

[Research]

A comparative study on body shape of the genus *Alburnus* (Rafinesque, 1820) in Iran, using geometric morphometric analysis

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ABSTRACT

Geometric morphometric method was used to examine body shape variations among all the seven valid species of the genus *Alburnus* in Iran. In total 409 specimens of *A. chalcoides*, *A. filippii*, *A. atropatena*, *A. caeruleus*, *A. mossulensis*, *A. hohenackeri* and *A. zagrosensis* were collected from Babolrud, Baleqlu-Chai, Miriseh, Sarabeleh, Gamasiyab, Mahabad-Chai Rivers and the Gandoman lagoon, respectively. Shape data was extracted by recording 15 landmark points on 2-D pictures of specimens. The PCA, DFA and CVA/MANOVA analysis were used to examine shape differences among the seven species. Significant differences were found among the species in term of body shape. The PCA and CVA/MANOVA showed separation of *A. hohenackeri* and *A. caeruleus* from the others. Results revealed that the studied species are divided into two categories; a group with great body depth and short caudal peduncle and the other one with shallow body depth and longer caudal peduncle. The obtained body shape properties can provide a shape-based identification key for the genus *Alburnus* in Iran, useful for fisheries and stock management or conservation programs.

Keywords: Geometric morphometrics, *Alburnus*, Cyprinidae, Landmark, Iran.

INTRODUCTION

Thirty-eight species of the genus *Alburnus* are presently recognized in the European and Western Asian waters (Bogutskaya *et al.*, 2000; Freyhof and Kottelat, 2007). The genus *Alburnus* has seven confirmed species in Iranian inland waters. *Alburnus alburnus* (Linnaeus, 1758), *A. chalcoides* (Güldenstaedt, 1772) and *A. filippii* (Kessler, 1877) are found in the Iranian part of the Caspian Sea basin. *Alburnus atropatena* (Berg, 1925) occurs in the Orumiyeh Lake basin (northwest of Iran) and also in the Ghareh-Chai River in the Namak basin (central Iran) (Khataminejad *et al.*, 2013). *Alburnus zagrosensis* described from a stream in the Karun River basin in the Zagros

Mountains (west of Iran), in Chahar Mahall-e-Bakhtiari Province (Coad, 2009). The Zagros Mountains have a series of rivers draining westward and southward to the Tigris River basin. Previously, only *A. mossulensis* (Heckel, 1843) had been recorded from these rivers although *A. caeruleus* (Heckel, 1843), reported from Iraq, may also occur there (Coad, 2013).

Morphological studies are strong and instrumental for determining discreteness of the similar species (Mousavi-Sabet *et al.*, 2011; Mousavi-Sabet *et al.*, 2012) and extensively used to identify differences between fish populations (Mousavi-Sabet and Anvarifar, 2013).

Geometric morphometrics (GM), a quantitative approach to analysis shape, is widely applied to compare and determine shape variations of biological structures (Sansom, 2009). Despite traditional approaches in GM, data is obtained from the coordinates of landmark points (Rohlf and Marcus, 1993; Adams *et al.*, 2004), which are morphological points of specimens that are of biological interest (Richtsmeier *et al.*, 2002). GM has been used in various studies in fish population biology such as stock identification and discrimination (Cadrin and Friedland, 1999; Heidari *et al.*, 2013; Mousavi-Sabet and Anvarifar, 2013). This method, which allows the study of shape and size, offers powerful analytical and graphical tools for the quantification and visualization of morphological variation within and among organisms (Slice, 2007).

Body shape is a major component of an organism's phenotype, and it bears important traits of its biological characters such as feeding efficiency, locomotors performance, vulnerability to predators, and reproductive success (Gill *et al.*, 2003). Fish body shape can be the result of evolutionary adaptations to environmental pressures (Gatz, 1979; Winemiller, 1991), particularly, food collection and hydrodynamic conditions (Matthews, 1998) making feasible more efficient utilization of available resources and improving fitness and performance (Pianka, 1994). Therefore, the body shape differences of populations are considered as essential steps in the process of speciation (Balon, 1993; Margurran, 1998).

