IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Karyotype Analysis In *Schizothorax Richardsonii* (Gray) From Kosi River In Uttrakhand (India)

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Abstract

Karyological characters of *Schizothorax richardsonii* in Kosi River in ,Uttrakhand (India) were studied by examining metaphase chromosome spreads from the kidney tissues.The examination of 80 metaphases spread prepared from 20 fingerling specimens indicated that the chromosome numbers of this species was found 2n=92 and the arm number was determined as NF=122. The prepared karyotype of this species consisted of 9 pairs of metacentric (m), 14 pairs submetacentric (sm) and 25 pairs subtelocentric (st) chromosomes. The chromosome formula can be stated as 2n = 9m + 14sm + 25st.Karyological parameters showed that centromeric index, arm ratio, relative length and so length variation range of chromosomes of the fish were between 25.00 – 49.24, 1.03 – 3.55, 0.88 – 4.13 and 14.57 – 67.70 respectively and total length of chromosome was 1639.16µm. The largest chromosome in this species was a pair of the submetacentric chromosome. The sex chromosomes were cytologically indistinguishable.With respect to the number of chromosomes and resistant of this fish to the environmental conditions, it seems to be tetra ploid origin fish.

Key words: Karyotype, Chromosome, *Schizothorax, richardsonii Kosi*.river

Introduction

The family Cyprinidae is the richest and most important family of fish, and its members are distributed throughout the world (Al-Sabti, 1991). The vast majority of boned fish belongs to this family in Iran, and these are distributed widely in freshwater resources (Abdoli, 1999). Although this family is represented by approximately 1500 species worldwide (Gül et al., 2004) there are only 38 Genera and over 80 species in Iran (Firouz, 2000).Systematically *Schizothorax. zarudnyi* belongs to teleostei class, cypriniformes order, cyprinidae family and schizothorax genus (Mostajeer and Vossoughi, 1994). The world distribution of *S. zarudnyi* is in semi-temporal freshwater of western Asia (Bianco and Banarescu, 1982). This fish is endemic in Iran and found in Sistan region mainly (Abdoli, 1999). The progress in increasing such knowledge has been closely related to the evolution of application methodologies (Rivlin et al., 1985). Studies of the chromosomes of fishes have not been as successful or widespread as in other vertebrate groups. Standard karyotypes are reported for less than 10% of more than 20000 extant species of fishes (Gold et al., 1990).Cytogenetic studies in fish have not been comprehensive when compared to other vertebrate grops in Iran. In this respect, the most important karyological studies of Cyprinids fishes in Iran are coomprise *Rutilus frisii kutum* **(**Nowruzfashkhami and Khosroshahi, 1995), *Abramis brama* (Nahavandi et al., 2001), *Ctenopharyngodon idella* (Nowruzfashkhami et al., 2002) and *Hypophthalmichthys molitrix* (Varasteh et al., 2002).As this species is a good candidate for other genetic investigations such as hybridization and chromosomal manipulation and so due to lack of their chromosomal data, this first report could provide the detailed information on the chromosome number and karyotype of this species.

Materials and Methods

Twenty *S. richardsonii,* weight 10 ± 1g, were caught in Kosi river(Nainital) is a famous hill station of the Central Kumaon Himalayas at an altitude of 1938 msl and 2126 msl. (Long. 29 0 23 'N, lat. 79 0 30 'E,). The fishes were transported live to the laboratory, and one year kept in a well-aerated aquarium at 20-24°C before analysis.

The stock solution of colchicine was made by dissolving 10 mg colchicine and 100 mg NaCl in 20 ml distilled water. The colchicine a dose of 25 and 50 µg/gr body weight (BW) was slowly injected into the intraperitoneal muscle. Then,fishes were left in aquaria at 20-24°C for 5-10 hours before sacrificing. After it, the fish were killed and their anterior kidneys removed, suspensioned and placed in hypotonic treatment (0.075M KCl and 1 % Sodium citrate solution) at two different temperature 4°Cand 25°C. Lasting time for hypotonisation treatment was 45-60 min.The swollen cell suspensions were fixed in 3:1 cooled Carnoy's fluid (3 parts methanol and 1 part glacial acetic acid) for 30 min, then, the old fixative was replaced with the fresh Carnoy's. Lasting time for fixation treatment was 60 min. The slides were stained in series of concentrations of Giemsa Merck solution in distilled water (5, 10 and 15%) and buffered by phosphate (40 mol Na_2HPO_4 and 26.6 mol KH_2PO_4) at PH 6.8 and were assessed at 7, 8, 9 and 10 min exposure times to determine optimum staining conditions. Slides were dipped into distilled water to wash off extra Giemsa solution and then were allowed to airdry at 25ºC for 2–3 h. Metaphases were examined under a microscope (Leca SER. NO. 990398, equipped with a green filter and digital camera) with an oil immersion lens at 1000 magnification. The chromosomes at the metaphase were photographed with a digital camera (Sony SSC-DC 58 AP) onto Kodak colour films (ASA 25). In the course of the microscopic examinations, the chromosome sets of 80 cells were counted and 10 of the best metaphases were used to measure karyotypes.

