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Morphometric and meristic comparisons of populations of Qanat tailor fish, *Alburnoides qanati* Coad & Bogutskaya, 2009 (Actinopterygii: Cyprinidae) in Kor River basin Iran

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Inter- and intra-population variations of a recently described endemic cyprinid fish *Alburnoides qanati* Coad and Bogutskaya, 2009 collected from three localities in the Kor River basin, Iran, were studied by applying morphometric and meristic characters using canonical discriminate function analysis (DFA), principal component analysis (PCA) and cluster analysis (CA). In DFA, the overall random assignment of individuals into their populations was 96.6% based on all the adjusted morphometric characters, 94.6% for meristic characters, and 100% based on both morphometric and meristic characters, indicating that the original grouped cases were correctly classified and they belonged to three morphologically distinct populations. The first principal component (PC) accounted for 16% and the second PC accounted for 15.8% of the shape variations among the samples. The Cluster analysis revealed a similar pattern of separation of one population from the others. The morphological heterogeneity observed in this species should be considered in conservation management and stock enhancement programs, especially during drought conditions in the region.

Key words: *Alburnoides qanati*, inter- and intra- population variations, morphological differences

INTRODUCTION

The family Cyprinidae, with 92 confirmed species (46% of those confirmed), is the most diverse freshwater fish family in Iran (Esmaeili et al., 2010). Certain nominal cyprinid species have been regarded as species complexes, some of which may be undescribed. The genus *Alburnoides* is one of those specifies. It comprises 11 species that were found in Europe, Asia Minor, and Central Asia. *Alburnoides bipunctatus* (Bloch, 1782) was the name applied to most populations across Europe and the Middle East from France north of the Alps eastwards to the Black, Caspian, and Aral Sea basins. The ongoing research reveals greater diversity in terms of the number of species in this taxon (Bogutskaya and Coad 2009; Coad and Bogustkaya 2009). Variations in the morphology and number of vertebra, branched anal fin ray counts, and other morphological measurements in *Alburnoides* indicate separate species.

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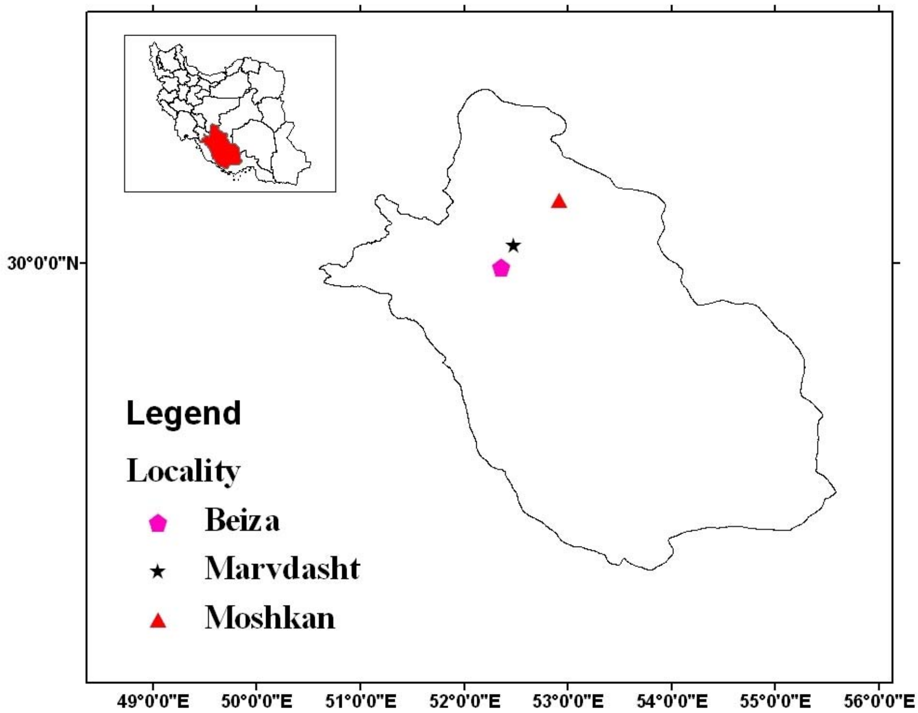

FIGURE 1. Collecting locations for *Alburnoides qanati* in the Kor River basin. 1, Moshkan; 2, Beiza; 3, Marvdasht.