All members of the genus *Alburnus* in Iran exhibit relatively similar body form and in this regard, it requires reference to

subtle meristic data to distinguish them. Within these characters, there is not a considerable degree of variation. GM methods have not been used to assess body shape variation among species of the genus *Alburnus*. Hence, this study was conducted to assess intraspecific body shape among the seven confirmed *Alburnus* species with visualization techniques afforded by the GM approach. Quantifying phenotypic differences between species may help better understanding in natural history across a species' geographic range, which would have implications for both theoretical and applied work in ecological and fisheries sciences.

MATERIALS AND METHODS

Study area, Sampling

In total 409 specimens of seven species of the genus *Alburnus* including *A. chalcoides* (62 specimens), *A. filippii* (48 specimens), *A. atropatenae* (58 specimens), *A. caeruleus* (56 specimens), *A. mossulensis* (73 specimens), *A. hohenackeri* (59 specimens) and *A. zagrosensis* (53 specimens) were collected from Babolrud (N 36 39 21.64 E 52 38 22.08), Baleqlu-Chai (N 38 02 21E 48 02 58), Miriseh (N 36 29 56.99 E 45 33 54.64), Sarabeleh (N 33 41 34.50 E 46 42 56.84), Gamasiyab (N 34 05 53 E 48 25 21), and Mahabad-Chai (N 36 48 56.8 E 45 44 15.66) rivers, and the Gandoman Lagoon (N 3150 09 E 51 06 03), respectively (Fig.1), using electrofishing with 200-300 V during the years 2010-2012. The specimens were preserved in 10% formalin and were transported to the laboratory for further examinations.

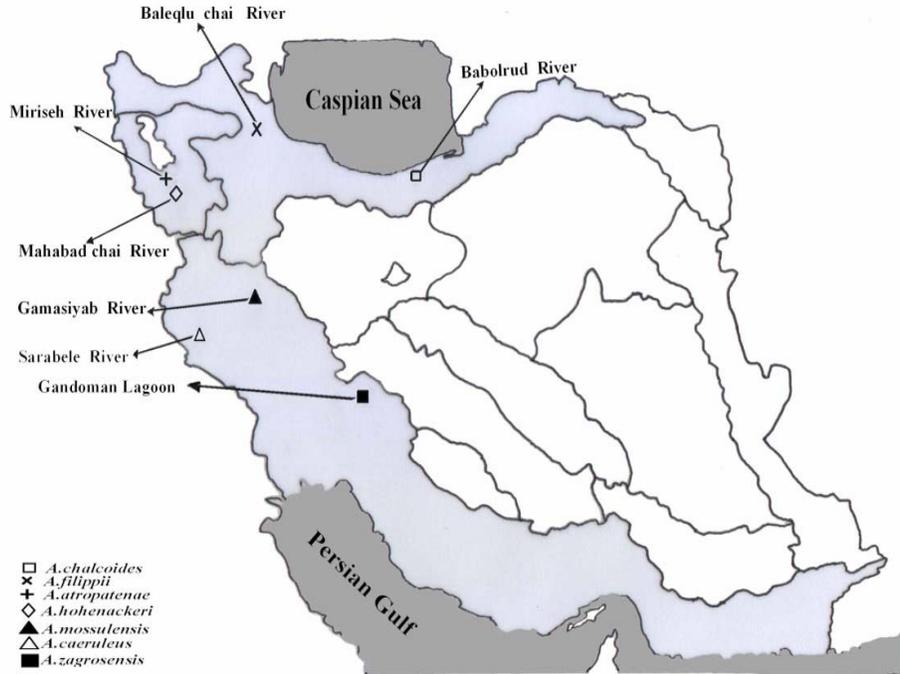


Fig. 1. The sampling sites in Iranian inland waters.

