

Morphometric and Meristic Variation in Twelve Different Populations of *Garra rufa* (Heckel, 1843) from Iran

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Abstract: This study is carried out to examine the meristic characteristics and variations in the body shape of the red stone lapper, *Garra rufa* (Heckel, 1843) living in many river systems including the Tigris, Jarrahi, Zohreh, and Mond Basins using geometric and classical morphometric methods. The morphological differences of twelve populations of red stone lapper, *G. rufa* from Iranian inland water basins were studied using meristic, morphometric, and geometric (Landmark-point) methods. For this aim, twelve meristic characters were counted, nineteen classical morphometrics were measured, and sixteen ratios were calculated. As for the geometric part, thirteen homologous landmark-points were digitized using tpsDig2 software. All analyses showed significant differences in seven meristic and nineteen morphometric characters among the populations. Further analyses including PCA, CVA, and CA of geometric data have shown that there are significant differences in the head region and dorsal-fin base among the *G. rufa* populations. These results suggest that classical and geometric morphometric methods can distinguish red stone lapper populations of the Iranian inland waters from each other; the differences in body shape suggest that habitat parameters including physicochemical parameters may have caused these patterns of variation in the body shape of fishes.

Keywords: *Garra rufa*, Variation, Habitat, Geography, Plasticity.

Introduction

Fishes are very sensitive to habitat conditions (Hossain *et al.*, 2010); therefore, living in many rivers and experiencing the different habitat conditions may cause variations in the body shape of fishes (Costa and Cataudella, 2007).

On the other hand, existing in many rivers with variable conditions has proven that fishes have a high adaptation ability and can respond to habitat situations by phenotype plasticity which guarantees their generation survival in many waters (Gelsvartas, 2005). Many studies have confirmed that different populations of fish species living in different habitats show variations in their body shape; for example *Cyprinion* (Nasri *et al.*, 2018); Trout Barb, *Capoeta trutta* (Keivany and Arab, 2017); *C. fusca* (Banimasani *et al.*, 2019); *Squalius turcicus* (Mouludi-Saleh *et al.*, 2020); Kura Barb (*Barbus lacerta*) (Zamani-Faradonbe and Eagderi, 2016; Zamani-Faradonbe *et al.*, 2015b) and *Alburnus mossulensis* (Keivany *et al.*, 2016a); Such differences appear to reflect the big ability of fish to survive under a range of conditions in rivers across Iran.

There are several techniques used to distinguish and compare the body shape of different populations including the geometric morphometric and classical morphometric methods. Geometric morphometrics is the Landmark-point method that is based on the digitalization and comparison of sampled body shapes (Bookstein, 1991; Zelditch *et al.*, 2004). In the classical morphometric method, researchers use meristic characters such as the number of scales and fin rays

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and other measurable characters (Naeem and Salam, 2005).

With at least thirteen valid species, the genus *Garra* Hamilton, 1822 is one of the most diverse genera of the Cyprinidae in Iranian waters that has a vast distribution area extending from the western to the eastern basins (Sayyadzadeh *et al.*, 2015; Mousavi-Sabet and Eagderi, 2016; Mousavi-Sabet *et al.*, 2019). The most widely distributed species of this genus is *Garra rufa* which lives in many tributaries flowing in the Tigris, Jarrahi, Zohreh, Fars, Maharlu, and the Mond basins; mostly, they live in lotic water, but sometimes they exist in lentic waters as well. *G. rufa* feeds on detritus, diatoms, algae, insects, and plants. Their vast distribution area suggests that this species is likely to have the ability to endure different habitat conditions which reflects their phenotype plasticity.

Coad (2010) and Kaya (2012) stated that *G. rufa* lives in moderate and fast rivers that are rocky and graveled, and mostly they have benthic characters.

The aim of this study is to assess the variations in the body shape of twelve populations of the red stone lapper, *G. rufa* from four basins in Iran. The results of this study can provide significant information for habitat conservation, and fish resource-management programs.

Materials and Methods

The Iranian drainage basins have been divided into 19-22 major basins based on fish distributions in different texts (Esmaili *et al.*, 2017; Keivany *et al.*, 2016b). For this study, a total of 223 individuals of *Garra rufa* were collected during November, 2017 using electrofishing equipment (Samus Mp750, 45 cm diameter, aluminium ring anode) and downstream stop-nets with a 0.2 cm mesh size from twelve different flowing waters and rivers, namely the Tigris, Jarrahi, Zohreh, and the Mond basins (Figure 1); The collected specimens were anesthetized in 1% clove oil, and were then fixed in 10% formalin and transferred to the laboratory

for meristic, morphometric and geometric studies.

The Classical Morphometric Method

A total of twelve meristic characters, including the number of lateral line scales (L.L), scales above L.L, scales bellow L.L to ventral fin, scales bellow L.L to the anal-fin, predorsal scales, circumpeduncular scales, dorsal, anal, pectoral, and ventral-fins soft rays, caudal-fin rays, and the number of barbells, were counted on the left side of the samples.

Also, nineteen morphometric characters were measured using a digital caliper (0.1 millimeter) on the left side of the specimens (Figure 2). Methods for the counting and measurements follow Kottelat and Freyhof, (2007). Some modification methods were used before the analysis of the morphometric data; three traits including snout length, orbital diameter, and postorbital length were used in the next analysis as the ratio of head length; predorsal fin length, dorsal fin base length, post dorsal fin length, preanal fin length, anal fin base length, post anal fin length, prepectoral fin length, Prepelvic fin length, pectoral-pelvic fins length, head length, head depth, body height, caudal peduncle depth were used as the ratio of standard length, and total, fork, and standard lengths were used in the further analysis without modification. After collecting data, the Kolmogorov– Smirnov test was used to test the normality of meristic and morphometric data, then the Kruskal–Wallis tests used for data following abnormal distribution and for data following normal distribution, one-way ANOVAs were applied (SPSS-19 software).

Geometric Morphometry and Body Shape

The left sides of all specimens were photographed using a Canon camera (12 MP resolutions); then to extract the body shape data, thirteen homologous landmark-points on the photographs were placed with the tpsDig2 software (Rohlf, 2003). In choosing

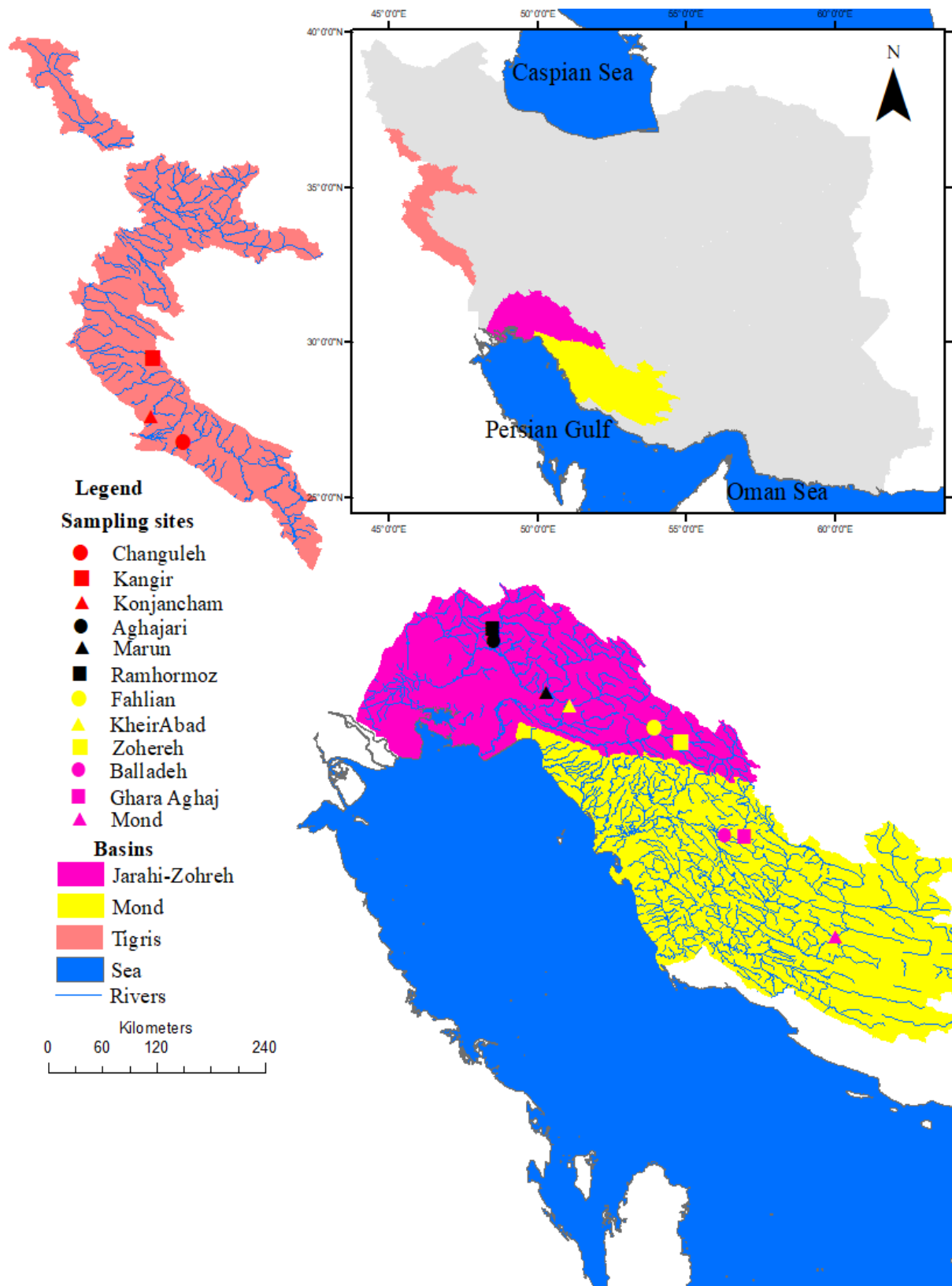


Figure 1. Map of Iran showing the sampling sites of the *Garra rufa* populations.

