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Morphological variations of *Alburnus mossulensis* (Heckel, 1843) populations in Iran

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Morphological variation of *Alburnus mossulensis* (Heckel, 1843) populations in Iran were investigated by collection of 705 specimens from 27 rivers of five basins, including Bushehr, Fars, Karkheh, Karun and tributaries of Tigris (Diyala) basin in Iran in 2010 by a seine net. The specimens were fixed in 10% formalin for further investigation after anesthetizing in 1% clove oil solution. Twenty-two morphometric and 11 meristic characters were examined. Morphometric characters in adjusted form and meristic characters in classified form were used for population comparison. Analysis of morphometric and meristic characters by ANOVA showed significant differences ($p<0.05$) in all basins for all characters except the number of dorsal and anal fin spines. Classification of meristic characters showed most specimens of all basins have eight soft rays in dorsal fin, 12 soft rays in anal fin, 19 soft rays in caudal fin, 15 soft rays in pectoral fin and nine soft rays in pelvic fin. Discriminant function analysis showed that populations from Fars and Karun Basins were different from each other and from other populations, on the other hand, populations from Diyala, Karkheh and Bushehr basins overlapped. This result may indicate that there are similar conditions in Diyala, Karkheh and Bushehr basins, resulting in more similarity amongst the populations of these basins.

Key words: *morphology, Alburnus mossulensis, morphometric, meristic.*

INTRODUCTION

Investigation of aquatic ecosystems, especially fishes are important from view point of evolution, ecology, behavior, conservation, stock identification, stock assessment of fishes and water resource management (Anvarifar *et al.*, 2011). The study of morphological characters with the purpose of identification or characterizing fish stock units for some time has been a valuable interest in ichthyology (Tudela, 1999). The study of morphometric and meristic characters are forceful tools for measuring and differentiation of the same species (Naeem & Salam, 2005). A sufficient amount of separation may result in notable morphological, meristic and genetic differentiation among stocks within a species, which may be recognizable as a basis for the management of different stocks (Mian *et al.*, 2014). Meristic characters are most commonly used for differentiation of species and populations (Sedaghat *et al.*, 2012). Morphometric measurements and its statistical relationships of fishes are essential for both fishery biology and taxonomic studies (Gharaei, 2012; Poria *et al.*, 2013; Sharma *et al.*, 2014; Zamani-Faradonbe *et al.*, 2015b). Investigation of morphometric and meristic

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characters remains the simplest and most direct way among methods of species identification. It is well-known that the examination of phenotypic differences in morphometric characters or meristic counts is the technique most commonly used to define stocks of fish (Samaradivakara *et al.*, 2015; Keivany & Arab, 2017; Mouludi-Saleh & Keivany, 2018). Despite the introduction of techniques which directly examines biochemical or molecular genetic variation, these conventional methods continue to have an important role in stock identification even to date (Swain & Foote, 1999; Jalili *et al.*, 2015; Keivany *et al.*, 2015).

The cyprinid genus, *Alburnus* Rafinesque, 1820 comprises about 39 recognized species distributed in Europe and West Asia (Falahatkar *et al.*, 2015). This genus has eight confirmed species in Iranian inland waters (Keivany *et al.*, 2016d), including *A. chalcoides* (Güldenstädt, 1772), *A. filippii* Kessler, 1877 and *A. hohenackeri* Kessler, 1877 in the south Caspian Sea basin, *A. atropatenae* Berg, 1925 in the Urmia Lake basin, *A. mossulensis* Heckel 1843 in the Tigris River, Esfahan, Kor River, Lake Maharlu, Bushehr and Hormuz basins, *A. zagrosensis* Coad, 2009 and *A. caeruleus* Heckel, 1843 in the Tigris River basin and *A. amirkabiri*, Mousavi-Sabet *et al.*, 2015 in the Namak Lake basin (Mousavi-Sabet *et al.*, 2014, 2015). In addition, *A. doriae* de Filippi, 1865 and *A. maculatus* Keyserling, 1861 have uncertain provenance and validity from Iran. Determination of fish stocks is too important for rational and effective fisheries management, because each stock needs separate management to aim of optimal harvest (Salini *et al.*, 2004; Erguden & Turan, 2005). Despite some works on the biology of this species (Keivany *et al.*, 2016a, b, c; 2017a, b), there is no thorough examination of the populations morphology. The aim of the present study was to examine the morphological variation of *Alburnus musselensis* from all basins in Iran to evaluate the differences among its populations.

MATERIAL AND METHODS

In total 705 specimens of *A. mossulensis* were caught from 27 rivers of five basins in Iran, including Bushehr, Fars and Tigris basins (Karun, Karkheh and Diyala river basins) (Fig. 1, Table 1) in 2010 by a seine net (5mm mesh sized), then the specimens were fixed in buffered 10% formalin after anesthetized in 1% clove oil solution at the sampling site and transferred to the laboratory for further examination. Twenty-two traditional morphometric characters were measured: total length, fork length, standard length, body height, head length, head width, snout length, eye diameter, cheek length, eyes distance, caudal peduncle length, caudal peduncle height, predorsal length, postdorsal length, preanal length, dorsal fin base length, dorsal fin height, anal fin base, length, anal fin height, ventral fin length, pectoral fin length and pectoral-ventral fin distance (Fig. 2).

