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RESEARCH ARTICLE **Open access**

Landmark-based geometric analysis of body shape variation and meristic plasticity among populations of *Alburnoides idignensis* from Tigris River Drainage, Persian Gulf Basin, Iran

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Abstract
To compare body shape variations and meristic plasticity among four populations of *Alburnoides idignensis* fish from Aab-barik, Aab-Sardeh, Darband and Sarab-e Kayvareh rivers in Tigris River Drainage, Persian Gulf Basin of Iran. Geometric morphometric method was used to compare shape data extracted by recording 15 landmark points on 2-D pictures of 94 specimens collected from the rivers by electrofishing and a seine net. The principal component analysis, Canonical Variate analysis and MANOVA analysis were used to examine shape differences among the populations. Eight meristic traits including number of lateral line scales (LL), scales above and below LL to ventral fin, pre-dorsal scales, dorsal, anal, pectoral and pelvic branched rays were counted under a stereomicroscope and mean number of the data were compared by Kruskal–Wallis and ANOVA in SPSS software. Significant differences were found among the four populations in all meristic traits but the number of LL scales and in their body shape, separating them from each other. Results revealed that the studied populations have some differences in meristic characters and in the shape and size of the head, body, caudal peduncle and ventral and anal fin position.

Key words: *Cyprinidae, Leuciscidae, river systems, Cypriniformes*

INTRODUCTION

Comparisons of anatomical traits of organisms have long been considered as the central component of biology Adams et al. (2004). The morphological accounts and descriptions of organisms turned out to be the basis for underpinning the understanding of life and classification of organisms Adams et al. (2004). Many geographical barriers to gene flow exist for fishes and, therefore, most species and populations have the opportunity to show natural variations (Marcil et al. 2006; Banimasani et al. 2018). Adaptive radiation has been defined as the process of extremely rapid species formation coupled with ecological, morphological, and behavioral diversity (Schluter, 2000). Moreover, phenotypic plasticity in morphometric traits may often be adaptive, reflecting the effects of anthropogenic impacts or prey-predator processes in

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the ecological niches of a population (Robinson & Parsons, 2000; Jalili et al. 2015; Ghorbani-Ranjbari & Keivany 2018a,b)

The “morphometrics” is a generally used word for the statistical analysis of a large number of distances, angles, or ratios. “Geometric morphometrics” deals directly with coordinates of anatomical landmarks, either in two or three dimensions, rather than with traditional distances or angles. Landmark points are loci that have names as well as Cartesian coordinates (Bookstein, 1991). Thus, we examined some populations of *Alburnoides idignensis* using this newer technique.

The genus *Alburnoides* De Filippi, 1863 with over 30 valid species has a distribution ranging from Europe to Asia Minor and Central Asia (Bogutskaya & Coad, 2009; Coad & Bogutskaya, 2009; Coad & Bogutskaya, 2012; Mousavi-Sabet et al. 2015; Mousavi-Sabet et al. 2015). Recently, 12 species are considered to occur in Iranian freshwaters (keivany et al. 2016; Esmaeili et al. 2017; Esmaeili et al. 2018), although Eagderi et al. 2013 synonymized some of these species. The species in the genus *Alburnoides* is distinguished by having a combination of morphological characters such as different fin ray counts and molecular traits (Jouladeh Roudbar et al. 2015; 2016). Differences in habitat and feeding ecology of the species can change body shape traits. Herein, we used some meristic traits and the geometric morphometrics approach [9] to explore body shape variations among four populations of *Alburnoides idignensis* Bogutskaya & Coad, 2009, collected from the Tigris River Drainage, Persian Gulf Basin in Iran.