the landmarks, the researchers concentrated on the description of the fish's body shape (Figure 3). To remove non-shape variations such as information on the isometric size of the objects, their position, and spatial

orientation from the data, the Generalized Procrustes Analysis was used (Dryden and Mardia, 1998).

Principal component analysis (PCA) was conducted to evaluate the structure and

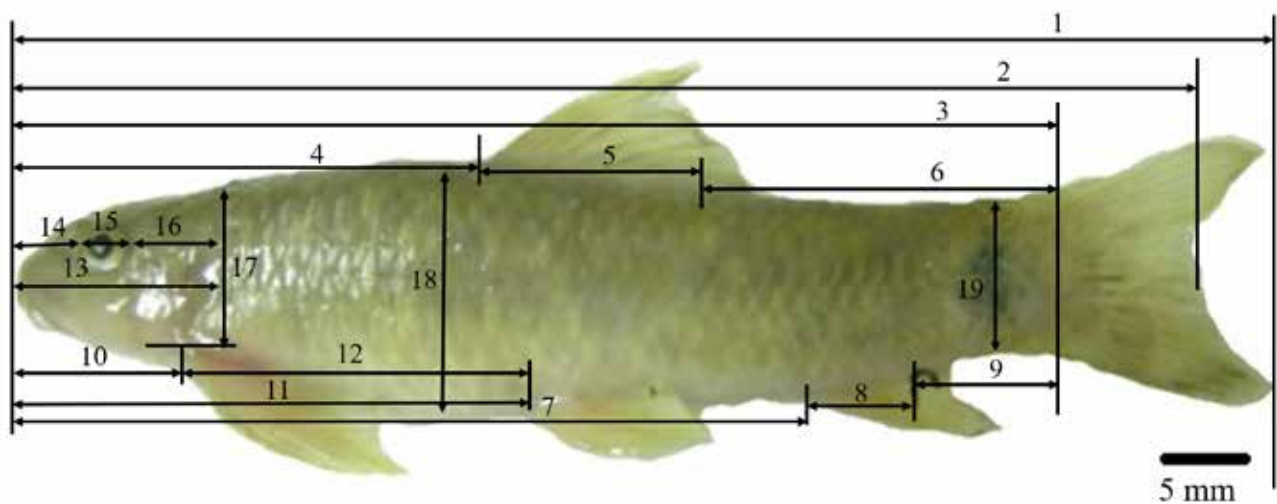


Figure 2. Morphometric measurements on *Garra rufa* body; 1: Total Length (TL); 2: Fork Length (FL); 3: Standard Length (SL); 4: Predorsal fin Length (PdL); 5: Dorsal fin Base Length (DfBL); 6: Post dorsal fin Length (PdL); 7: Preanal fin Length (PaL); 8: Anal fin Base Length (AfBL); 9: Caudal Peduncle Length (CPL); 10: Prepectoral fin Length (PpL); 11: Prepelvic fin Distance (PpD); 12: Pectoral-Pelvic fins Length (PVL); 13: Head Length (HL); 14: Snout Length (SnL); 15: Orbital (Eye) Diameter (OD); 16: Postorbital Length (PoL); 17: Head Depth at nape (HD); 18: Body Depth at dorsal fin origin (BD); 19: Caudal Peduncle Depth (CPD).

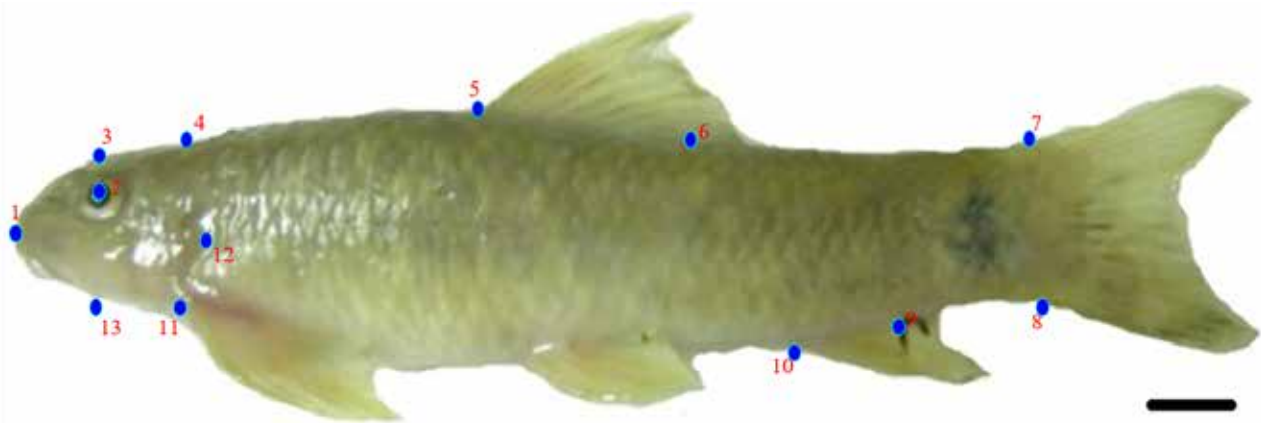


Figure 3. Selected landmark points' positions on the photos of the *Garra rufa* specimens, scale bar: 5 millimeters.

the contribution of the total variance of the data. Since the first three PCs encompass the largest portion of variability, the distribution of specimens on the grid, and the distribution of the portions of variability on them as well as the shape of deformation along the first three axes were analyzed. Deformation grids, on which the direction and amplitude of variability for every landmark points were denoted as a vector, were used to visualize the shape variability (Klingenberg, 2013). Canonical Variance Analysis (CVA) and Cluster Analysis (CA) were used to further quantify differences in shape (Bravi *et al.*, 2013). Mahalanobis distance (Md) and Procrustes distance (Pd) were used to report the CVA results statistically. These Md and

Pd measures are multivariate measurements of distance relative to the within-sample variation. All multivariate analyses were computed using PAST software (Hammer, 2012) and MorphoJ 1.01 (Klingenberg, 2011).

Results

The Classical Morphometric Method

Meristic Traits: After counting the meristic characters, the number of dorsal, anal, caudal-fin soft rays, and barbells were found to be fixed in all specimens (8, 5, 17, and 2, respectively); since predorsal scales have been embedded in the samples of some populations, statistical analysis was not performed on them. The results

of the Kolmogorov–Smirnov test showed that the number of lateral line scales (L.L), scales above L.L, scales below L.L to the ventral fin, scales below L.L to the anal-fin, predorsal scales, circumpeduncular scales, pectoral, and ventral-fin soft rays were nonparametric. The Kruskal–Wallis analysis results are presented in Table 1. According to Table 1, all studied populations have significant differences in meristic characters ($p < 0.001$).

Morphometric Traits: The results of the Kolmogorov–Smirnov test showed that ten morphometric characters including standard length, post-dorsal fin length\SL, dorsal fin base length\SL, post-dorsal fin length\SL, preanal fin length\SL, anal fin base length\SL, prepectoral fin length\SL, prepelvic fin distance\SL, pectoral-pelvic fins length\SL, head length\SL were parametric. Nine of these characters including total length, fork length, caudal peduncle length/SL, head depth at nape/SL, body depth at dorsal fin origin/SL, caudal peduncle depth/SL, snout length /HL, Orbital (Eye) diameter /HL, postorbital length /HL were nonparametric. The One Way ANOVA results for parametric distributed characters and the Kruskal–Wallis test for the non-parametric distributed characters showed that the morphometric characters were significantly different among the twelve studied populations of *G. rufa* samples ($p < 0.001$) (Tables 2 and 3).

