



Chromosomal characteristics of *Aplocheilus lineatus* (Cyprinodontiforms)

Reshma RS, Joelin Joseph, Sandeep Sreedharan, Dr. Mano Mohan Antony*

Molecular and Evolutionary Genetics division, Post Graduate and Research, Department of Zoology, University College, Palayam, Thiruvananthapuram, Kerala, India

Abstract

Chromosomal analysis on fishes has been advantageous for phylogenetic studies, taxonomic investigations and genetic advancement that register the extensive chromosomal variability and high degree of polymorphism among different species. The present study is a pioneer work that targets the chromosomal detailing and inter specific comparison of *Aplocheilus lineatus* (cyprinodontiforms). The 63.3% of chromosomal spreads of, *Aplocheilus lineatus* obtained reveals the modal chromosome number as 50 with the dominance of sub telocentric chromosomes that are exactly analogues to certain other genera that is closely related to the studied organism. From the karyological data obtained, the arm ratio was found to be between 1.010 ± 0.00 - 4.489 ± 0.30 and the karyotypic formula was $n = 8m + 2m + 15St$. The karyotype of a *Aplocheilus lineatus* obtained can be considered as a definite element, like any morphological character, for taxonomic purposes.

Keywords: karyotype, *Aplocheilus*, chromosome number, karyotype

1. Introduction

Aplocheilidae, belonging to the order cyprinodontiformes are popularly known aquarium fishes inhabiting freshwater, streams, reservoirs at high altitudes, rivers, wells of the plains, low-lying paddy fields, swamps and brackish waters. The natural distribution of these fishes is centred on Peninsular India and Sri Lanka. In fishes, the use of chromosomal information as tool in taxonomy has even an added importance, since classification is beset with many difficulties. Moreover since fishes are the originator of all the vertebrates, the karyotypic data in them can help in the understanding of the vertebrate evolutionary pathways. From the earliest known studies the modal number of the family Aplocheilidae is 50 and that are correlated to the other such genera (Manna and khudabuksh 1977) [1]. However, karyomorphological and molecular phylogenetic relationships is infrequently studied in Aplocheilidae and are not sufficient for explaining karyoevolution and speciation in these fishes. Hence the present study aims at karyomorphological investigations and comparisons of karyological parameters that are most potent for further investigation.

2. Materials and Methods

To perform karyotyping, the live adult specimens was collected from two ponds from Kadakkal village of Kollam district $8.8293^\circ N$, $76.9222^\circ E$. Colchicine (0.05%) was intramuscularly injected and the fishes which survived up to 2 hours was taken and the gills were dissected out prior to death. The cell suspension was prepared from homogenised gill tissue by following the chromosome preparation method of Barreto Netto *et al.*, and Nagpur *et al.*, (2007) [8] with slight modifications. The metaphase spread of chromosomes was observed in illuminating microscope (LABOMED model CXL MONO) under oil immersion. After calibration the clear

spreads was photographed using Mag Cam digital camera by Mag vision software. The karyotyping was performed by using smart type software. The classification of chromosomes was done by following the method of Levan *et al.* (1964) [7].

3. Results and Discussion

In the present study the chromosome spreads of *Aplocheilus lineatus* which is obtained from gill tissues was observed. 30 metaphase spreads from 15 mature species of *Aplocheilus* was observed. The frequency of diploid chromosome number ranged from 42- 52 per metaphase. In a total number of 30 metaphase spreads, 19 revealed their diploid chromosome number as 50 i.e., the modal number valid over 63.3% of metaphase cells. 10 % of spread shows chromosome number as 48, 6.6% of them as 52, 10% as 44, 3.3% as 46 and 6.6% as 42 consecutively. The graph (Fig.1) shows highest peak at 50, which could be suggested as the modal number in this family. In the present study the 63.3 % of metaphase spread revealed the modal number as 50. Only 6.6% of spreads shows the diploid number of 48 chromosomes and rest of the spreads vary in between 42 to 52. Altogether 187 species belong to the family cyprinodontidae have so far been cytologically worked out (Manna and khudabuksh 1977) [1]. According to the previous studies by Manna and khudabuksh, 17 species of *Aplocheilus* genus had variable numbers as *A.dagei*, had $n=25$ chromosomes similar with the karyotypes of *A. blocki*, *A.dayi*, *A.sexfasciatus* and *A.wernei* ($n=24$ chromosomes). *A.barmoiensis* and *A.spilargerins* had $n=17$ chromosomes. While *A.fasciolatus* was found to be chromosomally polymorphic having $n=18$ 19 or 20 chromosomes (Scheel 1966, 1972) [9, 10]. *A.panchax* the species was reported to have $n=18$ and $2n=36$ chromosomes by the same author ((Scheel 1966, 1972) [9] in the Thailand population while in the study, of the author khudabuksh

