Karyological analysis of small-scaled Damascus barbel, *Capoeta damascina* (Valenciennes, 1842) from Tigris Basin

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Introduction

Cyprinids are one of the major components of the freshwater fish diversity of Asia. Among different genera of this family; *Capoeta* shows wide distribution in the southwest area of this mainland. It contains about 20 species of which 8 occur in Iran (Keivany *et al*., 2015). Small-scaled Damascus barbel, *Capoeta damascina* (Valenciennes, 1842) is one of the most important species of *Capoeta* in Iran. It attains the greatest size and the highest density among all other *Capoeta* species in this region. Chromosome analysis is a valuable tool for systematic evaluation, biodiversity, conservation, stock assessment and aquaculture (Dorafshan and Kalbassi, 2006; Kalbassi *et al*., 2006; Pisano *et al*., 2007). Despite the importance of fish cytogenetics, when available data sets on fish karyotype are analysed, it is clear that they are still very incomplete (Gromicho and Collares-Pereira, 2007). In the *Cyprininae* subfamily, we can find evolutionary diploids (2n≈48-50) e.g. smallmouth lotak, *Cyprinion kais* (Nasri *et al*., 2010), tetraploids (2n≈96-100) e.g. common carp, *Cyprinus carpio* (Al-Sabti, 1986) and *Schizothorax zarudnyi* (Kalbassi *et al*., 2008) and hexaploid (2n≈ 148-150) e.g. *Barbus canis* (Gorshkova *et al*., 2002). Changes in polyploidy level may be a key factor in the cause of evolutionary changes in *Cyprinidae.* Some reports are available on the karyology of different species and/or subspecies of *Capoeta* like *C. trutta* and *C. capoeta ulma* from Tigris River, Turkey (Kiliç-Demirokand Ünlü, 2001), and *C. c. grasilis* from the Caspian Sea Basin, Iran (Darestani *et al*., 2006). However

the only report available on the karyology of *C. damascina* is based on the Wadi Karak stream population from the Kingdom of Jordan (Gorshkova *et al*., 2002).

The aim of this study was to investigate the karyotype of *C. damascina* for basic information for evaluation, conservation and/or aquaculture purposes.

Materials and methods

Fifteen specimens (11-17 g and 7-14 cm SL, 5 males and 10 females) of *C. damascina* were obtained on 15 June 2010 from the Monj River 50˚ 41' E and 31˚ 35' N, a tributary of the Karoon River, Tigris Basin, located in the Charmahal-o-Bakhtiari Province, west of Iran. The fish were delivered live to the lab, in 100 L well-aerated aquaria at 24-26˚C following guidelines for treating experimental fish approved by the Isfahan University of Technology Committee. Chromosome preparation was made following the standard method of Thorgaard and Disney (1990) with some modification. Briefly, the fish received two identical intraperitoneal (i.p.) injections of phytohaemaglutinin, PHA (Baharafshan, Iran) with an interval of 24-h, final dose 40 µg/gbw. 12 h after the final PHA injection, the fish received i.p. injection of 25-50 µg/g body weight of colchicine (Sigma, USA) as a mitogenic inhibitor. The head kidney and the gill filaments of each fish were extracted separately for each fish, 7-8 h after colchicine injection. The tissues were immersed in a cold (4°C) hypotonic solution of 0.1 M KCl for 45 min. The suspension was centrifuged at 1300 rpm for 10 min, supernatant removed and the rest was fixed with cold-fresh Carnoy's solution (3:1 methanol and glacial acetic acid) as a fixative. Three changes of fixative were made at 30 min intervals, followed by smear preparation on cold lamella using splash method. The slides were stained by 10% Giemsa.

A minimum of 4 metaphase spreads of the kidney and gill tissues were examined for each specimen using a Nikon microscope (Fujix HC-300zi, Japan) to account for the chromosome number. After chromosome number determination, the best spread was photographed using compact microscope (NTHCSM, Swiss) at 4000 X to provide the karyogram. The morphometric measurements were done by Image tools V.6 software.

Arm ratio (AR) expressed as the ratio of the long arm to the short arm length of each pair of chromosome. Relative length of chromosome (RL) was the absolute length of each chromosome pair divided by the sum of the absolute length of total chromosome expressed in percentage. The centromic index (CI) or form percentage (F%) calculated as the ratio of the length of the short arm of the chromosome to that of the total chromosome, ordinarily expressed as a percentage. While, r-value and total form percentage (TF%) were the ratio between the shortest to the longest chromosome pair and the

ratio of the length of the short arm of the total chromosome to the total length of all chromosome respectively (Levan *et al*.,1964; Macgregor and Varley, 1993).

