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Karyotype analysis of chub, *Squalius cephalus* (Linnaeus, 1758) (Teleostei: Cyprinidae) from Karasu River, Erzurum, Turkey

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ABSTRACT

The karyotypic characteristics of chub, *Squalius cephalus* have been investigated by examining metaphase chromosomes spreads obtained from gill and kidney tissues. The fish used in the study were caught with fishing nets from Dumlu Stream, one of the main tributaries of the Karasu River. The live fish were transported to the laboratory, kept in a well aerated aquarium before analysis and then were injected intraperitoneally with doses of phytohemagglutinin, 0.01 ml.g^{-1} BW of 1% solution with 48-h interval to induce cell divisions. At the end of the period, the fish were injected intraperitoneally with doses of colchicine (0.01 ml.g^{-1} BW of 6% solution) and left for 3 hours before anesthesia and sacrificing. The best treatment parameters for preparing good metaphase chromosome spreads from the gill and kidney cells were performed as hypotonic (0.075 M KCl) treatment for 50 minutes, fixation with cold Carnoy solution at 3:1 ratio (methanol: acetic acid) and a concentration of 5% Giemsa for 35 minutes. The diploid chromosome number of this species was 2n = 50. The fundamental arm number (FN) was 92. The karyotypes were composed of 5 metacentric, 11 submetacentric, 5 subtelocentric and 4 acrocentric chromosome pairs (10 M + 22 SM + 10 ST + 8 A). No sex chromosomes were cytologically detected in this fish.

Key words: Karyogram, Karasu Basin, Chromosomes, Fish

INTRODUCTION

The uses of chromosome cytologic information are many cytotaxonomy and phylogenetic relationship in insects, plants and mammals among other organisms. In fish, chromosome analysis based on variations in chromosome number and morphology are typically used to conduct genetic questions (Felip *et al.* 2009). A considerable wealth of cytogenetic information is now available for fish species (Molina *et al.* 2014), which makes possible general analyses of structural cytogenetic processes that are involved in karyotype evolution of the main groups. Karyotypes have been reported for 3,425 species/subspecies of fishes. Specifically, numbers of karyotyped fish species are 747 (21.8%) in Cypriniformes (Arai 2011).

The order Cypriniformes with 11 families and 4298 species is one of the large order of fishes around the world (Eschmeyer & Fong 2016). Cyprinidae, one of the families, are found in Eurasia, Africa and North America which is the most abundant family of freshwater fishes, comprising 3042 species (Eschmeyer & Fong 2016). The subfamily Leuciscinae is a member of this diverse family including the genus *Squalius* and about 46 species (Turan *et al.* 2013) which distributed widely throughout Eurasia from the Iberian Peninsula to the Amur River and from the Kolyma River to the Tigris-Euphrates basin (Bogutskaja & Naseka 2004). About 188 species of cyprinids are identified and reported from Turkey (Çiçek *et al.* 2015). There are 21 *Squalius* species known for Turkey, including *S. cephalus* (Linnaeus, 1758) (Çiçek *et al.* 2015). The species, commonly known as chub, is distributed in the North, Baltic, northern Black, White, Barents and Caspian Sea

basin (Freyhof 2014). It is also found in all major rivers of Turkey including Karasu, Euphrates, Tigris, Çoruh, Aras, Gediz, Asi (Kuru 2004; Fricke *et al.* 2007; Geldiay & Balık 2009). Although *S. cephalus* (Fig. 1) has been described and compared morphologically from Karasu River, but its karyotype has not been investigated so far. Hence the objective of the present study was to determine the karyotyping characteristics of chub, *S. cephalus* from Karasu River (Karasu Basin, Erzurum) in Turkey.

Fig. 1. *Squalius cephalus* from Karasu River, Erzurum.