Geometric Morphometry

The first three PCs that were higher than the cut-off point of the Joliffe line (Joliffe, 2002) accounted for the majority of shape variations (65.52%): PC1 explained 37.94%, PC2 14.55%, PC3 13.03%, while each of the twenty-three remaining components explained less than 8% (Figure 4). The PCA scatter plot and deformation grids showed a high morphological variation in the populations (Figures 5 and 6). The main part of the body shape deformation is attributed to the landmark points of the head region (1, 3, 4, 11-13) and Dorsal-fin base (5, 6) (Figure 6). In order to assess intra-population differences in the body shape,

CVA was performed on data of the *G. rufa* populations. The p -value of the permutation test in Canonical Variance Analysis (CVA) showed a significant difference (Wilks lambda = 0.0013, $F = 3.93$, $p < 0.001$) in the body shape among the populations. Based on the CVA scatter plot, the populations of the Changuleh, Mond, and Aghajari rivers were separated from other populations (Figure 7). The main part of the shape deformation was in the head region (1, 3, 4, 11-13) and dorsal-fin base (5, 6) (Figure 8). This analysis highlighted the significant differences among the populations; the highest values of Md (> 4.00) and Pd were among Fahlian-Balladeh and Changuleh and Marun; Mond-Aghajari; Marun- Changuleh and Mond; Ramhormoz- Balladeh and Mond and Kheir Abad (Tables 4 and 5).

The cluster analysis (CA) of the different sampled populations showed at least two main groups, each of which has two sub-groups based on body shape (Figure 9). In the first group, the Ramhormuz population (from the Jarrahi- Zohreh Basin) was located in a separate sub-group, while the populations of Aghajari, Kheir Abad (from Jarrahi- Zohreh Basin), Konjanchem, Changuleh (from the Tigris Basin), Ghara Aghaj, and Mond (from the Mond Basin) were in the same branch. As for the second group, the Fahlian population (from the Jarrahi- Zohreh Basin) was in a separate sub-group, while the populations of Kangir (from the Tigris Basin), Balladeh (from the Mond Basin), Marun, and Zohreh (from the Jarrahi- Zohreh Basin) were in the other branch of the second branch.

Discussion

In this study, three different methods, namely the meristic (countable), morphometric (measurable) and geometric (Landmarks-point based) methods were used to compare twelve populations of the red stone lapper, *Garra rufa* in the riverine waters of Iran. As mentioned before, the studied samples were captured from different waters flowing in the Tigris, Jarrahi, Zohreh and Mond basins. It is worth mentioning that all these rivers finally reach the Gulf.

Table 1. Meristic characters ranges, Minimum- Maximum (Mean \pm SD) and Kruskal-Wallis test results for abnormal distributed characters of *Garra rufa* populations of Iran

| | Aghajari | Balladeh | Changuleh | Fahlian | Ghara Aghaj | Kangir | Mond | Kheir Abad | Konjancham | Zohreh | Marun | Ramhormoz | p |
|----|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|------|
| 1* | 32-35 (33.3 \pm 0.9) | 32.0-36.0 (34.2 \pm 0.9) | 31.0-37.0 (34.1 \pm 1.4) | 31.0-35.0 (33.3 \pm 1.2) | 29.35 (32.4 \pm 1.5) | 32.0-36.0 (34.3 \pm 1.1) | 25.0-29.0 (27.5 \pm 1.3) | 30.0-35.0 (32.7 \pm 1.2) | 31.0-34.0 (32.4 \pm 0.9) | 30.0-35.0 (33.3 \pm 1.1) | 30.0-33.0 (31.8 \pm 1.0) | 32.0-34.0 (33.2 \pm 0.7) | 0.00 |
| 2 | 0.3-0.5 (3.7 \pm 0.6) | 0.3-0.4 (3.6 \pm 0.5) | 3.0-5.0 (3.9 \pm 0.3) | 3.0-5.0 (3.05 \pm 0.8) | 3.0-4.0 (3.3 \pm 0.5) | 3.0-4.0 (3.8 \pm 0.4) | 3.0-4.0 (3.0 \pm 2.0) | 3.0-4.0 (3.4 \pm 0.5) | 3.0-4.0 (3.3 \pm 0.5) | 3.0-3.0 (3.0 \pm 0.0) | 3.0-3.0 (3.0 \pm 0.0) | 3.0-4.0 (3.3 \pm 0.5) | 0.00 |
| 3 | 0.3-0.4 (3.3 \pm 0.4) | 3.0-4.0 (3.1 \pm 0.3) | 3.0-4.0 (3.9 \pm 0.4) | 3.0-3.0 (3.0 \pm 0.0) | 2.0-4.0 (3.1 \pm 0.4) | 3.0-3.0 (3.0 \pm 0.0) | 2.0-3.0 (3.0 \pm 0.3) | 3.0-3.0 (3.0 \pm 0.0) | 3.0-3.0 (3.0 \pm 0.0) | 3.0-3.0 (3.0 \pm 0.0) | 3.0-3.0 (3.0 \pm 0.0) | 3.0-3.0 (3.0 \pm 0.0) | 0.00 |
| 4 | 0.3-0.4 (3.2 \pm 0.4) | 3.0-4.0 (3.3 \pm 0.5) | 3.0-4.0 (3.5 \pm 0.5) | 3.0-3.0 (3.0 \pm 0.0) | 3.0-3.0 (3.0 \pm 0.0) | 3.0-4.0 (3.1 \pm 0.3) | 2.0-3.0 (3.0 \pm 0.2) | 3.0-3.0 (3.0 \pm 0.0) | 3.0-3.0 (3.0 \pm 0.0) | 3.0-4.0 (3.2 \pm 0.4) | 3.0-3.0 (3.0 \pm 0.0) | 3.0-3.0 (3.0 \pm 0.0) | 0.00 |
| 5 | 10.0-13.0 (11.9 \pm 0.8) | 12.0-15.0 (13.9 \pm 0.7) | 10.0-14.0 (11.9 \pm 0.8) | 9.0-12.0 (10.5 \pm 1.1) | 11.0-15.0 (12.7 \pm 0.8) | 11.3-13.0 (12.1 \pm 0.6) | 11.0-15.0 (12.8 \pm 1.1) | 10.0-13.0 (11.6 \pm 0.7) | 11.0-13.0 (11.9 \pm 0.7) | 11.0-13.0 (11.9 \pm 0.8) | 11.0-13.0 (12.3 \pm 0.8) | 11.0-14.0 (11.8 \pm 1.1) | 0.00 |
| 6 | 6.0-8.0 (7.7 \pm 0.6) | 6.0-9.0 (8.3 \pm 0.7) | 7.0-9.0 (7.8 \pm 0.4) | 6.0-8.0 (7.2 \pm 0.7) | 7.0-8.0 (7.8 \pm 0.4) | 6.0-9.0 (7.5 \pm 0.8) | 7.0-10.0 (8.2 \pm 0.9) | 7.0-8.0 (7.4 \pm 0.5) | 7.0-8.0 (7.8 \pm 0.4) | 6.0-9.0 (7.3 \pm 0.8) | 7.0-8.0 (7.8 \pm 0.4) | 7.0-8.0 (7.8 \pm 0.4) | 0.00 |
| 7 | 13-.016.0 (14.7-1.0) | 13.0-16.0 (14.6 \pm 1.0) | 11.0-15.0 (12.9 \pm 0.9) | 13.0-15.0 (14.3 \pm 0.7) | 12.0-16.0 (13.9 \pm 0.9) | 13.0-17.0 (14.9 \pm 1.2) | 11.0-15.0 (12.8 \pm 0.9) | 10.0-15.0 (13.1 \pm 1.3) | 11.0-14.0 (12.4 \pm 0.9) | 11.0-16.0 (14.5 \pm 1.2) | 11.0-15.0 (13.4 \pm 1.1) | 14.0-17.0 (14.7 \pm 1.1) | 0.00 |

* List of meristic character: 1) Lateral line scales, 2) scales above L.L., 3) scales below L.L. to ventral fin, 4) scales below L.L. to anal fin, 5) pectoral-fin soft rays, 6) ventral-fin soft rays, 7) circumpeduncular scales.

