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Karyotypic Descriptions of Five Cyprinid Fish Species from Western Ghats, India

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Abstract

The karyotypic and cytological characteristics of five native cyprinid fishes of Western Ghats of peninsular India were investigated by examining the metaphase chromosomes spreads obtained by Giemsa staining method. The diploid chromosome numbers of *Barilius canarensis, Chela fasciatus Puntius conchonius* and *Garra mullya* were 2n=50 and *Osteochilichthys nashii* has 2n=48. Chromosome number of *Puntius conchonius* and *Garra mullya* is in agreement with the previous published work but with different chromosome formula. This is the first report of karyotypic information on these species such as *Barilius canarensis, Chela fasciatus* and *Osteochilichthys nashii* from Western Ghats, Ideogram was prepared for each species by measuring the chromosome. The results may be useful for population study, taxonomic problems and conservation of this native fishes.

Keywords: Cyprinid fishes- Chromosome- Karyotyping- Western Ghats.

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1. Intruduction

Cypriniformes in peninsular India is represented by 4 families, 8 sub families, 40 genera and 166 species. Family Cyprinidae has 4 subfamilies, 27 genera and 135 species (Arunachalam *et al.,* 2008). Fishes are characterized either by the occurrence of stable karyotypic structure and chromosomes numbers or by divergent groups with an extensive chromosome diversity (De Rosa *et al.,* 2007). Polyploidization and chromosome rearrangements among and within fish families were common (Volckaert and Agnèse, 1996). Cytogenetic study was not successful or widespread as in other vertebrate groups because of the presence large number of small chromosome and the karyotypic data on fishes were available only for 10% of the species approximately 25,000 species (Ramasamy 2010). The typical karyotype for the cyprinids worked so far consists of 6-8 pairs of metacentric chromosomes, 12-17 pairs of submeta and subtelocentric chromosomes and 3-4 pairs of acrocentric chromosomes (Ràb, 1991; Ràb and Collares-Pereira, 1995).

Fontana *et al.,* (1997) showed that the diploid chromosome number of fishes varies from 2n=22 to 26 in some species and it may vary from 2n=240 to 260. 2n=50 is the chromosome number for most of cyprinid fishes and it is considered as model chromosome number for cyprinid fishes (Al-sabti, 1991; Gul *et al.,* 2004). Polyploidy chromosome state is also observed in some cyprinid fishes (Oellerman *et al.,*1990). The genus *Puntius* of the family Cyprinidae is karyologically well known with 26 species and it was the most extensively explored genus (Sharma *et al.,* 1990) and the intraspecific variation has also been noted (Sharma and Agarwal, 1981).

Chromosome study is useful in addressing ambiquituis in classification of fish species (Hamid Reza Esmaeili *et al.,* 2010). Chromosome polymorphism has well known effects on body size (Barbadilla *et al.,* 1994). Results from cytogenetic analysis of fishes have great importance in evolution, systematics, aquaculture and mutagenesis (Amemiya 1986, Al-Sabti 1991) has attention in recent years (Galetti 2000, Ozouf-Costaz 1992). Since morphological data classifications are no longer considered cutting edge, chromosomal analysis is used for genetic studies and for taxonomy and phylogeny research (Catalina Luca *et al.,* 2010).

The Western Ghats is one of the biodiversity hotspots that supports rare, endemic and threatened species of fauna and flora of which nearly 250 species were freshwater fishes. Increasing importance of chromosomal studies on fish and the lack of karyotypic data on endemic fishes forced us to this study. For karyotypic study five species fishes belongs to the family Cyprinidae namely *Barilius canarensis*, (Jerdon) *Chela fasciattus* Silas, *Puntius conchonius*, (Hamilton) *Osteochilichthys nashii*, (Day) and *Garra mullya* (Sykes) were selected. Information on the karyotype of *Barilius canarensis*, *Chela fasciatus*, and *Osteochilichthys nashii* is the first report.

2. Materials and Methods

Samples were collected using cast and gill nets from streams and rivers in different parts of Western Ghats and were transferred to Manonmaniam Sundaranar University laboratory. 10 healthy five specimens of each species were taken for this study and chromosome preparation was carried out by using the modified protocol of Arunachalam and Murugan (2007). Fishes were injected with 0.5% colchicine (1ml/100g of body weight) and allow them to swim in well aerated water. Fishes were sacrificed after 2-3 hrs. The kidney tissues were taken and placed in hypertonic KCl for 45 min and fixed in cornoys' reagent. Then the solutions were centrifuged for 10 min at 1000 rpm. Slides were heated at 50^{o} C and the spreads were made by direct air drying method and the slides were stained with 5% Giemsa stain. 50 good spreads were taken from the preparation and were photographed. Standard idiogram was prepared by following Levan *et al.,* (1964). Detailed karyological morphometry was taken by using an ocular micrometer. The average length of short and long chromosome and the centromeric index were calculated for each chromosome. Chromosome pairs were classified following Macgregor (1993).