MATERIAL AND METHODS

Geometric morphometric

A total of 94 specimens from four populations of *Alburnoides idignensis*, caught by electrofishing and a seine net, from Aab-barik at 48º15'34"E, 34º56'44"N, Aab-Sardeh at 48º39'24"E, 33º46'06"N, Darband at 49°15'59"E, 33°26'24"N and Sarab-e Kayvareh at 48º43'22"E, 33º48'59"N in Tigris River Drainage, Persian Gulf Basin during 2008-2009 and preserved at IUT museum, were measured (Table 1).

Each specimen was photographed on left side by a digital camera (13 megapixel) at the same light exposure condition. The digital photographs were then processed using tpsDig 2.10 software for landmark acquisition. Some 18 Landmarks (Fig. 1) were identified by conventional rules on biological homology basis (spatial congruence, ontogenetic and phylogenetic) (Bookstein, 1991). The landmarks were digitised then their position was related to systems of coordinates (the x and y coordinates) which were useful for transformation. Each set of co-ordinates were submitted separately to a generalized procrustes analysis (GPA) available in the tpsRelw software1.45. This procedure translated, rotated and scaled the original configurations in order to achieve the best superimposition of all shapes. The size of each specimen is represented by the "centroid size", a measure that is able to estimate the size in all directions in a body better than is possible by using univariate measures such as maximum length. The TpsSmall 1.20. software was used to assess the correlation between Procrustes and Kendall tangent space distances to ensure that the amount of shape variation in a data set is small enough to allow statistical analyses to be performed in the linear tangent space, approximating the Kendall shape space, which is non-linear (Hammer, 2001; Rohlf, 2003; 2007).

The software was used to introduce shape variables into a Principal Component Analysis (PCA), and to visualize the warping associated with the various principal components (PCs). These components represent relative warps in the context of a TPS (thin-plate spline) approach (Bookstein, 1991). Finally, Principal Component Analysis, Canonical Variate Analysis and Cluster Analysis were conducted using PAST software (Eagderi et al. 2019).

Meristic characters

Eight meristic traits including number of lateral line scales (LL), scales above and below LL to ventral fin, pre-dorsal scales, dorsal, anal, pectoral and pelvic branched rays were counted under a stereomicroscope and after a normality test, the mean number of the non-normal data were compared by Kruskal–Wallis and the mean number of the normal data by ANOVA in SPSS software.

TABLE 1. Mean±SD, Kruskal–Wallis and ANOVA results for the meristic characters in *A. idignensis* populations.

	Meristic traits	Aab-Barik	Aab-Sardeh	Darband	Sarab-e Kayvareh	*p*
1	Lateral line scales (LL)	42.00±2.87	44.44±4.16	42.70±2.26	42.84±3.02	0.06
2	Scales above LL	08.12±0.72	08.79±0.60	07.58±0.77	08.50±0.93	0.00
3	Scales bellow LL to ventral fin	04.95±0.45	05.03±0.88	04.52±0.49	05.34±0.87	0.01
4	Pre-dorsal scales	17.95±2.44	22.34±2.39	20.29±2.65	22.07±1.79	0.00
5	Anal branched rays	11.25±0.66	11.27±0.58	11.11±0.58	10.65±0.67	0.00
6	Dorsal branched rays	08.08±0.49	07.93±0.25	07.64±0.47	07.88±0.31	0.01
7	Pectoral branched rays	14.04±0.73	13.27±0.94	13.05±0.99	13.57±0.56	0.00
8	Pelvic branched rays	06.87±0.33	06.93±0.25	06.47±0.49	06.61±0.68	0.00