Table 2. Morphometric characters ranges, Minimum- Maximum (Mean \pm SD), and One way ANOVA results for normal distributed characters of *Garra rufia* populations of Iran

| | Aghajari | Balladeh | Changuleh | Fahlian | Ghara Aghaj | Kangir | Mond | Kheir Abad | Konjancham | Zohreh | Marun | Ramhormoz |
|----|--|---|---|--|--|--|--|--|--|--|---|---|
| 1* | 27.6-74.1 (47.3 \pm 11.5) ^{cd} | 38.3-79.6 (55.0 \pm 8.1) ^{de} | 26.1-58.4 (33.5 \pm 6.7) ^{ab} | 29.5-85.4 (51.7 \pm 22.3) ^{cd} | 32.9-90.7 (47.1 \pm 16.0) ^{cd} | 33.9-70.8 (57.0 \pm 10.5) ^{de} | 16.5-35.6 (27.7 \pm 4.7) ^a | 23.5-78.5 (46.2 \pm 13.6) ^{cd} | 30.1-84.7 (40.6 \pm 13.7) ^{bc} | 28.8-113.4 (68.2 \pm 25.4) ^f | 35.4-102.5 (62.3 \pm 24.1) ^{ef} | 24.6-33.6 (30.5 \pm 3.2) ^{ie} |
| 2 | 0.4-0.5 (0.5 \pm 0.0) ^d | 0.4-0.5 (0.5 \pm 0.0) ^a | 0.5-0.6 (0.5 \pm 0.0) ^c | 0.4-0.6 (0.5 \pm 0.1) ^c | 0.5-0.6 (0.5 \pm 0.0) ^{bc} | 0.4-0.5 (0.5 \pm 0.0) ^a | 0.5-0.6 (0.5 \pm 0.0) ^c | 0.4-0.5 (0.5 \pm 0.0) ^{ab} | 0.4-0.5 (0.5 \pm 0.0) ^{bc} | 04-0.9 (0.5 \pm 0.1) ^a | 0.4-0.5 (0.5 \pm 0.0) ^a | 0.5-0.5 (0.5 \pm 0.0) ^c |
| 3 | 0.2-0.2 (0.2 \pm 0.0) ^c | 0.1-0.2 (0.2 \pm 0.0) ^{bc} | 0.2-0.2 (0.2 \pm 0.0) ^c | 0.2-0.2 (0.2 \pm 0.0) ^{bc} | 0.1-0.2 (0.2 \pm 0.0) ^a | 0.1-0.2 (0.2 \pm 0.0) ^a | 0.1-0.2 (0.2 \pm 0.2) ^a | 0.1-0.2 (0.2 \pm 0.0) ^a | 0.1-0.2 (0.2 \pm 0.0) ^{ab} | 0.1-0.4 (0.2 \pm 0.1) ^{bc} | 0.2-0.2 (0.2 \pm 0.0) ^c | 0.1-0.2 (0.2 \pm 0.0) ^{ab} |
| 4 | 0.3-0.4 (0.3 \pm 0.0) ^{cde} | 0.3-0.4 (0.3 \pm 0.0) ^{cde} | 0.2-0.3 (0.3 \pm 0.0) ^{ab} | 0.3-0.4 (0.3 \pm 0.0) ^{cde} | 0.2-0.4 (0.3 \pm 0.0) ^{cde} | 0.3-0.4 (0.3 \pm 0.0) ^c | 0.2-0.4 (0.3 \pm 0.0) ^{bcd} | 0.3-0.4 (0.3 \pm 0.0) ^{de} | 0.3-0.4 (0.3 \pm 0.0) ^{abc} | 0.1-0.4 (0.3 \pm 0.1) ^{cde} | 0.3-0.4 (0.3 \pm 0.0) ^{bc} | 0.2-0.3 (0.3 \pm 0.0) ^a |
| 5 | 0.8-0.9 (0.8 \pm 0.0) ^{bc} | 0.8-0.9 (0.8 \pm 0.0) ^{ab} | 0.8-0.9 (0.8 \pm 0.0) ^c | 0.7-0.9 (0.8 \pm 0.0) ^a | 0.8-0.9 (0.8 \pm 0.0) ^c | 0.7-0.8 (0.8 \pm 0.0) ^a | 0.7-0.9 (0.8 \pm 0.0) ^{abc} | 0.8-0.9 (0.8 \pm 0.0) ^c | 0.8-0.9 (0.8 \pm 0.0) ^c | 0.3-0.9 (0.8 \pm 0.1) ^{abc} | 0.8-0.8 (0.8 \pm 0.0) ^{abc} | 0.8-0.8 (0.8 \pm 0.0) ^{bc} |
| 6 | 0.1-0.0 (0.1 \pm 0.0) ^{ab} | 0.1-0.1 (0.1 \pm 0.0) ^a | 0.1-0.1 (0.1 \pm 0.0) ^a | 0.1-0.1 (0.1 \pm 0.0) ^d | 0.1-0.1 (0.1 \pm 0.0) ^a | 0.1-0.1 (0.1 \pm 0.0) ^a | 0.1-0.1 (0.1 \pm 0.0) ^{cd} | 0.1-0.1 (0.1 \pm 0.0) ^{ab} | 0.1-0.1 (0.1 \pm 0.0) ^{abc} | 0.1-0.7 (0.1 \pm 0.1) ^{bcd} | 0.1-0.1 (0.1 \pm 0.0) ^d | 0.1-0.1 (0.1 \pm 0.0) ^{abcd} |
| 7 | 0.1-0.3 (0.2 \pm 0.0) ^{ab} | 0.2-0.3 (0.2 \pm 0.0) ^{ab} | 0.2-0.3 (0.3 \pm 0.0) ^c | 0.2-0.3 (0.2 \pm 0.0) ^b | 0.2-0.3 (0.3 \pm 0.0) ^c | 0.1-0.3 (0.2 \pm 0.0) ^a | 0.2-0.3 (0.3 \pm 0.0) ^c | 0.2-0.3 (0.2 \pm 0.0) ^b | 0.2-0.3 (0.2 \pm 0.0) ^b | 0.1-0.3 (0.2 \pm 0.0) ^b | 0.1-0.3 (0.2 \pm 0.0) ^{ab} | 0.2-0.3 (0.3 \pm 0.0) ^c |
| 8 | 0.5-0.6 (0.6 \pm 0.0) ^{defg} | 0.5-0.6 (0.6 \pm 0.0) ^{abc} | 0.5-0.6 (0.6 \pm 0.0) ^{defg} | 0.4-0.6 (0.5 \pm 0.1) ^a | 0.5-0.7 (0.6 \pm 0.0) ^g | 0.5-0.6 (0.5 \pm 0.0) ^{ab} | 0.5-0.7 (0.6 \pm 0.0) ^{efg} | 0.5-0.6 (0.6 \pm 0.0) ^{defg} | 0.5-0.6 (0.6 \pm 0.0) ^{cdef} | 0.2-0.6 (0.5 \pm 0.1) ^{bcd} | 0.5-0.6 (0.6 \pm 0.0) ^{cde} | 0.6-0.7 (0.6 \pm 0.0) ^g |
| 9 | 0.1-0.4 (0.4 \pm 0.1) ^b | 0.3-0.4 (0.3 \pm 0.0) ^b | 0.3-0.8 (0.3 \pm 0.1) ^b | 0.2-0.4 (0.3 \pm 0.1) ^a | 0.3-0.4 (0.3 \pm 0.0) ^b | 0.3-0.4 (0.3 \pm 0.0) ^b | 0.2-0.4 (0.3 \pm 0.0) ^b | 0.3-0.5 (0.4 \pm 0.0) ^b | 0.3-0.4 (0.3 \pm 0.0) ^b | 0.3-0.5 (0.3 \pm 0.1) ^b | 0.3-0.4 (0.4 \pm 0.0) ^b | 0.3-0.4 (0.3 \pm 0.0) ^b |
| 10 | 0.2-0.3 (0.2 \pm 0.0) ^{abcd} | 0.2-0.3 (0.2 \pm 0.0) ^{abc} | 0.3-0.3 (0.3 \pm 0.0) ^g | 0.2-0.3 (0.3 \pm 0.0) ^{cdef} | 0.2-0.3 (0.3 \pm 0.0) ^{defg} | 0.1-0.3 (0.2 \pm 0.0) ^a | 0.2-0.3 (0.3 \pm 0.0) ^{fg} | 0.2-0.3 (0.3 \pm 0.0) ^{bcd} | 0.2-0.3 (0.2 \pm 0.0) ^{bcd} | 0.2-0.3 (0.2 \pm 0.0) ^{abcd} | 0.1-0.3 (0.2 \pm 0.0) ^{ab} | 0.3-0.3 (0.3 \pm 0.0) ^{efg} |

* List of morphometric characters: 1) SL, 2) Pd\SL, 3) DfBL\SL, 4) PdL\SL, 5) PaL\SL, 6) AfBL\SL, 7) PpL\SL, 8) PpD\SL, 9) PVDL\SL, 10) HL\SL; a, b, c, d, e, f, g: Duncan results.