MATERIALS AND METHODS

In June 2013, 16 live individuals of *S. cephalus* (mean weight = 58.15 ± 5.8 g, mean length = 17.75 ± 1.2 cm) were caught in Dumlu Stream (40° 01′ 52K, 41° 18′ 49D alt. 1763 m), a main tributary of Karasu River (Fig. 2) by fishing nets. The fish were transported live to laboratory and kept in well aerated aquaria at 20 °C before analysis. Air-dried chromosome preparation method as described by Collares-Pereira (1992) with some modifications was followed. Fish received 0.01 ml 1% phytohemagglutinin (PHA) (Sigma) injections per gram of body weight using an insulin syringe, in a 48-h interval at 20 °C. After the injection, the fish were injected intraperitoneally with 0.01 ml of 6% colchicine (Sigma) per gram of body weight, and then were replaced in the aquarium for 4 hours. The specimens were anesthetized in benzocaine hydrochloride (50 $mg.l^{-1}$) and then killed. The fish were then dissected, and gill filaments and kidneys were removed and placed in hypotonic 0.075M KCl solution for 50 min at room temperature. The tissues were homogenized and mixed. Suspensions were centrifuged at 2000 rpm for 10 min. Supernatants were then discarded and 5 ml cold and fresh cold Carnoy fixative (3 : 1 methanol and glacial acetic acid) was added to sediments, mixed thoroughly. Suspensions with Carnoy were centrifuged at 2000 rpm for 10 min, and then supernatants were discarded and 5 ml Carnoy was added to sediments. This process was repeated two times. Smears were prepared on pre-chilled slides using the splash method from 45 cm height and air-dried for 12 h. The slides were stained with 5% Giemsa for 35 min. Chromosomes were observed, selected and photographed by Leica DM750 microscope model Leica ICC50 HD Camera with 100x magnification lens. Approximately thirty metaphase plates were counted from each gill and kidney. The best metaphase spread picture was selected among all metaphase plates for arranging the karyotypes. Karyotypes were prepared by arranging chromosomes in pairs by size. To determine chromosome formula, each arm of the chromosomes and centromeric index (CI) were measured. The morphometric measurements of chromosome were conducted with Leica LAS EZ 3.0 image analyzer software-

programme by determining the coordinate arms and centromere. Then the length of each arm was identified using line formula by Microsoft Office Excel 2007. Firstly, CI (length of the chromosome, short arm divided by its total length) was calculated. Finally, to determine homologous pairs and chromosome formula, the chromosomes were arranged based on CI in the descending order. The chromosome type was identified by method of Levan *et al.* (1964). The chromosome pairs were classified into Metacentric (M), Submetacentric (SM), Subtelocentric (ST) and Acro-(Telo) centric A (T), with CI ranges of 50.00 - 37.51, 37.50-25.01, 25.00 - 12.51 and 12.50 - 0, respectively.

For each chromosome, the average lengths of the short and long arms and arm ratio (the ratio of the long to short arm length of chromosomes) were calculated and fundamental arm number (FN) expressed as of twice the number of a telocentric plus the number of telocentric chromosomes.

Microsoft Office Excel 2007 software was used to calculate centromeric indices and to draw the ideogram.

