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Morphological variations among *Chondrostoma regium* populations in the Tigris River drainage

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Abstract. This study was conducted to determine suitable characters for separating eight different king nase *Chondrostoma regium* populations in different aquatic ecosystems from western Iran (Hamedan, Kermanshah and Ilam provinces). During the year 2011, a total of 131 individuals were caught using electrofishing and the specimens were evaluated for 26 morphometric and 7 meristic characters. Morphometric characteristics were standardized before analysis for reduce error of allometric growth. The obtained results of variance analysis (one-way ANOVA) and the non-parametric Kruskal-Wallis test showed that twenty modified morphometric and five meristic characteristics within the sampled were significantly different ($p > 0.05$), that indicates a relatively high phenotypic diversity in studied areas. Using principal component analysis (PCA) by PAST and SPSS software revealed populations separation. Within the morphometric characters, six factors accounted for about 65% of phenotypic variation between individuals, in the case of meristic characters, three factors accounted for 60% of variation within populations. The results of this study showed that morphometric characters which are more affected by habitat, could largely separate the populations than the meristic characters.

Key Words: Cyprinidae, morphometric variation, king nase, Persian Gulf basin, Iran.

Introduction. The study of morphological characters, whether morphometric or meristic, with the aim of defining or characterizing fish stock units, has for some time been a strong instrumental for determining discreteness of the similar species (Turan et al 2006; Mousavi-Sabet & AnvariFar 2013) and extensively used to identify differences between fish populations (Tzeng 2004). They are measurable features which are helpful for separating closely related genera, species and even populations within them (Cadrin 2000). In general, a fish stock is a local population adapted to a particular environment, having genetic differences from other stocks (Cadrin 2000; Tzeng 2004). Although genetic differences between stocks are a condition of this definition, phenotypic variations still continue to have an important role in stock identification among groups of fish (Swain & Foote 1999). Also, in order to rational and effective fishery management of resources it is important to know the stock structure of an explored species, as each stock must be managed separately to optimize their yield (Meng & Stocker 1984). It should be done for both large and small fishes.

The genus *Chondrostoma* of cyprinid fishes contains about 26 species, of which 2 species (*Chondrostoma regium* and *C. cyri*) occur in Iran (Coad 2014). This genus has an European and Middle Eastern distribution. Its relationships to other taxa are poorly known. *C. regium* is found in the Tigris-Euphrates basin and the Mediterranean basins of southeastern Turkey and the northern Levant. In Iran it is found in the Tigris River drainage. Additional localities are springs near Kermanshah and the Gamasiab River (Coad 2014). This species is found in both rivers and lakes but habitat requirements have not been studied in Iran. The species has been caught and used for food in Khuzestan. Ünlü (2006) reports that this species prefers stone grounds and still waters in rivers and lakes in Turkey. The morphometric characters between male and female sexes in this species are not different (Coad 2014). However biology of this species in Iran needs study and this may reveal conservation needs. Also the morphological studies of this species has not been investigated in Iran. Therefore, the propose of this study was to use a set of morphometric and meristic characters as a first step in analyzing potential fragmentation of *C. regium* populations among the major fishing grounds in the Tigris River drainage, and to examine whether specific ecological constraints, due to geographic variation, could affect the formation of stock separation for this species.

Material and Method. A total of 131 individuals of *C. regium* were collected from eight sampling sites, including the Seimareh River (47°25′25″N, 33°10′22″E; 14 individuals), Hashilan Wetland (46°52′42″N, 34°34′49″E; 13 individuals), Dinevar River (47°27′25″N, 36°62′33″E; 18 individuals), Alvand River (45°34′26″N, 34°30′10.59″E; 18 individuals), Kharchangroud River (47°49′25″N, 34°22′33″E; 16 individuals), Gamasiab River (48°58′25″N, 34°06′59″E; 21 individuals), Dehno River (48°20′21″N, 34°14′10″E; 18 individuals) and Aran River (47°55′30″N, 34°24′48″E; 13 individuals) in November 2011 by electrofishing with 200–300V (Figure 1).

The sampled fishes were fixed in 10% formaldehyde at the sampling site and transported to the Laboratory for further studies. A total of twenty nine morphometric characters were measured. To keep the morphological mistake stable, measurements were performed by the same person. The morphometric characters included: total body length (TL), forkal body length (FL), standard body length (SL), preanal length (PA), postanal length (POA), predorsal length (PD), postdorsal length (POD), head length (HL), head width (HW), head depth (HD), posthead length (PHL), preorbital length (prO), postorbital length (poO), inter-orbital distance (iOD), eye diameter (ED), dorsal fin base (DFB), dorsal fin height (DFH), anal fin base (AFB), anal fin height (AFH), pectoral fin length (PFL), ventral fin length (VFL), body depth (BD), minimum body depth (MBD), pectoral–anal distance (P–A), pectoral–ventral distance (P–V), ventral–anal distance (V–A), caudal fin length (CFL), caudal peduncle length (CPL) and mouth width (MW). In addition, seven meristic characters were numerated in each sample by use of stereomicroscope. Acronyms used for meristic characteristics are: dorsal fin soft rays (D1), dorsal fin spines (D2), anal fin soft rays (A1), anal fin spines (A2), lateral line scales (ll), scales above lateral line (squ.sup) scales below lateral line (squ.inf). Body weight (g) was taken on a digital balance with 0.01 g accuracy and morphometric measurements were made by digital caliper with an accuracy of 0.01 mm.