Table 3. Morphometric characters ranges, Minimum- Maximum (Mean \pm SD), and Kruskal-Wallis test results for non-parametric distributed characters of *Garra rufa* populations of Iran.

| | Aghajari | Balladeh | Changuleh | Fahlian | Ghara Aghaj | Kangir | Mond | Kheir Abad | Konjancham | Zohreh | Marun | Ramhormoz | p |
|----|--------------------------------|--------------------------------|-------------------------------|---------------------------------|---------------------------------|--------------------------------|-------------------------------|--------------------------------|---------------------------------|---------------------------------|---------------------------------|-------------------------------|------|
| 1* | 34.9-94.1 (60.2 \pm 14.7) | 45.2-95.0 (66.6 \pm 10.0) | 25.3-76.3 (43.9 \pm 9.1) | 38.5-107.5 (65.6 \pm 26.9) | 39.5-112.7 (57.3 \pm 19.6) | 41.4-85.4 (67.8 \pm 12.2) | 20.3-45.0 (35.3 \pm 5.9) | 30.5-96.9 (58.0 \pm 16.7) | 36.8-109.5 (50.5 \pm 18.0) | 38.4-135.0 (84.5 \pm 29.8) | 45.1-123.5 (78.0 \pm 27.9) | 32.0-43.2 (38.6 \pm 4.0) | 0.00 |
| 2 | 31.8-87.9 (54.8 \pm 13.7) | 42.3-90.1 (61.4 \pm 9.5) | 24.9-67.7 (39.4 \pm 8.1) | 35.0-98.0 (59.7 \pm 25.0) | 37.3-104.1 (53.3 \pm 18.1) | 39.0-78.5 (63.2 \pm 11.4) | 18.9-40.4 (32.2 \pm 5.2) | 27.5-88.5 (53.0 \pm 15.1) | 33.3-98.1 (45.9 \pm 16.1) | 34.3-127.4 (77.2 \pm 28.2) | 42.0-113.6 (71.1 \pm 25.7) | 29.0-39.0 (35.2 \pm 3.5) | 0.00 |
| 3 | 0.1-0.1 (0.1 \pm 0.0) | 0.1-0.3 (0.1 \pm 0.1) | 0.1-0.1 (0.1 \pm 0.0) | 0.1-0.2 (0.1 \pm 0.0) | 0.1-0.1 (0.1 \pm 0.0) | 0.1-0.1 (0.1 \pm 0.0) | 0.1-0.1 (0.1 \pm 0.0) | 0.1-0.1 (0.1 \pm 0.0) | 0.1-0.1 (0.1 \pm 0.0) | 0.1-0.1 (0.1 \pm 0.0) | 0.1-0.1 (0.1 \pm 0.0) | 0.1-0.1 (0.1 \pm 0.0) | 0.00 |
| 4 | 0.1-0.2 (0.2 \pm 0.0) | 0.0-0.1 (0.1 \pm 0.0) | 0.2-0.2 (0.2 \pm 0.0) | 0.2-0.2 (0.2 \pm 0.0) | 0.1-0.1 (0.1 \pm 0.0) | 0.2-0.2 (0.2 \pm 0.0) | 0.1-0.2 (0.2 \pm 0.0) | 0.2-0.2 (0.2 \pm 0.0) | 0.2-0.2 (0.2 \pm 0.0) | 0.1-0.2 (0.2 \pm 0.0) | 0.2-0.2 (0.2 \pm 0.0) | 0.2-0.2 (0.2 \pm 0.0) | 0.00 |
| 5 | 0.2-0.3 (0.3 \pm 0.0) | 0.0-0.1 (0.1 \pm 0.0) | 0.2-0.3 (0.2 \pm 0.0) | 0.2-0.3 (0.3 \pm 0.0) | 0.0-0.1 (0.1 \pm 0.0) | 0.2-0.3 (0.2 \pm 0.0) | 0.2-0.3 (0.3 \pm 0.0) | 0.2-0.3 (0.2 \pm 0.0) | 0.2-0.3 (0.2 \pm 0.0) | 0.2-0.3 (0.2 \pm 0.0) | 0.2-0.3 (0.3 \pm 0.0) | 0.2-0.3 (0.3 \pm 0.0) | 0.00 |
| 6 | 0.1-0.1 (0.1 \pm 0.0) | 0.1-0.1 (0.1 \pm 0.0) | 0.1-0.1 (0.1 \pm 0.0) | 0.1-0.2 (0.1 \pm 0.0) | 0.1-0.1 (0.1 \pm 0.0) | 0.1-0.1 (0.1 \pm 0.0) | 0.1-0.2 (0.1 \pm 0.0) | 0.1-0.1 (0.1 \pm 0.0) | 0.1-0.1 (0.1 \pm 0.0) | 0.1-0.2 (0.1 \pm 0.0) | 0.1-0.2 (0.1 \pm 0.0) | 0.1-0.1 (0.1 \pm 0.0) | 0.00 |
| 7 | 0.3-0.4 (0.4 \pm 0.0) | 0.6-1.0 (0.7 \pm 0.1) | 0.3-0.5 (0.4 \pm 0.0) | 0.2-0.4 (0.3 \pm 0.0) | 0.5-0.7 (0.6 \pm 0.1) | 0.3-0.4 (0.3 \pm 0.0) | 0.3-0.4 (0.3 \pm 0.0) | 0.3-0.7 (0.4 \pm 0.1) | 0.3-0.4 (0.4 \pm 0.0) | 0.3-0.9 (0.4 \pm 0.1) | 0.3-0.4 (0.4 \pm 0.0) | 0.3-0.4 (0.4 \pm 0.0) | 0.00 |
| 8 | 0.3-0.4 (0.3 \pm 0.0) | 0.9-1.7 (1.1 \pm 0.2) | 0.2-0.3 (0.3 \pm 0.3) | 0.2-0.3 (0.3 \pm 0.0) | 0.7-1.2 (0.9 \pm 0.2) | 0.2-0.3 (0.3 \pm 0.0) | 0.2-0.3 (0.3 \pm 0.0) | 0.2-0.3 (0.3 \pm 0.0) | 0.3-0.4 (0.3 \pm 0.0) | 0.2-0.3 (0.2 \pm 0.0) | 0.2-0.3 (0.3 \pm 0.0) | 0.3-0.4 (0.4 \pm 0.0) | 0.00 |
| 9 | 0.3-0.4 (0.4 \pm 0.0) | 0.5-0.8 (0.6 \pm 0.1) | 0.3-0.5 (0.4 \pm 0.0) | 0.4-0.5 (0.4 \pm 0.0) | 0.4-0.7 (0.5 \pm 0.1) | 0.4-0.5 (0.4 \pm 0.1) | 0.4-0.5 (0.4 \pm 0.0) | 0.3-0.4 (0.4 \pm 0.0) | 0.3-0.4 (0.4 \pm 0.0) | 0.2-0.4 (0.4 \pm 0.1) | 0.3-0.7 (0.4 \pm 0.1) | 0.4-0.4 (0.4 \pm 0.0) | 0.00 |

* List of morphometric characters: 1) TL, 2) FL, 3) CPL, 4) HD, 5) BD, 6) CPD, 7) SnL, 8) OD, 9) PoL.

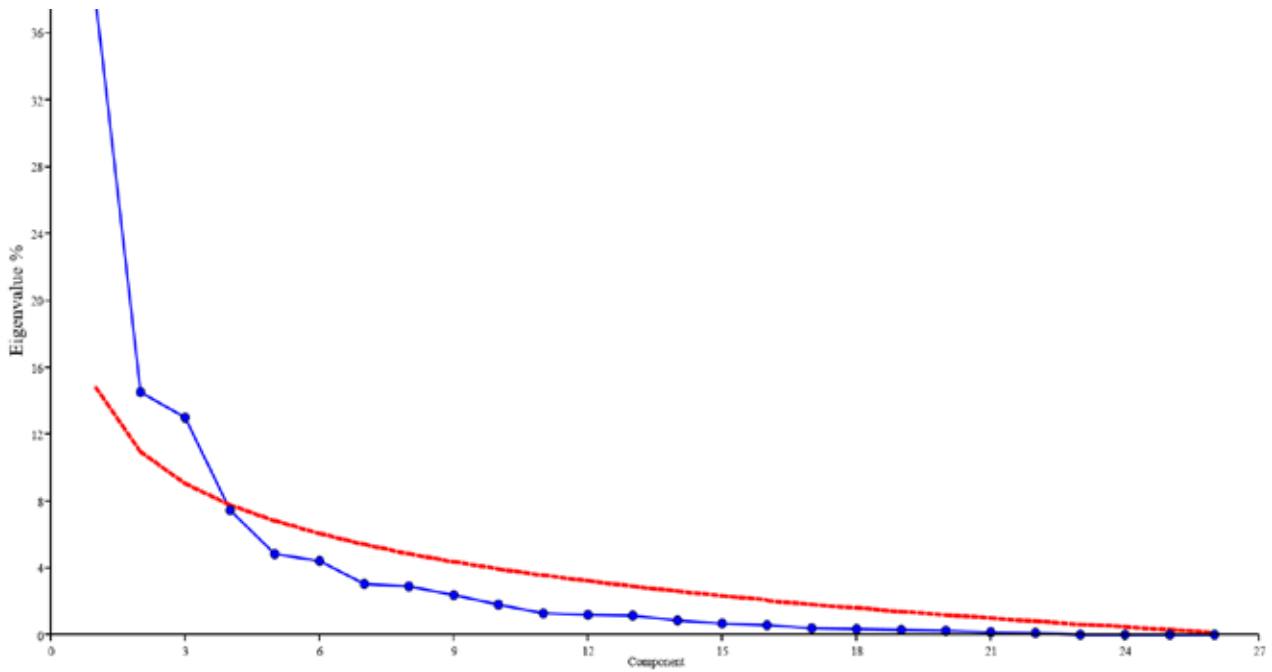


Figure 4. The plot of the Joliffe cut-off point in PCA of *Garra rufa* populations of Iran.

