for the development and maintenance of an organism. Chromosomes are the elements that differentiate one species from another and allow genetic information to be passed down from generation to generation. Chromosomes are the vehicles that allow a species to replicate and sustain itself Ciccone et al. (2005) and De Ravel et al. (2006).

## METHODS OF CHROMOSOME PREPARATION

The study of fish cytogenetics started as early as the last decade of the nineteenth century, when some idea of chromosomes was made possible by the studies of Retziat (1989) on agnathan *Myxine glutinosa*, using histologically cut gonadal material. It was later understood that chromosome preparation could be obtained from all the tissues in which mitosis occurs. Since the 1960s, several methods have been used to study the chromosome of fish. Those employed involves colchicine injections and squashes of the testes or haematopoietic tissues Ohno et al. (1965), corneal and conjunctival epithelium Drewry (1964), gill epithelium Chen and Ebling (1968), embryonic material Simon and Dollar (1963), in vitro tissue growth Roberts (1967), blood leukocytes in culture Ojima et al. (1970) and epithelium scale Denton and Howell (1969). Improved techniques for the preparation of fish chromosomes were developed after the 1970s Nagpure et al. (2001).Tissue cultures Roberts (1964), squashing technique of the testis, and other karyotypic techniques have accompanied the advancement in cytogenetical studies of teleostean fishes. Roberts (1964); Ohno et al. (1965), embryonic tissues or haematopoetic materials Simon (1963); Yamada (1967), smearing technique from gill epithelium McphaiL and Jones (1966); Stewart and Levin (1968), solid tissues like kidney Ojima et al. (1972); Arai (1973); Ueno and Ojima (1977), from regenerating fin tissue Cattin and Ferreira (1989); Volker et al. (2005) and air drying techniques Eicher (1966); Bertollo et al. (1978); Thode et al. (1988), together with colchicine treatment Yamazaki (1971). Dropping method was used in most previous studies to distribute cells from different tissues for chromosome preparation. For spreading and flattening metaphase chromosomes, the squash technique is the oldest process Denton (1973). The air drying process, on the other hand, is the most commonly used method for preparing animal chromosomes Evans et al. (1964). According to Chourrout and Happe (1986), modern chromosome preparation techniques using air drying after colchicine injection in young fish resulted in sufficient metaphase spreads Mcphail and Jones, (1966); Kligerman and Bloom (1977). However, these methods yielded some results, it was discovered that a large number of cells were lost during the cell dropping. Furthermore, dropping the cells precisely on the preheated slides requires a high degree of technical ability. Some researchers have also attempted to prepare chromosome spreads by lowering cells from a certain height onto frozen slides Ojima et al. (1964); Ida et al. (1978). For chromosome preparations, researchers also tried incorporation of hot steam and metal plates with a temperature gradient across their surface Henegariu et al. (2001).

### *Squash method*

Squashes have been the most widely used methods of providing karyotyping material. Although an ancient technique, it is still very effective because preparations are possible from small pieces of tissue that can be separated without causing serious injury to the animal. For example, chromosome slides can be made from epithelium of scale, gill epithelium, marginal barbels and fins, and corneal tissue. One can even greatly improve mitotic activity in these tissues by triggering local injury and allowing it to heal. Squashing is typically performed in some stain-fixatives, such as aceto-carmine or aceto-orcein, after pre-treatment with hypotonic solution. In place of potassium chloride, sodium citrate, tap water or distilled water or even a 1.0 per cent tissue culture medium may be used. The ideal treatment time is between 20 and 30 minutes. A good metaphase spread from the kidneys and gills of *Labeo rohita* and *Cirrhinus mrigala* following the squash method was obtained by Khuda-Bukhsh and Chakraborty (1994).

### *Cell culture method*

In terms of chromosome preparation, cell culture yielded promising results. Successful attempts have been made by Amemiya et al. (1984). However, Chen and Ebling (1975) contended that this method requires the killing of fish before tissues can be excised. Blood lymphocyte culture helps to overcome many of the limitations mentioned above, as fish do not need to be killed,

so repeated samples can be taken if necessary and the number of mitoses is increased due to the need to stimulate mitogen lymphocytes Blaxhall (1983). The author also identified the use of phytohaemagglutinin-purified blood lymphocyte culture for karyotyping of *Salmo trutta* L. and *Cyprinus carpio* L. (PHA-P).