For each chromosome, the average lengths of the short and long arms, and the centromeric index (CI, expressed as the ratio of the short arm length to the total length of chromosomes) were calculated. The relative length of each pair was expressed by the percentage of the absolute length of each chromosome pair divided by the sum of the absolute length of total chromosomes. The Excel application paired up all the chromosomes using criteria of maximum resemblance based on the total length and the centromere position. The homologous chromosome pairs were classified according to the increasing differences between the homologous chromosomes. The total length of chromosome was computed by summing up the average chromatid lengths of each diploid complement. The length was recorded in pixels by the Colour image. Analysis System Video Pro 32 (Leading Edge) was converted into micrometers after the scale factor was calibrated with a stage micrometer.The chromosome pairs were classified following the recommendations of Macgregor (1993) into metacentric (M), submetacentric (SM), subtelocentric (ST) and telocentric(T), with CI ranges of 46-49, 26-45, 15-26 and less of 15, respectively.

Results

Relatively small and high numbers of chromosomes were observed in *S.richardsonii*.The scattering of the diploid chromosome number values is shown in Fig. 1. The counts of chromosome ranged from 95 to 101 per metaphases with a mode at 96, representing 92.94% of the metaphases. In 85 metaphases from the anterior kidney cells of 20 *S. richardsonii* specimens, the diploid chromosome number was 2n = 96 (Fig.2), which is valid over 90 of metaphases cells. Cells lacking a normal number of chromosomes values (2n=95–101) were probably caused by losses during preparation or additions from nearby cells. All chromosomes in the karyotype have a homologous pair. Homologous pairs of chromosomes were arranged in decreasing size and centromeric indexes. The investigation of metaphases showed notable difference in size and type of chromosomes in *S. richardsonii* In addition to, the sex chromosomes could not be distinguished in this species.The representative karyotype for *S. richardsonii* is shown in Fig. 3. The karyotype of *S.richardsonii* has 9 pairs metacentric, 14 pairs submetacentric and 23 pairs acro-telocentric chromosomes. The number of chromosome arms was determined NF=140 and chromosome formula can be expressed as 2n=9m + 12sm + 265a-t. The morphological and numerical data are summarized in Table 2.The centromeric index, arm ratio, relative length and lengthvariation range of chromosomes are between 25.00 – 49.24, 1.03 – 3.55, 0.88 – 4.13 and 14.57 – 67.70 respectively. Total length chromosomes was 1439.20µm. The largest chromosome is a pair of submetacentric chromosome.

The ideogram of the *S. richardsonii* was made on the basis of the karyotype (Fig. 4).In this study, the optimum colchicine concentration for *S. richardsonii* was determined to be 50 µg/g BW of colchicine solution

for five hours. This concentration effectively arrested the dividing cells in metaphase. In addition to, the best chromosomal spread quality (well-spread metaphase) was obtained from treatment of cells with 1% sodium citrate solution at 4°C for 45min and high of dropping in 120cm.The other hypotonic solution tested, 0.075M KCl, did not result in many scorable metaphases.