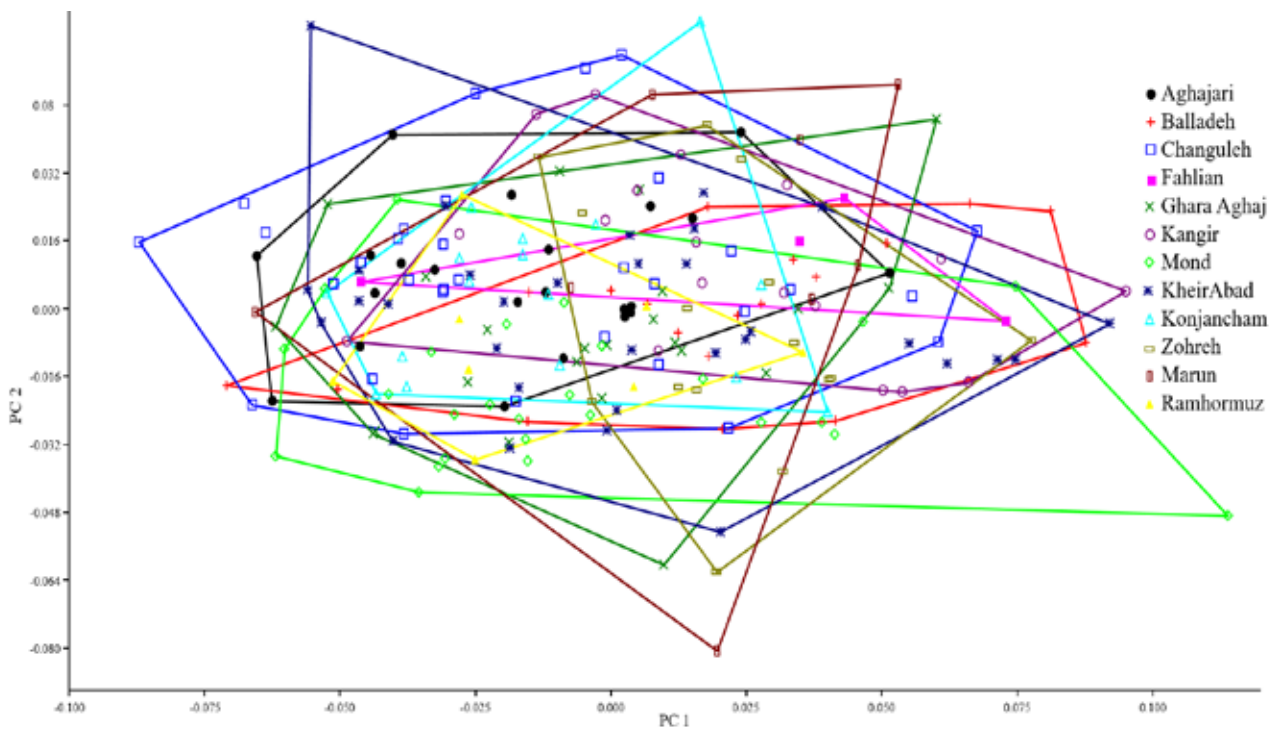


Figure 5. The scatter plot of Joliffe cut-off point in Principal Components Analysis of *Garra rufa* populations of Iran.

Despite the impressive development and progress made in new sciences such as genetics, the use of measurable and countable characters (morphometric and meristic) plays an important role in the study of fish species identification (Nelson *et al.*, 2016) and new species introductions (Strauss and Bond, 1990).

In regards to the meristic characters, four

were fixed in all of the specimens and these include the number of branched dorsal-fin rays (it was 8 in all specimens), branched anal-fin ray count (5 in all specimens), branched caudal-fin rays (17 in all specimens), and barbells (2 in all specimens). Such characters are keys to the identification and distinction of *G. rufa* from some other species of the genus *Garra* which exist in the Middle

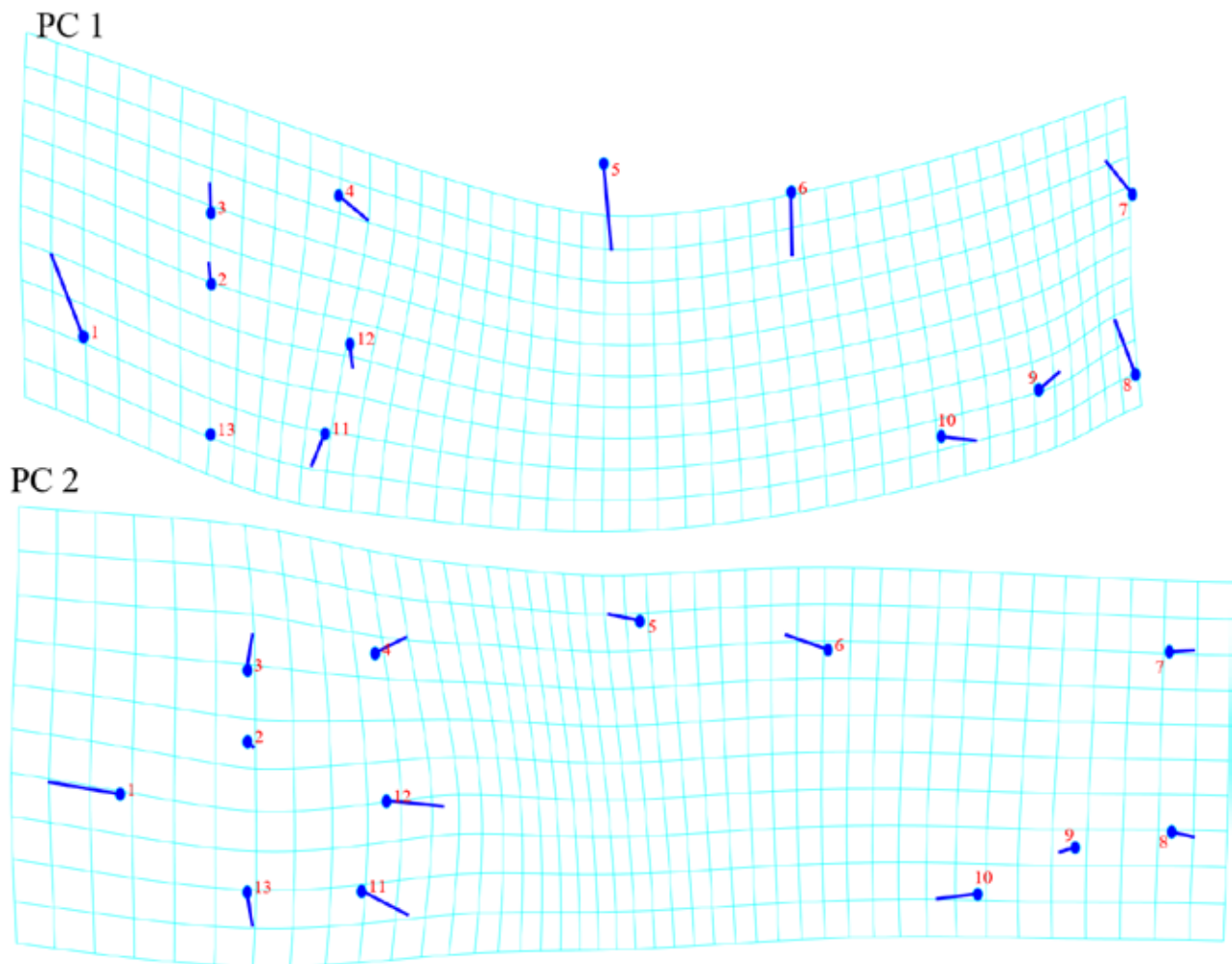


Figure 6. The body shape variation in the *Garra rufa* populations. Deformation grids are associated with the most positive values of the first two factors (PC1 and PC2) obtained by performing the ordination analysis of the Principal Components (PC).