As variation should be attributable to body shape differences, and not related to the relative size of the fish, an allometric method (Elliott et al 1995) was used to remove size-dependent variation in morphometric characters:

$$Madj = M (Ls / L0)b$$

where M is the original measurement, Madj the size adjusted measurement, L0 the standard length of the fish, Ls the overall mean of the standard length for all fish from all samples in each analysis, and b was estimated for each character from the observed data as the slope of the regression of log M on log L0 using all fish in any group. The sex of specimens was determined macroscopically, and there were no significant differences of tested variables between the sexes within the same stock. Univariate analysis of variance (ANOVA) was performed for each morphometric character to evaluate the significant differences among the eight populations (Garcia-Rodriguez et al 2010). Also principal component analysis (PCA) and cluster analysis (CA) by adopting the Euclidean square distance as a measure of dissimilarity and the UPGMA (Unweighted Pair Group Method with Arithmetical average) method as the clustering algorithm (Yakubu et al 2011) were employed to discriminate the eight populations. To test whether meristic characters were significantly different among populations, the non-parametric Kruskal-Wallis test was used. Statistical analyses for morphometric data were performed using the SPSS 16 software, PAST 2.17c software and Excel 2013 software package.

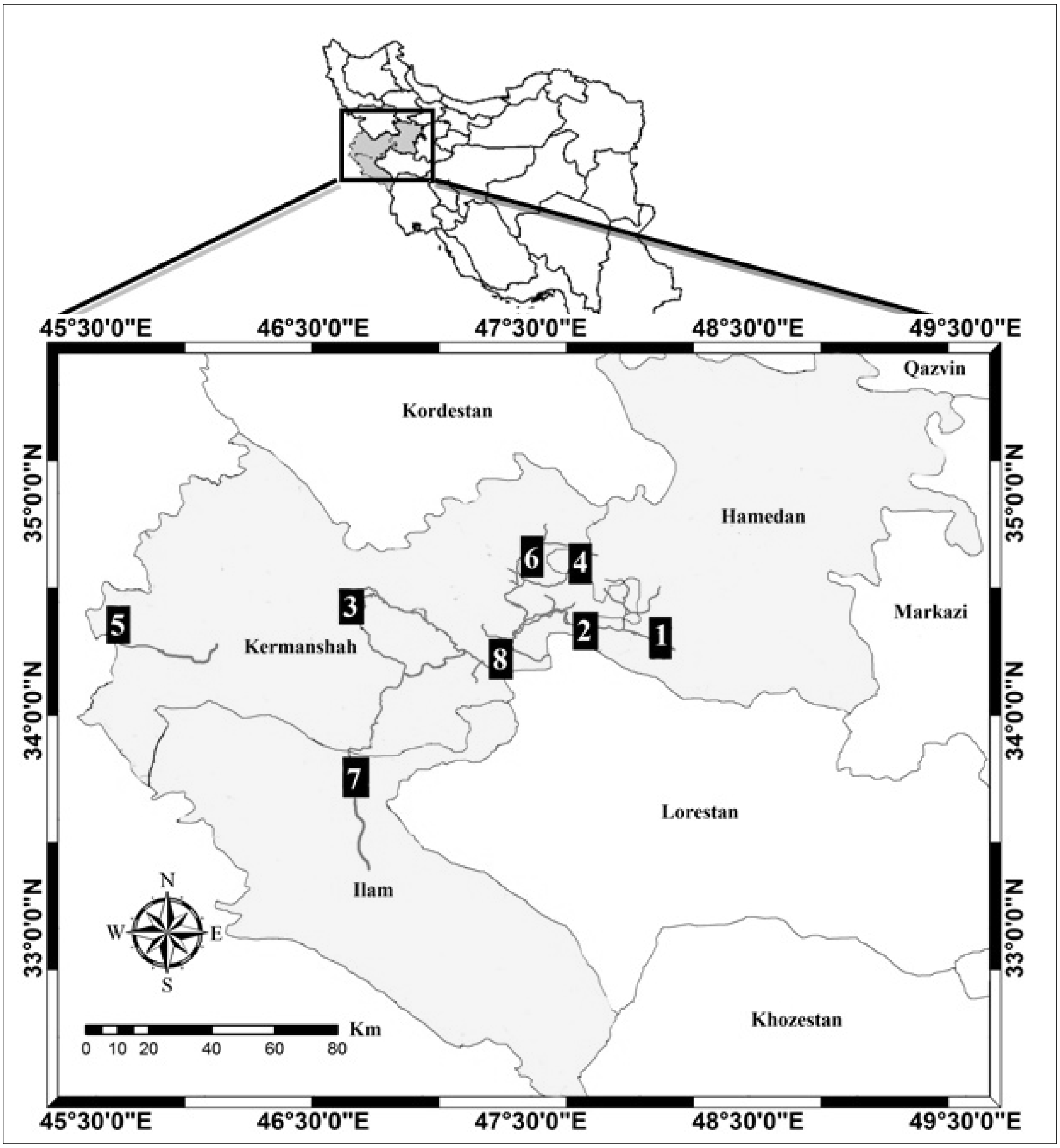

Figure 1. Sampling sites of *C. regium* in the Tigris River drainage. 1: Dehno River, 2: Kharchangroud River, 3: Hashilan Wetland, 4: Aran River, 5: Alvand River, 6; Dinevar River, 7: Seimareh River and 8: Gamasiab River.

Results. ANOVA revealed significant differences among means of the eight studied populations of *C. regium* for 20 of the 29 measured morphological characteristics ($p < 0.05$) (Table 1) which these 20 characters were subjected to multivariate analyses. As the HL, prO, poO and ED were not significantly different ($p > 0.05$), they were excluded. The non-parametric Kruskal-Wallis test revealed significant differences for 5 of the 7 meristic characters ($p < 0.05$) (Table 2).