Recently, five new species were reported from Iran: *A. petrubanarescui* (type locality Qasemlou-Chay, Orumiyeh Lake basin), *A. namaki* (type locality a qanat at Taveh, Namak Lake basin), *A. nicolausi* (type locality Simareh River in the Karkheh River system, Tigris River drainage), *A. idignensis* (type locality Bid Sorkh River, Gamasiab River system, Tigris River drainage), and *Alburnoides qanati* (type locality a qanat in the Pulvar River, Kor Basin) (Bogutskaya and Coad 2009; Coad and Bogutskaya 2009). *Alburnoides bipunctatus eichwaldii* was the name applied to most populations across the south Caspian Sea basin of Iran. Bogutskaya and Coad (2009) resurrected *A. eichwaldii* (De Filippi, 1863), present at least in the western part of the Caspian Sea basin, west of the Safid River.
Alburnoides qanati, the qanat tailor fish, was named after the major qanat system in Iran that taps groundwater to supply desert regions and also provides habitats for fishes (Coad and Bogutskaya, 2009). This is the southernmost *Alburnoides* species and may have entered the Kor River basin through its past connection to the Tigris-Euphrates River basin (Coad, 2010). Recent efforts made by the first author show that *A. qanati* is also found in the Sirjan basin (Yazd Province). Information on its biology, ecology, and distribution is limited to its original description (Coad and Bogutskaya, 2009). The aim of this study was to investigate the population structure of *A. qanati* from three localities in the Kor River basin, by analysis of morphometric and meristic characters.

MATERIAL AND METHODS

Specimens were collected by electro-fishing in 2005-2006 from three locations covering its distribution area in the Kor River basin: Safashahr, Moshkan village, (location 1, n = 75); Beiza spring (location 2, n = 32); and Marvdasht (location 3, n = 41) (Fig. 1). Twenty-eight morphometric characters were measured with digital calipers and these are given in Fig. 2.