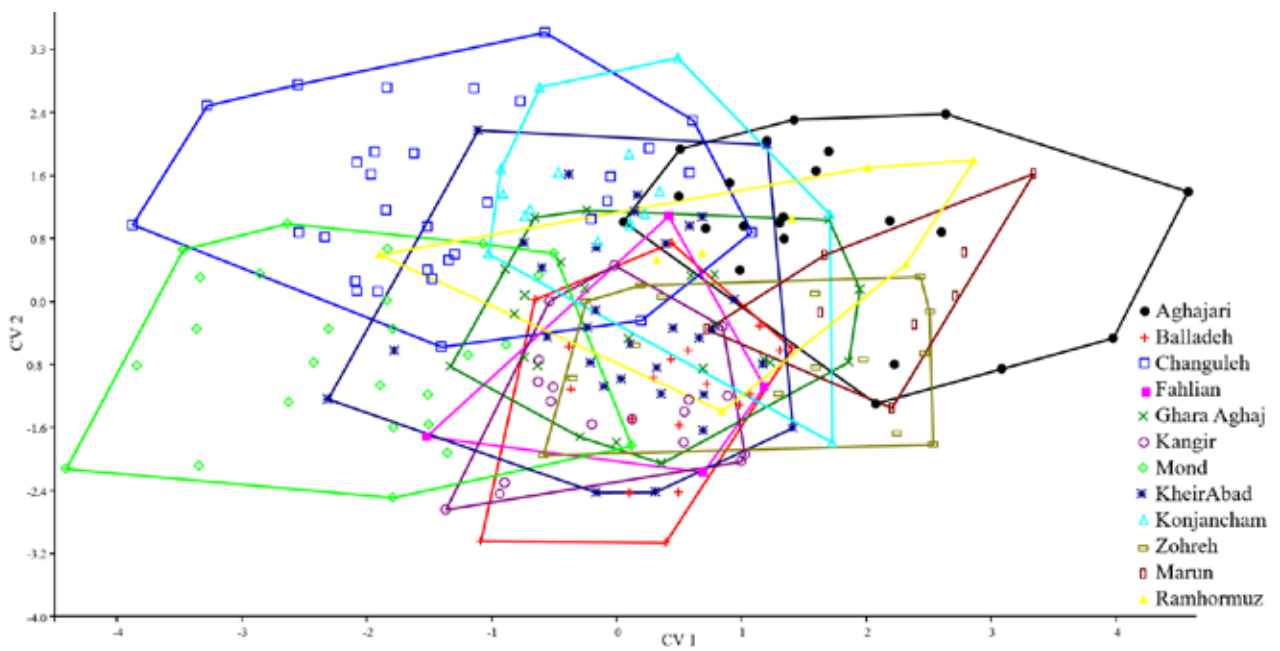


Figure 7. Graph of Canonical Variance Analysis results of body shape of *Garra rufa* populations of Iran.

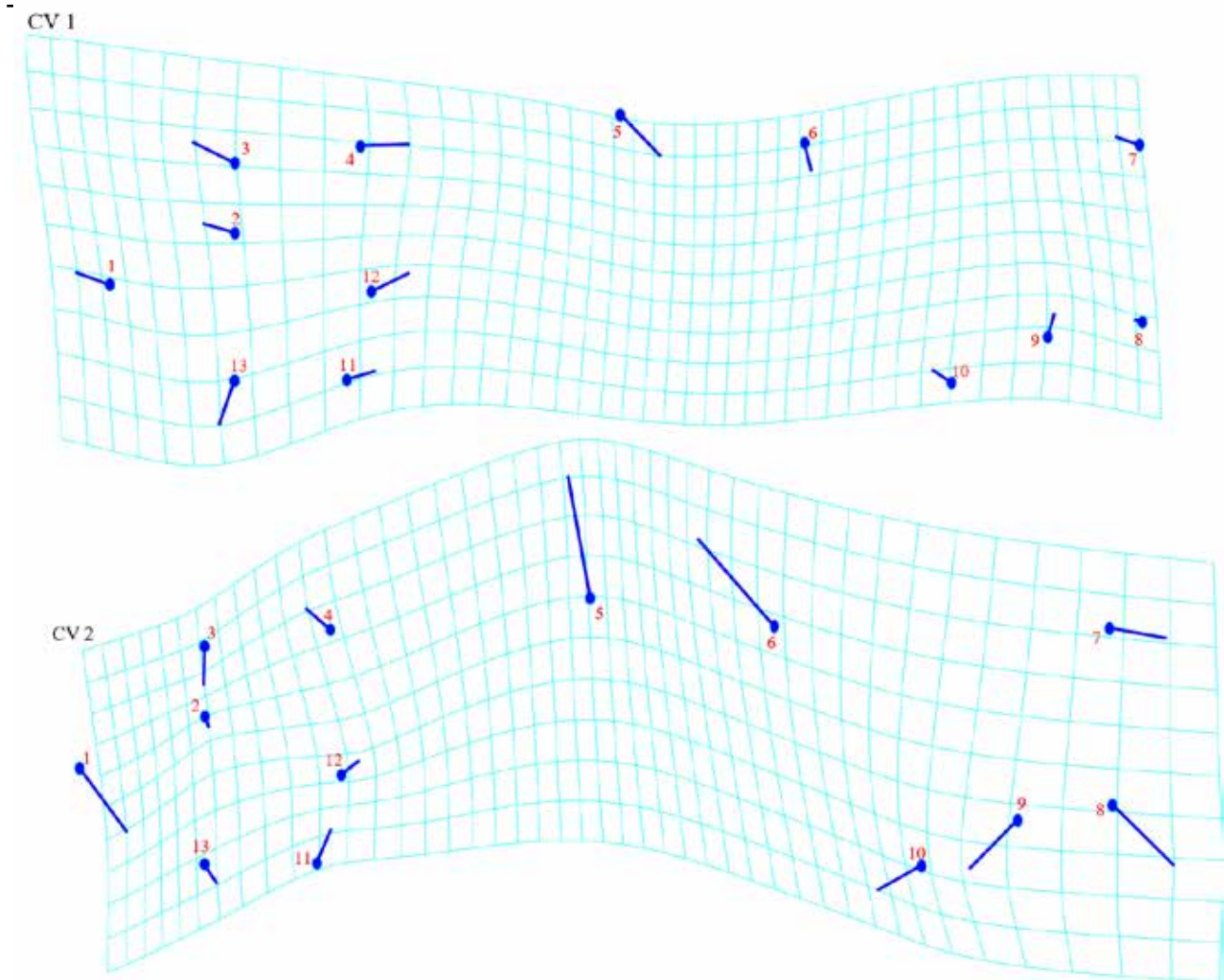


Figure 8. The body shape variation in all samples of *Garra rufa*. Deformation grids are associated with the most positive values of the first two factors (CV1 and CV2) obtained by performing the ordination analysis of the Canonical Variance (CV).

Table 4. Mahalanobis distance results of *Garra rufa* populations

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---------------|------|------|------|------|------|------|------|------|------|------|------|
| 1 Aghajari | | | | | | | | | | | |
| 2 Balladeh | 3.04 | | | | | | | | | | |
| 3 Changuleh | 3.52 | 3.53 | | | | | | | | | |
| 4 Fahlian | 3.92 | 4.28 | 4.30 | | | | | | | | |
| 5 Ghara Aghaj | 3.04 | 3.20 | 2.96 | 3.41 | | | | | | | |
| 6 Kangir | 3.53 | 3.29 | 3.41 | 3.58 | 2.52 | | | | | | |
| 7 Mond | 4.27 | 3.59 | 2.97 | 3.78 | 3.58 | 3.49 | | | | | |
| 8 Kheir Abad | 2.63 | 2.72 | 2.53 | 3.53 | 2.19 | 1.92 | 3.10 | | | | |
| 9 Konjan Cham | 2.47 | 3.11 | 2.02 | 4.00 | 2.92 | 3.20 | 3.25 | 2.16 | | | |
| 10 Zohreh | 2.82 | 2.50 | 3.64 | 3.55 | 3.18 | 3.02 | 3.74 | 2.59 | 2.73 | | |
| 11 Marun | 2.46 | 3.65 | 4.22 | 4.35 | 3.82 | 4.01 | 4.75 | 3.45 | 3.50 | 2.53 | |
| 12 Ramhormoz | 3.57 | 4.20 | 3.99 | 3.78 | 3.63 | 4.43 | 4.46 | 3.63 | 3.75 | 3.33 | 3.72 |

Table 5. Procrustes distance results of *Garra rufa* populations of Iran.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 1 Aghajari | | | | | | | | | | | |
| 2 Balladeh | 0.044 | | | | | | | | | | |
| 3 Changuleh | 0.036 | 0.046 | | | | | | | | | |
| 4 Fahlian | 0.056 | 0.040 | 0.048 | | | | | | | | |
| 5 Ghara Aghaj | 0.034 | 0.038 | 0.027 | 0.042 | | | | | | | |
| 6 Kangir | 0.045 | 0.033 | 0.051 | 0.039 | 0.039 | | | | | | |
| 7 Mond | 0.042 | 0.040 | 0.035 | 0.051 | 0.028 | 0.050 | | | | | |
| 8 KhairAbad | 0.027 | 0.028 | 0.034 | 0.044 | 0.022 | 0.027 | 0.032 | | | | |
| 9 Konjancham | 0.021 | 0.038 | 0.021 | 0.049 | 0.026 | 0.043 | 0.032 | 0.023 | | | |
| 10 Zohreh | 0.044 | 0.017 | 0.050 | 0.038 | 0.039 | 0.031 | 0.041 | 0.029 | 0.040 | | |
| 11 Marun | 0.037 | 0.027 | 0.045 | 0.037 | 0.039 | 0.032 | 0.045 | 0.031 | 0.037 | 0.023 | |
| 12 Ramhormoz | 0.043 | 0.060 | 0.031 | 0.051 | 0.029 | 0.061 | 0.035 | 0.041 | 0.036 | 0.051 | 0.051 |

Eastern region, especially in Iranian waters (Esmaeili *et al.*, 2016; Zamani-Faradonbe and Keivany, 2020).