Distances were measured using digital caliper to the nearest 0.1 mm. As variation should be attributable to body shape differences and not related to the relative size of the fish; in order to eliminate any variation resulting from allometric growth, all morphometric measurements were standardized according to Elliott *et al.* (1995):

$$M_{adj} = M (L_s / L_0)^b$$

Where M is the original morphometric measurement, M_{adj} the size adjusted measurement, L_0 the standard length of fish and L_s is the overall mean of standard length for all fish from all samples for each variable. The parameter b was estimated for each character from the observed data as the slope of the regression of log M on log L_0, using all specimens. The results derived from the allometric method were confirmed by testing significance of the correlation between transformed variables and standard length. Eleven meristic characters including pectoral fin soft ray, ventral fin soft ray, anal fin hard ray, anal fin soft ray, dorsal fin hard ray, dorsal fin soft ray, caudal fin soft ray, scales on lateral line, scales above lateral line, scales below lateral line, Circumpeduncle scales, predorsal scales, keel scales and gill arch spines, on the left side whenever possible were counted. Counts and measurements were done under a stereomicroscope. Also weight was measured with a Digital scale to the nearest 0.1 gram. Univariate Analysis of Variance (ANOVA) was performed for each morphometric and meristic character to evaluate the significant difference between the five basins. Morphometric characters that had significant differences were used for principal component analysis (PCA), discriminant function analyses (DFA) and cluster analysis (CA). In the present study linear discriminant function analyses (DFA), principal component analysis (PCA) and cluster analysis (CA) were employed to discriminate the five populations. The Wilk's lambda was used to compare the difference among all groups. The DFA was used to calculate the percentage of correctly classified (PCC) fish. A cross-validation using PCC was done to estimate the expected actual error rates of the classification functions. As a complement to discriminant function analysis, morphometric and meristic distances between the populations of five basins were inferred to Cluster analysis (CA) (Veasey *et al.*, 2001). Statistical analyses were performed using the PAST version 2.17b, SPSS version 19 software package and Excel 2013.

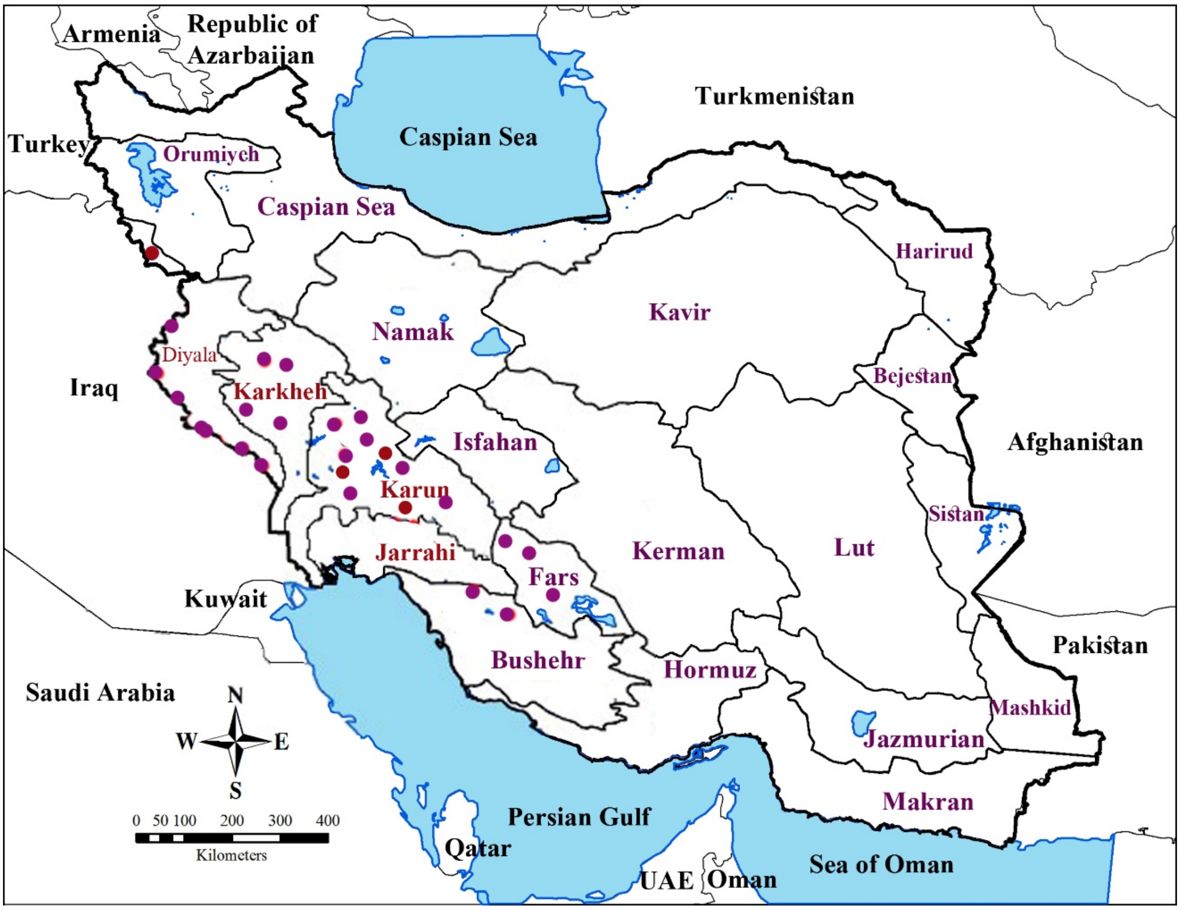

FIGURE 1. Iran map, basins and sampling sites (Based on Keivany *et al.*, 2016d).

TABLE 1. Number, mean total length and weight of *A. mossulensis* from different basins of Iran.