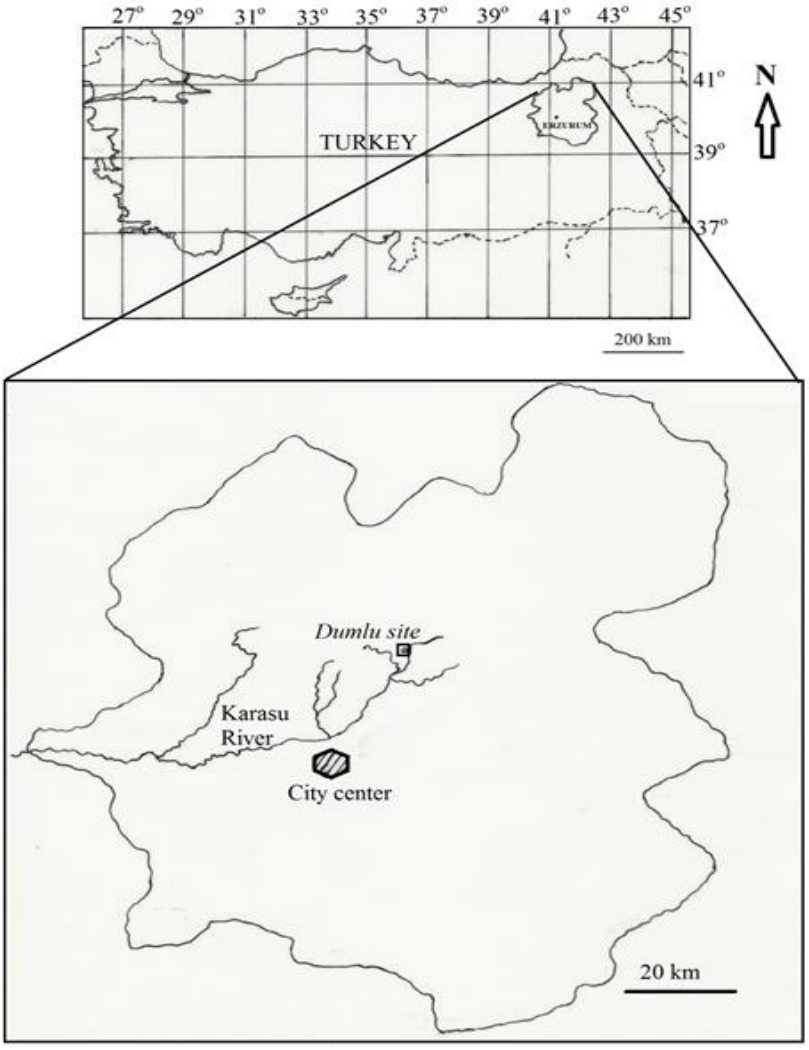

Fig. 2. Map of Turkey showing sampling site of *Squalius cephalus* in Dumlu Stream.

RESULTS

In gills 467 and in kidney 378 metaphase plates of 16 specimens of *S. cephalus* were counted. The observed diploid number per each metaphase plate ranged between 42 and 52. A diploid number of 2n = 50 constituted 85% in gill and 86% in kidney of the counted metaphase plates (Table 1). Metaphase spreads

of *S. cephalus* gill and kidney are given in Fig. 3. The diploid chromosome number in this species was found as 2n = 50 (Fig. 4).

The quantitative data of the different measurements used to classify chromosomes and ideogram are given in Table 2 & Fig. 5. The karyotype consisted of 10 metasentric (10 M), 22 submetacentric (22 SM), 10 subtelocentric (10 ST) and 8 acrocentric (8 A), and the fundamental number was FN = 92. The shortest and longest chromosomes were a acrocentric and a submetacentric one, 0.04 and 0.23 µm, respectively (Table 2). Based on the chromosomal indicators (Table 2), the ideogram was depicted (Fig. 5). Karyotype of gill and kidney cells was the same. No sex chromosomes were cytologically detected in the examined fish.

Table 1. Analysis of frequency of chromosome numbers in gill and kidney of *S. cephalus*.

	Gill tissue				**Kidney tissue**			
Number of chromosomes in each metaphase plate	46	48	50	52	46	48	50	52
Number of metaphase plates	14	48	399	6	8	40	326	4
Frequency (%)	3	10	85	2	2	11	86	1