Table 1

Descriptive data [mean ± standard deviations (SD) (mm), minimum - maximum (mm) and body weight (g)] of 29 morphometric characters calculated for the eight studied populations of *C. regium* and significant differences among the localities

Characters	*Localities*							
	Gamasiab	*Dehno*	*Aran*	*Dinevar*	*Alvand*	*Seimareh*	*Hashilan*	*Kharchangroud*
TL	139.97±12.03	158.58±20.37	160.53±24.59	166.52±28.89	169.14±24.39	159.88±32.82	75.29±65.167	166.98±24.57
	164.94-121.3	93.73-113.84	96.80-191.08	95.44-207.6	138.68-213.92	109.33-229.92	128.82-220.84	111.51-201.9
FL	129.14±11.86	147.01±18.22	150.13±23.75	153.84±27.79	157.57±23.34	148.19±31.49	33.29±91.155	154.43±23.93
	110.84-153.38	104.5-173.78	89.34-183.74	87.60-190.44	127.27-200.14	100.83-216.47	116.46-210.10	100.78-87.82
SL	117.83±10.89	134.58±17.22	138.05±21.92	140.34±26.21	144.39-21.71	136.37±30.32	52.26±09.142	140.83±22.21
	100.78-139.80	93.50-160.11	84.24-169.92	77.77-176.48	115.29-184.45	92.36-206.69	106.70-187.23	90.34-172.56
PA	82.27^{bc}±7.56	93.46^{bc}±12.09	95.19^{c}±14.53	96.23^{cd}±17.01	100.64±14.82	96.11±20.09	99.65^{a}±18.23	95.85^{d}±15.48
	70.18-99.86	66.25-113.95	58.66-116.33	55.34-119.32	80.35-127.26	65.42-140.48	76.54-132.13	62.60-117.07
POA	46.54^{b} ±4.00	52.71^{b} ±6.26	51.76^{c} ±8.02	57.58^{a} ±9.85	55.26^{bc} ±8.50	52.12^{bc} ±10.41	55.33^{b} ±9.48	56.97^{a} ±8.58
	41.00-53.14	40.50-66.40	35.29-65.08	34.30-75.69	43.77-70.44	37.59-73.31	44.10-70.75	39.57-73.60
PD	57.89^{bc} ±5.20	65.67^{bc} ±7.93	66.80^{bc} ±9.58	68.04^{bc} ±11.77	70.27^{ab} ±8.97	67.68^{a} ±14.01	69.14^{ab} ±12.36	67.28^{c} ±10.39
	47.75-68.21	47.93-80.23	41.50-79.81	40.76-84.49	57.33-84.50	45.93-96.99	53.71-90.44	45.47-84.17
POD	69.13^{a}±6.53	76.68^{b}±9.70	77.79^{bc}±12.26	81.82^{a}±13.81	81.34^{ab}±11.79	75.55^{a}±11.79	79.88^{bc}±13.87	82.47^{a}±12.49
	58.25-80.58	53.24-91.36	44.22-94.70	47.29103.28	64.50-102.29	64.50-102.29	58.79-108.52	55.68-102.09
HL	26.27±2.17	29.38±3.42	29.84±4.19	30.46±5.33	31.38±3.60	29.74±5.16	31.58±5.04	30.53±4.92
	23.05-30.76	21.47-36.16	18.30-35.90	18.96-38.31	27.01-38.51	20.71-40.49	25.21-40.69	20.12-37.48
HW	14.52^{bc}±1.31	16.54^{b}±1.91	16.4^{c}±2.38	17.81^{a}±3.50	18.21^{a}±2.25	17.34^{a}±3.62	18.13^{a}±3.02	16.85^{bc}±2.70
	12.30-17.09	12.13-19.44	10.37-20.08	9.72-22.95	15.24-22.49	11.39-25.50	14.40-24.06	11.28-21.60
HD	18.96^{abc}±1.35	20.92^{abc}±2.30	20.92^{bc}±2.70	21.80^{abc}±3.77	22.30^{bc}±2.72	21.32^{c}±3.62	22.17^{a}±3.43	21.20^{c}±3.08
	16.64-21.36	15.55-25.57	14.07-24.48	13.09-27.77	18.48-27.56	15.71-28.79	17.66-30.10	14.68-26.59
PHL	21.53±1.74	24.21±2.95	24.69±3.12	25.17±4.28	25.57±2.81	24.51±4.07	25.44±3.43	24.62±3.68
	18.20-24.86	18.16-32.06	16.49-28.66	15.58-31.67	21.70-29.60	17.58-33.00	20.60-32.24	16.70-29.83
prO	7.79±0.88	9.00±1.17	9.01±1.18	9.16±1.71	9.43±1.08	9.17±1.42	9.41±1.39	8.98±1.38
	6.65-9.34	6.76-11.56	5.81-10.70	5.61-12.08	8.25-11.05	6.49-11.83	7.53-11.46	6.05-10.73
poO	12.31±1.39	14.12±1.86	14.31±2.42	14.98±3.05	14.92±2.56	14.57±30.6	15.33±2.68	14.81±2.78
	9.48-14.61	9.24-17.73	8.23-18.65	8.81-20.03	9.54-20.20	9.75-21.68	12.07-20.71	9.58-19.15
iOD	8.45^{d}±0.84	10.16^{abc}±1.26	10.10^{cd}±1.71	10.73^{a}±2.00	10.60^{bcd}±1.66	10.32^{ab}±2.15	10.86^{a}±1.93	10.31^{bcd}±1.75
	7.30-10.08	7.49-12.56	5.91-12.68	6.05-13.44	7.41-13.29	6.66-15.56	8.20-14.11	6.84-13.96
ED	5.45±0.45	6.03±0.71	6.14±0.68	6.10±1.06	6.39±0.79	5.80±0.98	6.37±0.71	6.16±0.85