Laboratory works

The left side of the specimens was photographed using a digital camera (Canon Power Shot SX 30 IS). Fifteen homologous landmark-points were defined and digitized on 2-D images using tpsDig2 software version 2.16 (Rohlf,

2004). The landmark-points were chosen to the best represent the external shape of the body (Fig. 2). The landmark-points were chosen at the specific points, in which a proper model of fish body shape was extracted (Bookstein, 1991).

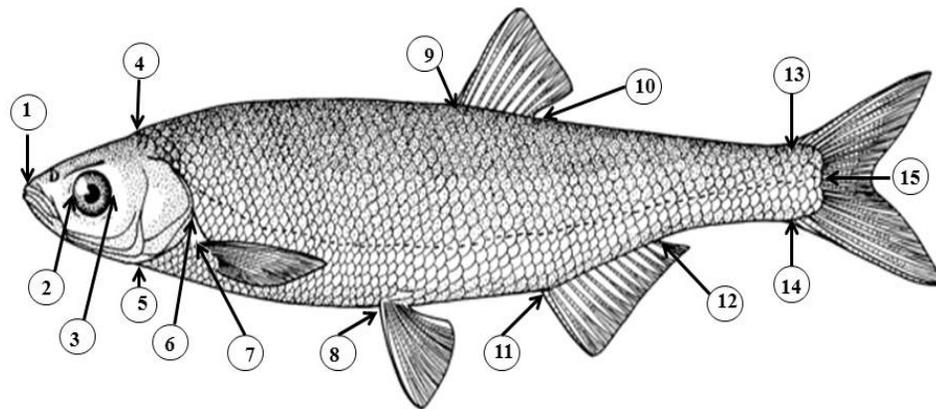


Fig. 2. Defined landmark points to extract body shape. 1. Anterior tip of the premaxilla; 2. Front of the eye; 3. End of the eye; 4. Beginning of the scales at the dorsal side; 5. The lower beginning of operculum; 6. End of operculum; 7. Base of the pectoral fin; 8. Base of the pelvic fin; 9 & 10. Anterior and posterior insertion of the dorsal fin; 11 & 12. Anterior and posterior insertion of the anal fin; 13. Upper margin of caudal peduncle; 14. Lower margin of caudal peduncle; 15. End of the medial region of caudal peduncle.

Data analysis

The extracted landmark-points (body shape data) were submitted to a generalized procrustes analysis (GPA) to remove non-shape data in PAST software.

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 (Tzeng, 2004). In the present study, the size effect was removed successfully by procrustes action in PAST software. To achieve the recommended ratio of the number of organisms measured (N) to the parameters included (P) in the analysis of at least 3–3.5 (Kocovsky *et al.*, 2009), in order to obtain a stable outcome from multivariate analysis.

Principal component analysis (PCA) was performed to summarize the variation among the specimens as few dimensions as possible. Canonical variant analysis CVA/MANOVA was accomplished to investigate power of distinction among different species by PAST statistical and MorphoJ software (Klingenberg, 1998). As a complement to discriminant analysis, morphometric distances among the seven species were inferred to cluster analysis by adopting the Euclidean square distance as a measure of dissimilarity method as the clustering algorithm (Sneath and Sokal, 1973). The patterns of taxon's body shape were illustrated in transformation grids in relation to consensus configuration of all specimens presented, depicting relative shape differences among the species.

RESULTS

Harris (1975) indicates that if the number of individuals minus the number of variables is greater than 30, then the sample can be considered large. In accordance with the results obtained in this study all had ratios greater than 30.

A common problem with many fish morphology studies that use multivariate analysis is potentially inadequate sample size. For decades, authors of theoretical works on PCA and DFA recommended that the ratio of the number of organisms measured (N) relative to the parameters included (P) in the analysis should be at least 3–3.5 (Kocovsky *et al.*, 2009). Small N values may fail to adequately capture covariance or morphological variation, which may lead to false conclusions regarding differences among groups (McGarigal *et al.*, 2000). In this study, the N:P ratios for *A. chalcoides*, *A. filippii*, *A. atropatena*, *A. zagrosensis*, *A. mossulensis*, *A. caeruleus*, and *A. hohenackeri* were 4.13, 3.20, 3.86, 3.53, 4.86, 3.73 and 3.93, respectively.