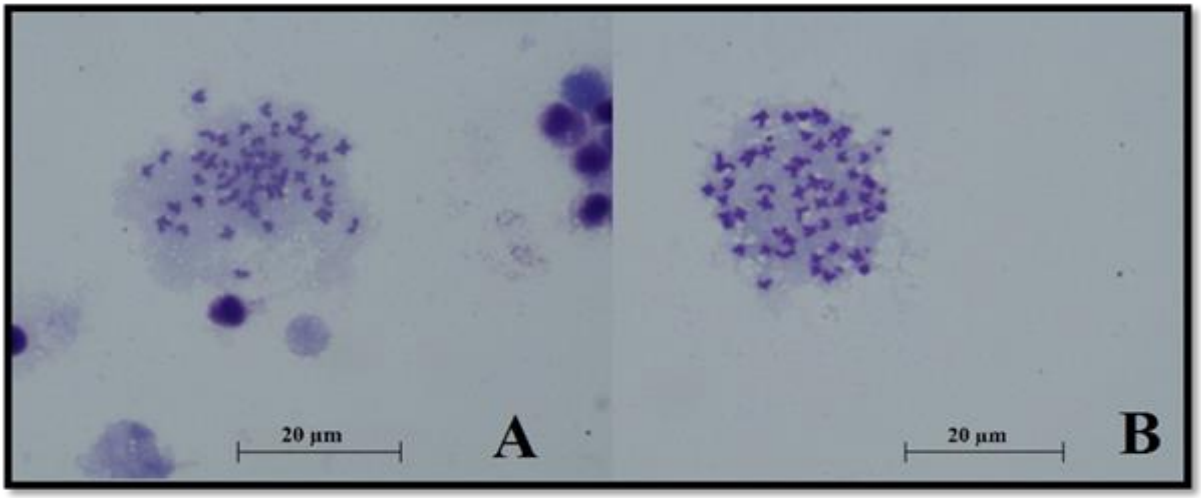

Fig. 3. Metaphase spreads of *S. cephalus* A) Metaphase spread of gill epithelial cells, B) Metaphase spread of kidney cells.

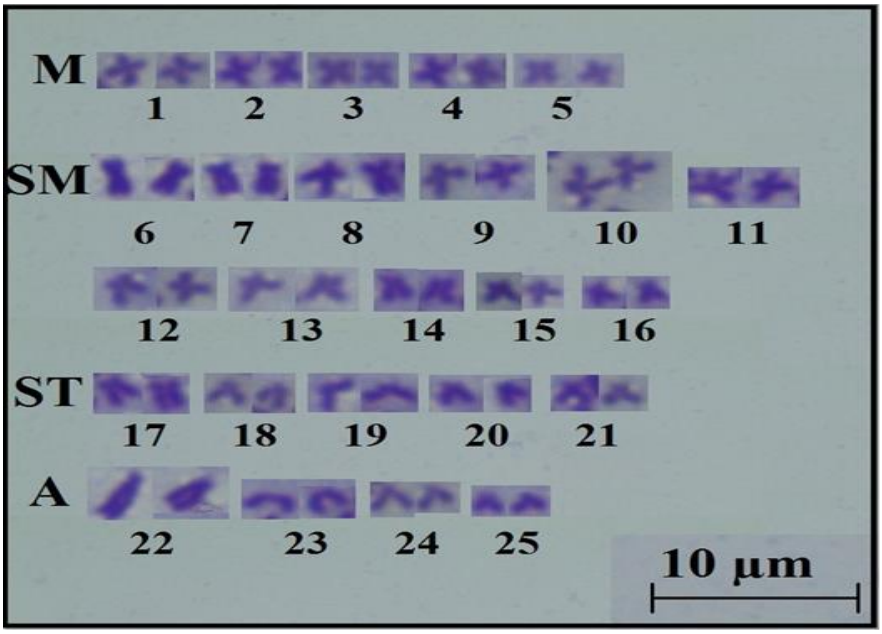

Fig. 4. Karyotype of *S. cephalus*: 10M+22SM+10ST+8A, FN = 82.

Table 2. Chromosome measurements (in) and classification of *S. cephalus* chromosomes.