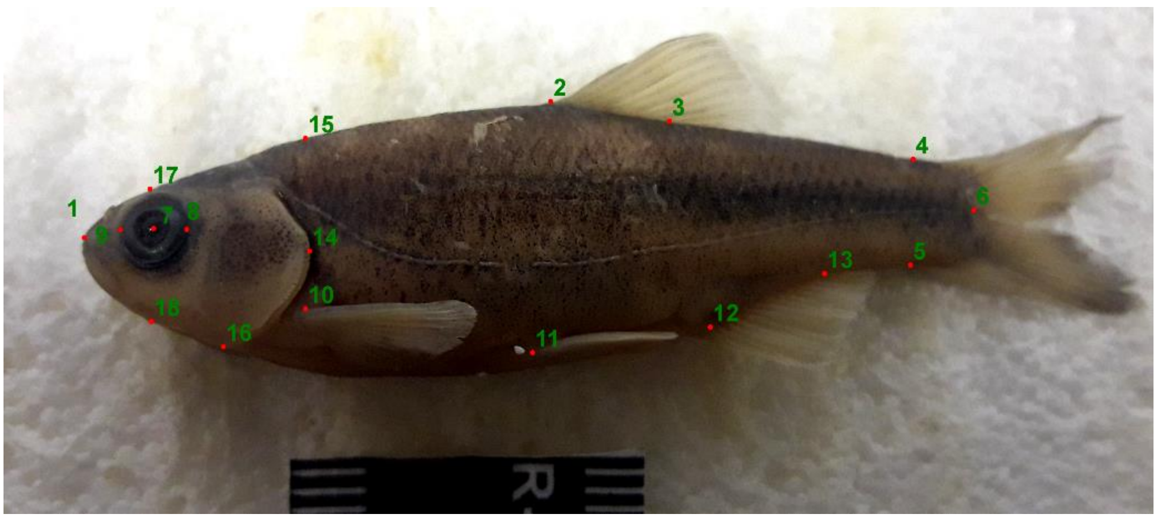

FIGURE 1. The location of the landmarks used for geometric morphometric analysis of *A. idignensis* populations in Tigris River Drainage, Persian Gulf Basin.

RESULTS

Geometric morphometric

Our results of principle component analysis (PCA) with landmark-points data showed that the three first PCs accounted for 64.19 % of variances (35.175, 18.01 and 12.8 % for PC1, PC2 and PC3, respectively, Fig. 2). The scree plot is used to determine the number of factors to retain in principal components to keep in a principal component analysis (PCA). Investigations of deformation grids along the CV1 and CV2 showed that the populations along PC1 (positive side) have smaller head, lower snout length, shorter base of anal fin, higher body height and longer of caudal peduncle. The populations along PC2 (positive side) have lower body height, pelvic and anal fins in anterior position and longer caudal peduncle length. Figure 5 shows the results of cluster analysis of the landmark-points data of *A. idignensis* populations and shows that Aab-Sardeh, Darband and Aab-Barik populations are in the same group and Sarab-e Keyvareh is in another group.

