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Full Length Article

Morphological variation among the Caspian roach (*Rutilusrutilus caspicus*) populations from the Southern Caspian Sea using Geometric Morphometrics technique

Fariborz Ghojoghi[1], Abolghasem Kamali[1], Soheil Eagderi[2], Mehdi Soltani[3], Iraj Hashemzadeh Segherloo[4]

[1]Department of Fisheries Sciences, Science and Research branch, Islamic Azad University,Tehran, Iran.
[2]Department of Fisheries, Faculty of Natural Resources and Agricultural, University of Tehran, Karaj, Iran.
[3]Departmnt of Aquatic Animal Health, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
[4]Department of Fisheries and Environmental Sciences, Faculty of Natural Resources and Earth Sciences, University of ShahreKord, ShahreKord, Iran.
fariborzghojoghi@yahoo.com

ABSTRACT

This study was conducted to compare the morphological variation of three reported Caspian roach (Rutilusrutiluscaspicus) population sin southern Caspian Sea using landmark-based geometric morphometric. A total number of 120 specimens from Bandar-e-Turkmen shore, Anzali wetland and Aras River were sampled. The left side of specimens were photographed and 14landmark-points were digitized on 2D imagesusingTpsDig2 software. Then GPA analysis was applied to eliminate non-shape variations. DFA, PCA, CVA and Cluster analysis were used to examine shape differences among populations and their sexes. The results showed the presence of sexual dimorphism in Aras and Kura population, but not in Turkmen one. The significant morphological differences were found among the body shape of three studied populations suggesting that they should be considered as distinct stocks. The result found precence of sexual dimorphism in Caspian roach showing better ability of GM approach to study the stock identification in Caspian roach.

Keywords: Generalized Procrustes Analysis, Shape Variation, Caspian roach.

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Introduction

The Caspian roach (*Rutilusrutilus caspicus*) have a wide distribution in southern Caspian Sea [1] and displaying morphological variation among populations [2,3,4,5]. Hence, the populations of this species are recognized as the morpha-migratorius, perhaps a first steps toward speciation [6,1]. Various populations of this species has retained as a distinct species i.e. *Rrutilus caspicus* with three varieties includingthe Astrakhan or Northern, Turkmenian (natio*knipowitschi*, Pravadin 1927) and Kura (natio*Kurensis*, Berg, 1932) Caspian roach based on morphometrics and meristic characteristics [7,1].

Some works showed morphological differences between various populations of Caspian roach based on traditional morphometrics [2,3,4,8,9,10]. In addition, the molecular studies using microsatellite marker revealed controversial results [11,12,13,14], displaying a higher gen flow and non-significant differences among the southern population of Caspian roach. Therefore, despite genetic similarities, the morphological differences may be considered as response to various environmental conditions [4].

These attempts to differentiate Caspian roach populations have been led to various definitions of its geographical groups to recognize their stocks concerning to fishing management and restocking programmed. However due to any distinctive morphological characteristics among its wide geographical distribution, there is no consensus on its populations. Hence our objective is to compare the body shape of three southern populations of Caspian roach using landmark-based geometric morphometrics (GM) as a new method. This method has a wide application to study the shape of organisms in different biological fields including fisheries and Ichthyology [15]. This technique allows the study of shape, offering powerful

analytical and graphical tools for the quantification and visualization of morphological variation within and among organisms.

Phenotypic difference in shape within a species may exist because of sexual dimorphism and ecological specialization [16]. Since, there is no reported sexual dimorphism in Caspian roach. The present study aimed to reveal potential sexual dimorphism and morphological differences among Caspian roach populations in the southern Caspian Sea for its fisheries management purpose. However other morphological and genetic criteria for stock delimitation was not found adequate to distinguish their stocks. Since phenotypic diversity between populations can be considered as essential step in ongoing steps in process of speciation [17].

MATERIALS AND METHODS

In total 180 specimens were collected from three distinguished populations of the Caspian roach from southern Caspian Sea (60 specimens per population, including 30 male and 30 female) using gill net. The Sampling regions were Bandar-e-Turkmen shore, Anzali wetland and Aras River (Figure 1). Specimens were anesthetized in clove solution, fixed into 4% buffered formalin and transformed to 72% ethanol after 96 hour for further examination.