Basin	River	Counts
Bushehr	Fahlian	12
	Sheshpir	39
Fars	Safid	25
	Tang Boragh	13
	Zangiabad	40
Karun	Khersan	46
	Marbor	15
	Eivan Abbasi	30
	Bonestan	17
	Shirvan	11
	Dopolan	14
	Aligodarz	18
	Sepidar	15
	Darband	12
	Beshar	17
Karkheh	Gamasiab	14
	Sarab Gamasiab	20
	Malayer	62
	Kashkan	56
Diyala	Abdanan	11
	Haran	40
	Kangir	25
	Leileh	17
	Little Zab	16
	Rabat	86
	Shoshir	10
	Sirvan	24

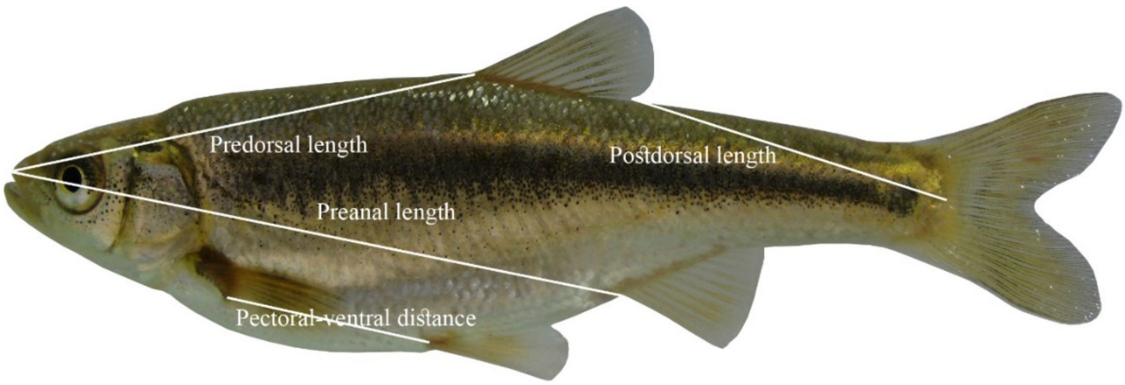

FIGURE 2. A photo of *Alburnus mossulensis* with some morphometric measurements.

RESULTS

Morphometric characters

The results of ANOVA for morphometric characteristics among five populations of *A. mossulensis* from the five basins of Iran are shown in **Error! Reference source not found.**. Significant differences ($p<0.05$) among populations of Bushehr, Fars, Karkheh, Karun and Diyala basins were observed for all the 22 morphometric characters (**Error! Reference source not found.**). These significant variables (except standard length and weight) were used for further multivariate analysis (PCA, DFA and CA). In order to determine which morphometric measurement most effectively differentiates populations, the contributions of variables to principal components (PC) were examined. Being KMO coefficients approximately more than 0.6 indicate that PCA method will suitable for our data (Kaiser, 1974). For morphometric characters, the KMO coefficient were obtained as 0.946 and for morphometric characters that is explaining of appropriation of this test at good and medial level, and the Bartlett's Test of sphericity is significant ($P\leq0.01$) (**Error! Reference source not found.**). In PCA analysis, the characters with an eigenvalues more than 1 were included and others discarded (**Error! Reference source not found.**).

The Wilks' lambda test of discriminant analysis indicated significant differences in morphometric characters of the five populations from basins. In this test, all functions were highly significant ($P\leq0.01$) (

). Nimalathasan (2009) mentioned out that factor loading greater than 0.30 are considered significant, 0.40 are considered more important and 0.50 or greater are considered very significant. Principal component analysis of 22 morphometric characters showed that PC 1 accounts for 68.556% of the variation, PC II for 13.274% and PC III for 7.395% (**Error! Reference source not found.**) and that the most significant weightings on PC I were from B.H, H.L, H.W, Sn.L, E.D, Po.L, E.W, C.L, C.H, Pr.D.L, Po.D.L, D.H, A.B, A.H, V.L, P.L and P-V.L, on PC II were from T.L, F.L, B.H, H.W, E.W, C.H and on PC III were from Pr.A.L and D.B (**Error! Reference source not found.**). The rotated (Varimax) component loadings for the three components (factors) are presented in **Error! Reference source not found.**.

For the discriminant analysis, the averages of percentage of correctly classified (PCC) were 80.5% for morphometric characters. High classification success rates were obtained for Bushehr (72.5%), Fars (98.7%), Karkheh (66.7%), Karun (100%) and Diyala (64.6%) stocks indicating a high correct classification of individuals into their original populations with respect to morphometric characters (**Error! Reference source not found.**). **Error! Reference source not found.** indicates the coordinates of the five populations in the two first axes of DFA. In this analysis there was a low degree of separation among three populations from Bushehr, Karkheh and Diyala basins and high degree of separation among Fars and Karun basins population with selves and with other populations of *A. mossulensis*.

Cluster analysis based on Euclidean distances in morphometric characters among the groups of centroids in *A. musselensis* populations resulted into grouping of populations in three clusters, the Fars basin, the Karun basin and the other remaining three basins (**Error! Reference source not found.**). This grouping pattern was similar to discriminant functions analysis (DFA) in **Error! Reference source not found.**.