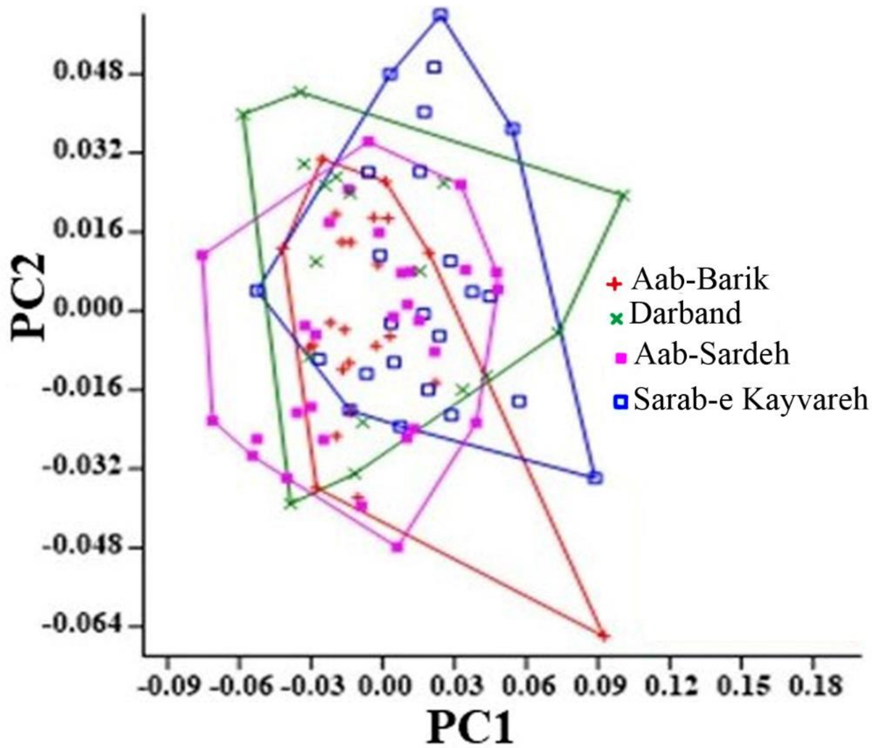

FIGURE 2. Principal Component Analysis (PCA) of *A. idignensis* populations in Tigris River.

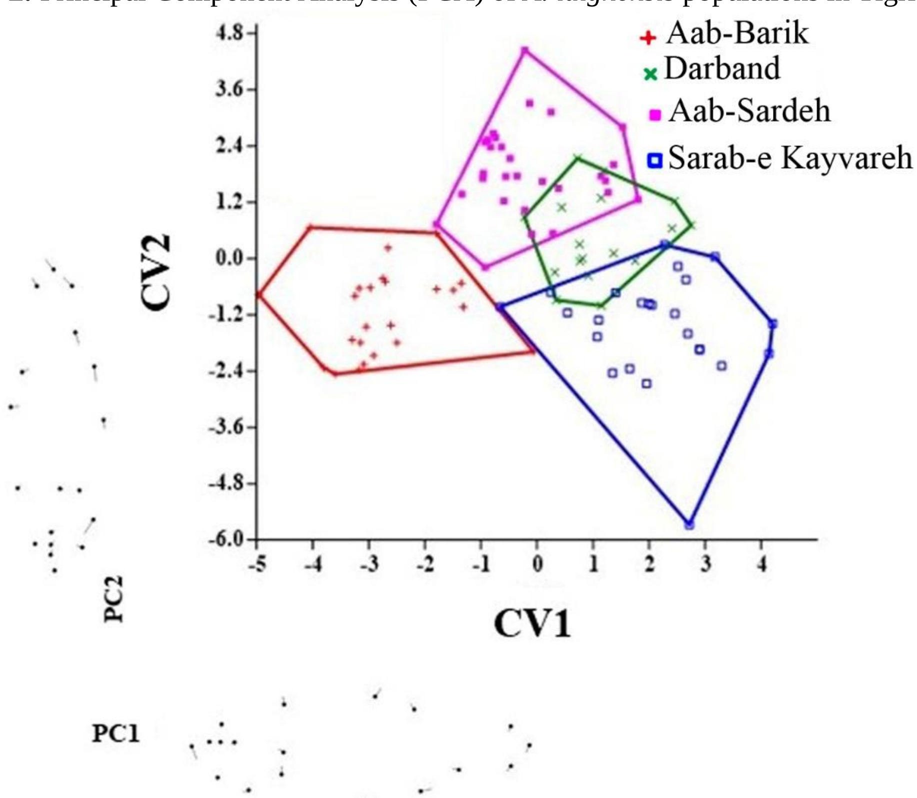

FIGURE 3. Canonical Variance Analysis (CVA) of *A. idignensis* populations in Tigris River Drainage, Persian Gulf Basin and TPS-deformation grid along the CV1 and CV2.