	4.86-6.35	4.88-7.62	4.35-7.40	3.96-7.57	5.39-8.01	3.76-7.42	5.46-7.92	4.38-7.25
DFB	23.93^{ab}±1.87	26.00^{abc}±2.69	25.57^{c}±2.99	27.92^{a}±4.66	27.5^{abc}±2.94	25.56^{bc}±3.58	27.92^{a}±4.72	27.78^{a}±4.38
	20.97-27.13	20.47-31.32	17.96-31.01	16.83-35.74	23.28-32.76	19.04-33.00	22.60-39.34	19.65-35.63
DFH	14.92^{abc}±1.34	16.35^{c}±2.64	16.51^{c}±2.84	18.32^{ab}±4.07	17.99^{abc}±2.58	16.71^{bc}±3.50	18.78^{a}±4.85	16.92^{c}±2.77
	12.59-17.20	11.10-21.04	10.00-21.26	9.30-23.95	14.69-23.63	11.95-24.29	13.00-26.69	10.86-22.30
AFB	18.46^{ab}±1.34	19.97^{b}±2.37	20.52^{b}±3.20	21.82^{a}±3.64	21.50^{ab}±2.89	20.04^{b}±3.37	21.37^{ab}±4.10	22.00^{a}±3.78
	16.37-21.63	15.10-24.48	12.45-24.54	13.38-27.51	18.32-26.27	14.46-26.98	16.23-30.15	15.48-29.32
AFH	12.50±1.62	13.70±2.11	14.65±3.29	14.72±3.14	15.23±3.41	14.45±3.57	15.45±3.58	14.78±2.75
	9.06-16.07	9.72-17.04	7.76-19.89	8.03-20.01	10.11-21.54	9.40-22.84	8.90-20.60	9.68-21.06
PFL	19.46^{abc}±1.62	21.55^{abc}±2.53	21.59^{c}±3.53	23.09^{ab}±4.14	23.39^{ab}±2.80	21.60^{bc}±4.00	23.39^{a}±4.29	23.30^{a}±3.69
	17.35-23.20	16.83-27.41	14.26-28.03	14.39-28.97	18.82-28.75	14.83-30.21	18.86-33.22	16.18-31.14
VFL	18.00^{ab}±1.79	19.89^{abc}±2.94	19.56^{c}±3.03	20.95^{ab}±3.71	21.20^{abc}±3.13	19.66^{bc}±3.61	20.93^{abc}±4.30	21.53^{a}±3.47
	14.44-20.94	14.46-27.10	13.34-25.09	12.0126.76	17.38-28.24	13.92-27.54	15.92-30.94	14.46-27.07
MBD	28.11^{c}±2.87	33.14^{b}±3.95	31.14^{cd}±4.19	34.37^{b}±6.06	35.79^{b}±5.73	34.04^{b}±7.82	36.48^{b}±6.03	31.19^{d}±3.34
	23.20-35.25	24.73-38-73	19.93-35.73	19.12-42.50	28.63-44.23	22.58-53.43	29.05-45.82	21.87-39.16
BD	10.93^{b}±1.36	13.2^{a}±1.89	12.65^{b}±1.90	13.72^{a}±2.88	14.08^{a}±2.22	13.58^{a}±2.85	14.08^{a}±2.56	12.85^{b}±2.00
	8.95-13.73	9.22-16.20	8.64-15.39	7.17-18.85	11.29-18.48	9.03-20.33	10.53-18.37	8.93-17.34
P–A	56.44^{bc}±6.40	63.89^{c}±9.07	65.69^{c}±10.75	66.09^{c}±11.38	69.62^{abc}±11.80	66.99^{a}±15.07	69.47^{ab}±14.11	64.70^{d}±9.74
	48.33-71.59	44.15-76.88	39.06-81.79	37.96-83.60	53.89-91.63	43.91-99.95	52.12-95.11	43.28-78.13
P–V	33.03^{abc}±3.51	37.17^{c}±5.41	37.96^{c}±6.48	39.08^{bc}±7.09	40.53^{abc}±6.81	39.08^{ab}±9.08	40.93^{a}±7.56	37.29^{d}±5.77
	27.67-41.30	26.18-46.69	21.95-50.36	22.07-49.96	31.91-53.12	25.23-59.09	31.15-53.55	24.52-45.81
V–A	23.58^{abc}±3.05	26.78^{abc}±4.11	27.68^{abc}±4.58	27.06^{bc}±4.28	29.46^{ab}±5.28	28.32^{a}±6.60	28.56^{abc}±6.44	27.11^{c}±4.39
	20.12-30.48	18.32-32.43	17.21-35.16	15.36-33.52	21.44-39.07	18.67-42.45	20.77-42.78	19.20-34.21
CFL	25.11^{bc}±1.54	27.79^{bc}±3.56	27.44^{c}±3.59	30.19^{a}±4.39	29.30^{bc}±3.52	27.40^{c}±5.32	29.80^{ab}±4.87	29.99^{ab}±4.75
	22.61-27.80	22.11-36.55	20.00-32.58	18.54-36.50	23.33-34.24	19.66-37.57	24.37-41.19	20.95-37.76
CPL	23.65^{bc}±2.80	27.69^{bc}±3.95	27.80^{bc}±5.00	30.55^{a}±6.92	29.34^{bc}±5.50	27.30^{c}±6.75	28.82^{bc}±5.95	29.90^{ab}±5.71
	17.96-27.58	19.57-34.14	17.57-37.19	16.69-48.79	22.39-38.92	18.37-42.45	21.61-40.83	19.5-41.58
MW	5.97^{bc}±0.68	6.83^{abc}±0.89	6.67^{c}±1.11	7.04^{abc}±1.28	7.32^{ab}±0.88	6.85^{abc}±1.31	7.39^{a}±1.16	6.90^{bc}±1.06
	4.93-7.36	5.06-8.45	4.21-7.97	4.35-8.93	6.31-8.79	4.56-10.04	5.63-9.38	4.50-8.39
W	31.23-9.15	47.93±15.90	46.87±18.00	59.89±30.26	64.15±28.97	58.67±39.34	68.92±35.20	48.04±18.75
	18.23-52.59	17.05-74.32	10.75-81.37	9.46-117.96	32.24-124.38	17.39-170.79	30.78-135.45	13.92-85.5