Chromosome number	Short arm	Long arm	Chromosome length (µm)	Arm ratio	Centromeric index	Relative arm length (%)	Chromosome type	Arms no.
1	0.11	0.12	0.23	1.09	47.82	6.72	M	4
2	0.11	0.11	0.22	1.0	50	6.43	M	4
3	0.11	0.11	0.22	1	50	6.43	M	4
4	0.09	0.09	0.18	1.0	50	5.26	M	4
5	0.07	0.08	0.15	1.14	46.66	4.38	M	4
6	0.08	0.14	0.22	1.75	36.36	6.43	SM	4
7	0.08	0.14	0.22	1.75	36.36	6.43	SM	4
8	0.07	0.12	0.19	1.71	36.84	5.55	SM	4
9	0.05	0.10	0.15	2.0	33.33	4.38	SM	4
10	0.06	0.10	0.16	1.6	37.50	4.67	SM	4
11	0.05	0.09	0.14	1.8	35.71	4.09	SM	4
12	0.04	0.08	0.12	2.0	33.33	3.50	SM	4
13	0.04	0.08	0.12	2	33.33	3.50	SM	4
14	0.04	0.07	0.11	1.75	36.36	3.21	SM	4
15	0.03	0.06	0.09	2.0	33.33	2.63	SM	4
16	0.03	0.06	0.09	2.0	33.33	2.63	SM	4
17	0.04	0.10	0.14	2.5	28.57	4.09	ST	4
18	0.03	0.08	0.11	2.6	27.27	3.21	ST	4
19	0.02	0.07	0.09	3.5	22.22	2.63	ST	4
20	0.02	0.06	0.08	3	25.00	2.33	ST	4
21	0.02	0.05	0.07	2.5	28.57	2.04	ST	4
22	0	0.14	0.14	∞	0	4.09	A	2
23	0	0.09	0.09	∞	0	2.63	A	2
24	0	0.05	0.05	∞	0	1.46	A	2
25	0	0.04	0.04	∞	0	1.16	A	2
Total	1.19	2.23	3.42	-	-	-	-	92

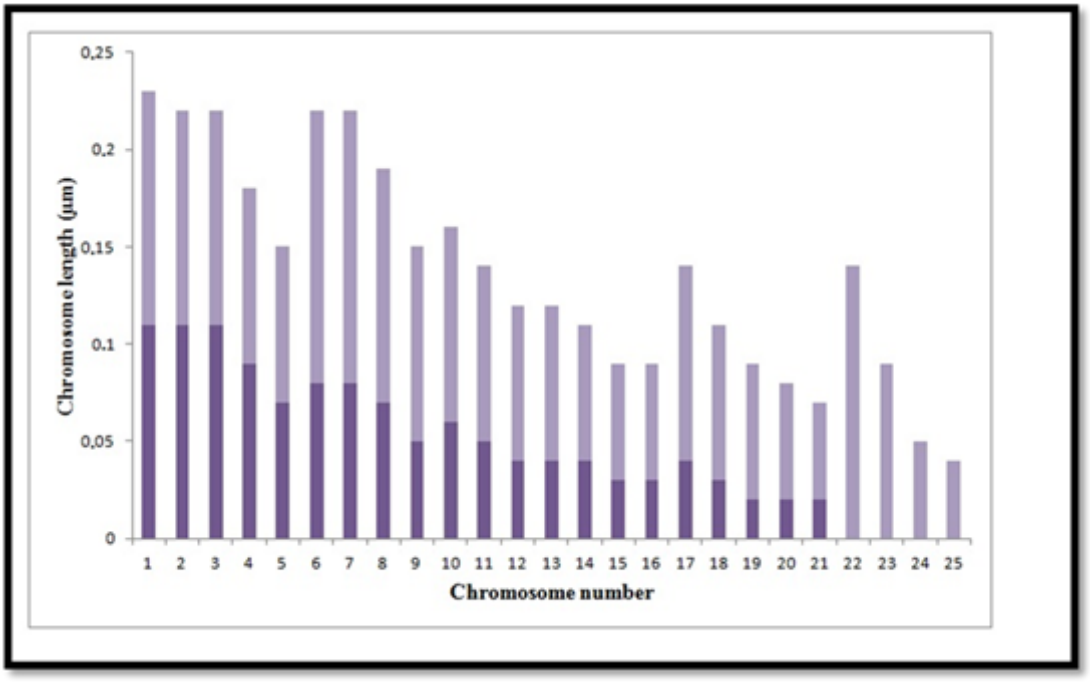

Fig. 5. Haploid ideogram of *S. cephalus*.