Discussion

Several techniques have been developed to examine chromosomes in tissues of various adult fish. These include squashed blood leucocyte culture and cell suspensions from tissues such as gill, kidney ,intestine, scales. Each of these procedures was optimized to obtain large numbers of well-spread metaphases and was used regularly for karyotypeic analysis that in present lecture we utilized from anterior kidney. Karyological study of teleost fishes presents technical difficulties which are not encountered in the study of other vertebrates, and these difficulties are due to the small size and high number of chromosomes. Different techniques are presently being used to perform such studies: direct, *in vivo*; and indirect, *in vitro*. With those forms employing direct techniques, the preparation of slides for optical microscopy is quite easy.Karyological study has been in different steps. Each of the steps involved in the preparation of tissues and slides for cytogenetic analysis was important in attaining large number of well-spread metaphases. The first step in the procedure was treatment of the cells with colchicine, which arrests cell division at metaphase (Baski and Means, 1988). High concentration and long period of colchicine treatment effect on chromosome, causes to aggregate and smallish of chromosome and their arms, so it is difficult to identify short arm of acrocentric chromosomes and other chromosomes.This study suggests that colchicines concentrations of 50 µg/gr BW can effectively arrest dividing cells in metaphase in kidney tissues. But the maintenance periods may vary according to species.Type of hypotonic treatment and the length of exposure to the tissue affect on the degree of chromosome spreading. In this study, 0.075M KCl hypotonic treatment was ineffective in obtaining well-spread metaphases. Although condensed chromosomes could be observed, they were often seen inside an intact cell or only slightly spread.Fixative treatment was not found to be as important as hypotonic treatment in obtaining well-spread metaphases.The main difficulty in working with fish chromosomes is in obtaining high quality metaphase spreads. A few studies have used fish standard karyotypes to examine taxonomic or systematic problems (Bolla, 1987). The major difficulty encountered is the morphological variation existing even between homologous chromosomes in the same nucleus (Al-Sabti, 1991 and Levan et al., 1964). Sometimes it could happen that some chromosomes are more contracted than others, so chromosome measurement are very small chromosomes compared to those of man and mammals. Another problem is that fish karyotypes are not identical, as in human being or other animal species, so we can have a standard karyotype for fish because not only are there differences between species, but polymorphism often occurs within the same fish species (Al-Sabti, 1991).Several incomplete metaphases were encountered in the preparation, and these probably resulted from hypotonic over treatment (Nanda et al., 1995). The majority of authors classify uni-armed and bi-armed chromosomes according to the guidelines of Macgregor (1993). Where differences in the number of chromosome arms was seen, which, this is usually the result of a difference in the scoring of subtelocentric chromosomes by different authors (Philips and Rab, 2001).The majority of cyprinid species have 2n = 50 chromosome (Al-Sabti, 1985), while *Cyprinus carpio* has 2n = 98-100 (Demirok and Ünlü, 2001) and polyploidy Barbus species from southern Africa has 2n = 148 or 150 chromosome (Oellerman & Skelton, 1990), and fishes which have several chromosome series(2n > 50) are called polyploids. The role of polyploidy in evaluation and survive of fishes is very important because it provide from natural selection pressure (Oellerman and Skelton, 1990). Khuda et al., in 1982, noticed that *S. nigar* was caught in India is a polyploidy fish. In other species of cyprinids such as *Tor putitora, Tor khudree* and *Tor tor* polyploidy was reported, too (Anonym, 1982). So, with respect to the number of *S. richardsonii* chromosomes and their resistant to the environmental conditions, it seems to be polyploidian fish.Until now, karyotype of some member of Schizothorax genus was determined like as *S. kumaonensis* (2n=98, NF=126, 2n=18m+10t+70a) (Lakara et al., 1997) but there isn't any report about *S. richardsonii* and our study is the first report of the karyotype of *S. richardsonii* The karyotype analysis is a key step toward the stock improvement by polyploidy manipulation, hybridisation and related genetic engineering (Tan et al., 2004).

Conclusion

As in other animals, comprehensive genetic researches are needed for this fish.The present paper is the first to provide the detailed information on the chromosome number and karyotype of *S. richardsonii*. We are optimistic that our research will serve to provide a path for advancing the study of fish.

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Table 1. Numeral characteristics of the karyotype of *Schizothorax richardsonii* showing the mean values of measurements from ten best mitotic metaphases