Meristic characters

The results of ANOVA for meristic characteristics among five populations of *A. mossulensis* from the five basins of Iran are shown in **Error! Reference source not found.**. Significant differences ($p<0.05$) among populations of Bushehr, Fars, Karkheh, Karun and Diyala basins were observed for all the meristic characters except counts of anal and dorsal fin spine (**Error! Reference source not found.**). So, these significant variables were used for further multivariate analysis (PCA, DFA and CA). In order to determine which meristic measurements most effectively differentiate populations, the contributions of variables to principal components (PC) were examined. Being KMO coefficients approximately more than 0.6 indicate that PCA method will be suitable for our data (Kaiser, 1974). For meristic characters, the KMO coefficient were obtained as 0.722 and for meristic characters which explains the appropriation of this test at good and medial level, and the Bartlett's Test of sphericity was significant (P≤0.01) (**Error! Reference source not found.**).

TABLE 2. The mean±SD and results of ANOVA for morphometric characters of *A. mossulensis* from the five basins of Iran.

No.	Abb.	Characters	Bushehr 51	Fars 78	Karkheh 152	Karun 194	Diyala 229
1	T.L	Total Length	89.1 ± 13.2^{b}	77.3 ± 26.7^{d}	82.0 ± 20.4^{c}	85.3 ± 24.1^{a}	73.9 ± 27.4^{d}
2	F.L	Fork Length	80.8 ± 12.2^{b}	70.2 ± 24.6^{c}	75.0 ± 19.1^{c}	79.0 ± 20.9^{a}	67.5 ± 25.4^{d}
3	S.L	Standard Length	73.5±11.4	63.3±22.9	67.4±17.4	71.7±17.4	60.9±23.5
4	B.H	Body Height	16.6 ± 2.7^{d}	13.1 ± 5.3^{a}	14.2 ± 3.9^{b}	16.4 ± 5.3^{c}	12.8 ± 5.5^{a}
5	H.L	Head Length	18.7 ± 2.8^{c}	17.0 ± 5.6^{c}	16.8 ± 4.0^{b}	17.6 ± 4.5^{a}	15.2 ± 5.4^{a}
6	H.W	Head Width	8.9 ± 1.4^{c}	7.5 ± 2.6^{ab}	7.7 ± 1.9^{ab}	8.5 ± 2.3^{b}	7.2 ± 2.6^{a}
7	Sn.L	Snout Length	5.0 ± 0.8^{c}	4.4 ± 1.2^{b}	4.2 ± 1.1^{b}	4.4 ± 1.3^{b}	3.8 ± 1.5^{a}
8	E.D	Eye Diameter	5.1 ± 0.6^{c}	4.3 ± 1.1^{b}	4.6 ± 0.9^{b}	4.4 ± 1.0^{a}	4.0 ± 1.3^{a}
9	Po.L	Cheek Length	9.6 ± 1.5^{b}	8.8 ± 3.2^{b}	8.5 ± 2.2^{a}	9.2 ± 2.4^{a}	7.9 ± 2.9^{a}
10	E.W	Eyes Distance	5.6 ± 0.8^{c}	4.5 ± 1.6^{a}	4.9 ± 1.2^{b}	5.2 ± 1.4^{b}	4.4 ± 1.8^{a}
11	C.L	Caudal Length	18.5 ± 2.9^{b}	15.6 ± 5.4^{a}	16.8 ± 4.3^{b}	17.8 ± 5.0^{a}	15.2 ± 5.8^{a}
12	C.H	Caudal Height	7.3 ± 1.1^{c}	5.7 ± 2.4^{a}	6.3 ± 1.7^{b}	7.1 ± 2.1^{c}	5.7 ± 2.4^{ab}
13	Pr.D.L	Predorsal length	38.1 ± 6.0^{d}	33.7 ± 12.1^{cd}	35.0 ± 9.3^{c}	37.8 ± 11.1^{a}	31.3 ± 11.9^{b}
14	Po.D.L	PostDorsal Length	29.0 ± 4.5^{c}	23.8 ± 8.9^{a}	25.9 ± 6.8^{b}	27.2 ± 8.5^{a}	23.5 ± 9.3^{a}
15	Pr.A.L	PreAnal Length	47.8 ± 7.6^{d}	41.6 ± 15.4^{a}	44.1 ± 11.5^{d}	47.3 ± 14.1^{b}	39.8 ± 15.2^{c}
16	D.B	Dorsal fin Base length	8.1 ± 1.4^{b}	6.8 ± 2.3^{c}	7.3 ± 2.0^{ab}	7.7 ± 2.4^{a}	6.7 ± 2.5^{a}
17	D.H	Dorsal fin Height	15.0 ± 2.2^{d}	12.5 ± 3.8^{b}	13.5 ± 3.2^{c}	13.1 ± 3.3^{a}	12.1 ± 4.2^{b}
18	A.B	Anal fin Base length	10.0 ± 1.6^{c}	8.1 ± 3.1^{a}	9.0 ± 2.4^{b}	8.8 ± 2.6^{a}	8.0 ± 3.0^{a}
19	A.H	Anal fin Height	11.8 ± 1.7^{e}	10.0 ± 3.0^{c}	10.8 ± 2.5^{bc}	10.5 ± 2.6^{d}	9.5 ± 3.2^{b}
20	V.L	Ventral fin Length	11.4 ± 1.8^{c}	9.6 ± 3.6^{a}	10.4 ± 2.7^{b}	10.2 ± 2.9^{a}	9.1 ± 3.5^{a}
21	P.L	Pectoral fin length	14.8 ± 2.3^{c}	11.9 ± 4.4^{b}	13.1 ± 3.4^{ab}	13.0 ± 3.5^{b}	11.6 ± 4.2^{a}
22	P-V.L	Pactoral-Ventral Distance	16.9 ± 2.7^{b}	13.9 ± 5.4^{a}	15.5 ± 4.4^{b}	17.6 ± 5.8^{c}	14.1 ± 5.6^{b}
23	W	Weight	6.4 ± 3.3^{b}	4.8 ± 5.4^{a}	4.7 ± 3.4^{a}	7.4 ± 7.8^{b}	4.7 ± 4.7^{a}

No.: Numbers, Abb.: Abbreviation. a, b, c, d and e are result of Duncan grouping.