The PCA analysis for all specimens explained 48.42% of shape variations by the first two PC axes extracted from the variance-covariance matrix (PC1=34.61% and PC2=13.81%). Plotting the first and second PCA showed separation of *A. hohenackeri* and *A. caeruleus* from the others in the plot of factor scores for PC1 and PC2 (Fig. 3). The CVA/MANOVA revealed significant different in body shape among the studied species ($P < 0.05$). The Mahalanobis distances among the groups are represented in Table 1.

The CVA plot, also, displays the separation between *A. hohenackeri* and *A. caeruleus* and the other species. *A. chalcoides* had a low degree of overlapping in respect to body shape with *A. mossulensis* (Fig. 4). Discriminant analysis (DA) on relative warps classified 94.4% in origin data and 91.6% in cross-validation of specimen into the correct groups (Table 2).

The dendrogram derived from cluster analysis of Euclidean square distances among groups of centroids showed that the seven species of the genus *Alburnus* were partly distinct from each other with respect to morphometric characters (Fig. 5).

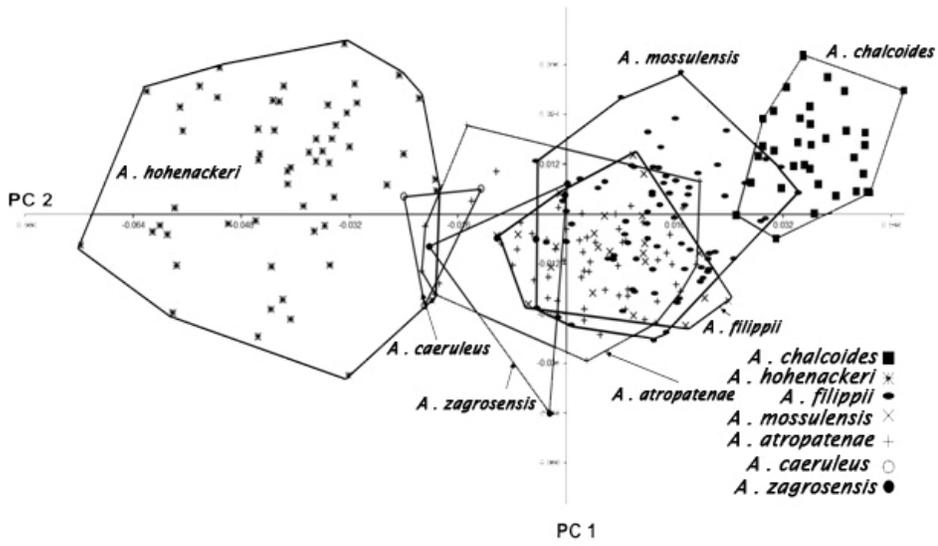


Fig. 3. Plot of the factor scores for PC1 and PC2 of the seven species of the genus *Alburnus* body shape.