Chromosome Pair no.	**Total length(µm)**	**Long arm(µm)**	**Short arm(µm)**	**Relativelength (%)**	**Centromericindex**	**Armratio**	**Classification**
1	51/26	32/83	26/43	3/71	47/02	1/08	Metacentric
2	54/75	27/86	23/80	3/20	43/29	1/28	Metacentric
3	43/69	23/76	22/93	2/84	44/95	1/13	Metacentric
4	43/34	23/32	20/02	2/64	46/19	1/16	Metacentric
5	43/01	22/92	20/09	2/62	46/95	1/13	Metacentric
6	41/16	20/93	20/23	2/51	49/24	1/03	Metacentric
7	41/02	22/00	19/02	2/50	46/48	1/15	Metacentric
8	40/95	22/00	18/95	5/49	46/40	1/16	Metacentric
9	36/95	19/85	17/10	9 /25	46/27	1/16	Metacentric
10	66/70	34/20	26/50	4/13	43/46	1/34	Submetacentric
11	52/16	33/24	22/92	3/48	36/01	1/49	Submetacentric
12	55/75	37/50	18/25	¾	32/73	2/05	Submetacentric
13	53/48	32/26	21/22	3/26	36/66	1/52	Submetacentric
14	52/77	29/43	23/34	3/21	44/26	1/26	Submetacentric
15	41/73	23/34	18/39	2/54	43/83	1/03	Submetacentric
16	37/91	25/18	12/73	2/31	33/48	1/99	Submetacentric
17	37/37	26/88	10/49	2/27	28/68	3/55	Submetacentric
18	37/35	22/07	15/28	2/27	41/70	1/47	Submetacentric
19	37/18	26/99	10/19	2/26	27/43	2/64	Submetacentric
20	36/22	20/31	15/91	2/20	43/26	1/50	Submetacentri
21	35/64	35/64	9/47	2/17	26/56	2/49	Submetacentric
22	28/20	21/15	7/05	1/72	25/00	25/00	Submetacentric
23	27/20	27/20	6/90	1/65	25/36	2/94	Submetacentric
24	33/95	33/95	0/00	2/07	0/00	∞	Subtelocentri

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25	32/00	32/00	0/00	1/95	0/00	∞	Subtelocentric
26	30/24	30/24	0/00	1/85	0/00	∞	Subtelocentric
27	28/69	28/69	0/00	1/75	0/00	∞	Subtelocentric
28	28/58	28/58	0/00	1/74	0/00	∞	Subtelocentric
29	28/30	28/30	0/00	1/72	0/00	∞	Subtelocentric
30	28/02	28/02	0/00	1/70	0/00	∞	Subtelocentric
31	28/01	28/01	0/00	1/70	0/00 ic	∞	Subtelocentric
32	27/16	27/16	0/00	1/66	0/00	∞	Subtelocentric
33	26/60	26/60	0/00	1/62	0/00	∞	Subtelocentric
35	25/48	25/48	0/00	1/55	0/00	∞	Subtelocentric
36	25/25	25/25	0/00	1/54	0/00	∞	Subtelocentric
37	24/81	24/81	0/00	1/51	0/00	∞	Subtelocentric
38	24/76	24/76	0/00	1/51	*0/00*	∞	Subtelocentric
39	24/37	24/37	0/00	1/48	0/00	∞	Subtelocentric
40	24/05	24/05	0/00	1/46	0/00	∞	Subtelocentric
41	23/75	23/75	0/00	1/44	0/00	∞	Subtelocentric
42	23/91	23/91	0/00	1/45	0/00	∞	Subtelocentric

43	22/64	22/64	0/00	1/38	0/00	∞	Subtelocentric
44	21/93	21/93	0/00	1/33	0/00	∞	Subtelocentric
45	21/01	21/01	0/00	1/28	0/00	∞	Subtelocentric
46	16/29	15/70	0/00	0/99	0/00	∞	Subtelocentric
47	15/70	14/57	0/00	0/99	0/00	∞	Subtelocentric
48	15/70	14/57	0/00	0/88	0/00	∞	Subtelocentric