*Staining of chromosomes*

To distinguish the colourless chromosomes from the similarly colourless cytoplasm, staining is needed. For chromosome visualisation, the slides are stained with the required solution. Most commonly, the Giemsa stain is used to stain slides. The use of filtered acetoricine as a stain was recorded by Mc Phail and Jones (1966). Arcement and Rachlin (1976) experimented with various stains, including Giemsa (normal or buffered), aceto-orcein, and aceto-cannine, and found that Giemsa standard provided the best results. However, the majority of the workers proposed diluting Giemsa with phosphate buffer.

*Chromosome banding techniques*

The chromosomal band is defined as a segment of chromosomes, which can be differentiated from adjacent segments by being either lighter or darker depending on the staining technique involved. Chromatin is the substance that makes up chromosomes, and there are two types: euchromatin, which stains softly, and heterochromatin, which stains darkly. The arrangement and organisation of chromosomes can be better understood with chromosome banding. The unambiguous identification of chromosomes in the karyotype, as well as the study of heteromorphism between and within organisms, is two of the most important applications of the banding technique. Banding techniques may also be used to identify chromosome rearrangements that have taken place during the course of evolution. The different banding techniques used today for the cytogenetic study are C-, G-, R-, Q-, and NOR-banding. Among these, NOR- and C-banding are commonly used in fish Hartley and Horne (1985). G-banding was also tried, but only with little success Blaxhall (1983).The approach to slide preparation is vital, as incorrect spreading strategies can result in the chromosomes being washed out during the staining process. After slide preparations, various staining techniques such as classic staining (e.g., aceto-orcein, haematoxylin, giemsa, wright and leishman stains) or banding techniques are employed to stain chromosomes for various purposes e.g., Q-banding, G-banding, R-banding, C-banding and High Resolution banding Calado et al. (2013); Moore and Best (2001); Wang et al. (2010). Concentrated staining solutions and/or over incubation result in a dark background filling the space between chromatids, whereas diluted staining solutions and/or a short incubation period produce chromosomal spreads that are indistinguishable.

*Use of Colchicine*

The most common microtubular poison is colchicine. Colchicine inhibits spindle microtubules and disperses metaphase chromosomes in the cytoplasm before nuclear envelope breakdown (NEB) in metaphase cells Caperta et al. (2006), whereas in *D. rerio* both colchicine concentration and incubation period were significantly influenced by larval age, and in *C. gariepinus* only colchicine incubation period was significantly influenced by larval age. It has previously been demonstrated that microtubule polymers are sensitive to physical and chemical parameters Tilney and Porter (1967); Weisenberg (1972). Therefore, age- and species-dependent cellular parameters may influence the sensitivity of cells towards depolymerizing effects of colchicine. A spindle poison (e.g., colchicine) is used to arrest the cells at their metaphase stage in conventional chromosome preparation processes Kligerman and Bloom (1977). In order to achieve clear and identifiable metaphase chromosome spreads, it is essential to select the appropriate concentration and incubation time for the poison Rieder and Palazzo (1992). While the cells cannot be stopped at metaphase stage with insufficient concentration and/or time spindle poisons exposure, extremely high concentrations or excessively long durations of exposure might lead to chromosome condensation Rieder and Palazzo (1992); Wood et al. (2001). Cells or larvae must be incubated in a hypotonic solution following mitotic spindle inhibition to swell the nuclei and disperse the chromosomes on slides Moore and Best (2001). Using an appropriate hypotonic solution is the other critical element that has been emphasized in the current study. Potassium chloride (KCl 0.075 M) is one of the most commonly used hypotonic solutions in chromosomal preparation protocols. Analogously, the efficacy of distilled water as a hypotonic treatment has been shown in some other protocols. When distilled water was used instead of KCl, the amount of clear metaphase chromosome spreads in *C. gariepinus* increased significantly. The use of KCl resulted in a lot of cell burst and chromosomal loss. Changes in the hypotonic solution, on the other hand, had no impact on the amount of metaphase chromosome spreads in *D. rerio*. Karami et al. (2015) also