(1977) [1] in the India population of the same species differed in having $n=19$ and $2n=38$ chromosomes. A direct comparison of karyotypes from all related genera makes it clear that a succession of rearrangements such as translocation and fission/fusion has occurred. This is one of a main limitation of the karyotypical study, that mostly concerning and affecting the total compliment study on a species. The variations of the chromosome number may also be due to the scientific error or by the evolutionary processes.

From the comparative cytogenetics studies of Valente *et al.*, (2012) [11] *Squalius aradensis* (teleost) and *S.torgalensis* (cyprinid) have the same compliment of $2n=50$, a chromosome set with mainly biarmed elements (dominated by some metacentric and submetacentrics). In the present study we have the diploid number of 50 chromosome, the same number has identified in cytogenetic divergent studies on some teleost and characiform fish (Silva *et al.*, 2006) [11]. The modal number of some catfish family is considered as 56, that valid over 86 % of spreads (Singh *et al.*, 2013) [12]. Cells differ than $2n=56$ were probably caused by losses during preparation or addition from nearby cells (Singh *et al.*, 2013) [12]. The cyprinodontidae family have a peak value of diploid chromosome number is 48 (Manna and khudabuksh 1977) [1]. While the present study have variation in between 42 to 52, the statistical confirmation shows the peak at $2n=50$ (Fig.1). Comparisons of chromosomes types between different species studied by different authors may present some problems due to possible mistakes in classifying the elements which are at the boundaries of two distinct morphological categories (LeGrande *et al.*, 1976) [6].

The metaphase spreads of *Aplocheilus lineatus* shows the different types of chromosomes with reference to arm ratio, the ideogram revealed that they possess 8 pairs of sub metacentric(sm), 2 pairs of metacentric (m), and 15 pairs of sub telocentric (st) chromosomes (Fig.3). With a formula of $n = 8m + 2sm + 15St$. In accordance with the studies on *Aplocheilus* genera, karyotypic analysis of chromosomes in female panchax revealed 18 homomorphic pair comprising 6 pairs of metacentric, 4 pairs of sub metacentric, 2 pairs of sub telocentric and 6 pairs of telocentric chromosomes, then one heteromorphic pair consists a small metacentric, near metacentric and a fairly large sub metacentric chromosomes (Manna and khudabuksh 1977) [1]. While the *A. lineatus* ideogram shows only three types of chromosomes not possess telocentric one. This lack of telocentric(t) chromosomes may be attribute to the fact that in describing karyotypes of fishes some authors prefer to group together the st and t types to avoid mistakes and facilitate comparison (Gomez *et al.*, 1994). The karyotypic analysis of chromosomes in females of *Aplocheilus panchax* showed that all the 38 chromosomes could be aligned comprising 7 pairs of metacentric, 4 pairs of sub metacentric, 2 pairs of sub telocentric and 6 pairs of telocentric chromosomes. The karyotype in male was therefore essentially similar to that in female except to that one small metacentric or near metacentric chromosomes. The chromosome formula determined was for male panchax as $n=7m+4sm+2st+2t+4T$ (Manna and khudabuksh 1977) [1]. This formula is quite different from the observations of present study of the same family. In the karyotypic study of *Aplocheilus parvus* we have found that they do not have any

additional micro chromosomes and such type of inclusions doesn't report in this family yet.