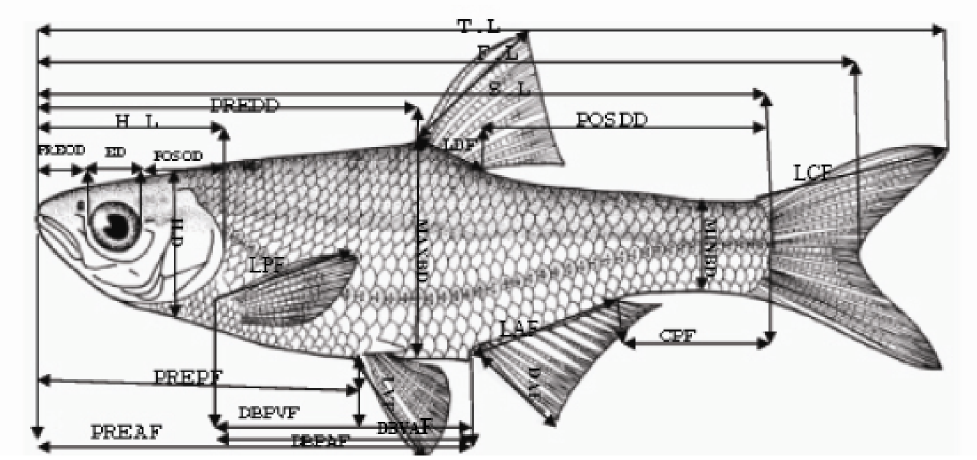

FIGURE 2. Morphometric characters measured in *Alburnoides qanati.* Total length (tl), fork length (fl), standard length (sl), head length (hl), head depth (hd), head width (hw), preorbital distance (preod), postorbital distance (posod), interorbital distance (inord), eye diameter (ed), predorsal distance (predd), postdorsal distance (posdd), length of dorsal fin (ldf), depth of dorsal fin (ddf), length of anal fin (laf), depth of anal fin (daf), preanal fin (pread), length of pectoral fin (lpf), length of pelvic fin (lvf), prepelvic length (prevfd), maximum body depth (maxbd), minimum body depth (minbd), distance between pectoral fin and anal fin (dbpaf), distance between pectoral fin and pelvic fin (dbpvf), distance between pelvic fin and anal fin (dbvaf), length of caudal fin (lcf), length of caudal peduncle (lcp), and mouth width (mw). Meristic counts were: unbranched dorsal fin ray (dfru), branched dorsal fin ray (dfrb), unbranched pectoral fin ray (pfru), branched pectoral fin ray (pfrb), branched ventral fin ray (vfr), unbranched anal fin ray (afru), branched anal fin ray (afrb), lateral line scale (ll), scales between dorsal fin and lateral line (sall), scales between ventral fin and lateral line (sbll), gill raker (gr), and caudal peduncle scale (cpsale).

As there were significant linear correlations among all morphometric characters and standard length (Table 1), length effects in the data set were eliminated using the allometric formula given by Beacham (1985)

$$Madj= M\ (Sl\ mean/SL)^{b}$$

Where M is the original measurement, Madj is the size adjusted measurement, SL is the standard length, Sl is the overall mean standard length, and b is the slope of the regression of log M on log SL in each basin.

The effectiveness of size adjustment transformations was assessed by testing the significance of correlations between transformed variables and standard length (Turan et al., 2005). Meristic characters are fixed early in ontogeny and remain stable throughout life (Barlow, 1961), so no size correction was applied.

Univariate analysis of variance (ANOVA) and Duncan *post hoc* multiple comparisons were carried out to test the significance of morphometric and meristic differences among populations. In addition, size-adjusted data were standardized and principal component analysis (PCA), canonical discriminate function analysis (DFA), and cluster analysis (Turan et al., 2005) was applied using SPSS v.17. Means of ratios of the original morphometric measurements were calculated to compare the degree of differentiation of each character for each population.

TABLE 1- Regression models: Linear relationships between standard length (dependent variable) and 26 morphometric characters (independent variables) in *A. qanati* (See Fig. 2 for key to symbols).

Characters	Equation	R^2	P
tl	Y=0.847x-1.480	0.994	0.001
fl	Y=0.917x-1.438	0.996	0.001
hl	Y=3.963x-2.822	0.978	0.001
hd	Y=4.543x+3.890	0.972	0.001
hw	Y=7.025x+1.984	0.961	0.001
preod	Y=12.461x+2.287	0.898	0.001
posod	Y=7.843x+1.961	0.949	0.001
ed	Y=16.641x-17.614	0.894	0.001
inod	Y=12.493x-2.421	0.936	0.001
predd	Y=1.876x+1.489	0.992	0.001
posdd	Y=1.820x-2.167	0.975	0.001
ldf	Y=6.489x+5.325	0.932	0.001
ddf	Y=4.272x-1.434	0.953	0.001
laf	Y=5.531x+6.891	0.896	0.001
daf	Y=5.960x-2.650	0.910	0.001
lpf	Y=4.778x-.784	0.917	0.001
lvf	Y=5.444x+5.078	0.932	0.001
prevfd	Y=2.002x+1.976	0.982	0.001
maxbd	Y=2.958x+10.348	0.966	0.001
minbd	Y=7.296x+8.576	0.968	0.001
dbpaf	Y=20270x+4.592	0.974	0.001
dbpvf	Y=3.848x+5.617	0.954	0.001
dbvaf	Y=4.755x+8.476	0.893	0.001
lcf	Y=4.217x-4.958	0.898	0.001
lcp	Y=4.136x+.856	0.948	0.001
mw	Y=10.881x+5.795	0.842	0.001

RESULTS

After the allometric transformation, there was no significant correlation between the standard length and the adjusted morphometric measurements, indicating that the allometric transformation effectively removed size effects from the data. ANOVA revealed significant differences among the three populations in all measured characters ($P<0.001$) except post orbital distance, length of dorsal fin, number of unbranched pectoral fin ray and number of pelvic fin ray ($P>0.05$). Duncan *post hoc* multiple comparisons revealed important differences among characters in the three populations (Table 2).