Chromosomes were classified into metacentric (M), submetacentric (SM), subtelocentric (ST) and acrocentric (A) based on the Levan *et al*. (1964) recommendation when the AR were in the range of 1-1.7, 1.7-3, 3-7 and >7, respectively. The karyogram and ideogram were provided using Adobe Photoshop 6.0 and Microsoft Excel 2003 respectively.

Results and discussion

The count of chromosomes ranged from 147 to 152 per metaphases, with a mode of 150 representing 67% of the metaphases (Table 1). The sizes of the chromosomes were in the range of 1.54-4.10 µm. The largest and smallest chromosomes were a pair of SM and A, respectively. The long arm and short arm ranges were 1.03-3.47 and 0-1.45µm, respectively (Table 2). The ranges of AR, RL and CI or F were in the ranges of 1.08-∞, 0.79-2.12% and 0.00-48.19%, respectively (Table 2). The r-value and TF index were calculated as 0.37 and 24.36%, respectively. There were 9 pairs of M, 30 pairs of SM, 22 pairs of ST and 14 pairs of A chromosomes providing the chromosome number and formula of *C. damascina* as 2n=150 and 2n= 9M+30SM+22ST+14A (Table 2). The chromosome spread, karyogram and ideogram of *C. damascina* are presented in Figs. 1 to 3, respectively. The homologous pairs of chromosomes were arranged according to the classification. The NF was 228, which was calculated by assigning a value of two arms for M/SM chromosomes and one arm for the A/T chromosomes. No sex chromosomes were clearly observed.

Fontana *et al*. (1997) stated the range between 2n=22-26 for *Nototheniidae* to 2n=240-260 in *Acipensereidae*. While, Hallerman (2003) reported the lowest chromosome number as 2n=16 in *Sphaerichthys osphramenoides* (*Belontidea*) to 2n=446 *in Datchus dipogon*. Nevertheless, it is well documented that most of the cyprinid fish have 2n=50, although some of them have higher chromosome number such as 2n=96-100 in common carp (Al-Sabti, 1986).

Table 1: Chromosome complement of small- scaled Damascus barbel, *Capoeta damascina* Valenciennes, 1842), based on observed frequency, 2n = 150

Number of fish	Sex*	Chromosome number					Total metaphases	Karytype** (2n=150)		
		147	148	149	150	152		M-SM	ST-A	NF
1	M	1			4	1	6	78	72	228
2	M			1	4	2	7			
3	F		2		5		7			
4	F		1		4	1	6			
5	F		1		5		6			
6	F	1			4	1	6			
7	F		1		5	2	8			
8	M		1		4		5			
9	M			1	3		4			
10	F		1		4	1	6			
11	F		1	1	4		6			
12	F				5	1	6			
13	M		1		3		4			
14	Immature		1		3		4			
15	M				3	1	4			
Totals		**2**	**10**	**3**	**60**	**10**	**85**			

*- M: Male; F: Female. **- M-SM: Metacentric-Submetacentric; ST-A: Subtelocentic-Acrocentric. NF: Number of Fundamental.

The diploid chromosome number of *C.damascina* was determined from Tigris Basin for the first time and defined as 2n=150 including 18 M, 60 SM, 44 ST and 28 A. In general, fish can survive and reproduce actively even with some chromosomal rearrangements which maybe pernicious to other vertebrates like mammals. Based on available information (Table 3), 2n = 150 might be acceptable as a diploid chromosome number and this genus of *Cyprinidae* could be categorised as hexaploid cyprinids. It has been reported that different fish species can undergo different levels of ploidy such as diploid, tetraploid and hexaploid levels which has been observed in some Cyprinids (Tsigenopoulos *et al*., 2002), Salmonids (Gromicho and Collares-Pereira, 2007) and Acipenserids (Fontana *et al*., 2007). Changing in ploidy levels can be categorised as an important speciation force in many groups of fish (Fontana *et al*., 2008).

Table 2: Numeral characteristics of the karyotype of small-scaled Damascus barbel.