From 1st to 25th pair, the total length of chromosome ranges from 7.284 ± 0.50 to $19.875 \pm 0.06 \mu\text{m}$ (Table.1). From the above observation, arm ratio ranges between 1.010 ± 0.00 to 4.489 ± 0.30 . The total length of the chromosome revealed from the present study is $7.284 \pm 0.50 - 19.875 \pm 0.06 \mu\text{m}$. There is little variation in between these range. Compared to that *L. calcifer* have short range in between $2.4-0.9 \mu\text{m}$, and the arm ratio ranged from 1.28 to 4.14. Instead in *A. lineatus* the arm ratio is in between $1.010 \pm 0.00 - 4.489 \pm 0.30$. *G. chapra* the chromosomes gradually seriated and measured between 23 and $1.0 \mu\text{m}$ in length (Manna and khudabuksh 1977) [1]. In the case of *N.barba* the total length of the chromosome ranging from 6.29 to $1.81 \mu\text{m}$, that is little bit close to the length measured in Aplocheilidae. Studies on Neotropical cichlids that is close related to the family Aplocheilidae revealed that arm ratio is very close to 3.0, which is the border line value between the M-SM and ST-A groups (Feldberg *et al.*, 1985), with the consequent need to opt for one of the groups considered. Obviously this criterion may lead to errors in chromosome classification, a fact that also has been pointed out by Thompson (1979). In cat fish *M. ngasep* the total arm length and arm ratio were between 6.16-18.36 and 1.11-∞ respectively, that is more or less close to the cyprinodontiforms. This type of similarities may show in several studies in Aplocheilidae family, that attribute to definite phylogenetic relationship.

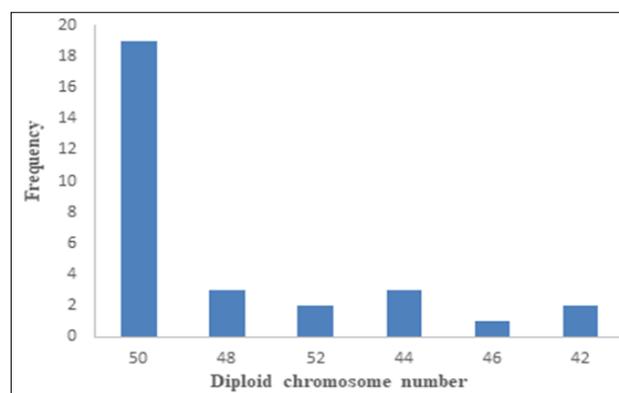


Fig 1: Frequency of diploid chromosome number recorded in metaphase spreads

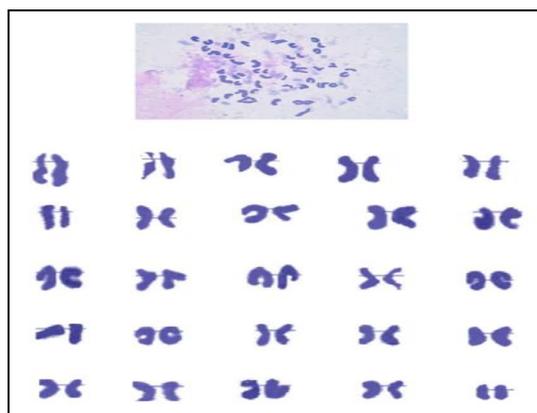
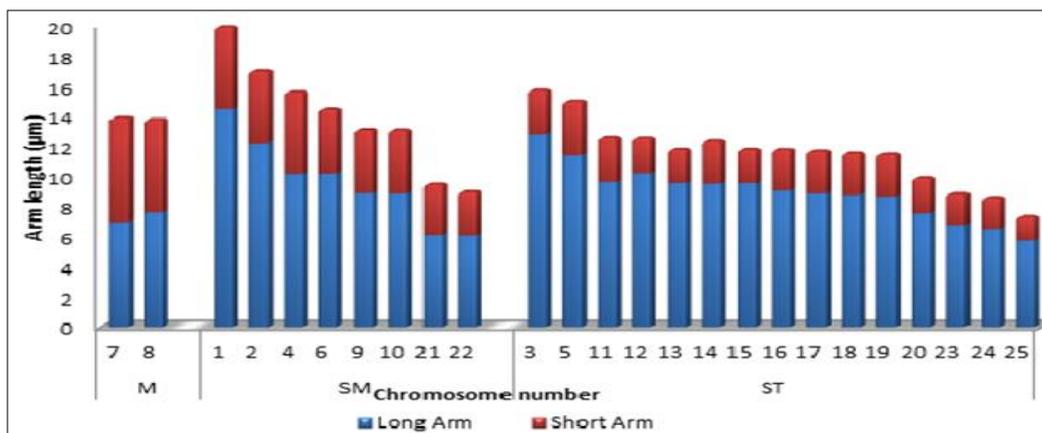