TABLE 3. Eigenvalues, percentage of variance, percentage of cumulative variance and Canonical Correlation for the Canonical Discriminant Functions in case of morphometric characters of *A. mossulensis* from the five basins of Iran.

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	65.964	82.5	82.5	0.993
2	13.456	16.8	99.3	0.965
3	0.378	0.5	99.8	0.524
4	0.183	0.2	100.0	0.393

TABLE 4. Result of Wilks' lambda test for verifying difference among five populations of *A. mossulensis* from the five basins of Iran. When morphological measurements are separately compared using discriminant function analysis.

Test of Function(s)	Wilks' Lambda	Chi-square	Df	Sig.
1 through 4	0.001	5081.079	84	0.000
2 through 4	0.042	2180.215	60	0.000
3 through 4	0.613	337.143	38	0.000
4	0.845	115.877	18	0.000

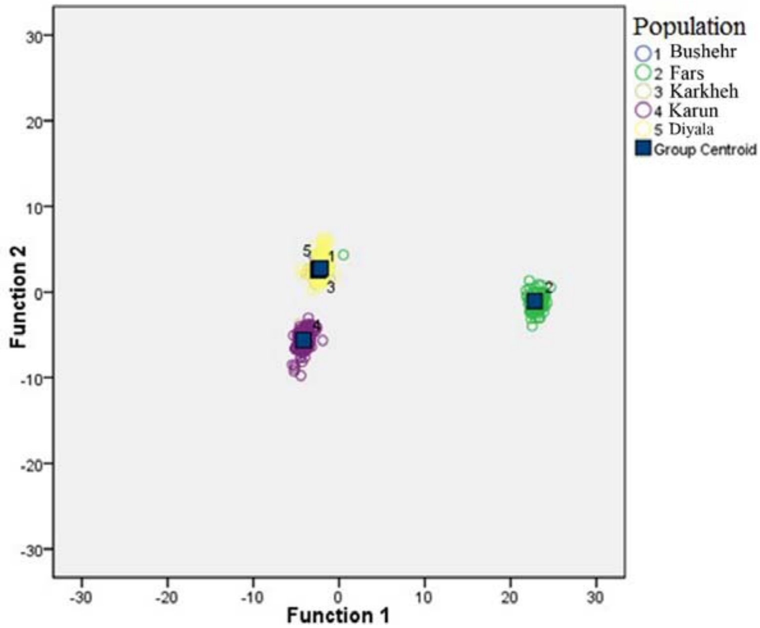

FIGURE 3. Coordinate plot of *A. mossulensis* from the five basins of Iran according to the first two discriminant functions from morphometric data analysis.

TABLE 5. KMO test and Bartlett's Test in case of morphometric characters for of *A. mossulensis* from the five basins of Iran.

Kaiser-Meyer-Olkin (KMO) Measure of Sampling Adequacy.		0.946
Bartlett's Test of Sphericity	Approx. Chi-Square	28692.264
	Df	210
	Sig.	0.000

TABLE 6. Eigenvalues, percentage of variance and percentage of cumulative variance for the principal components in case of morphometric characters for of *A. mossulensis* from the five basins of Iran.

Component	Eigenvalues	% of Variance	Cumulative %
1	14.397	68.556	68.556
2	2.788	13.274	81.830
3	1.553	7.395	89.226

TABLE 7. Factor loadings for the principal components and correlations between the morphometric characters for *A. mossulensis* from the five basins of Iran.

	PC 1	PC 2	PC 3		PC 1	PC 2	PC 3
Adj-T.L		-0.931		Adj.Pr.D.L	0.904		
Adj-F.L		-0.911		Adj.Po.D.L	0.898		
Adj.B.H	0.818	0.498		Adj.Pr.A.L			-0.949
Adj.H.L	0.897			Adj.D.B			0.959
Adj.H.W	0.871	0.405		Adj.D.H	0.919		
Adj.Sn.L	0.891			Adj.A.B	0.882		
Adj.E.D	0.886			Adj.H	0.909		
Adj.Po.L	0.888			Adj.V.L	0.959		
Adj.E.W	0.877	0.421		Adj.P.L	0.949		
Adj.C.L	0.819			Adj.P-V.l	0.794	0.451	
Adj.C.H	0.845	0.492					

Adj: adjusted

TABLE 8. Percentage of specimens classified in each group and after cross validation for morphometric characters for *A. mossulensis* from the five basins of Iran.

basins	Bushehr	Fars	Karkheh	Karun	Diyala
Original %					
Bushehr	76.5	0.0	19.6		3.9
Fars		98.7			1.3
Karkheh	15.8	0.0	69.1	0.7	14.5
Karun				100.0	
Diyala	13.5		20.1		66.4
Cross-validated %					
Bushehr	72.5		21.6		5.9
Fars		98.7			1.3
Karkheh	16.4		66.4	0.7	16.4
Karun				100.0	
Diyala	14.8		20.5		64.6

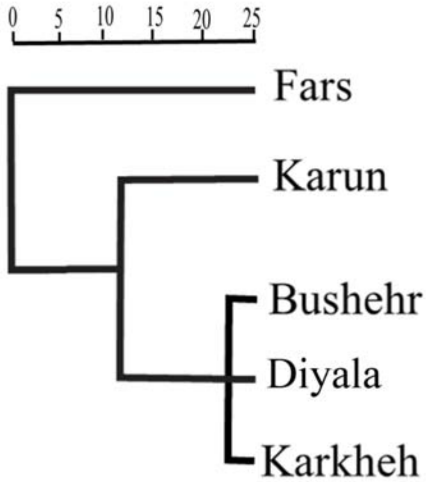

FIGURE 4. Dendrogram derived from cluster analyses of 21 morphometric characters on the basis of Euclidean distance for *A. musselensis* populations.