reported that the type of hypotonic solution used can be changed depending on the fish species and/or larval age in order to obtain a desired number of consistent chromosome spreads. Besides these aforementioned elements, the researchers attempted to alter other essential aspects of chromosomal preparation protocols in their research. Their preliminary research also showed that the larvae should be killed before being incubated in colchicine solution, as incubating live larvae in the solution did not result in chromosome spread. Furthermore, the yolk sac should be extracted prior to incubation in colchicine to obtain direct chromosome spread Hussain and McAndrew (1994); Pradeep et al. (2011), because the yolk's high lipophilicity can limit the penetration of colchicine or hypotonic solution into the cells Hussain and McAndrew (1994); Pradeep et al. (2011).

*Hypotonic Treatment*

Hypotonic treatment is an important and crucial factor in improving the chromosome spreads. This treatment helps in removal of lipid and denatures proteins. Hypotonic treatment allows the swelling of the cell, which facilitates cell disruption and the dispersion of chromosomes when the cell contents are spread on slides. Ida et al. (1978) reported that the use of potassium chloride showed the best chromosome spreads as compared to other two hypotonic solutions of sodium citrate and distilled water. Chourrout and Happe (1986) reported that the chromosome spreading was insufficient at 0.56% KCl for hypotonic treatment at a lower temperature in the rainbow trout. However, the same concentration of KCl showed slightly better results when the experiments were performed at ambient temperature. According to the same author trisodium citrate as hypotonic treatment gave significant improvement in chromosome spreading. Pradeep et al. (2011) used 50% acetic acid during the chopping of tissues, but simple distilled water, produced better suspensions. In the modified technique, different durations of staining along with different concentrations of giemsa stain were also tried. A concentration of 5% giemsa stain for 20 minutes of treatment as described by Bayat and Woznicki (2006) was not very effective. Moreover, counting of the chromosomes was found difficult at a concentration of 20% as suggested by Don and Avtalion (1986). Changing timing and concentrations of giemsa stain significantly affected the visibility and brightness of the spreads on the slides. A concentration of 10% giemsa stain prepared in 0.01 M phosphate buffer of pH 7 for 20 minutes, as described by Hussain and Mcandrew (1994) was very effective in obtaining clear images.

The majority of genetic defects are caused by chromosomal abnormalities. Cytogenetics is the diagnostic study of chromosome structure and properties, as well as cell division, using a number of techniques, one of which is "karyotyping." It refers to a method of photographing a stained preparation in which the chromosomes are organised in a uniform pattern. The advancement of newer techniques such as "karyotyping" has made it possible to see previously undetected chromosomal abnormalities such as small chromosome segments and chromosome translocations, Veerabhadrappa (2016).

Colchicine injections and squashes of the testes or haematopoietic tissues are among the techniques used Roberts (1964); Ohno et al. (1965), corneal and conjunctival epithelium Sick et al. (1962); Drewry (1964), gill epithelium McPhail and Jones (1966); Chen and Ebling (1968), embryological material Simon (1963); Simon and Dollar (1963), sectioning of testes Nogusa (1960), growth of various tissues in vitro Roberts (1964); (1966); (1967), blood leukocytes in culture Heckman and Brubaker (1970); Ojima et al. (1970), scale epithelium Denton and Howell (1969). A good quality review of some of these methods was made by Roberts (1967).

In several classes of plants and animals, karyological characteristics have proved to be a useful tool in taxonomic and evolutionary studies. Fish cytology has been used by few ichthyologists because the chromosomes are tiny and the available techniques have often yielded distorted counts and limited morphological information. The use of squash preparations of gill arch epithelial cells in karyological methods produced satisfactory results. The gill arch technique defined by McPhail and Jones (1966) was used with modifications that improved the performance Lieppman and Hubbs (1969).

In aquaculture, the study of karyotype is also significant because of the use of chromosome manipulation techniques such as induction of polyploidy, gynogenesis, androgenesis, and inter or intra-specific hybridization Wu et al. (1986); Diter et al. (1993). Karyological studies can help resolve a number of evolutionary and genetic questions about animals Macgregor (1993), and chromosomal analysis can help determine changes that transformed an ancestral karyotype as it transformed into new lines Winkler et al. (2004). Chromosomal analysis is also important for genetic regulation, taxonomy, and evolutionary studies Macgregor and Varly (1993); Fister et al. (1999); Suleyman et al. (2004) and is widely use in various investigations Pisano et al. (2007).