Chromosome pair	Short arm (µm)	Long arm (µm)	Total length (µm)	AR[a]	RL[b] (%)	Cl[c] (%)	Classification *
1	1.42	1.62	3.04	1.14	1.57	46.68	M
2	1.32	1.53	2.85	1.16	1.47	46.27	M
3	1.31	1.52	2.84	1.16	1.46	46.35	M
4	1.22	1.40	2.63	1.15	1.35	46.58	M
5	0.98	1.52	2.50	1.55	1.29	39.23	M
6	1.12	1.31	2.43	1.17	1.25	46.09	M
7	1.03	1.40	2.43	1.36	1.26	42.33	M
8	0.89	1.13	2.03	1.27	1.04	44.00	M
9	0.96	1.03	1.98	1.08	1.02	48.19	M
10	1.45	2.66	4.10	1.83	2.12	35.31	SM
11	1.31	2.25	3.56	1.72	1.83	36.71	SM
12	1.08	2.34	3.42	2.17	1.76	31.58	SM
13	1.21	2.16	3.37	1.79	1.74	35.90	SM
14	0.91	2.37	3.28	2.60	1.69	27.75	SM
15	0.97	2.27	3.24	2.33	1.67	30.00	SM
16	1.14	2.01	3.14	1.77	1.62	36.16	SM
17	0.84	1.96	2.80	2.35	1.44	29.89	SM
18	1.01	1.76	2.76	1.74	1.42	36.48	SM
19	0.99	1.76	2.75	1.78	1.42	35.95	SM
20	0.86	1.87	2.74	2.17	1.41	31.58	SM
21	0.84	1.85	2.69	2.22	1.39	31.10	SM
22	0.88	1.80	2.69	2.04	1.39	32.91	SM
23	0.72	1.93	2.65	2.67	1.37	27.26	SM
24	0.84	1.80	2.64	2.15	1.36	31.72	SM
25	0.86	1.73	2.59	2.00	1.34	33.36	SM
26	0.76	1.74	2.50	2.29	1.29	30.38	SM
27	0.65	1.81	2.46	2.77	1.27	26.51	SM
28	0.70	1.70	2.40	2.42	1.24	29.21	SM
29	0.82	1.57	2.39	1.90	1.23	34.44	SM
30	0.62	1.73	2.35	2.80	1.21	26.29	SM
31	0.63	1.68	2.31	2.65	1.19	27.37	SM
32	0.75	1.55	2.30	2.06	1.19	32.71	SM
33	0.74	1.54	2.28	2.09	1.18	32.37	SM
34	0.75	1.49	2.24	1.99	1.15	33.47	SM
35	0.55	1.55	2.10	2.82	1.08	26.18	SM
36	0.60	1.50	2.10	2.48	1.08	28.71	SM
37	0.61	1.47	2.07	2.42	1.07	29.28	SM
38	0.56	1.45	2.01	2.58	1.04	27.93	SM
39	0.68	1.27	1.95	1.88	1.00	34.74	SM
40	0.95	3.03	3.98	3.20	2.05	23.83	ST
41	0.96	2.96	3.92	3.09	2.02	24.74	ST
42	0.67	3.12	3.79	4.65	1.95	17.70	ST
43	0.76	2.49	3.24	3.28	1.67	23.34	ST
44	0.76	2.51	3.27	3.29	1.69	23.31	ST
45	0.43	2.67	3.10	6.20	1.60	13.89	ST

Continued Table 2:

Chromosome pair	Short arm (μm)	Long arm (μm)	Total length (μm)	Ara	RLb (%)	Clc (%)	Classification*
46	0.62	2.47	3.10	3.96	1.60	20.16	ST
47	0.64	2.25	2.89	3.50	1.49	22.24	ST
48	0.56	2.26	2.82	4.07	1.45	19.71	ST
49	0.59	2.10	2.68	3.58	1.38	21.81	ST
50	0.57	1.95	2.52	3.43	1.30	22.58	ST
51	0.54	1.93	2.47	3.55	1.28	21.97	ST
52	0.42	2.02	2.45	4.77	1.26	1732	ST
53	0.37	1.94	2.31	5.24	1.19	16.04	ST
54	0.33	1.95	2.28	5.95	1.18	14.39	ST
55	0.36	1.91	2.28	5.28	1.17	15.93	ST
56	0.37	1.81	2.18	4.91	1.12	16.92	ST
57	0.46	1.67	2.13	3.61	1.10	21.71	ST
58	0.29	1.76	2.05	6.04	1.06	1421	ST
59	0.34	1.71	2.05	4.99	1.06	16.69	ST
60	0.38	1.65	2.02	4.35	1.04	18.67	ST
61	0.29	1.65	1.94	5.67	1.00	14.99	ST
62	0.00	3.53	3.53	∞	1.82	0.00	A
63	0.00	3.47	3.47	∞	1.79	0.00	A
64	0.00	2.81	2.81	∞	1.45	0.00	A
65	0.00	2.65	2.65	∞	1.37	0.00	A
66	0.00	2.55	2.55	∞	1.31	0.00	A
67	0.00	2.44	2.44	∞	1.26	0.00	A
68	0.00	2.18	2.18	∞	1.12	0.00	A
69	0.00	2.06	2.06	∞	1.06	0.00	A
70	0.00	1.96	1.96	∞	1.01	0.00	A
71	0.00	1.77	1.77	∞	0.91	0.00	A
72	0.00	1.75	1.75	∞	0.90	0.00	A
73	0.00	1.62	1.62	∞	0.84	0.00	A
74	0.00	1.56	1.56	∞	0.80	0.00	A
75	0.00	1.54	1.54	∞	0.79	0.00	A

[a]: Arm ratio, [b]: Relative length, [c]: Centromic index, [d]: The chromosomes (75 pairs) are classified as M, metacentric; SM, submetacentric; ST, subtelocentric; A, acrocentric; according to Levan *et al.* (1964). Refer to the material and methods for detailed information.

Table 3: Some recent studies on karyotype of *Capoeta* spp. from different rivers/basins.

Species	River/Basin	2n	Classification*	NF	References
C. trutta	Tigris River	150	70M/SM + 80ST/A	220	Demirok and Ünlü, 2001
C. capoeta umbla	Tigris Rriver	150	86M/SM + 64ST/A	236	Demirok and Ünlü, 2001
C. damascina	Wadi Karak	148	78M/SM + 32ST + 38A	258	Gorshkova *et al*., 2002
	Stream/Dead Sea	149-150	76M/SM + 24ST + 49-50A	250	
		150-154	76M/SM + 32-34ST + 42-44A	260	
C. capoeta gracilis	Sefidrood River /Caspian Sea	150	24M + 60SM + 14ST + 52T	234	Darestani *et al*., 006
C. capoeta gracilis	Madarsoo River /Caspian Sea	150	24M + 56SM + 14ST + 56T	230	Darestani *et al*., 2006
C. damascina	Monj Rriver /Tigris	150	18M + 60SM + 44ST + 28A	228	Present study

*- M, metacentric; SM, submetacentric; ST, subtelocentric; A, acrocentric.

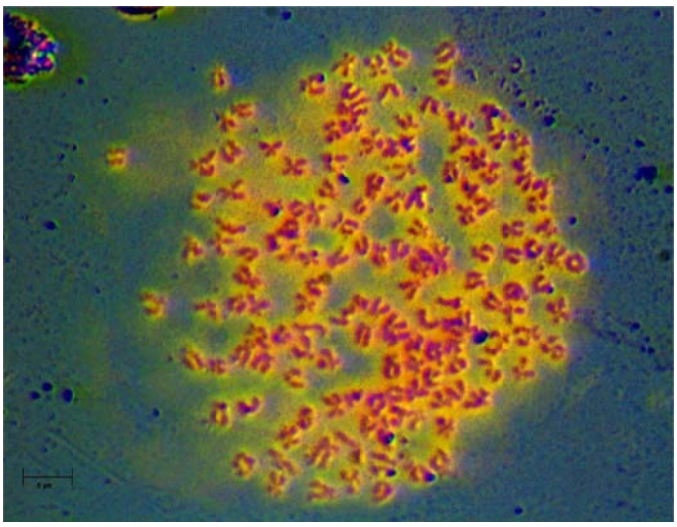

Figure 1: Chromosome spread (2n=150) of head kidney tissue from small-scaled Damascus barbel, *Capoeta damascina*. Bar = 5 µm.