PCA of the correlation matrix, generated by the normalization procedure, produced six eigenvalues>1 (results not shown). Based on these results, the first principal component (PC) accounted for 16.3% and the second PC accounted for 15.8% of the variations among the samples. Examination of ratios (in percent of sl) of the measurements among samples revealed that head depth, pre-anal distance, pre-ventral fin distance, maximum body depth, minimum body depth, distance between pectoral and anal fins, and mouth width were lowest in the Marvdasht population, differentiating this population from the other two populations.

A canonical discriminant function analysis (DFA) was plotted to allow visual examination of the distribution of each population along the function axis that differentiated the populations. The overall random assignment of individuals to their population was 96.6% based on all adjusted morphometric characters (Fig. 3), 94.6% for meristic characters (Fig. 4), and 100% for both morphometric and meristic characters (Fig. 5), demonstrating that the original groups were correctly assigned.

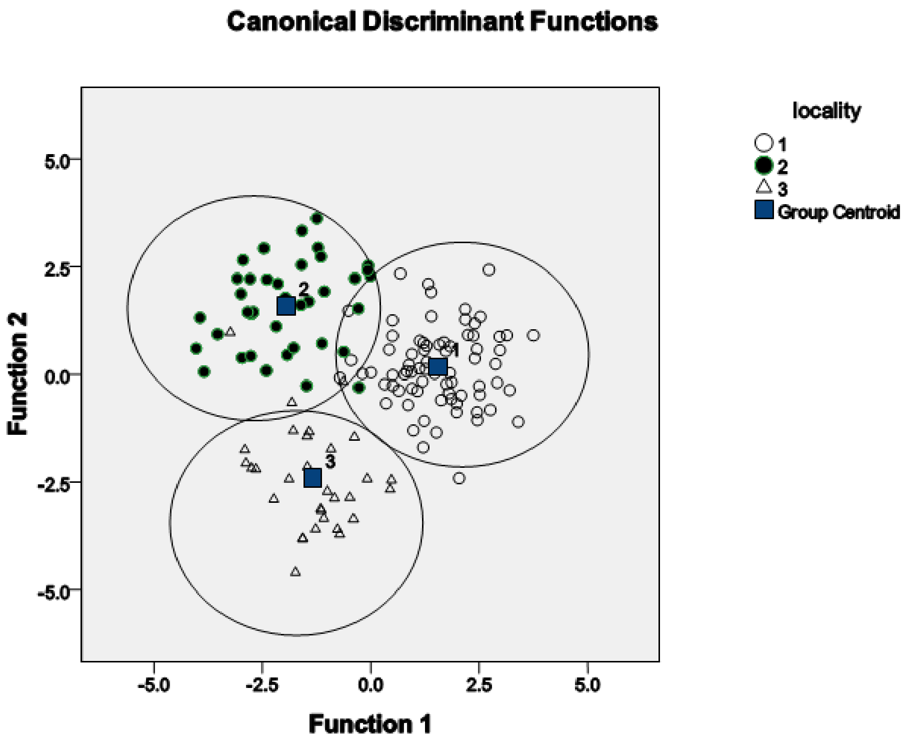

FIGURE 3. Canonical discriminate function analysis of morphometric characters of *Alburnoides qanati*. ○ , Moshkan; ● , Beiza; △ , Marvdasht.