When comparing karyotypes among related fishes, chromosome number, arm number, and DNA volume can be exemplified. When one is viewed alone, it may

lead to erroneous conclusions. Centromeric fusion can minimise chromosome number without affecting chromatin content fundamentally. Similarly, unequal reciprocal translocations may change arm numbers but have little impact on chromatin Booke (1968). Polyploidy can result in substantial changes, suggesting greater phylogenetic effects than previously thought Ohno et al. (1967).

In India, the analysis of fish chromosomes began in the 1960s, and of the approximately 2000 species of inland and marine fish studied for karyological information, over 200 species are native to the region, including both freshwater and marine species. Das and Barat (1995) for instance, *Schizothorax richardsonii* Sharma et al. (1992); Lakara et al. (1997); Barat et al. (1997), *Schizothorichthys prograstus* Rishi et al. (1983), *S. kumaonensis*, Lakara et al. (1997); Rishi et al. (1998), *Catla catla* and *Mystus vittatus* John et al. (1992), *Labeo* John et al. (1993), *Tor khudree* and *Tor mussullah* Kushwaha et al. (2001), *Heteropneustes fossilis* Kushwaha et al. (2002), *Labeo rohita* Nagpure (1997), *Clarias gariepinus* Nagpure et al.(2000), *Labeo rohita, Catla catla and Cirrhinus mrigala* Nagpure et al. (2001). *Labeo dussumieri, Horabagrus brachysoma and Puntius filamentosus* Nagpure et al. (2004), *Horabagrus nigricollaris, Puntius denisonii and Puntius sarana subnasutus* Nagpure et al. (2004). Though their taxonomy has been studied by several workers in the past Heckel (1838); Mcclelland (1839); Silas (1960); Talwar and Jhingran (1991); Kullander et al. (1999), there is still a lot of uncertainty about the exact number of species occurring in different aquatic ecosystems of the valley. This is complicated even further by the fact that hybrids of some of these species have been reported Heckel (1838) and Hora (1936). As a result, despite its value as a food fishery, this species complex has not been studied for its nutritional and biochemical components, nor has it been commercially cultured.

Ganai et al. (2011) studied five recognized species of *Schizothorax* viz., *Schizothorax niger*, *S. esocinus*, *S. curvifrons*, *S. plagiostomus* and *S. labiatus* for various karyological features. Somatic complement of *Schizothorax niger* showed a diploid number of 98 chromosome pairs, including 12 metacentric pairs, 16 sub-metacentric pairs, 11 sub-telocentric pairs, and 10 telocentric pairs. The diploid complement of *Schizothorax esocinus* was 98, with 15 metacentric chromosome pairs, 11 sub-metacentric pairs, 5 sub-telocentric pairs, and 18 telocentric pairs. The diploid complement of *Schizothorax labiatus* was 98, with 12 metacentric pairs, 10 sub-metacentric pairs, 1 sub-telocentric pair, and 26 telocentric pairs. The somatic complement of *Schizothorax plagiostomus* was 96, with 12 metacentric pairs, 9 sub-metacentric pairs, and 27 telocentric pairs. *Schizothorax curvifrons* had a diploid chromosomal complement of 94 chromosomes: 13 metacentric pairs, 10 submetacentric pairs, 10 subtelocentric pairs, and 14 telocentric pairs. *S. niger, S. esocinus*, and *S. labiatus,* three of the five species examined, had a diploid number of 98 and a fundamental arm number of FN of 154, 150, and 142, respectively. Intra-chromosomal changes involving pericentric and paracentric inversion, as well as centromeric shifts, could explain the difference in the fundamental arm number without a change in the 2n Rishi et al. (1998).