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3. Results

Five images of species were shown in (Fig 1). More than 80% of observed fifty metaphase images prepared from the kidney cells for all the above five species support the following results. Chromosome spread (a) and karyotype (b) of the five species shown in (Fig 2). NF and the maximum distance between two adjacent chromosomes were shown in (Table 1). The diploid chromosome number of *Barilius canarensis* was 2n=50 with the karyotypic formula of 2 metacentric, 9 submetacentric, 2 subtelocentric and 12 telocentric chromosomes and their total length ranged from 0.2 to 1.1 µm and the idiogram was shown in (Fig 3). The karyotype of *Chela fasciatus* was 2n=50 comprised of 5 metacentric, 17 submetacentric and 3 telocentric chromosomes and their total length was ranged from 0.2 to 1.5 µm. (Fig. 4).

The diploid chromosome number of *Osteochilichthys nashii* was 2n=48, comprised of 18 subtelocentric and 6 telocentric chromosomes. The chromosome total length ranged from 0.3 to 1.4 µm calculated from the mean values of the measurements of best mitotic metaphase spreads. (Fig 5). Diploid chromosome number of *Puntius conchonius* was 2n=50 comprised of 17 metacentric, 2 submetacentric, 4 subtelocentric and 2 telocentric with a total length range from 0.2 to 3.4 µm. (Fig. 6). The karyotype of *Garra mullya* showed 2n= 50 with a karyotypic formula of 9 metacentric, 5 submetacentric, 3 subtelocentric and 8 telocentric chromosomes and the chromosome total length ranged from 0.3 to 1.1 µm. (Fig. 7).

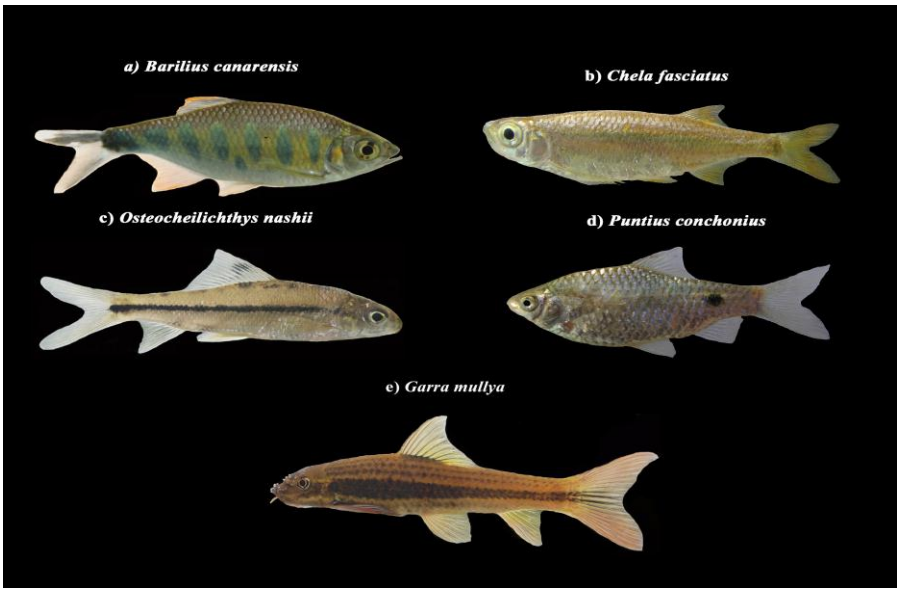

Fig. 1: The experimental fishes

Tab1. Chromosome formula of the four species.