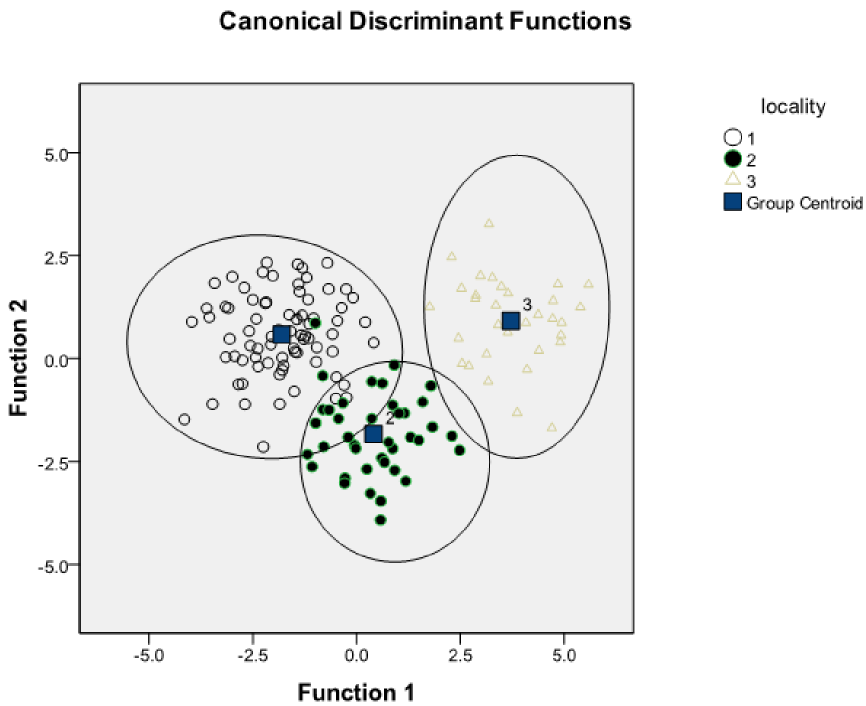

FIGURE 4. Canonical discriminate function analysis of meristic characters of *A. qanati*. ○ , Moshkan; ● , Beiza; △ , Marvdasht

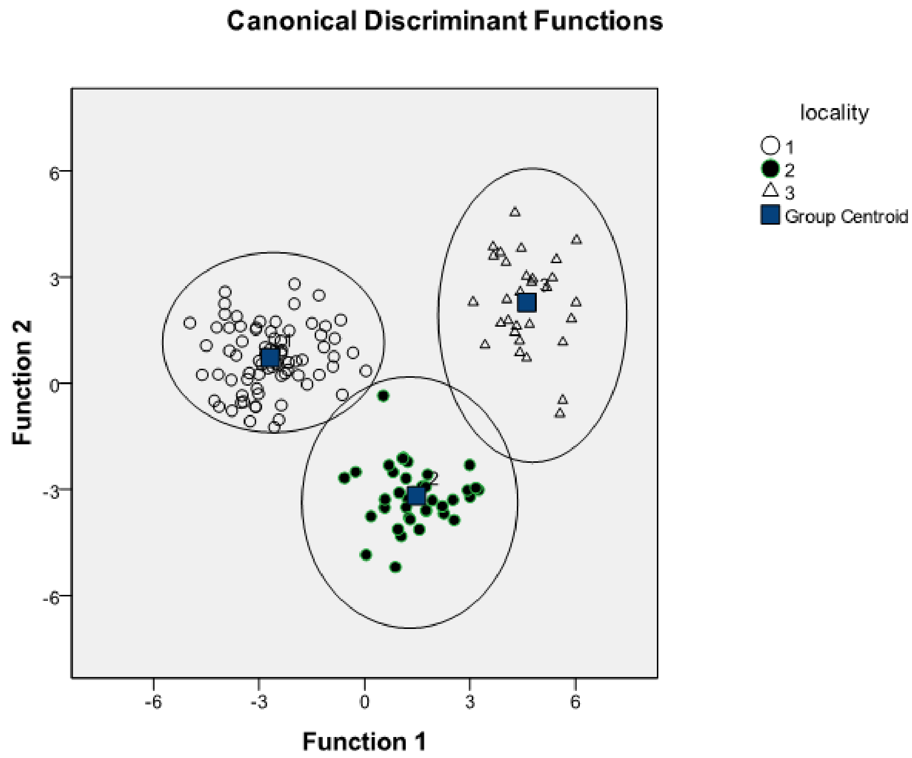

FIGURE 5. Canonical discriminate function analysis of both morphometric and meristic characters of *A. qanati.* ○ , Moshkan; ● , Beiza; △ , Marvdasht

The cluster analysis (UPGMA–average linkage clustering method) based on the three data sets including all adjusted morphometric characters and meristic characters, and on the combined morphometric and meristic characters, showed similar patterns. The cluster for morphometric and meristic characters is illustrated in Figure 6. In the dendrograms, the Moshkan and Beiza populations were separated from the Marvdasht population showing a distinct cluster.