The Wilks' lambda tests of discriminant analysis indicated significant differences in meristic characters of the five populations. In this test, all functions were highly significant (P≤0.01) (**Error! Reference source not found.**). In PCA analysis the characters with an eigenvalues more than 1 were included and others discarded (**Error! Reference source not found.**). Principal component analysis of 14 meristic characters showed that PC1 accounts for 21.23% of the variation, PC II for 17.87%, PCIII for 9.39% and PC IV for 8.82% (**Error! Reference source not found.**3) and that the most significant weightings on PC I were from scales on lateral line, scales above lateral line, Circamucaudal scales and Predorsal scales, on PCII were from anal fin soft ray, pectoral fin soft ray, ventral fin soft ray and keel scales, on PC III were from caudal fin soft ray and gill arch spines and on PCIV were from dorsal fin soft ray, scales down lateral line and keel scales (**Error! Reference**

source not found.). The rotated (Varimax) component loadings for the three components (factors) are presented in **Error! Reference source not found.**.

For the Discriminant Factor Analysis, the averages of percentage of correctly classified (PCC) were 93.0% original data and 92.3% for Cross-validated data for meristic characters. High classification success rates were obtained for Bushehr (100%), Fars (100%), Karkheh (92.9%), Karun (86.2%) and Diyala (95.1%) stocks indicating a high correct classification of individuals into their original populations with respect to meristic characters (**Error! Reference source not found.**). **Error! Reference source not found.** indicates the coordinates of the five populations in the two first axes of DFA. In this analysis there was a low degree of separation among three populations from Bushehr, Karkheh and Diyala basins and high degree of separation among Fars and Karun basins population with selves and with other populations of *A. mossulensis*. Clustering analysis based on Euclidean distances in meristic characters among the groups of centroids in *A. musselensis* populations resulted into grouping of populations, first, Bushehr basin separated, then Karkheh and other three basins were in one group (Fig. 6).

TABLE 9. The results of ANOVA for morphometric characters of *A. mossulensis* from the five basin of Iran.

	Pectoral fin soft ray							Ventral fin soft ray				
		% Frequency of each count							% Frequency of each count			
basins	mean±SD	13	14	15	16	17	18	mean±SD	7	8	9	10
Bushehr	15.2±0.8[b]	0	18	47	29	6	0	8.6±0.5[b]	2	37	61	0
Fars	15.6±1.1[c]	0	15	33	31	17	4	8.7±0.5[b]	0	33	67	0
Karun	14.6±0.8[a]	21	34	15	11	3	1	8.3±0.2[a]	2	70	28	0
Karkheh	15.3±1.1[bc]	0	3.3	23	34.9	25	13.8	8.7±0.6[b]	0	29	71	0
Diyala	15.0±1.5[b]	14	28	28	13	7	10	8.6±0.9[b]	2	41	56	1

TABLE 9 Continued. The results of ANOVA for morphometric characters of *A. mossulensis* from the five basin of Iran.

	Anal fin hard ray			Anal fin soft ray					
		% Frequency of each count			% Frequency of each count				
basins	mean±SD	2	3	mean±SD	9	10	11	12	13
Bushehr	3.0±0.0[a]	0	100	11.3±1.5[c]	0	5.9	41.2	47	5.9
Fars	3.0±0.0[a]	0	100	10.9±0.8[b]	3	28	49	19	1
Karun	3.0±0.0[a]	1	99	14.6±0.8[a]	21	36	16	11	1
Karkhe	3.0±0.1[a]	0.7	99.3	11.1±0.6[bc]	0	11.8	64.5	21.7	2.0
Diyala	3.0±0.0[a]	0	100	11.0±0.7[b]	1	26.5	23.4	37	7

TABLE 9 Continued. The results of ANOVA for morphometric characters of *A. mossulensis* from the five basin of Iran.

	Dorsal fin hard ray			Dorsal fin soft ray			
	mean± SD	% Frequency of each count		mean±SD	% Frequency of each count		
basins		2	3		7	8	9
Bushehr	2.7±0.5[a]	15	85	7.9±0.4[ab]	2	14	84
Fars	3.0±0.0[a]	0	100	7.9±0.3[b]	8	1	91
Karun	3.0±0.1[a]	3	97	7.9±0.1[ab]	12	85	3
Karkhe	3.0±0.0[a]	4.6	95.4	8.0±0.2[b]	4	95	1
Diyala	3.0±0.1[a]	1	99	7.8±0.4[a]	21	78	1

TABLE 9 Continued. The results of ANOVA for morphometric characters of *A. mossulensis* from the five basin of Iran.