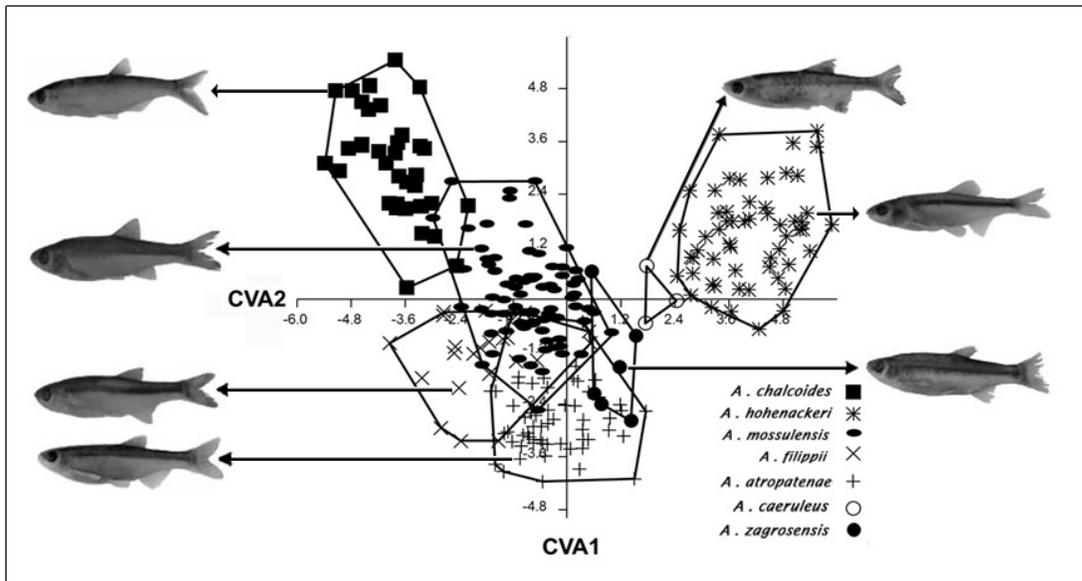


Fig. 4. The results of Canonical discrimination analysis (CVA) of the seven species of the genus *Alburnus* body shape with respect to the first two canonical variables.

Table 1. Mahalanobis distance analysis for the seven studied species of the genus *Alburnus*.

Species	<i>A. atropatena</i>	<i>A. caeruleus</i>	<i>A. chalcoides</i>	<i>A. fillippi</i>	<i>A. hohenackeri</i>	<i>A. mossulensis</i>
<i>A. caeruleus</i>	9.9879					
<i>A. chalcoides</i>	6.1761	11.6326				
<i>A. fillippi</i>	4.0329	10.408	5.2572			
<i>A. hohenackeri</i>	7.1762	9.7439	9.4343	8.0464		
<i>A. mossulensis</i>	4.1003	11.1689	5.0075	4.9691	7.4141	
<i>A. zagrosensis</i>	3.9281	10.542	7.0353	4.7073	5.9791	4.8297

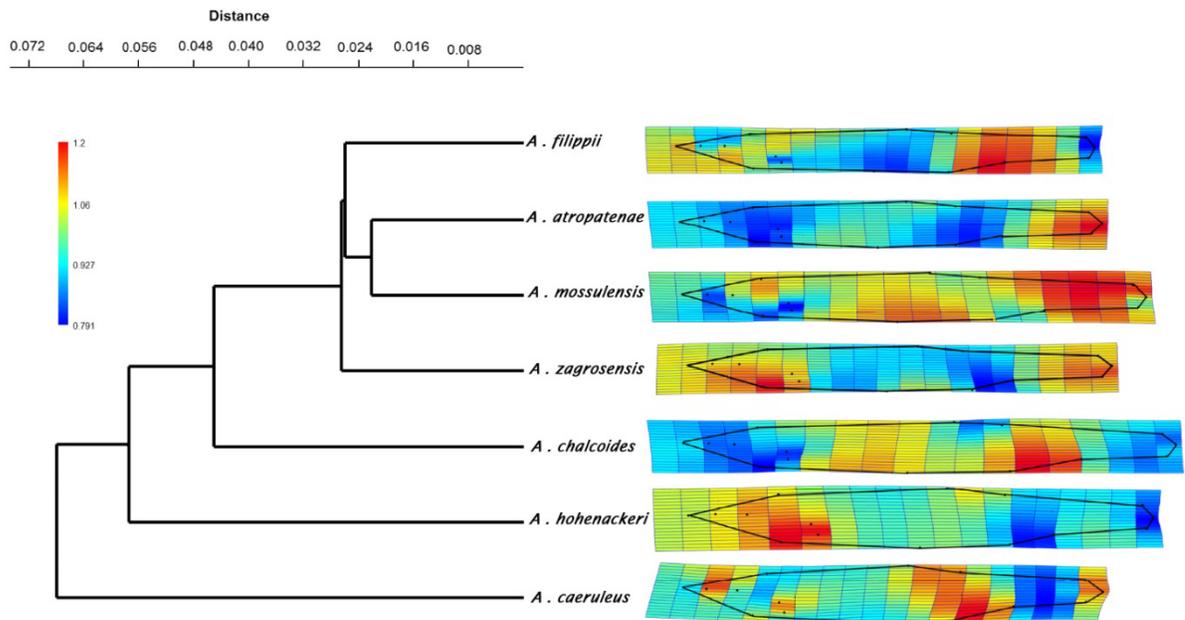
**Fig. 5.** Dendrogram derived from cluster analyses of morphometric variables on the basis of Euclidean distance of the seven *Alburnus* species in Iran. Mean shape of species in relation of consensus shape of the genus *Alburnus* are represented.