Fig 2: Karyotype of *Aplocheilus lineatus*

Table 1: The decreasing order of total arm length of chromosomes

Chromosome Number	Chromosome type	Long Arm (μm)	Short Arm (μm)	Total Length(μm)	Arm Ratio
1	SM	14.442 \pm 0.03	5.433 \pm 0.09	19.875 \pm 0.06	2.659 \pm 0.05
2	SM	12.201 \pm 0.09	4.787 \pm 0.06	16.988 \pm 0.15	2.549 \pm 0.02
3	ST	12.768 \pm 0.05	2.909 \pm 0.06	15.677 \pm 0.11	4.391 \pm 0.08
4	SM	10.147 \pm 0.10	5.413 \pm 0.03	15.560 \pm 0.13	1.874 \pm 0.01
5	ST	11.410 \pm 0.02	3.536 \pm 0.03	14.946 \pm 0.01	3.227 \pm 0.03
6	SM	10.189 \pm 0.08	4.22 \pm 2 \pm 0.04	14.412 \pm 0.09	2.413 \pm 0.03
7	M	6.963 \pm 0.03	6.896 \pm 0.02	13.860 \pm 0.05	1.010 \pm 0.00
8	M	7.595 \pm 0.13	6.144 \pm 0.11	13.739 \pm 0.22	1.236 \pm 0.02
9	SM	8.862 \pm 0.15	4.185 \pm 0.09	13.048 \pm 0.19	2.118 \pm 0.06
10	SM	8.844 \pm 0.08	4.188 \pm 0.14	13.032 \pm 0.21	2.113 \pm 0.05
11	ST	9.612 \pm 0.20	2.913 \pm 0.17	12.526 \pm 0.25	3.306 \pm 0.20
12	ST	10.209 \pm 0.06	2.281 \pm 0.16	12.490 \pm 0.22	4.489 \pm 0.30
13	ST	9.544 \pm 0.145	2.239 \pm 0.18	11.784 \pm 2.68	4.081 \pm 0.86
14	ST	9.501 \pm 0.05	2.807 \pm 0.03	12.308 \pm 0.06	3.386 \pm 0.04
15	ST	9.545 \pm 0.15	2.239 \pm 0.19	11.784 \pm 0.08	4.285 \pm 0.41
16	ST	9.054 \pm 0.26	2.707 \pm 0.02	11.761 \pm 0.25	3.344 \pm 0.11
17	ST	8.846 \pm 0.08	2.818 \pm 0.09	11.665 \pm 0.17	3.140 \pm 0.07
18	ST	8.763 \pm 0.12	2.769 \pm 0.07	11.532 \pm 0.19	3.165 \pm 0.03
19	ST	8.663 \pm 0.07	2.786 \pm 0.05	11.448 \pm 0.11	3.110 \pm 0.06
20	ST	7.519 \pm 0.15	2.362 \pm 0.08	9.881 \pm 0.23	3.185 \pm 0.06
21	SM	6.092 \pm 0.05	3.371 \pm 0.02	9.463 \pm 0.04	1.808 \pm 0.02
22	SM	6.064 \pm 0.02	2.891 \pm 0.27	8.955 \pm 0.26	2.109 \pm 0.19
23	ST	6.764 \pm 0.03	2.055 \pm 0.01	8.820 \pm 0.02	3.291 \pm 0.03
24	ST	6.500 \pm 0.10	2.0530.01 \pm	8.554 \pm 0.10	3.166 \pm 0.06
25	ST	5.722 \pm 0.29	1.562 \pm 0.21	7.284 \pm 0.50	3.691 \pm 0.31