Note: Values followed by different letters within the same row are significantly different ($p < 0.05$).

Table 2

Descriptive data [mean ± standard deviations (SD) (mm) and minimum - maximum (mm)] of 7 meristic characteristics calculated for the eight studied populations of *C. regium* and significant differences among the localities

Characters	Localities							
	Kharchangroud	*Hashilan*	*Seimareh*	*Alvand*	*Dinevar*	*Aran*	*Dehno*	*Gamasiab*
L.L	$55.31^{a}\pm1.35$	$69.23^{a}\pm1.59$	$67.14^{b}\pm0.95$	$62.89^{c}\pm2.00$	$58.28^{d}\pm1.23$	$54.85^{e}\pm1.07$	$57.72^{d}\pm0.96$	$58.05^{d}\pm1.36$
	54.00-57.00	66.00-71.00	66.00-69.00	60.00-67.00	57.00-60.00	54.00-57.00	56.00-59.00	57.00-61.00
squ.inf	$10.06^{bc}\pm0.57$	$8.85^{d}\pm0.38$	$8.86^{d}\pm0.36$	$11.17^{a}\pm0.62$	$9.72^{c}\pm0.46$	$10.15^{b}\pm0.69$	$9.78^{bc}\pm0.43$	$9.90^{bc}\pm0.44$
	9.00-11.00	8.00-9.00	8.00-9.00	10.00-12.00	9.00-10.00	9.00-11.00	9.00-10.00	9.00-11.00
squ.sup	$6.88^{a}\pm0.34$	$5.92^{bc}\pm0.49$	$6.14^{b}\pm0.36$	$6.11^{bc}\pm0.32$	$5.89^{bc}\pm0.32$	$6.69^{a}\pm0.48$	$5.83^{c}\pm0.38$	$5.95^{bc}\pm0.22$
	6.00-7.00	5.00-7.00	6.00-7.00	6.00-7.00	5.00-6.00	6.00-7.00	5.00-6.00	5.00-6.00
D2	3.13 ± 0.34	3.08 ± 0.28	3.07 ± 0.27	3.06 ± 0.24	3.06 ± 0.24	3.00 ± 0.00	3.06 ± 0.24	3.05 ± 0.22
	3.00-4.00	3.00-4.00	3.00-4.00	3.00-4.00	3.00-4.00	3.00-3.00	3.00-4.00	3.00-4.00
A2	3.06 ± 0.25	3.08 ± 0.28	3.07 ± 0.27	3.06 ± 0.24	3.06 ± 0.24	3.00 ± 0.00	3.00 ± 0.00	3.05 ± 0.22
	9.00-10.00	3.00-4.00	3.00-4.00	3.00-4.00	3.00-4.00	3.00-3.00	3.00-3.00	3.00-4.00
D1	$9.94^{a}\pm0.25$	$9.77^{a}\pm0.44$	$7.86^{c}\pm0.36$	$8.06^{c}\pm0.24$	$9.22^{b}\pm0.43$	$9.77^{a}\pm0.44$	$9.28^{b}\pm0.46$	$9.24^{b}\pm0.54$
	9.00-10.00	9.00-10.00	7.00-8.00	8.00-9.00	9.00-10.00	9.00-10.00	9.00-10.00	8.00-10.00
A1	$10.00^{b}\pm0.00$	$20.54^{a}\pm0.52$	$8.21^{c}\pm0.89$	$9.89^{b}\pm0.32$	$9.94^{b}\pm0.73$	$9.85^{b}\pm0.38$	$9.94^{b}\pm0.73$	$9.86^{b}\pm0.65$
	10.00-10.00	10.00-11.00	7.00-9.00	9.00-10.00	9.00-11.000	9.00-10.00	9.00-11.00	9.00-11.00

Note: Values followed by different letters within the same row are significantly different ($p < 0.05$).