Table 2. Classification matrix showing the number and percentage of individuals that were correctly classified

	<i>A. atropatena</i>	<i>A. caeruleus</i>	<i>A. chalcoides</i>	<i>A. filippii</i>	<i>A. hohenackeri</i>	<i>A. mossulensis</i>	<i>A. zagrosensis</i>	Total
Original (%)								
<i>A. atropatena</i>	94.6	0.0	0.0	1.8	0.0	0.0	3.6	100
<i>A. caeruleus</i>	0.0	100	0.0	0.0	0.0	0.0	0.0	100
<i>A. chalcoides</i>	0.0	0.0	94.3	0.0	0.0	5.7	0.0	100
<i>A. filippii</i>	5.0	0.0	0.0	95.0	0.0	0.0	0.0	100
<i>A. hohenackeri</i>	0.0	1.7	0.0	0.0	98.3	0.0	0.0	100
<i>A. mossulensis</i>	4.2	0.0	2.8	0.0	0.0	91.7	1.4	100
<i>A. zagrosensis</i>	16.7	0.0	0.0	0.0	0.0	0.0	83.3	100
Cross-validate (%)								
<i>A. atropatena</i>	92.9	0.0	0.0	1.8	0.0	1.8	3.6	100
<i>A. caeruleus</i>	0.0	100	0.0	0.0	0.0	0.0	0.0	100
<i>A. chalcoides</i>	0.0	0.0	94.3	0.0	0.0	5.7	0.0	100
<i>A. filippii</i>	5.0	0.0	0.0	95.0	0.0	0.0	0.0	100
<i>A. hohenackeri</i>	0.0	1.7	0.0	0.0	96.6	0.0	1.7	100
<i>A. mossulensis</i>	9.7	0.0	2.8	1.4	0.0	84.7	1.4	100
<i>A. zagrosensis</i>	16.7	0.0	0.0	0.0	0.0	0.0	83.3	100

Depicting the differences in body shape between each seven studied species and consensus are presented in Fig. 5. Inspection of the grids of Fig. 5, shows a longer caudal peduncle for *A. atropatena*, small head, shallow body depth and longer anal fin base for *A. chalcoides*, a shallow body depth for *A. filippii*, shorter caudal peduncle, great body depth in midsection and large head for *A. hohenackeri* and deep and great body depth, short and deep caudal peduncle, deep head and upper mouth for *A. caeruleus* in relation to their consensus body shape. *A. zagrosensis* and *A. mossulensis* have shown almost similar body shapes (Fig. 5).

The morphometric characters between two sexes did not differed significantly ($P > 0.05$), because sampling times were outside of the breeding season, at this time, the sexes of related cyprinids have similar morphometrical characteristics (Anvarifar *et al.*, 2011).