	Caudal fin soft ray				Scales on lateral line	Scales up lateral line	Scales down lateral line	Circamu caudal scales	Predorsal scales	Keel scales	Gill arch spines
		Frequency of each count (%)									
basins	mean±sd	18	19	20	mean±sd	mean±sd	mean±sd	mean±sd	mean±sd	mean±sd	mean±sd
Bushehr	18.9 ± 0.4^{ab}	10	86	4	64.2 ± 3.4^{a}	12.7 ± 1.0^{a}	4.9 ± 0.4^{a}	19.8 ± 1.9^{a}	30.2 ± 2.5^{a}	9.8 ± 1.6^{c}	11.3 ± 0.8^{a}
Fars	19.0 ± 0.3^{b}	3	94	4	71.2 ± 4.3^{c}	14.7 ± 0.8^{c}	5.3 ± 0.8^{c}	21.8 ± 2.9^{b}	35.1 ± 2.1^{c}	10.0 ± 1.4^{c}	13.4 ± 1.3^{e}
Karun	18.8 ± 0.1^{a}	15	85	0	65.7 ± 13.8^{b}	13.3 ± 2.5^{b}	4.9 ± 0.9^{c}	20.4 ± 3.9^{b}	31.1 ± 6.1^{b}	7.5 ± 1.4^{a}	11.7 ± 0.5^{b}
Karkheh	19.0 ± 0.2^{b}	2.6	95.4	2	75.0 ± 4.8^{d}	14.8 ± 0.6^{c}	5.2 ± 0.5^{bc}	22.0 ± 1.2^{b}	34.4 ± 2.8^{c}	9.2 ± 1.2^{b}	12.1 ± 0.9^{c}
Diyala	18.9 ± 0.4^{a}	11	88	1	69.9 ± 4.9^{bc}	14.3 ± 0.9^{b}	5.1 ± 0.5^{b}	21.3 ± 1.0^{b}	33.6 ± 3.1^{b}	8.9 ± 1.1^{b}	12.6 ± 1.1^{d}

TABLE 10. Eigenvalues, percentage of variance, percentage of cumulative variance and Canonical Correlation for the Canonical Discriminant Functions in case of morphometric variables of *A. mossulensis* from five basins of Iran.

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	18.231^{a}	84.3	84.3	0.974
2	2.102^{a}	9.7	94.0	0.823
3	0.953^{a}	4.4	98.4	0.699
4	0.342^{a}	1.6	100.0	0.505

TABLE 11. Result of Wilks' lambda test for verifying difference among five populations of *A. mossulensis* from the five basins of Iran. When morphological measurements are separately compared using discriminant Function analysis.

Test of Function(s)	Wilks' Lambda	Chi-square	df	Sig.
1 through 4	0.006	2895.057	68	0.000
2 through 4	0.123	1200.955	48	0.000
3 through 4	0.381	552.271	30	0.000
4	0.745	168.586	14	0.000

TABLE 12. KMO test and Bartlett's Test in case of morphometric variables for of *A. mossulensis* from the five basin of Iran.

Kaiser-Meyer-Olkin (KMO) Measure of Sampling Adequacy.		0.722
Bartlett's Test of Sphericity	Approx. Chi-Square	1161.644
	df	66
	Sig.	0.000

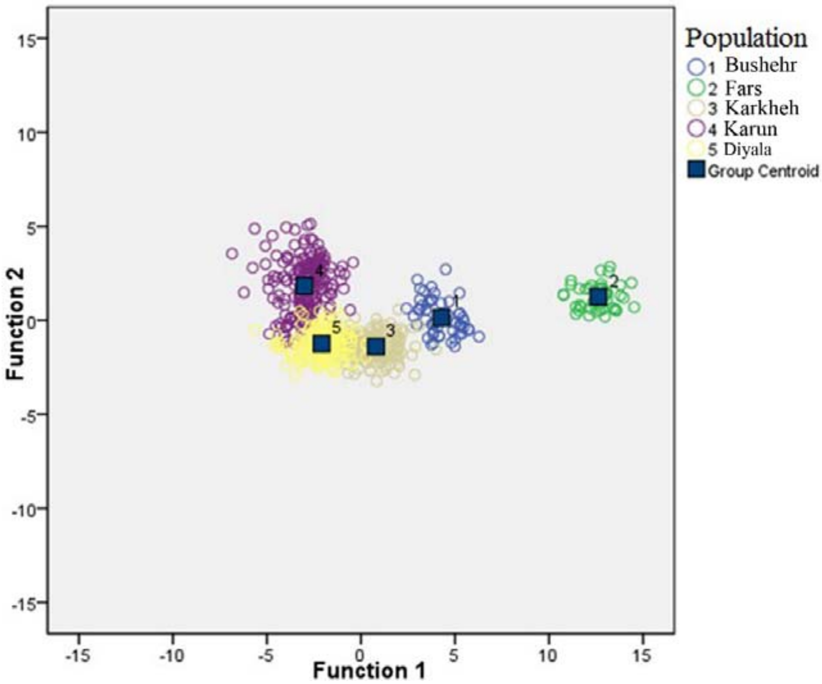

FIGURE 5. Coordinate plot of *A. mossulensis* from the five basins of Iran according to the first two discriminant functions from morphometric data analysis (1: Bushehr, 2: Fars, 3: Karkheh, 4: Karun, 5: Diyala).

TABLE 13. Eigenvalues, percentage of variance and percentage of cumulative variance for the principal components in case of morphometric variables for of *A. mossulensis* from the five basins of Iran.