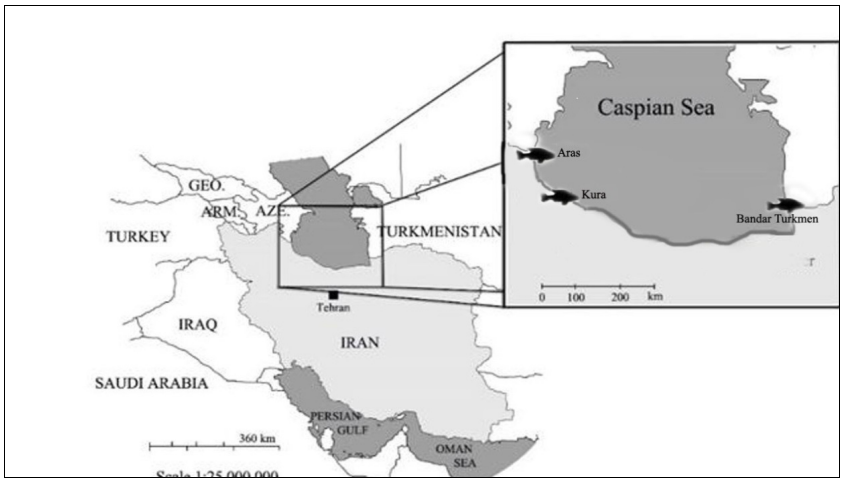

Figure 1. Sampling stations in south Caspian Sea.

To extract the body shape data in landmark based GM method, digital images were taken using a copy-stand equipped to digital camera (Kodak 6.MP) from their left side. Then fourteen landmark-points were defined and digitized using TPSDig2 software version 2.16 (18) (Figure 2). Further investigation performed in two stages including (1) comparison the body shape between male and female of each population and (2) comparison of body shape among all populations.

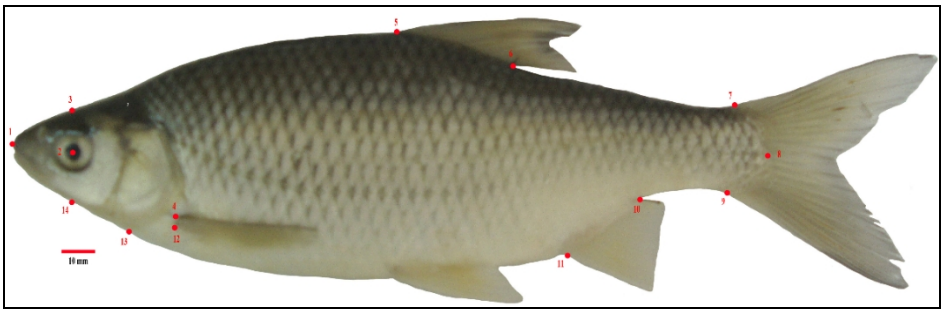

Figure 2. Used landmark-points to extract shape data of *Rutilusrutiluscaspicus*. 1. Tip of the snout; 2. The center of eye; 3. Dorsal edge of head perpendicular; 4. Anterior base of the pectoral fin; 5, 6. Anterior and posterior base of dorsal fin; 7. Lower margin of caudal peduncle; 8. End of the medial region of caudal peduncle; 9. Upper margin of caudal peduncle; 10, 11. Anterior and posterior insertion of the anal fin; 12. Ventral base of the pectoral fin; 13. Ventral end of opercular slit; 14. Ventral edge of head perpendicular.

Correlation coordinate data between the procrustes and tangent shape distances using tpsSmall software were calculated [19] and landmarks were superimposed using generalized procrustes analysis (GPA) to remove non-shape data including size, position and orientation (20). Discriminate functional analysis

(DFA) and T-test hoteling were performed to compare body shape between sexes using PAST software. Visualization of shape difference as wireframe graph were performed using MorphoJ software (21). Principal component analysis (PCA) was performed to summarize the variation among the data of three populations [22]. Canonical variant analysis (CVA) was used to investigate power of distinction of three populations with P-value obtained from permutation test with 10000 replication in MorphoJ software. Finally, a cluster analysis by adapting the Euclidean square distance a measure of similarity and selecting 100 bootstrapping was performed [23]. The Mahalanobis distances between populations and their sexes was extracted using MorphoJ software [21].