DISCUSSION

Among several geometric approaches to morphometrics, the landmark based is one the most widespread and the best understood in its mathematical and statistical properties (Bookstein, 1996; Small, 1996; Dryden and Mardia, 1998). GM provides the opportunity to get new insights in the variety of morphological characteristics, discriminating genera, species, populations, and morphs or even individuals. One important advantage of the GM approach in relation to traditional ones is that GM does not need to decide a priori which measurements are likely to display differences (Rohlf and Marcus, 1993).

The present study on the seven species of the genus *Alburnus* shape variation demonstrated significant variation among them. Differences among the studied species can be divided into two categories; (1) great body depth and short caudal peduncle in *A. hohenackeri* and *A. caeruleus* as compared to the other species and, (2) shallow body depth and longer caudal peduncle in the other species.

The obtained results revealed an association between deep body and short and deep caudal peduncle among the species. This result is consistent with previous studies on other fishes such as darters (Page and Swofford, 1984) and this situation can be an adaptation for inhabiting in stream riffles. In contrast the relatively shallow bodies may be useful for benthic pool fishes as pointed out by Wood and Bain (1995). Adult morphology may be determined by a diversifying selection and ecological adaptation.

Observed differences in head shape of *A. chalcoides* and *A. hohenackeri* are indirectly related to feeding behaviors (Andersson *et al.*, 2005). Changes in head and mouth shapes can be considered as reflective of differences in selection of food items and direction of feeding (Langerhans *et al.*, 2003). Costa and Cataudella (2007) found that shape differences were related to trophic ecology for several species of the family Sparidae, thus indicating local adaptation and possibly ecological radiations (Schluter and McPhail, 1992; Langerhans *et al.*, 2003). If shape is related to either environmental influences on larval development (Cadrin and Silva, 2005) or diversifying selection and ecological adaptation at a trophic level (Costa and Cataudella, 2007), then spatially or latitudinally different environmental factors (e.g., temperature and resource availability) may explain the variations in body shape among the studied species.

Our results showed significant body shape differences among the species of the genus *Alburnus* in Iranian inland waters. Hence, it can be interpreted as differences at the species level suggesting possibility of well-differentiation species based on their body shape. These results provides an identification key based on body shape to the genus *Alburnus* in Iran, which can be useful for further fisheries and stock managements or conservation programs in the region.

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مطالعه مقایسه‌ای شکل بدن در ماهیان جنس *Alburnus* (Rafinesque, 1820) در ایران، با استفاده از روش ریخت‌سنجی هندسی

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چکیده

روش ریخت‌سنجی هندسی جهت بررسی تفاوت‌های شکل بدن میان تمام هفت گونه از جنس *Alburnus* در ایران مورد استفاده قرار گرفت. همه 409 نمونه از *A. caeruleus*، *A. atropatenae*، *A. filippii*، *A. chalcoides* و *A. zagrosensis* و *A. hohenackeri*، *A. mossulensis* گاماسیاب، مه‌بادچای و تالاب گندمان جمع‌آوری شدند. داده‌های شکلی از نقاط 15 لندمارک روی تصاویر دو بعدی نمونه‌ها استخراج شدند. آزمون‌های PCA، DFA و CVA/MANOVA جهت بررسی تفاوت‌های شکلی میان هفت گونه مورد استفاده قرار گرفتند. تفاوت‌های معنی‌دار در رابطه با شکل بدن بین گونه‌ها تشخیص داده شد. PCA و CVA/MANOVA جدایی گونه‌های *A. caeruleus* و *A. hohenackeri* از دیگر گونه‌ها را نشان دادند. نتایج نشان داد که گونه‌های مورد مطالعه به دو دسته تقسیم شدند: یک گروه با پهنای زیاد بدن و ساقه دم کوتاه و دیگری با پهنای کم بدن و ساقه دم بلند. مشخصات بدست آمده بر اساس شکل بدن جنس *Alburnus* در ایران می‌تواند یک کلید شناسایی فراهم آورد که برای مدیریت ذخایر شیلاتی و برنامه‌های حفاظتی مفید است.

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