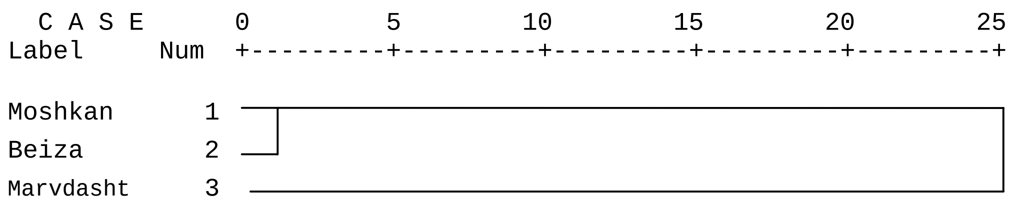

FIGURE 6. Cluster dendrogram using average linkage (between groups) for meristic and morphometric characters of *Alburnoides qanati* populations from the Kor River basin.

TABLE 2- Mean values of significantly different morphological characters (Duncan *post-hoc* test, one way ANOVA analysis, p<0.05) of *Alburnoides qanati* from Kor river basin (See Fig. 2 for key to symbols).

Localition	udfr	sall	gr	cpsale	tl	hl	ed
Moshkan	2.1467	8.6250	5.9750	15.0000	57.8291	12.3872	3.5184
Beiza	2.5750	9.2400	6.9067	16.2500	58.5622	12.8124	3.8305
Marvdasht	2.9688	9.9063	7.4375	19.3750	59.2172	13.1980	4.1749

DISCUSSION

The morphometric analysis of *A. qanati* in the Kor River basin revealed three distinct populations with inter-population variable phenotypic characteristics. Morphological characters are commonly used in fish biology, including at the systematic level, to measure discreteness and relationships among taxonomic categories (Clayton, 1981) and have been a central element of biology for centuries (Verep et al., 2006). Both taxonomic classification of organisms and understanding of species diversity were historically based on morphological descriptions (Dean et al., 2004). In fish, morphometric characters represent one of the major keys determining their systematics, growth variability, ontogenetic trajectories (Kovac and Copp, 1999), and/or population parameters (Verep et al., 2006). However, the major limitation of morphological characters at the intra-specific level is that phenotypic variation is not directly under genetic control but subject to environmental modification (Clayton, 1981).

Phenotypic plasticity of fish allows adaptation to environmental change by modification of their physiology and behavior to mitigate the effects of environmental variation, which leads to changes in morphology, reproduction, and survival (Stearns, 1983; Meyer, 1987). Environmentally induced phenotypic variation may have advantages for stock identification, especially when the timescale is insufficient for significant genetic differentiation of populations. Phenotypic markers may detect morphological differentiation due to environmental differences in the habitats of partially isolated stock.

According to Winfield and Nelson (1991) diversity in meristic characters is independent of environmental change and is driven by genetic factors. However, there are reports of differences in meristic characters of species and subspecies of fishes living at different latitudes (Bianco and Banarescu, 1982).

The present morphometric study provides evidence of significant morphometric heterogeneity among three populations of *A. qanati* in the Kor River basin, especially between Marvdasht and the other two populations.

Results of ANOVA and Duncan *post hoc* multiple comparison showed that some characters, including total length head length, eye diameter, distance between pectoral fin and pelvic fin, number of unbranched dorsal fin ray, number of scales between dorsal fin and lateral line, gill raker and caudal peduncle scale vary among the three populations. The mean values of all these characters in the Marvdasht population are greater than those observed in the Beiza and Moshkan populations. The impact of these morphometric and meristic parameters in differentiation of populations varies. According to Ihssen et al. (1981) morphometric analysis is based on a set of measurements that represent size and shape variation and are continuous data, in contrast to meristic characters, which are discrete.