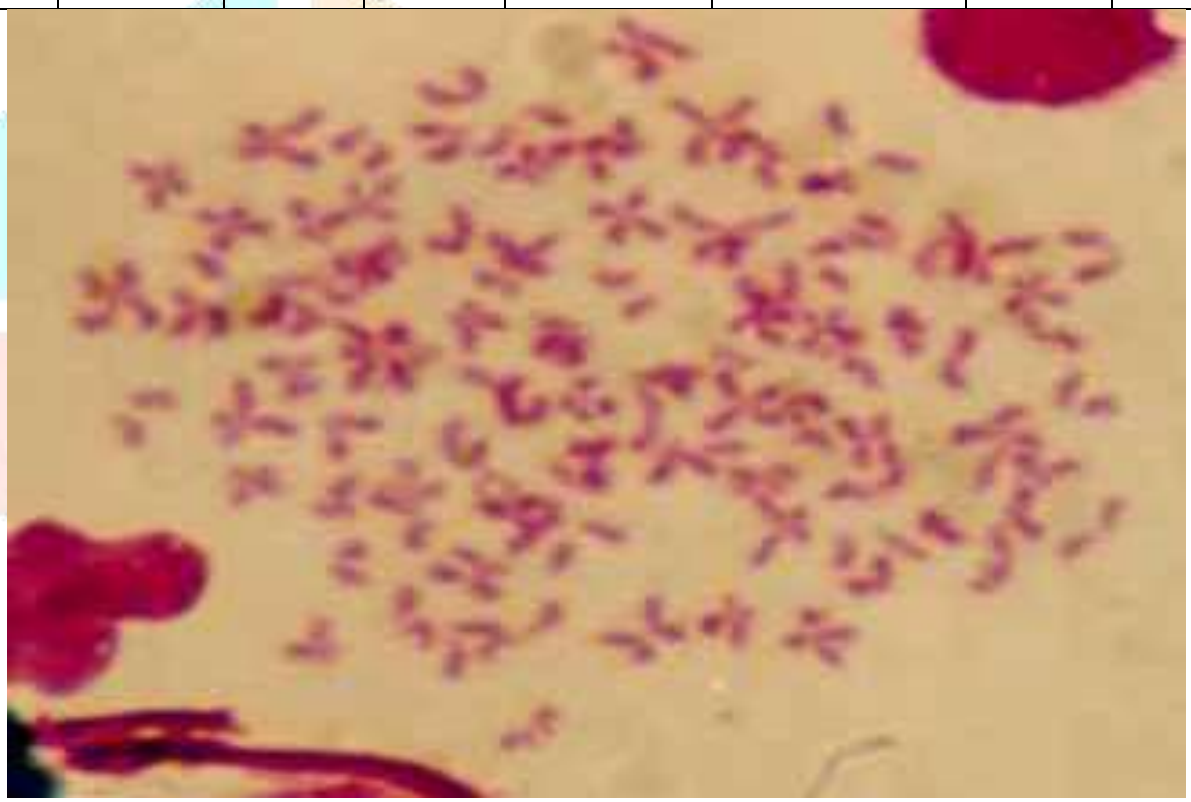

Fig.1. Metaphase spread from kidney tissue of *S. Richardsonii* ×1000,

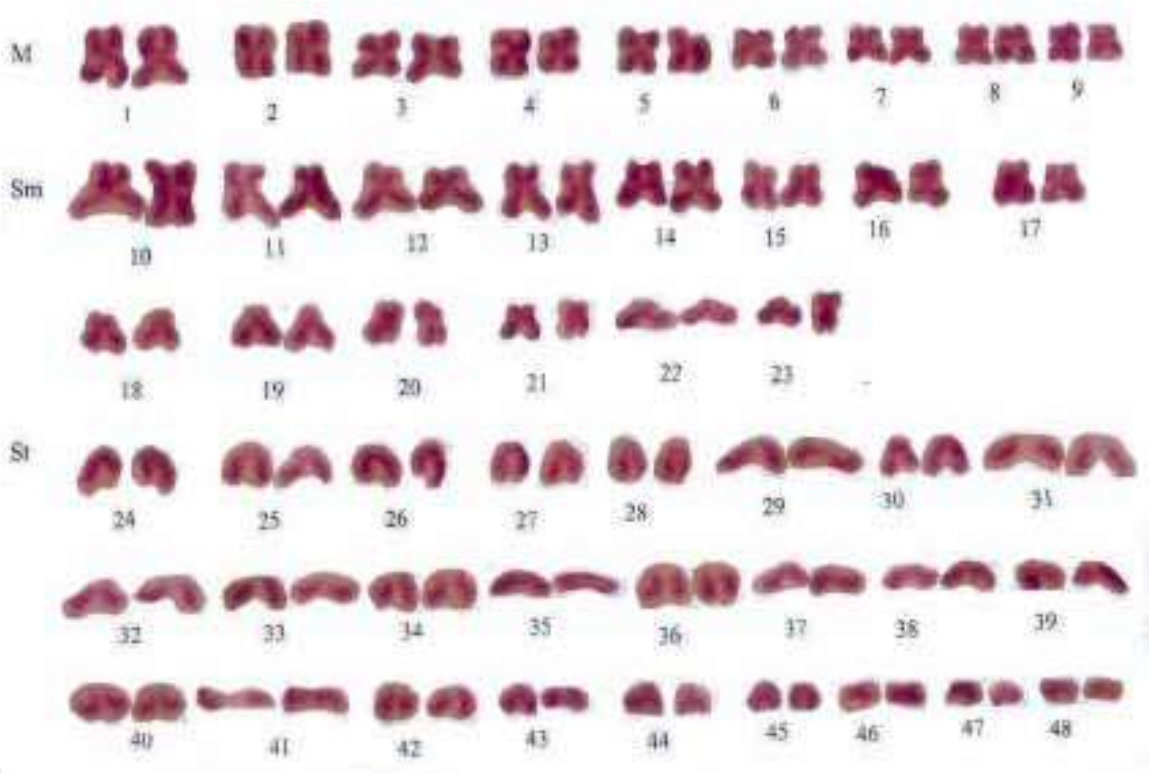