RESULTS

The discriminate analysis (DA) functions showed the body shape differences between two sexes in Aras (*P*=0.0005) and Kura (*P*=0.0428) populations, whereas there was no sexual dimorphism in Bandar-e-Turkmen one (Figure 3). In firsttwo populations, female has deeper body and lower anterior position of snout. Also, female of Aras population has a smaller head than that of male (Figure 4).

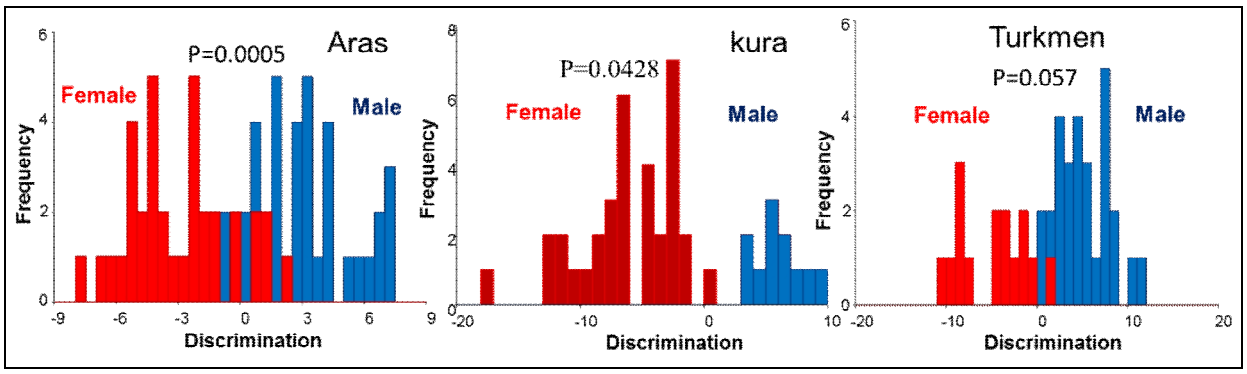

Figure 3.DFA graph of the frequency and distribution of partial warp scores of body shape variation between sexes of tree studied populations of *Rutilusrutiluscaspicus*.

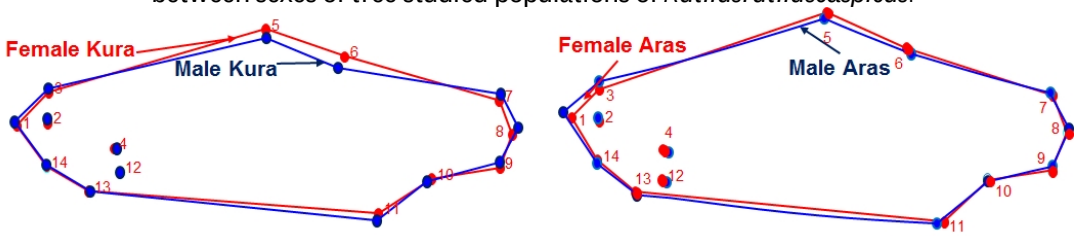

Figure 4. Deformation wireframe graph showing body shape difference between sexes related in Kura and Aras populations of *Rutilusrutiluscaspicus*.

Since, the results showed presence of sexual dimorphism in terms of body shape in Kura and Aras populations, therefore the statistical analysis subjected for two sexes as separate. PCA analysis of female and male specimens explained 64.6% (PC1=33.8% and PC2=30.8%) and 63.5% (PC1=42 % and PC2=21.5%) of shape variation, respectively.Plotting of first and second PCs displayed distinction of the Aras population than two others, particularly in male specimens (Figure 5).