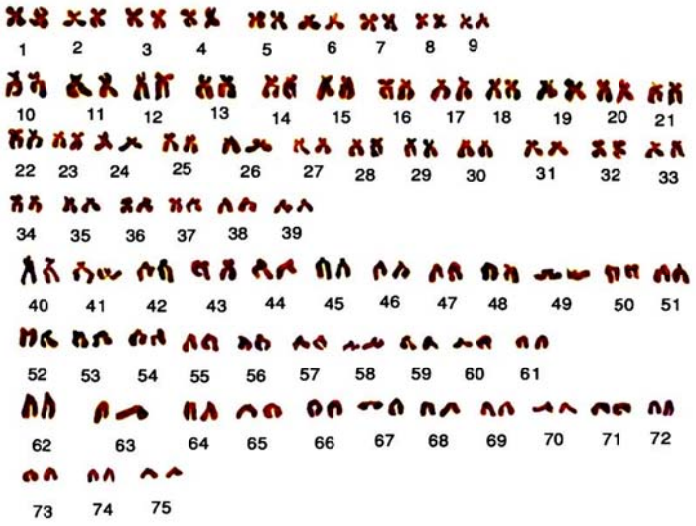

Figure 2: Standard karyotype of small-scaled Damascus barbel, *Capoeta damascina* (Valenciennes, 1842) (2n=150). 1-9 (metacentric); 10-39(submetacentric); 40-61 (subtelocentric) and 62-75 (acrocentric) according to Levan et al. (1964).

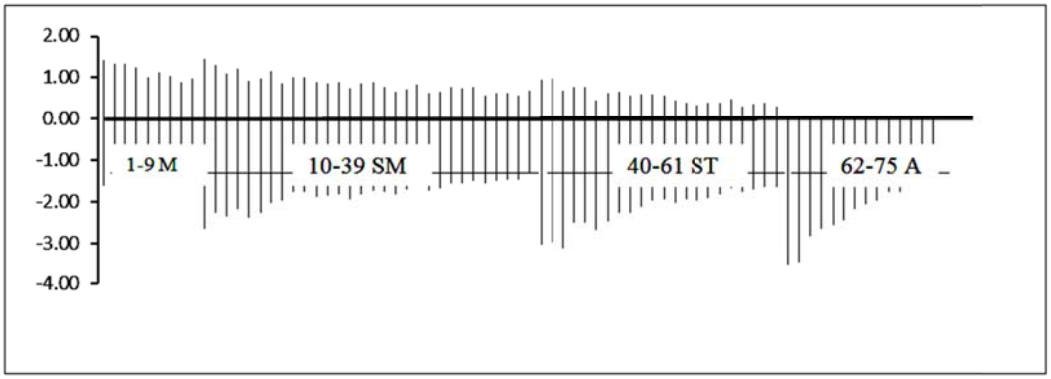

Figure 3: Standard ideogram of small-scaled Damascus barbel, *Capoeta damascina*. The longest and shortest chromosomes are number 10 (SM) and the last one (no.75, A) with total length of 4.10 and 1.54 µm, respectively.

Other available reports about diploid chromosome numbers of *Capoeta* species from different basins indicated 2n as 148 in *C. damascina* from the Dead Sea Basin, the kingdom of Jordan (Gorshkova *et al*., 2002), 2n=150 in *Capoeta trutta* and *C. capoeta* from Tigris River, Turkey (Kiliç-Demirok and Ünlü, 2001) and *C. C. grasilis* from the Caspian Sea Basin, Iran (Darestani *et al*., 2006). Polymorphism in chromosome number as well as its classification is very common phenomena in fish (Gorshkova *et al*., 2002; Nasri *et al*., 2010; Table 3). These differences may be caused by evolutionary phenomena, exposure to contaminated water, hybridization and meiotic and mitotic disjunctions (Hartly, 1998). Chromosomal rearrangements such as pericentric inversion and Robertsonian fusions are other factors which can vindicate different chromosome classification and NF for the same species.

Comparison of these data might relay close phyletic connections of *Capoeta* genus in different areas of the Middle East, but it is necessary to consider the chromosome number of other species of this genus. Because of the large number of small chromosomes in all studied *Capoeta* species including *C. damascina*, it would be recommended to use other staining techniques such as G- or C-banding or Ag-NOR. These data would be more helpful in cytotaxonomy and phylogenetic studies of *Capoeta*.

It could be concluded that *C. damascina* from the Monj River, Tigris Basin has 2n=150 chromosome and could be categorized as a hexaploid species. However more detailed studies would be recommended to find out the ploidy origin and exact chromosome kind and NF.

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