Fig. 2. Karyotype of S. *Richardsonii*, 2n = 48

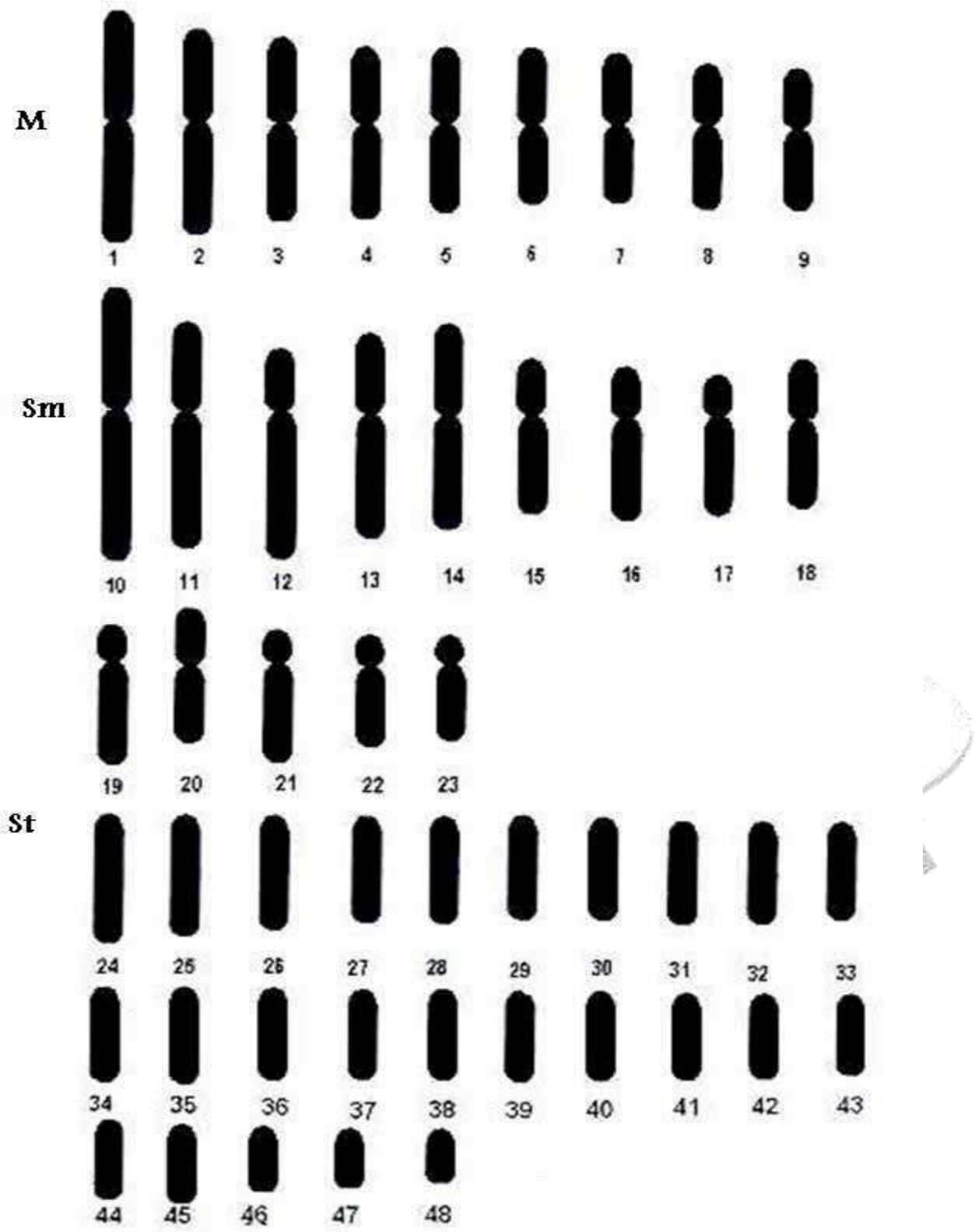

Fig. 4. Idiogram of *S., Richardsonii* n = 48