S.No	Species	Chromosome category					NF	Max. distance between adjacent chromosome
		2n	m	sm	st	t		
1	*Barilius canarensis*	50	2	9	2	12	76	0.2
2	*Chela fasciata*	50	5	17	-	3	94	0.1
3	*Osteochilichthys nashii*	48	-	-	18	6	88	0.4
4	*Puntius conchonius*	50	17	2	4	2	100	0.2
5	*Garra mullya*	50	9	5	3	8	90	0.1

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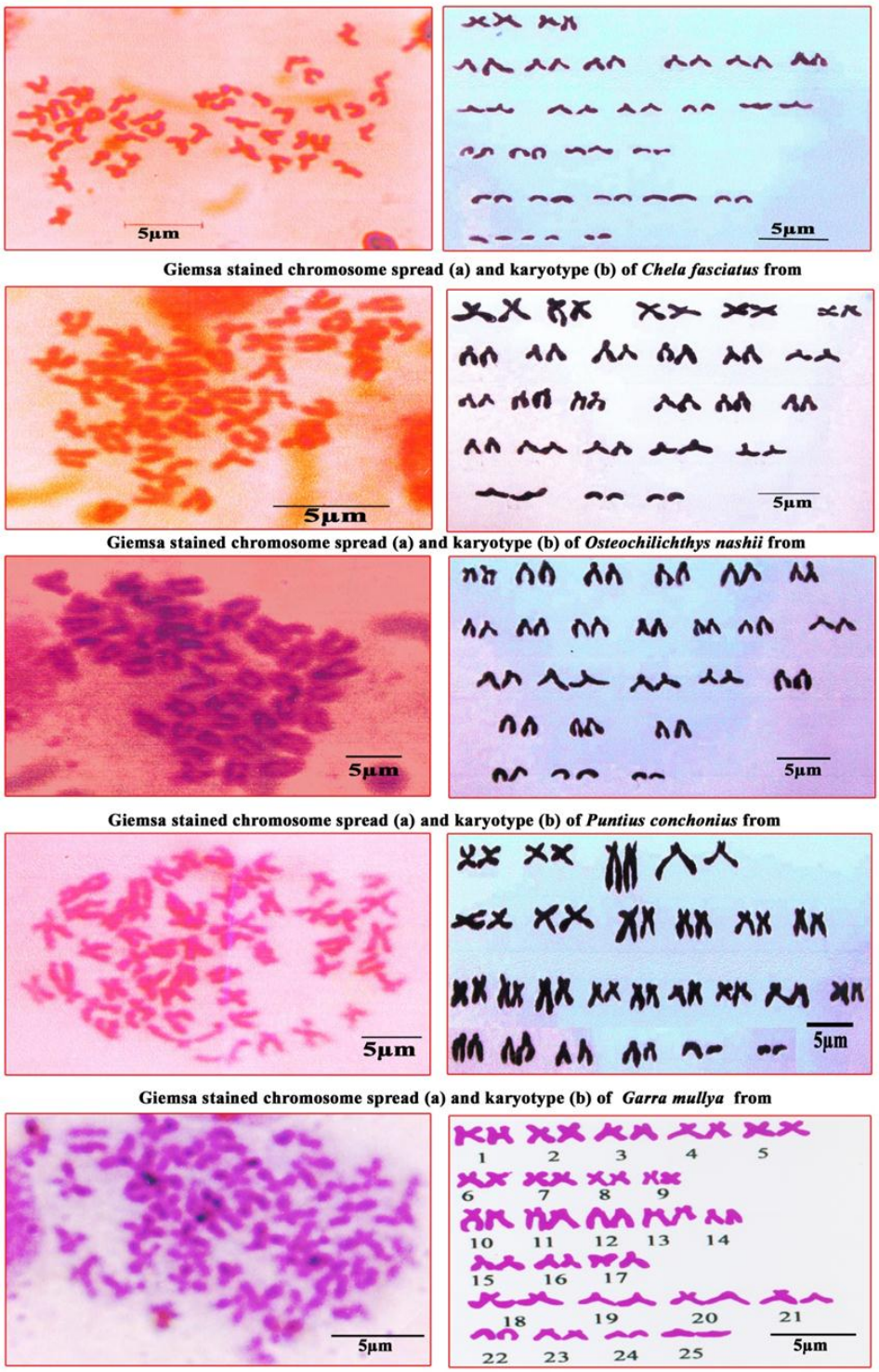

Fig.2: Giemsa stained chromosome spread and karyotype of studied fish species

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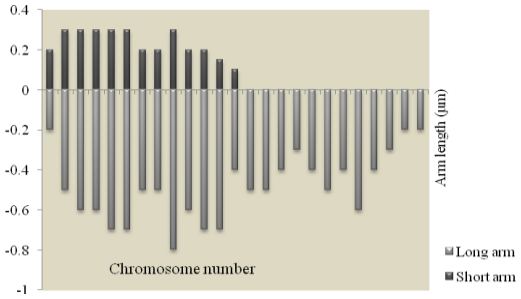