For determine which morphometric measurement most effectively differentiates populations, the contributions of variables to principal components (PC) were examined. To examine the suitability of the data for PCA, Bartlett's Test of sphericity and the Kaiser–Meyer–Olkin (KMO) measure were performed. The Bartlett's Test of sphericity tests the hypothesis that the values of the correlation matrix equal zero and the KMO measure of sampling adequacy tests whether the partial correlation among variables is sufficiently high (Nimalathasan 2009). The KMO statistics varies between 0 and 1. Kaiser (1974) recommends that values greater than 0.5 are acceptable. In this study, the value of KMO for overall matrix is 0.722 and the Bartlett's Test of sphericity is significant ($p < 0.01$). The results of KMO and Bartlett's suggest that the sampled data is appropriate to proceed with a factor analysis procedure. Principal component analysis of 20 morphometric measurements extracted 6 factors with eigenvalues > 1, explaining 65% of the variance (Table 3). The first principal component (PC1) accounted for 16.8% of the variation, the second principal component (PC2) for 14.1%, the third principal component (PC3) for 10.8%, the four principal component (PC4) for 10.7%, the five principal component (PC5) for 7.4% and the six principal component (PC6) for 5.2% (Table 3) and the most significant loadings on PC1 were HL and PHL, on PC2 were PFL, VFL and AFL, on PC3 were P–A and P–V, on PC4 was MBD, on PC5 were PA and CPL and on PC6 was DFH. In this analysis, the characteristics with an eigenvalues exceeding 1 were included and others discarded. Principal component analysis of 7 meristic measurements extracted 3 factors with eigenvalues >1, explaining 60% of the variance (Table 3). The first principal component (PC1) accounted for 24.4% of the variation and the most significant loadings on PC1 was dorsal fin soft rays.

Visual examination of plots of PC1 and PC2 scores for morphometric characteristic revealed that the specimens of Seimareh River were deviated from Kharchangroud River and Hashilan Wetland and have some degree of overlap with other populations (Figure 2). Also visual examination of plots of PC1 and PC2 scores for meristic characteristic revealed that the specimens of Seimareh River were deviated from other populations (Figure 3).

The dendogram derived from CA of Euclidean square distances among groups of centroids showed two clusters which for morphometric characteristic indicating the Alvand River, Dehno River, Gamasiab River, Aran River, Hashilan Wetland and Seimareh River populations are in the first clade and Kharchangroud River and Dinevar River populations are in the second clade (Figure 4). Also the dendogram derived from CA for meristic characteristic indicating the Dinevar River, Dehno River, Gamasiab River, Seimareh River and Alvand River populations are in the first clade and Kharchangroud River, Hashilan Wetland and Aran River populations are in the second clade (Figure 5).

Table 3
Eigen values, percentage of variance and percentage of cumulative variance of morphometric and meristic measurements for the studied *C. regium* populations in the Tigris River drainage

Factor	*Eigenvalues*	*Percentage of variance*	*Percentage of cumulative variance*
		Morphometric characters	
1	4.361	16.773	16.773
2	3.668	14.109	30.881
3	2.814	10.825	41.706
4	2.782	10.698	52.405
5	1.925	7.403	59.807
6	1.349	5.189	64.996
		Meristic characters	
1	1.710	24.434	24.434
2	1.340	19.149	43.583
3	1.163	16.614	60.198

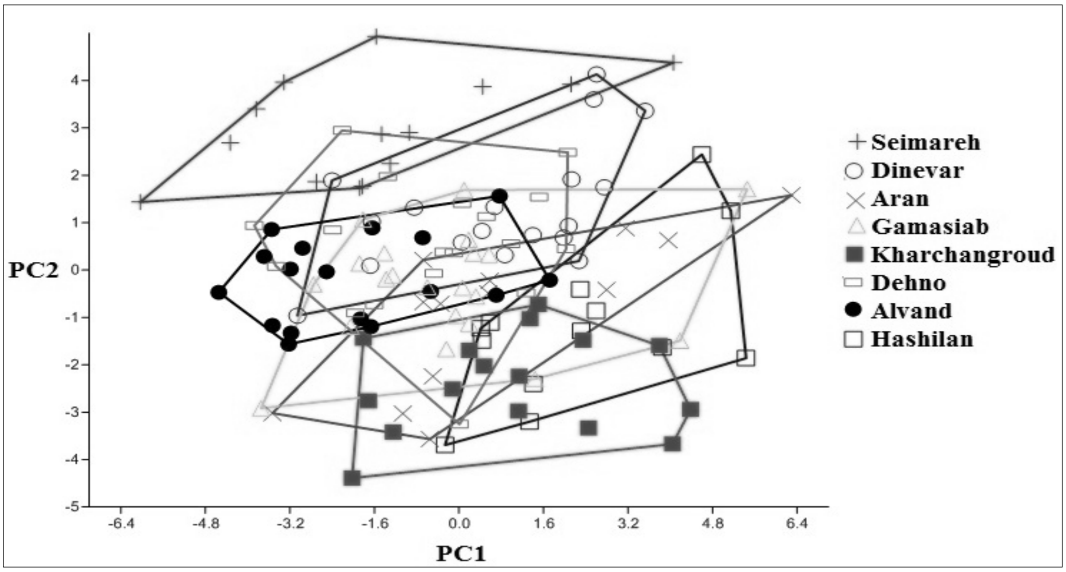

Figure 2. Plot of the factor scores for PC1 and PC2 of morphometric measurements for the studied *C. regium* populations in the Tigris River drainage.