DISCUSSION

The chromosomes of the family Cyprinidae have been well studied (Rab & Collares-Pereira 1995). The clear dominant mode of 2n = 50 chromosomes seems to reflect the plesiomorphic chromosome number for the family. The karyotype of cyprinids is usually characterized by relatively high number of biarmed (meta- and submetacentrics) compared to uniarmed (subtelo- and

acrocentrics) chromosomes (Sola & Gornung 2001). The largest chromosome pair is characteristically a subtelo-/acrocentric element, which is a cytotaxonomic marker in Leuciscinae cyprinids (Rab & Collares-Pereira 1995; Rab *et al.* 2000). On the whole, cyprinid sex chromosomes appear to have remained morphologically undifferentiated (Sola & Gornung 2001). *S. cephalus* also displays the cyprinid properties mentioned above. Moreover, Anatolian cyprinids, *Acanthobrama marmid*, *Chalcalburnus mossulensis* (now *Alburnus mossulensis*), *Cyprinion macrostomus* (now *Cyprinion macrostomum*) (Gaffaroğlu 2003), *Alburnoides bipunctatus* (Kılıc-Demirok & Unlu 2004), *Pseudophoxinus firati* (Karasu *et al.* 2011) were found to have 2n = 50 chromosomes, like *S. cephalus*. About 20 among 35 putative species of the genus *Squalius,* as well as other taxa in the subfamily Leuciscinae, cytogenetically investigated so far, indicated a considerable great karyological similarity (Collares-Pereira *et al.* 1998, Bianco *et al.* 2004). Their karyotypes were characterized by Boron (2001) and Ra'bova *et al.* (2003) as 2n = 50. According to our observations, the diploid chromosome number of *S. cephalus* was 2n = 50. *S. cephalus* karyotypes were determined as being composed of 5 metacentric, 11 submetacentric, 5 subtelocentric and 4 acrocentric chromosome pairs (10 M + 22 SM + 10 ST + 8 A).

The chromosome preparation from both tissues (gill and kidney) and their karyotypes were similar. The basic diploid chromosome number (2n), for *S. cephalus*, was reported to be 50 from all the previous studies (Table 3) (Boron *et al.* 2009).

Table 3. Chromosomal data of *S. cephalus* in different locations.

2n	Karyotype	FN	Location	References
50	20 MSM + 30 A	70	Bosnia and Herzigovina	Berberovic & Sofradzja 1974
50	16 M + 12 SM + 12 ST + 10 A	78	Italy (Savuto River)	Cataudella *et al.* 1977
50	34 MSM + 16 A	84	Bosnia and Herzigovina	Sofradzija 1977
50	18 M + 20 SMST + 12 A	88	France (Garonna River)	Hafez *et al.* 1978
50	10 M + 16 SM + 14 ST + 10 A	90	Yugoslavia (Danube River)	Vujosevic *et al.* 1983
50	34 MSM + 16 STA	84	Slovenia	Al-Sabti 1986
50	20 M + 12 SM + 18 ST-A	80	Turkey (Kastamonu dam lake)	Pekol 1999
50	14 M + 20 SM + 16 ST-A	84	Turkey (Tigris River)	Kılıç-Demirok 2000
50	16 M + 26 SM + 8 ST/A	92	Italy (Sele River)	Bianco *et al.* 2004
50	10 M + 22 SM + 10 ST + 8 A	82	Poland (Wislok River)	Boron *et al.* 2009
50	10 M + 22 SM + 10ST + 8 A	92	Turkey (Karasu River)	Present study