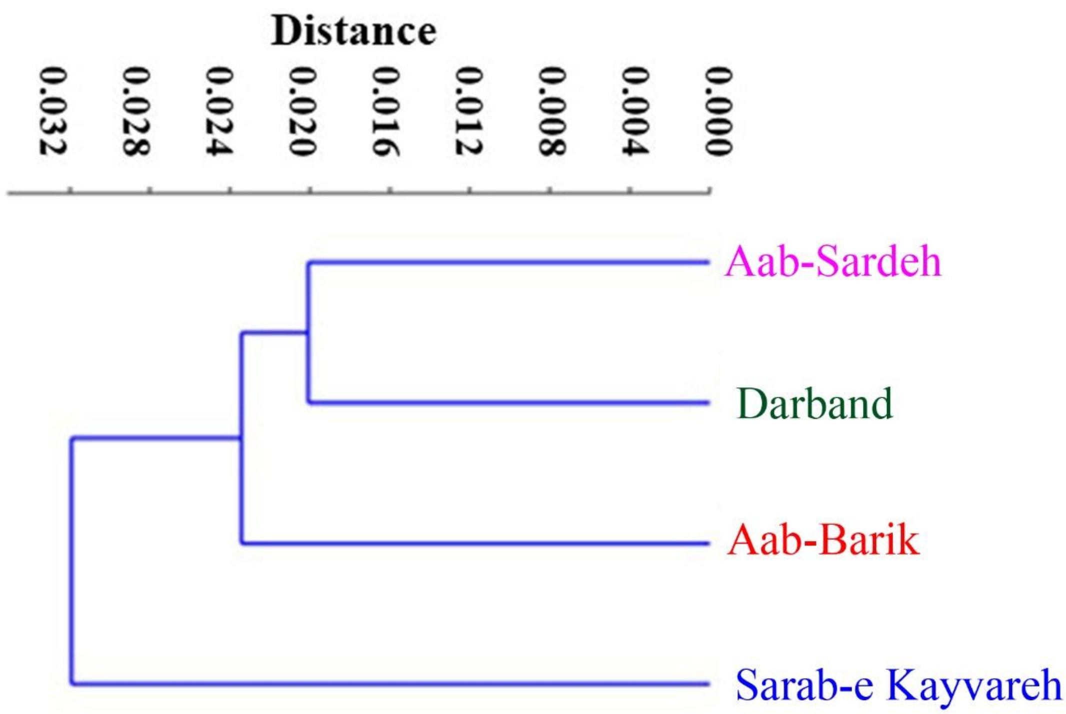

FIGURE 4. Cluster analyses of *A. idignensis* populations in Tigris River Drainage, Persian Gulf Basin.

Meristic characters

Based on normality test, all meristic characters were nonparametric except lateral line scales (LL) and pre-dorsal scales, so they were analysed with Kruskal–Wallis and ANOVA, respectively (Table 2) and showed that studied populations were different in all meristic character (p<0.05) except in the LL scales.

DISCUSSION

The four studied populations were different in most meristic traits (7 out of 8) and body shape. Aab-Sardeh, Darband and Aab-Barik populations are in the same group and Sarab-e Keyvareh is in another group. In a study on *Alburnoides eichwaldii*, it was indicated that the body shape of fish populations inhabiting different habitats and rivers are different and these differences show the habitat-specific separation in the studied populations (Eagderi et al. 2013; Rohlf, 2010; Mouludi-Saleh e al. 2017; 2018; Tajik & Keivany; 2018). Moreover, results of PCA and CVA in the morphometric characteristics of Tajan, Babolrud and Aras rivers, somewhat separated from each other. The meristic characteristics of *Alburnoides eichwaldii* were very close together and there was high degree of overlap in their characteristics. In some northern rivers *Alburnoides eichwaldii* showed Intra-population variations in their morphology while morphometric and relative morphometric characters were not useful for separating the two populations and sexes. However, the meristic characters could relatively separate these two populations (Haghighy, 2015).