**Fig 3:** Ideogram showing different types of chromosomes in *Aplocheilus lineatus*

4. Conclusion

The karyograms are purely concerned with the comparative chromosome studies. The significance of chromosome morphology always known to be important in cases of closely related taxa with same chromosome number that can leads to the intense studies of chromosome evolution and karyological studies. Chromosomal data about the organisms are most convenient for species identification. These are very powerful attribute to the phylogenetic variations and any mutational effects happened in generations. The present study encompasses cytogenetic analysis of *Aplocheilus* species by using chromosomal data such as information on the number, size and arm ratio of chromosomes to indicate the crucial genetic identity of that species, marking a great step on further karyological studies.

5. References

1. Manna AGK, Khuda-Bukhsh R. A check list of chromosomes in cyprinid fishes. J zool. Res. 1977; 1(2):34-43.
2. Barreto Netto MRDCB, Pauls E, de Mello Affonso PRA. A standard protocol for obtaining fish chromosomes under post-mortem conditions. Micron. 2007; 38(3):214-217.
3. Feldberg E, Bertollo LAC. Nucleolar organizing regions in some species of Neotropical cichlid fish (Pisces, Perciformes). *Caryologia*. 1985; 38(3-4):319-324
4. Feldberg I, Cronin N, Evans M, Sharp N, Smith S. U.S. Patent No. 6,026,331. Washington, DC: U.S. Patent and Trademark Office, 2000.
5. Gomes V, Phan VN, Passos MJDAC. Karyotypes of three

- species of marine catfishes from Brazil. *Boletim do Instituto Oceanográfico*. 1994; 42(1-2):55-61.
6. LeGrande WH, Fitzsimons JM. Karyology of the mullets *Mugil curema* and *M. cephalus* (Perciformes: Mugilidae) from Louisiana. *Copeia*, 1976; (2):388-391.
 7. Levan A, Fredga K, Sandberg AA. Nomenclature for centromeric position on chromosomes. *Hereditas*. 1964; 52(2):201-220.
 8. Nagpure NS, Kushwaha B, Singh PJ, Kumar R, Srivastava SK, Lakra WS. Genotoxicity assessment in Fishes- a Practical Approach. *NBFGR Publication*, 2007.
 9. Scheel JJ. Taxonomic studies of African and Asian toothcarps (Rivulinae) based on chromosome numbers, haemoglobin patterns, some morphological traits and crossing experiments. *Vidensk. Medd. Dansk. naturh. Foren*. 1966; 129:123-148.
 10. Scheel JJ, Simonsen V, Gyldenholm AO. The karyotypes and some electrophoretic patterns of fourteen species of the genus *Corydoras*. *Journal of Zoological Systematics and Evolutionary Research*. 1972; 10(1):144-152.
 11. Silva J, Chambers I, Pollard S, Smith A. Nanog promotes transfer of pluripotency after cell fusion. *Nature*. 2006; 441(7096):997.
 12. Singh SK, Tang WZ, Tachiev G. Fenton treatment of landfill leachate under different COD loading factors. *Waste management*. 2013; 33(10):2116-2122.
 13. Thompson KW. Cytotaxonomy of 41 species of Neotropical Cichlidae. *Copeia*, 1979, 679-691.
 14. Valente GT, de Andrade Vitorino C, Cabral-de-Mello DC, Oliveira C, Souza IL, Martins C, *et al*. Comparative cytogenetics of ten species of cichlid fishes (Teleostei, Cichlidae) from the Araguaia River system, Brazil, by conventional cytogenetic methods. *Comparative cytogenetics*. 2012; 6(2):163.