**Table 1.** Nomenclature for designating chromosome type Levan et al. (1964).

| Centromeric Position | Arm Ratio | Chromosome type | Symbol |
|---|---|---|---|
| Median | 1.00-1.70 | Metacentric | M |
| Sub-median | 1.71-3.00 | Sub-metacentric | Sm |
| Sub-terminal | 3.01-7.00 | Sub-telocentric | St |
| Terminal | >7.01 | Acrocentric | A |

The karyotypes of the two species indicate that in *S. esocinus*, there was simultaneous fusion of telocentric and fission of metacentric chromosomes, resulting in the karyotype of *S. niger*. This is due to the fact that *S. niger* has more biarmed chromosomes than *S. esocinus*, and a karyotype of biarmed chromosomes is generally considered to reflect a derived condition Ohno et al. (1968); Ohno (1970); Denton (1973); Gold (1979). The karyotype of *S. labiatus* tends to be characterized by the same forms of chromosomal rearrangements. Except for *S. plagiostomus*, the chromosomes of all five Schizothorax species were divided into four groups: metacentric, submetacentric, subtelocentric, and telocentric, according to Levan et al. 1964. The overall similarity in chromosome number and morphology suggested that Schizothorax species are closely related in that they have not been separated as evolving organisms long enough for random chromosome changes to have occurred and become set, and that a particular karyotype will be selected implies an adaptive advantage for that specific configuration. For chromosome differences observed in Fundulus Chen (1971) and rivulines, this hypothesis has been proposed Scheel (1972). Cyprinid karyotypes have had systematic implications Joswiak et al. (1980) since comparative karyology has been a useful method in fish systematic studies Arai (1982); Buth et al. (1991) because chromosome number and morphology indicate changes that altered an ancestral karyotype as it developed into new lines Winkler et al. (2004) and are useful for addressing a range of genetic, genetological, and evolutionary genetic and cyto-taxonomic questions about animals Kirpichnikov (1981); Mcgregor (1993).

**Table 2.** Chromosome classification of various Schizothorax species, worked out in Kashmir valley (m = metacentric; Sm = sub-metacentric; St = sub-telocentric; t = telocentric; NF = fundamental arm number).

| S. No. | Name of the species | 2n | m | Sm | St | t | NF value | Author and Year |
|---|---|---|---|---|---|---|---|---|
| 1 | *Schizothorax niger* | 98 | 24 | 32 | 22 | 20 | 154 | Ganaie et al. (2011) |
| 2 | *Schizothorax esocinus* | 98 | 30 | 22 | 10 | 36 | 150 | Ganaie et al. (2011) |
| 3 | *Schizothorax labiatus* | 98 | 24 | 20 | 2 | 52 | 142 | Ganaie et al. (2011) |
| 4 | *Schizothorax plagiostomus* | 96 | 24 | 18 | | 54 | 138 | Ganaie et al. (2011) |
| 5 | *Schizothorax curvifrons* | 94 | 26 | 20 | 20 | 28 | 140 | Ganaie et al. (2011) |