Jouladeh Roudbar et al. showed that *A. idignensis* cannot be distinguished from other three studied species because the species overlapped in morphometric and meristic traits (Jouladeh Roudbar et al. 2015; 2016). These shape changes are probably related to differences in habitat and feeding habits among the populations (Keivany & Arab, 2017). The onset of adaptive radiation often requires two conditions to be met, the formation of a new habitat or a dramatic change of an already existing habitat and the owning of a key innovation, such as, a set of traits allowing for rapid adaptation towards new niches (Sturmbauer, 1998; Yoder, 2010). Generally, morphological changes seen in the rivers may be the result of phenotypic plasticity, local adaptation, ecological character displacement, genetic divergence or the interaction of any of these factors (Nicieza, 1995). In the species level, morphological differences among the species are often discussed as genetic divergent as results of competition and ecological preferences, so that different species exploiting different resources (Ehlinger & Wilson, 1988; Dynes et al. 1999). However, differences among-population are often considered to be the result of acclimation to local environmental conditions (Mittelbach et al. 1992). As a result, and in a general concept, variation in morphology resulted from environmental effects on phenotypic characters or by counteracting genetic differences between populations (Marcil et al. 2006).

Evaluation and explanation of the phenotypic plasticity among isolated population as well as the pattern of these variations has always been a great and difficult subject for evolutionary biologist. It is particularly true in the case of geographically widely distributed species. In nature, water flow and temperature vary considerably along streams and are very important in influencing the structure and morphological traits of fish communities. Even within streams, the range of temperature can be different from one part of the stream to the next. This variation in conditions eventually results in the adaptation of different populations to a given range of temperatures and water flow conditions [38,39]. Body depth showed differences among the individuals of three populations. Variation in body depth could affect the overall fusiform shape of the fish; therefore, it may change the hydrodynamic power and swimming ability of the fish [40] and can be considered as eco-morphological variation [41]. For example, we observed a lower caudal peduncle length in the populations that is probably caused by a high-water flow in Darband and a moderate flow in Aab-Sardeh and Sarab-e Keyvareh in Lorestan province. Aab-Barik with a low water flow is in Hamedan province. Moreover, Langerhans and Reznick (Langerhans, 2008). hypothesized that increasing water flow regimes may lead to increases in fin areas.

The relationship between morphology and ecology in fishes has long been known, and a few studies have applied multivariate morphometric methods to investigate ecomorphological patterns in multi-species fish communities (Douglas, 1992). Variation and differences in body shapes are important if lead to adaptations to environmental conditions and increases survival rates in aquatic habitats. Such adaptations are related to the need for compatibility with hydrodynamic forces to saving energy during biological behaviours (Nacua et al. 2010). The main reason for the separation of these populations in different rivers is probably due to the geographical separation of these populations which often leads to a decrease in the gene flow between them (Hellberg, 1994). In other words, genetic and body shape differences among populations increases with geographical barriers or isolation (Zamani-Faradonbe et al. 2015). Differences in the body forms of populations from different habitats and even in different species of same habitat, indicate the differences in the type of acclimation of fish to the type of habitat (like pools or riffles) (Tabatabaei et al. 2014). Differences in head shape and position of the mouth probably is related to feeding behaviours (Langerhans et al. 2003). Also, morphological variability and evolutionary proses of body shape of various populations of fish indicate that the habitat conditions along with the geographical separation are the key factors that change the phenotypic characters of the fish inhabiting that area. Different distribution of some populations in canonical discriminant analysis graph might be based on the habitat characters; geographical distance and isolation mechanisms (Cicek et al. 2016).

The phenotypic variability of the populations of a species in various environments is a phenomenon that results from the effects of environmental factors on this generation and previous generations in terms of adaptation and speciation. So these differences in body shapes among populations of *A. idignensis* probably reflects differences in environmental conditions and genetic variation. Since the purpose of this study was to compare the body shape of different populations of *A. idignensis* inhabiting different rivers, the results showed that studied populations differed in meristic characters and in the shape and size of head, body, caudal peduncle and ventral and anal fin position. Further studies are needed to provide a better genetic relationship in these populations.

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