Fig. 3. Idiogram of *Barilius canarensis*

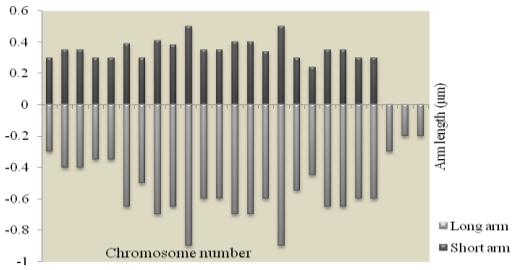

Fig. 4. Idiogram of *Chela fasciatus*

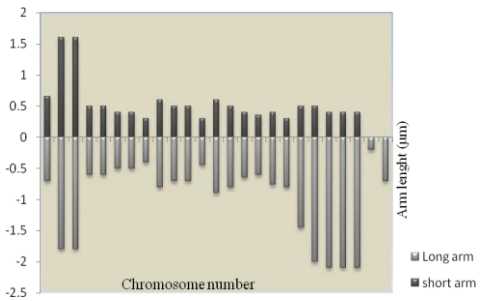

Fig. 5. Idiogram of *Puntius conchonius*

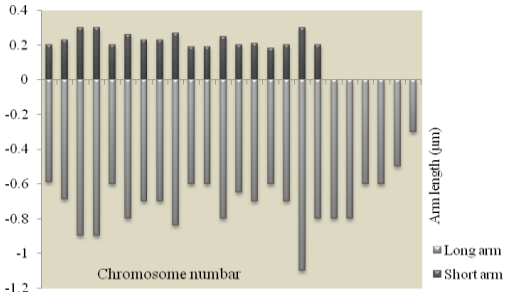

Fig. 6. Idiogram of *Osteochilichthys nashii*

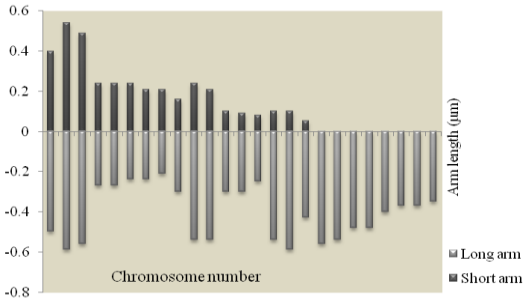

Fig. 7. Idiogram of *Garra mullya*

4. Discussion and Conclusion

This study is the first karyotypic report for the species, *Barilius canarensis*, *Chela fasciat*and *Osteochilichthys nashii*. Nayyar (1964) reported the diploid chromosome number 2n=52 for *Salmophasia bacaila*. Diploid chromosome number of *P. conchonius* is 2n=50 and this is in agreement with the earlier report (Nayyar 1964) for the same species but with varied chromosome formula. This result also agrees with the 2n chromosome number for other species of this genus such as *P. sophore P. conchonius P. stigma*, *P. ticto* (28m+16sm+6st) *P. chola* (2m+2sm+46t) (Prabhati Kumari Sahoo *et al.*, 2007), *P. arulis* and *P. tambraparniei* (Arunachalam and Murugan, 2007). This may be accountable that the chromosome number is conserved among the species of the genus *Puntius*.

Nagpue *et al.*, (2006) reported diploid chromosome number for *Garra mullya* was 50 with the chromosome formula 9m+7sm+5st+4t from Chalakkudy River, Kerala, India. The result for *Garra mullya* showed the same chromosome number but different karyotypic formula which indicates that different karyotypic forms exist. Karyotype analysis Sahoo *et al.*, (2007) was reported as 2n= 50 for *Garra gotyla gotyla*, *G. kempi* and *G. lissorhynchus* with different karyotypic formulae. Gorshkova *et al.*, (2011) reported the same diploid number of chromosomes for *G. rufa* and *G. ghorensis*. Khuda Buksh *et al.*, (1980) reported the same chromosome number for *Garra lamta* (3 m+9 sm+6 st+7 t). Esmaeili *et al.*, (2009) reported that

G. persica (15 m+8 Sm+1 st) an endemic species from Iran has 2n=48. This indicates that the diploid number of chromosome is not conserved in this genus. This information may be helpful to study the species which have taxonomic ambiguities.

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