**Table 3.** Karyotypic analysis of various fresh water fish species.

| Species | Family | Diploid (2n) | Chromosome formula (2n) | Authors |
|---|---|---|---|---|
| *1. Oncorhynchus mykiss* | Salmonidae | 56-65 | 24 M +20 SM + 16 T | Vasave et al. (2016) |
| *2. Cyprinus carpio* | Cyprinidae | 97 | 24 M +24 SM + 52 T | Khuda-Bukhsh and Barat (1987) |
| 3. *Ctenopharyngodon idella* | Cyprinidae | 48 | 14M+20SM+8St+6T | Manna (1983) |
| *4. Botia birdi* | Botiidae | 98 | 14 M+18 SM+ 4St + 62 T | Khuda-Bukhsh and Nayak (1982) |
| *5. Tor tor* | Cyprinidae | 100 | 24 M+ 24SM+ 6 St + 46 A | Khuda-Bukhsh (1980) |
| *6. Schizothorax curvifrons* | Cyprinidae | 94 | 26M+20SM+20St+28T | Ganai et al. (2014) |
| *7. Schizothorax niger* | Cyprinidae | 98 | 22 M +26 SM + 8 St + 42T | Khuda-Bukhsh and Nayak (1982) |
| *8. Tor putitora* | Cyprinidae | 100 | 10 M+24 SM+ 14St + 52 T | Khuda-Bukhsh (1980) |
| *9. Schizothorax esocinus* | Cyprinidae | 98 | 30M+22SM+10St+36T | Ganai et al. (2014) |
| *10. Schizothorax plagiostomus* | Cyprinidae | 96 | 24M+18SM+54T | Ganai et al. (2014) |
| *11. Cirrhinus mrigala* | Cyprinidae | 50 | 12 M+ 18SM + l0St + l0 T | Zhang and Reddy (1991) |
| *12. Crossocheilus dipiocheilus* | Cyprinidae | 48 | 12M+36A | Manna (1983) |
| *13. Carassius carassius* | Cyprinidae | 98/<br>100 | 24 M +26 SM + 12St + 36A<br>20 M +36 SM + 44 (St + A) | Singh (1983)<br>Spoz et al. (2014) |
| *14. Carassius auratus* | Cyprinidae | 96 | 12 M +36 SM + 48 A | Rishi (l981) |
| *15. Garra gotyla* | Cyprinidae | 50 | 14 M +10 SM + l0 St + 16 T | Khuda-Bukhsh (1984) |
| *16. Hypophthalmichthys molitrix* | Cyprinidae | 48 | 20 M +12 SM + 6St+ l0T | Manna and Khuda-Bukhsh (1977) |
| *17. Puntius conchonius* | Cyprinidae | 48<br>50 | 10 M +20 SM + 10St + 8 T<br>16 M +24 SM + 2 St + 8 T | Barat (1985); Khuda-Bukhsh et al. (1986) |
| *18. Nemacheilus moreh* | Nemacheilidae | 50 | 24 M + 22 SM + 4 T | Chanda (1989) |
| *19. Puntius ticto* | Cyprinidae | 50 | 14M+ 18SM + 14ST + 4T | Bano et al. (2015) |
| *20. Schizothorax richarsonii* | Cyprinidae | 96 | 18 M +16SM +12ST+ 50T | Vasave et al. (2016) |

## VARIATION IN THE CHROMOSOMAL NUMBER & CLASSIFICATION

Ganai and Yousuf (2011) observed diploid number per metaphasic plate ranged from 47 to 50. A modal diploid number of 2n = 50 constituted 72.5% (22 m+16 Sm+12 t) and 2n = 48 constituted 20% of the counted metaphase plates. Other diploid numbers other than 2n = 50 are usually the result of losses or additions during the karyotype preparation, including splashing due to their downfall from various heights from nearby cells, as reported in other studies (Suleyman et al. (2004); Esmaeli and Piraver (2006); Nasri et al. (2010). Ganai and Yousuf (2011) obtained proper metaphasic plate chromosomal indicators including eleven metacentric, eight sub-metacentric and six telocentric pairs respectively and fundamental number as FN = 88. Comparison with already worked out species of *P. conchonius* in Jammu and other parts of the country Sharma and Agarwal (1981); Tripathi and Sharma (1987) reveals that it is a new cytotype, inhabiting Dal lake, Kashmir. The most commonly occuring diploid number in family cyprinidae is 50, considered to be the modal number in case of this family Manna (1984); Rishi (1989). According to the studies performed by various workers on Puntius species of India Tripathi and Sharma (1987), it seems that 2n

= 50 in the genus Puntius, as in many other cyprinids. Despite the similarity of the diploid number in species of Puntius, there are differences in their karyotype formulae. Nayyar (1964) reported the presence of all acrocentric chromosomes in *P. conchonius*. Barman (2003) also confirms the presence of both biarmed and acrocentric chromosomes. The primitive teleost karyotype is thought to have consisted of 46 to 48 acrocentrics, Nayyar (1966); Ohno et al. (1968); Ohno (1970); Fitzsimons (1972); LeGrande (1975). Karyotypes with biarmed chromosomes are generally regarded to represent a derived condition Ohno et al. (1968); Ohno (1970); Denton (1973); Gold (1979).