Component	Eigenvalues	% of Variance	Cumulative %
1	2.547	21.228	21.228
2	2.145	17.873	39.101
3	1.127	9.392	48.493
4	1.058	8.817	57.310

TABLE 14. Factor loadings for the principal components and correlations between the morphometric variables for *A. mossulensis* from the five basins of Iran.

	Component					Component			
	1	2	3	4		1	2	3	4
Dorsal fin soft ray				-0.685	Scales up lateral line	0.739			
Anal fin soft ray		0.538			Scales down lateral line				0.598
Pectoral fin soft ray		0.709			Circamucaudal scales	0.794			
Ventral fin soft ray		0.697			Predorsal scales	0.709			
Caudal fin soft ray			0.775		Keel scales		0.701		0.415
Scales on lateral line	0.752				Gill arch spines			0.713	

TABLE 15. Percentage of specimens classified in each group and after cross validation for morphometric characters for *A. mossulensis* from the five basins of Iran.

	Bushehr	Fars	Karun	Karkheh	Diyala
Original (93.0%)					
Bushehr	100.0				
Fars		100.0			
Karkheh				93.6	6.4
Karun			87.0		13.0
Diyala			0.6	4.3	95.1
Cross-validated (92.3%)					
Bushehr	100.0				
Fars		100.0			
Karkheh				92.9	7.1
Karun			86.2		13.8
Diyala				4.9	95.1

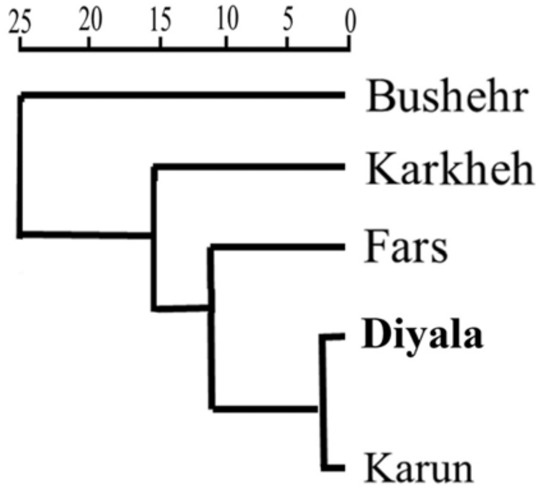

FIGURE 6. Dendrogram derived from cluster analyses of 12 meristic characters on the basis of Euclidean distance for *A. musselensis* populations.

DISSCUSSION

The multivariate analysis of morphometric and meristic characters in this work indicates the existence of morphologically differentiated groups of *A. mossulensis* and classified populations along the basins into five distinct groups. Body size is a major source of error in statistical analyses of data and plays a major role in morphometric analysis (Tzeng, 2004); however, in this study, allometric transformation was successfully removed the body size effect, so all significant variations showed the body shape differences when it was tested using ANOVA and other multivariate analysis.

In the present study, highly significant morphological variations were detected among *A. mossulensis* populations from Bushehr, Fars, Karkheh, Karun and Diyala. The results obtained from ANOVA analysis in our study showed that all of 21 transformed morphometric and 12 meristic characters were significantly different in populations of *A. mossulensis* existing in five basins of Iran that exhibits a high phenotypic variation among these populations.

Discriminant Function Analysis (DFA) is a useful method to distinguish different stocks of a same species, concern to stock management programs (Karakousis *et al.*, 1991). The results of DFA confirmed a high differentiation among the five populations of *A. mossulensis* in the study areas. This separation pattern was greatly confirmed by PCA, where showed that the populations were distinct from each other.

The reasons of morphological differences between populations are often quite difficult to explain (Bookstein, 1991; Poulet *et al*., 2004). It has been suggested that the morphological characteristics of fish are determined by genetic, environment and the interaction between them (Swain & Foote, 1999; Pinheiro *et al*., 2005; Anvarifar *et al*., 2010; Keivany *et al*., 2016b). The population from Fars basin (in the central part of the Iran) and Karun basin (in the southwest part of the Iran) are more distinct from each other and other populations, on the other, populations from Bushehr, Karkheh and Diyala basins were overlapping. It is well known that morphological characteristics can show high plasticity in response to differences in environmental conditions. This raises the probability that phenotypic plasticity may itself be adaptive, allowing fish populations to change their appearance to match their ecological environments (Swain & Foote, 1999; Mousavi, 2011; Zamani-Faradonbe *et al*., 2015b). The environmental factors that are dominant during the early development stages, when individual's phenotype is more amenable to environmental influence, is of particular importance (Pinheiro *et al*., 2005).

Therefore, the distinctive environmental conditions in divers rivers that flowing in Bushehr, Fars, Karkheh, Karun and Diyala basins, might cause the morphological differentiation between these five populations. The phenotypic variability may not necessarily reflect population differentiation at molecular level (Ihssen *et al*., 1981). In overall, fishes reveal greater difference in morphological characters both within and between populations than other vertebrates, and are more susceptible morphological variations that were effected by environmental conditions (Thompson, 1991; Wimberger, 1992; Turan *et al*., 2006; Zamani-Faradonbe *et al*., 2015c), so these difference might reveal different physicochemical conditions, diets, prey types, food availabilities, feeding pattern or other features (Rezaei *et al*., 2012).

The present study proposes high morphological differentiation among *A. mossulensis* populations along the five basins in Iran. The results also suggest these morphologically different populations should be considered as distinct stocks in the basins in fisheries management and commercial exploitation of this species and any stock enhancement program. Nevertheless, future studies on determination of population structure will be elucidated using biochemical and molecular genetics methods.

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