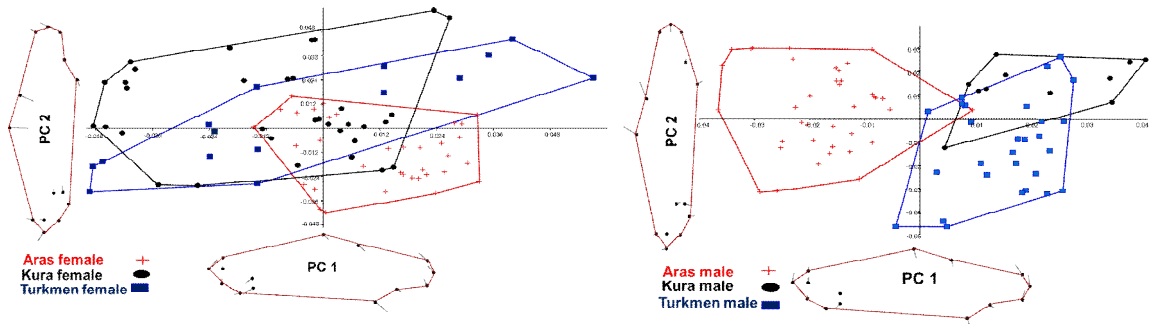

Figure 5. Scatter of individuals' body shape on principal component 1 and 2for male and female oftree studied populations of *Rutilusrutiluscaspicus* (the wireframe graphs showing mean shape as wireframe and vectors pointing in the direction of the axis loading for both male and female).

CVA analysis with obtained p-value from permutation test revealed a significant differences in body shape of studied populations in both sexes (P<0.05). The three populations separated from each other in CVA graphs (Figure 6).

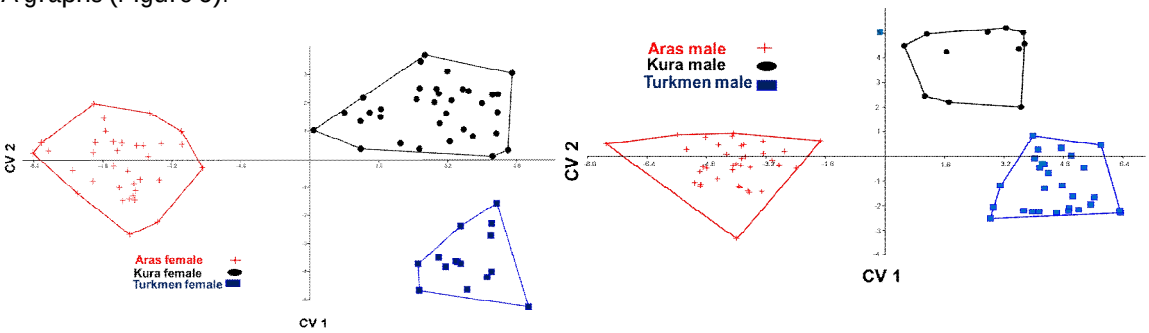

Figure 6. Canonical variant analysis (CVA) of body shape of male and female of *Rutilusrutiluscaspicus*

The mahalanobis distances among population and sexes are represented in Table 1. Based on results, the smallest and greatest distances found between two sexes of Aras population and females of Turkmen and Aras populations, respectively (Table 1).

Table 1. Mahalanobis distance analysis of males and femalesof *Rutilusrutiluscaspicus*

	Female Aras	Female Turkmen	Female Kura	Male Aras	Male Turkmen
Female Turkmen	6.885				
Female Kura	6.2782	4.5086			
Male Aras	2.1753	6.7284	5.9224		
Male Turkmen	6.793	2.6383	3.6819	6.6823	
Male Kura	6.829	4.7349	2.5436	6.6145	4.3823

Visualizing of differences in body shape of studied populations of male and female presented in Figure 6. The results showed that Aras population has a greater body depth than others. The Turkmen population has a shorter dorsal and anal fin base, whereas the Kura population poses longer caudal pedunclewhich considered as their distinguishable morphological traits.

The cluster analysis of studied populations showed in figure (7), dividing into two major distinct branches based on body shape. The first branch includes the Kura and Turkmen population and the second branch is included the Aras population.