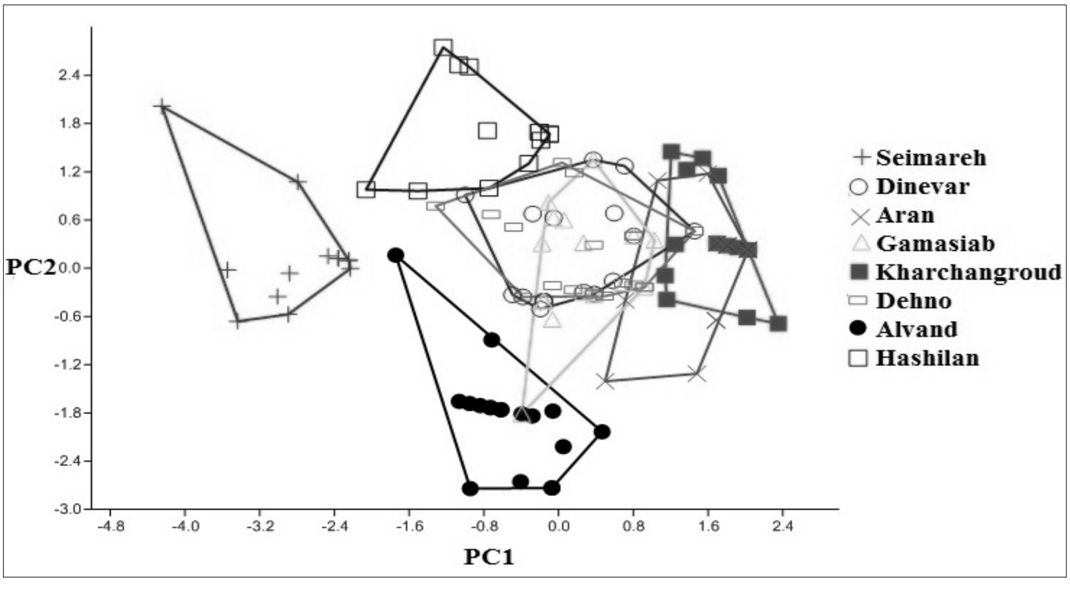

Figure 3. Plot of the factor scores for PC1 and PC2 of meristic measurements for the studied *C. regium* populations in the Tigris River drainage.

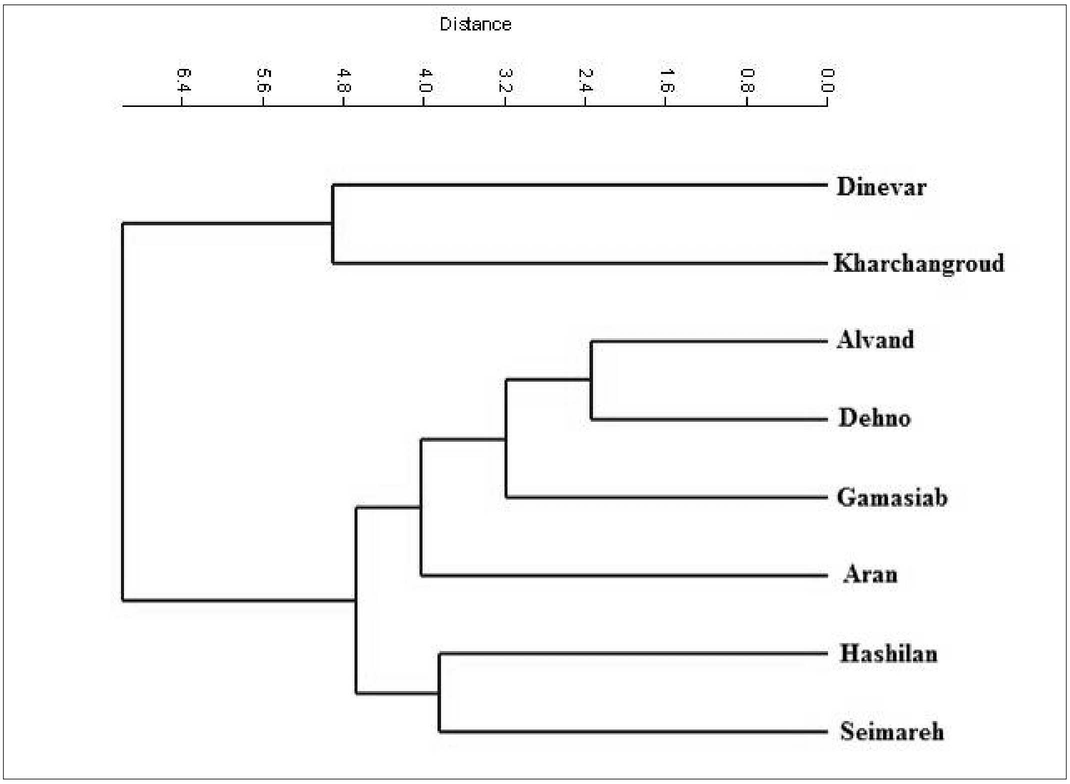

Figure 4. Dendrogram derived from cluster analyses of morphometric measurements on the basis of Euclidean distance for the studied *C. regium* populations in the Tigris River drainage.