However, the karyotype formula of *S. cephalus*, varied considerably from different geographical locations, such as: 16 M + 26 SM + 8 ST/A (FN = 92) from European freshwaters (Bianco *et al.* 2004); 10 M + 22 SM + 10 st ST + 8 S (FN = 82) from Wislok and Vistula River Basin-Poland (Boron *et al.* 2009); 20 M + 12 SM + 18 ST-A (FN = 80) from dam lake Kastamonu, Turkey (Pekol 1999); 14 M + 20 SM + 16 ST-A (FN = 84) from Tigris River,Turkey (Kılıç-Demirok 2000). Heteromorphic sex chromosomes have been reported in *S. cephalus* (Vujosevic *et al.* 1983). In the present study, however, no sex chromosomes were detected in the species examined, suggesting that the previously reported heteromorphic chromosomes might be a local polymorphism rather than a true sex chromosome.

Despite the similarities in chromosome numbers between this study and the previous studies, differences in chromosome formula and number of arms (FN) were observed (Table 3). This may be due to various factors including differences in population and also subspecies in sampling region, or may be related to interspecific polymorphism. It may also depend on technical and procedural experimental condition, loss of spreads, incorrect moving of fixed cells during spread preparation, addition of chromosomes form adjacent cells, unrecognizable micro arms in chromosomes, inadequate number of samples,

variety of population and subspecies in each region, errors in measuring chromosome arms and determining chromosome type, etc. (Khuda-Bukhsh *et al.* 1986; Arai 2011; Khosravanizadeh *et al.* 2011). The present study is the first to describe chromosomal characteristics of *S. cephalus* from Karasu River. These results, along with other taxonomic features such as morphological, anatomical and molecular data, could be used to enlighten the taxonomic status of this species for management and conservation programs.

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کاریوتایپ ماهی چاب *Squalius cephalus* (Linnaeus, 1758) از رود قره سو ، ارزروم، ترکیه

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چکیده

خصوصیات کاریوتایپ ماهی چاب با آزمایش بر روی گسترش های کروموزومی مرحله متافاز حاصل از بافت های آبشش و کلیه مورد تحقیق قرار گرفت. ماهی مورد استفاده توسط تورهای صیادی از رودخانه دولمو، یکی از انشعابات اصلی رود قره سو صید شد. ماهیان زنده به آزمایشگاه منتقل شدند و قبل از آزمایش در آکواریوم های هوادهی شده نگهداری شدند و سپس توسط مقادیری از فیتوهماگلوتینین ۰/۰۱ میلی لیتر به ازای گرم وزن بدن از محلول ٪۱ به صورت داخل صفاقی با فاصله ۴۸ ساعت جهت القای تقسیم سلولی تزریق شدند. در انتهای دوره ماهیان به صورت داخل صفاقی با مقادیری از کولشیسین (۰/۰۱ میلی لیتر به ازای گرم وزن بدن از محلول ۶٪ مورد تزریق قرار گرفتند و سه ساعت بعد بیهوش و قربانی شدند. بهترین پارامترهای تیمار برای تهیه گسترش های کروموزومی متافاز مناسب از سلول های آبشش و کلیه، تیمار هایپوتونیک کلرید پتاسیم ۰/۰۷۵ مول به مدت ۵۰ دقیقه، تثبیت با محلول کانوی سرد به نسبت ۱:۳ (متانول : اسید استیک) و غلظت ۵٪ گیمسا به مدت ۳۵ دقیقه بود. تعداد کروموزوم دیپلوییید این ماهی $2n = ۵۰$ بود. تعداد بازوهای اصلی (FN) ۹۲ عدد بود. کاریوتایپ این ماهی شامل زوج های کروموزومی به شرح زیر بود: ۵ زوج متاسنتریک، ۱۱ زوج ساب متاسنتریک، ۵ زوج ساب تلوسنتریک و ۴ زوج آکروسنتریک (10M + 22SM + 10ST + 8A) بود. در این ماهی هیچ کروموزوم جنسی از بعد سلول شناسی تشخیص داده نشد.

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