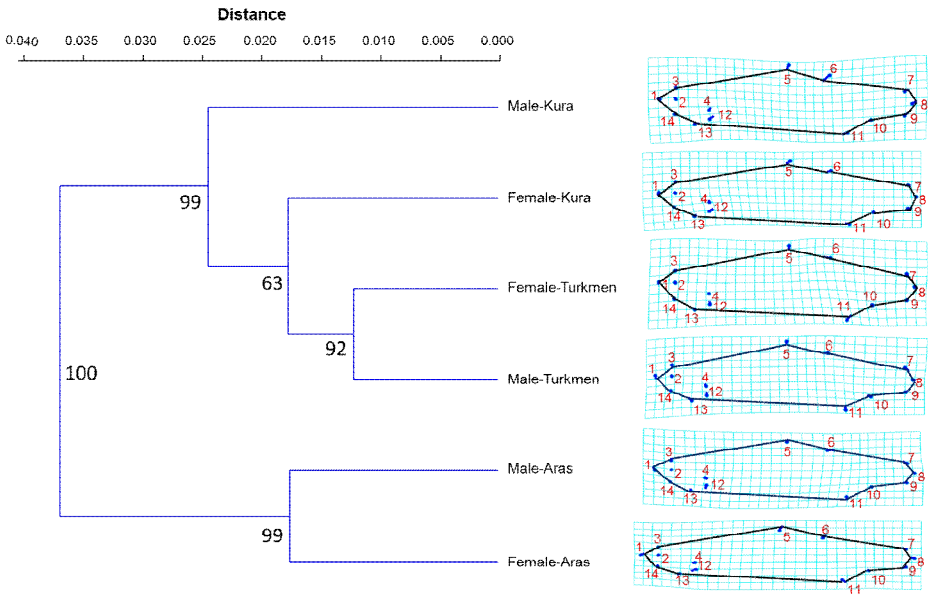

Figure 7. Cluster analysis of male and female studied populations

DISCUSSION

In Iranian waters of Caspian Sea, three distinct population of *Rutilusrutiluscaspicus*are reported [1]. A subspecies of the genus *Rutilus* (*R. rutilusschelkounikovi*Derkhavin 1926) was described from Aras River [1]. The second population with two types belongs to subspecies *R. rutiluscaspicus*natio*kurensis* from Anzali and Kura which lives in Anzali wetland. The recent subspecies is relatively small and the Kura type migrates between Anzali wetland and Kura River in Azerbaijan. The third population lives in south-east of the Caspian Sea known as *R. rutiluscaspicus*natio*knipowitschi*. Bogustkaya and Naseka [24] recognize all these varieties and their types as *R. rutiluscaspicus*(25) as the semi species in the Caspian Sea. In the present study, landmark-based geometric morphometrics tool was applied to compare and visualize the body shape changes among these three populations and the results showed a significant morphological difference in terms of body shape. Although these differences has no taxonomic value, but the results suggested that they should be considered as distinct stocks. It is now accepted that morphological variations has both environment and genetic component [26] and may reveal different growth, mortality or reproductive rate that are relevant for definition of stocks [27,28].

The results showed the presence of sexual dimorphism in Aras and Kura population, but not in Turkmen one. Their differences were related to body depth and head size which may be as result of niche dimorphism or feeding competitions concerned to the characteristics of their habitats [29] to decrease competition between sexes [30].The morphometric stock identification of a species with a sexual dimorphism and geographic differences were reported in many finfish stocks [31,32,33].Other works showed morphological differences between various populations of Caspian roach based on traditional morphometrics [2,3,4,8,9,10]. The results of this study showed presence of sexual dimorphism in two studied populations suggesting a revision in earlier morphometric works with considering their sexual dimorphism.

The results of Mahalanobis distances and cluster analysis showed two main branches, including the Kura and Turkmen population in the first branch and Aras in the second one. The first branch is divided into two groups comprising (a) male Kura and (b) the female Kura and both sexes of Turkmen population. This results reveal less divergent in terms of body shape between Turkmen and Kura populations may be related to the similar condition of their habitats i.e. Caspian Sea compared to riverine system of Aras.Many fish species show morphological differences among habitats [34,35,36,37] and intraspecific polymorphism is typically believed to arise from divergent selection pressures among various environments [34,35,16]. It is common that morphological characteristics can show high plasticity in response to different environmental circumstances [38].

Based on the results Aras population has a greater body depth, Turkmen population has a shorter dorsal and anal fin base and Kura population poses longer caudal peduncle which considered as their distinguishable morphological traits suggesting that they should be considered as distinct stocks. Morphometric stock discrimination studies are common, but the reasons of differences in shape are rarely interpreted. Here we applied advanced image processing techniques and appropriate methods to study morphological differences between Caspian roach populations from a wide geographic area. Geometric morphometric technique is a powerful tool for stock identification, than traditional method to better stock identification as our results could showed the presence of sexual dimorphism that was not reported before in this species.

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