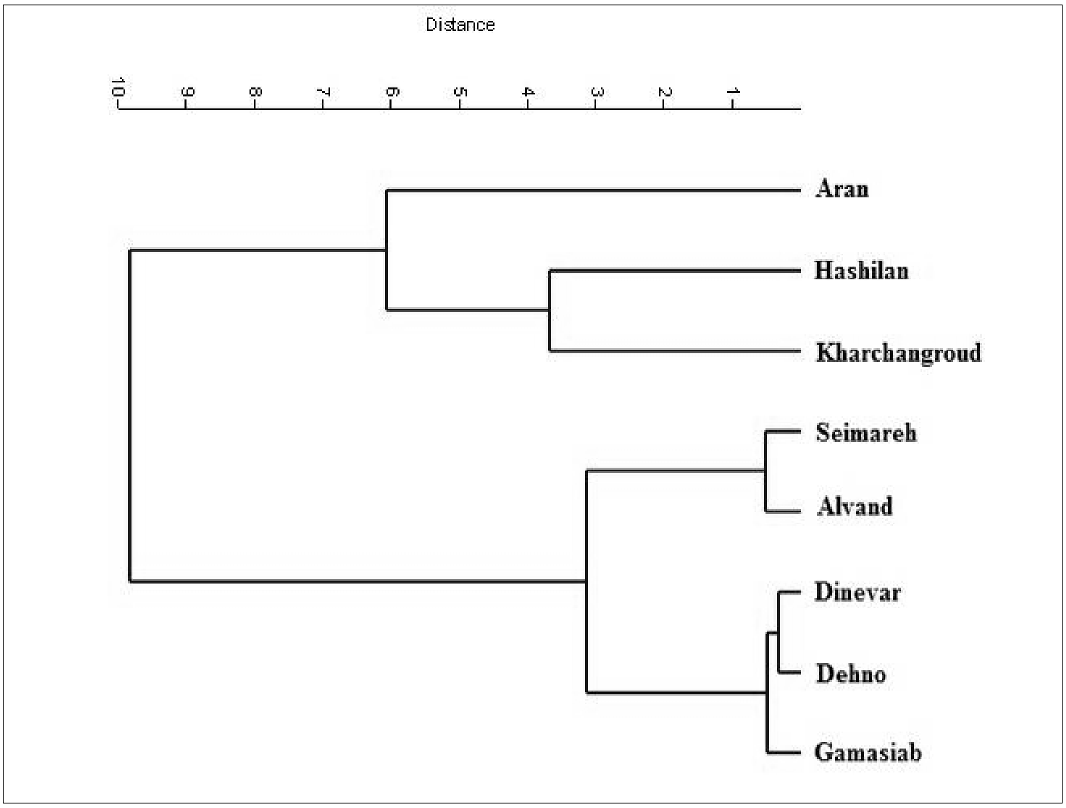

Figure 5. Dendrogram derived from cluster analyses of meristic measurements on the basis of Euclidean distance for the studied *C. regium* populations in the Tigris River drainage.

Discussion. The present study demonstrated an intra-specific morphological variation of the individuals among eight localities. This variation is markedly shown in morphometric characters compared to meristic characters. Studies have shown that morphometric characters are more suitable than meristic characters to describe intra specific variation (Murta 2000). However, in some studies meristic characters have been successfully used for stock identification (Melvin et al 1992). Better separation (in 100% accuracy) of fish in these sampling sites by morphometric characters show that they are more suitable in discrimination of different fish groups.

In Iran several researchers studied morphological differences between different freshwater and marine fish species populations e.g., *Liza aurata*, *Capoeta gracilis*, and genus *Alburnus* and genus *Cobitis* (Kohestan-Eskandari et al 2013; Heidari et al 2013; Khataminejad et al 2013; Habibi et al 2013; Mousavi-Sabet & AnvariFar 2013).

Meristic characters are fixed in early embryonic life of the individual and remain unchanged thereafter. Thus, they respond to environmental factors only a short time period during embryonic development. These could result in wide variations among members of the same and even in different year classes of a single stock (Lindsey 1988). In contrast, morphometric characters varied according to the changing environmental conditions of the habitat throughout their life and the phenotypic plasticity have been shown in many freshwater fish species (Peres-Neto & Magnan 2004). In the present study, meristic counts of all localities samples ranged 8-10 soft rays for the dorsal fin and 8-12 soft rays for the anal fin. Also lateral line scales ranged 50-69 which these results are similar to those reported by Coad (2014) for *C. regium* but for Hashilan Wetland, lateral line scales ranged 66-71.

In the present study, highly significant morphological variations were found among the eight studied populations and these variations were found in fins lengths, which may resulted by water current. In faster current the fishes have longer fins. In non-flowing waters for example Hashilan Wetland, fishes have shorter fins.

The phenotypic discreteness suggests a direct relationship between the extent of phenotypic divergence and geographic separation, which indicates that geographic separation is a limiting factor to migration among stocks. Turan et al (2004) also found similar results for *Liza abu* populations from the Tigris River in Turkey.

Morphometric differences among stocks are expected, because they are geographically separated and may have originated from different ancestors. Therefore, it is not unlikely that obvious environmental variations exist in these eight habitats. Fish are very sensitive to environmental changes and quickly adapt themselves by changing necessary morphometric. It is well-known that morphological characters can show high plasticity in response to differences in environmental conditions, such as food abundance and temperature (Wimberger 1992). In general, fish demonstrate greater variances in morphological traits both within and between populations than any other vertebrates, and are more susceptible to environmentally induced morphological variations (Wimberger 1992).

The multivariate analysis of morphometric characteristics classified the *C. regium* samples in the Tigris River drainage into eight distinct groups, differentiated to varying degrees (Figure 3). This results obtained successfully demonstrate that there are morphologically distinct populations of *C. regium* in these localities particularly for Seimareh River (Figures 2 and 3). Also in the present study, 65% of individuals were correctly classified into their respective groups by PCA (Table 3), indicating a high differentiation among the populations of *C. regium* in the studied areas. These morphological differences may be solely related to body shape variation and not to size effects which were successfully accounted for by allometric transformation. On the other hand, size-related traits play a predominant role in morphometric analysis and the results may be erroneous if not adjusted for statistical analyses of data (Bookstein 1991; Torres et al 2010). In the present study, the size effect was removed and the significant differences among the populations are due to the body shape variation when tested using ANOVA and multivariate analysis.

Geographical isolation can also affect growth pattern and reproductive strategy of fish species. The importance of such factors on producing morphological differentiation in fish species is well known (Yamamoto et al 2006).

Conclusions. Overall, the present analysis suggests high morphological differentiation among *C. regium* populations in the Tigris River drainage. The differentiation detected between populations may resulted by different environmental and habitat conditions, such as temperature, turbidity, food availability, and water depth. The present study provides basic information about the differentiation of *C. regium* populations in the studied areas using morphological parameters, and suggests that morphometric characters which are more affected by habitat could largely have discriminated the populations in compare with meristic characters.

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