The mean head depth, pre-anal distance, pre-ventral fin distance, maximum body depth, minimum body depth, distance between pectoral and anal fins, and mouth width are smaller in the Marvdasht population than in the other two populations. The inter-population variation in body depth may be attributed to the swimming ability. The Marvdasht population inhabits areas of rapid water flow, and exhibit shallower and more streamlined bodies. This may be related to possible differences in adaptations to swimming in fast currents for prolonged periods. Thus, selection should favor a more fusiform body shape that minimizes energy expenditure. This agrees with the conclusions of Taylor and McPhail (1985). The smaller mouth in fish from the Marvdasht population may be another adaptation to rapid currents, as more rapid current increases available oxygen, even with a smaller mouth. This morphometric difference might also reflect adjustments to the feeding environment and prey types. The other two populations inhabit areas of slow water currents with high levels of vegetation.

Other differences among the populations may be related to habitat characteristics such as temperature, turbidity, food availability, and water depth and flow (Turan, 2005). For example, eye diameter was greater in the Marvdasht populations, which may be due to differences in turbidity among the habitats (Matthews, 1988). Position of eyes may also be related to vertical habitat preference (Aleev, 1969).

Results obtained from DFA analysis using both morphometric and meristic characters demonstrate that there are three morphologically distinct populations of *A. qanati* in the Kor River basin, and these morphological differences may be solely related to body shape variation and not to size effects. However, the results of cluster analysis showed that fish populations from Moshkan and Beiza were similar and differed from Marvdasht, perhaps due to similar habitats in Moshkan and Beiza.

Comparison of our results with the original description of *A. qanati* showed that meristic characters of these three populations (Table 3) are similar to the original description by Coad and Bogutskaya (2009), although the Marvdasht population possessed a different number of branched anal fin rays.

The present study used morphological parameters to provide basic information about the differentiation of *A. qanati* populations in the Kor River basin and suggests that morphological variations observed in this species should be considered in conservation management and in any stock enhancement program. These variations may be due to ecological conditions, as have been reported in other fish species (Clayton, 1981; Shariati, 1999). Future studies of the Kor and also the Sirjan populations using molecular markers are recommended.

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TABLE 3- Comparison of meristic characters of *Alburnoides qanati* based on original description and three populations from the Kor River basin (See Fig. 2 for key to symbols).

Locality / Characters	Original description	Moshkan	Beiza	Marvdasht
udfr	3, 4	2(64), 3(11)	2(17), 3(23)	2(1), 3(31)
bdfr	7½, 8½	7(14), 8(61)	6(1), 7(12), 8(27)	7(3), 8(21)
bpfr	13(4), 14(20) or 15(6)	11(1), 12(6), 13(29) 14(33), 15(6)	12(1), 13(19), 14(19) 15(1)	11(2), 12(10), 12(10), 13(14), 14(5), 15(1)
bvfr	7	6(5), 7(62), 8(8)	7	7
uafr	3	2(39), 3(36)	2(14)	3(26)
bafr	10½ (3), 11½ (22), 12½ (5)	9(4), 10(42), 11(29)	10(8), 11(26), 12(6)	8(1), 10(8),11(17), 12(6)
ll	41(1), 42(1), 43(5), 44(6), 45(3), 46(7), 47(5) 48(1) or 49(1)	37(1), 40(10), 41(3), 42(6), 43(7), 44(12) 45(13), 46(9), 47(2) 48(6), 49(3), 50(1), 51(1), 52(1)	42(4), 43(5), 44(9), 42(4), 43(5), 44(9), 48(2)	40(1), 41(1), 42(3), 43(1), 44(4), 45(7), 46(3), 47(3), 48(2), 49(2), 50(4), 51(1)
sall	9(9), 10(18) or 11(3)	7(1), 8(11), 9(36), 10(23), 11(4)	7(1), 8(14), 9(24), 10(1)	8(1), 9(18), 10(16), 11(7)
sbll	3(4), 4(19) or 5(7)	3(12) 4(48), 5(15)	3(22), 4(18)	3(3), 4(20), 5(8), 6(1)
gr	6(4), 7(4), 8(21) or 9(1)	4(1), 6(14), 7(50), 8(10)	5(7), 6(29), 7(2), 8(2)	6(3), 7(12), 8(17)
cpsale		12(2),13(6) 14(17),15(24),16(19), 17(5),18(2)	14(2), 15(5), 16(17), 17(13), 18(3)	17(1), 18(5), 19(10), 20(13), 21(3)

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