Ganai et al. (2011) reported both the species of *Schizothorax* analysed cytologically, revealed a high number of chromosomes ranging from 94 to 98. All the *Schizothorax* species studied karyologically till date *S. richardsonii* Gray and *S. kumaonensis* Menon, Lakara et al. (1997); *S. zarudnyi,* Nikolskii, Kalbassi et al. (2008); *S. plagiostomus* and *S. esocinus* Ganai et al. (2011) show a high chromosome number ranging from 96 to 98. Species with high numbers are considered to have resulted through polyploidy from ancestral 2n= 48 or 50 Rishi et al. (1998). Such genomic enlargements have been hypothesised as key factors that enable or even drive diversification in various vertebrate groups Holland et al. (1994); Meyer and Malaga-trillo (1999); Navarro and Barton (2003a, b); Ohno (1970). Variation in the karyotypic configuration of *S. Niger* (24m + 32sm + 22st + 20t and FN=154) and *S. curvifrons* (26m+20sm+20st+28t) and FN=140 can easily be explained by centric fusion and fission events. Both centric fission and fusion probably provide important mechanisms to explain the diverse range of chromosome numbers observed in many mammalian and non-mam-malian animal taxa Todd (1970); Imai et al. (1986), Kolnicki (2000). Decrease in 2n and FN in *S. curvifrons* may be attributed to Robertsonian arrangements and pericentric inversion Choudhury et al. (1982); Ganai et al. (2011) also reported despite overlap in the general morphological features, the two species of Schizothorax investigated are genetically different and hence definite species as the chromosomal differentiation in animal species usually precedes strong morphological differentiation Howell and Villa (1976). Most morphologic features of fishes have been shown to have the potential of being modified by the environmental conditions Svardson (1965); Fowler (1970). Therefore, a morphologically based classification should be tested by the features not likely to be environmentally false and chromosome structure is best suited for this purpose as it reflects genetic divergence and is least affected by environmental distortion, Campos (1972).

Barat et al. (2012) reported the majority (85%) of cells had metaphase complements containing 2n = 50 chromosomes, though a few metaphases had a range of 46 to 52 chromosomes. The karyotypic formula was detected as 2n = 12m (metacentric) + 14sm (sub-metacentric) + 10st (subtelocentric) + 14T (telocentric) with a fundamental arm number (NF) of 80. However, most of the members of the family Cobitidae had a diploid chromosome number (2n) of 50, with just a few species – *Botia birdi*, *B. macroracantha* and *B. Dario* – with a diploid chromosome number of 90–98 Khuda-Bukhsh et al. (1986). Therefore, the modal chromosome number in this family could be ascertained as 50 Barat et al. (2012).

## ADVANTAGES & APPLICATIONS

- Karyological studies have made a substantial contribution to various fields in fisheries like systematics, evolution, mutagenesis, aquaculture, phylogenetic relationship and hybridization Kligermann and Bloom (1977).
- Chromosomal analysis is important for fish breeding from the viewpoint of genetic control Kirpichnikov (1981).
- Besides, Karyological studies also generate information about genetic diversity in natural fish population, which is imperative in the conservation and stock management Kligermann and Bloom (1977).
- Karyological studies have provided basic information on the number, size and morphology of chromosomes that is important to undertake chromosome manipulations in fish Khan et al. (2000).
- Since 1960s, karyological studies in teleost fish have made noteworthy contributions to increasing knowledge in the fields of genetics, taxonomy and environmental toxicology Cucchi and Baruffaldi (1990).
- Karyotyping helps in analyzing the entire genome. It can visualize individual cells and individual chromosomes.
- Many cytogenetic techniques are useful in fish breeding and culture practices such as:
- Ploidy determination Rishi and Haobam (1984)
- Hybrid identification Manna (1989)
- Sex determination Manna (1989)
- Genotoxicity study of the pollutants Rishi (1989).
- Further cytogenetic characterization of threatened species is useful in drawing programmes for conservation and stock management John et al. (1994).

## CONCLUSION

Karyological studies have provided basic information on the number, size and morphology of chromosomes that is important to undertake chromosome manipulations in fish. The development of newer techniques such as "karyotyping" has made it possible to visualize undetected chromosomal anomalies such as small portions of chromosomes and translocations of tiny parts of chromosomes to one another. Because such procedures also enabled each pair of chromosomes to be distinguished individually, it has helped to further our understanding of chromosomal basis of certain important genetic disorders. Chromosomal analysis is important for fish breeding from the viewpoint of genetic control. Indigenous species of Kashmir (Schizothorax sps.) analysed cytologically, revealed a high number of chromosomes ranging from 94 (*Schizothorax curvifrons*) to 98 (*Schizothorax niger*). The NF value of Schizothorax species of Kashmir valley ranged from 138 (*Schizothorax plageostomus*) to 154 (*Schizothorax niger*).

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