Other meristic characters, namely the lateral line (L.L) scales, scales above L.L, scales below L.L to the ventral fin, scales below L.L to the anal fin, pectoral fin, ventral-fin soft rays, and the circumpeduncular scales were significantly different among the studied populations. This diversity in the meristic traits probably reflects the genetic diversity and also the different conditions of the habitats (Esmaeili *et al.*, 2016; Keivany *et al.*, 2016b).

The results of the comparison of the morphometric characters showed that there was some diversity in the body shape of the *G. rufa* populations. The sampling was carried out across a large area from the west to the south, and there could be different habitats with different conditions (Keivany *et al.*, 2016b) affecting the body shape of fishes.

Ghalenoey *et al.* (2010) studied thirteen populations of *G. rufa* from Gamasiab, Dez, Karun, Kol, Khoramrud, Dalaki, Mond, Zohreh, Jarrahi, and Kashkan in Iran. Their results showed that the Mond river population was separated from the others, but other populations overlapped with each other in terms of the studied characteristics ($p < 0.05$). Aquatic environmental factors such as

water temperature and water velocity play important roles in changing body shape characters including morphometric traits which are so sensitive to environmental changes. Fishes can quickly adapt themselves to new conditions (Brraich and Akhter, 2015), and the genetic structure of fish mostly controls the meristic characters (Brraich and Akhter, 2015). Also, the morphology of fish can affect some important biological and physiological attributes in them including reproductivity, swimming performance, maneuvering ability, feeding, and avoidance from the hunter (Sfakiotakis *et al.*, 1998).

Due to allometric growth patterns in the first stages of life history, morphological measurements change throughout that period (Elliott *et al.*, 1995), but meristic characters do not in relation to the size of fish (Helfman *et al.*, 2009), so before using the morphometric characters in the analysis, it is important to remove the size effects from them. In this study, the ratio method followed by Cicek *et al.* (2016) has been used; in this method the morphometric characters in the head area were modified as the ratio of head length and the morphometric characters in the body area were modified as the ratio of standard length.

The geometric morphometric results of this study showed significant shape differences among the populations of *G. ruffa*. In the deformation grid, the most differences

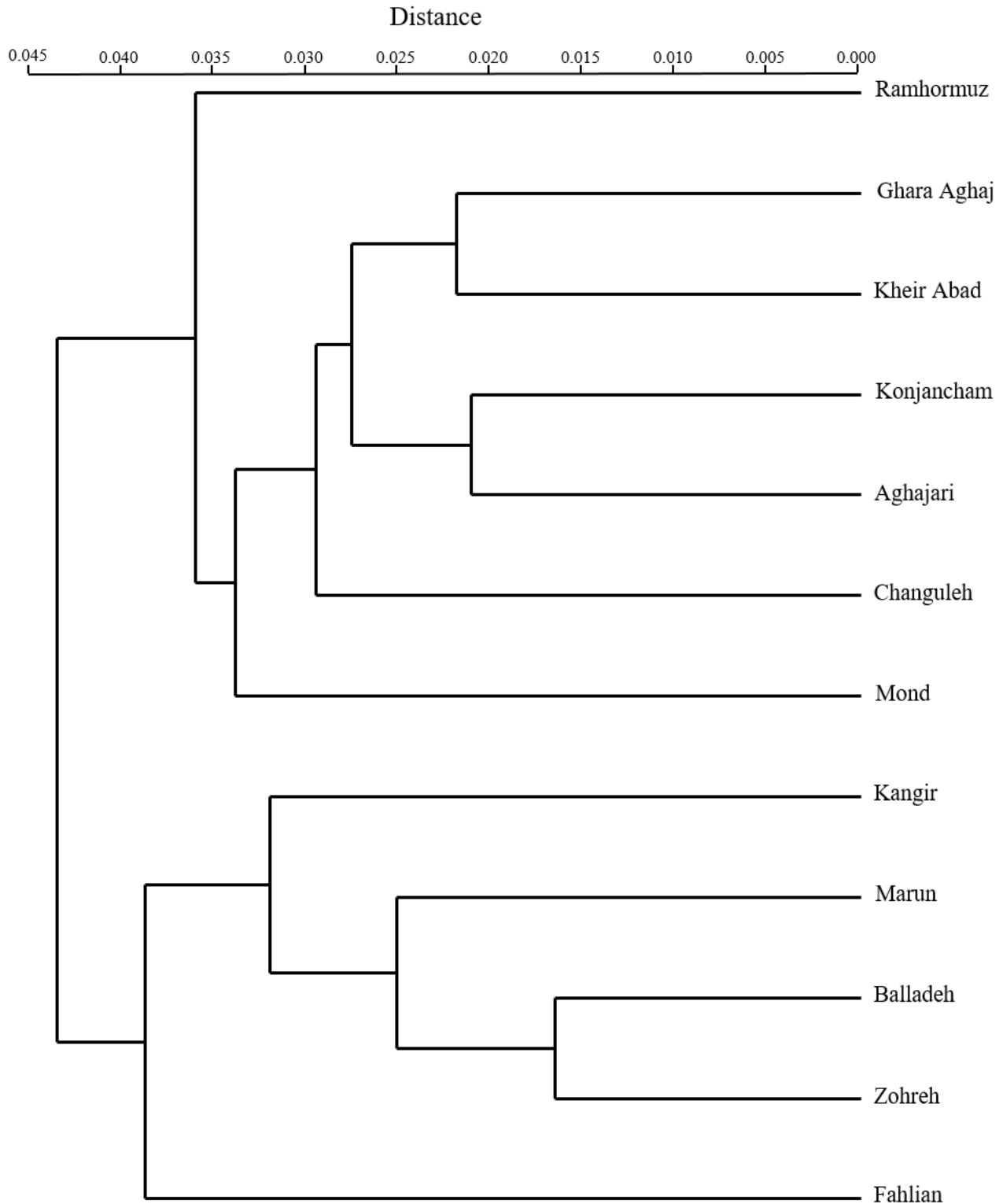


Figure 9. Dendrogram of Cluster analysis of the studied populations of *Garra rufa*

were in the head height and length, dorsal-fin position, and body depth. The morphological variations observed among the populations might have been influenced by the genetic makeup of the specimens as Anvarifar *et al.* (2012) found a relationship between RAPD

genetic markers and morphology in *Capoeta gracilis* in Tajan River. This could be a reflection of the adaptation strategies that fish populations have against a mixture of environmental factors (chemical, physical, and biological factors). Chemical factors

include salinity and dissolved oxygen, and physical factors include temperature, radiation, water depth, and the current flow influence. Biological factors include food availability, the feeding mode, and habitat use (Spoljaric and Reimchen, 2011; Antonucci *et al.*, 2012; Bravi *et al.*, 2013).

The studied populations by Ghalenoei *et al.* (2010), Keivany *et al.* (2015), Cicek *et al.* (2016), Esmaili *et al.* (2016 b), and this study showed that *G. rufa* has a vast habitat extending from at least the west to the east of Iran. Karahan (2007) and Çiçek (2009) suggested that the variations in the locations of *G. rufa* are quite high, and that there are many differences between them in terms of morphometric and meristic characteristics.

One of the important habitat factors is food availability which can affect fish morphology particularly feeding-related characters such as the head shape (Nicieza, 1995). Functional relationships which have been proven between morphology and the main ecological dimension are between the type of food (vegetable versus animal) and gut length which is different between herbivores and carnivores (Sturmbauer *et al.*, 1992) and between prey size and mouth gape (Wainwright and Richard, 1995) which could finally affect the size of the head.

In lentic to low-velocity waters, deeper body is useful for rapid turning and maneuvering (Moyle and Cech, 2004); based on the body shape shown in the deformation grids, this kind of body shape can be seen in the population in which the dorsal-fin landmarks are upper than the consensus position such as Fahljan, Zohreh, Balladeh, Marun, and Kangir. In lotic and fast-flowing waters, the streamlined body shape would be useful; the population with this body shape, such as changuleh, can decrease being dragged in the water currents and hence reduces energy consumption to keep the position (Keast and Webb, 1966; Webb, 1984). Also, the fusiform body shape is advantageous in water currents for constantly moving and searching out prey (Keast and Webb, 1966; Webb, 1984).

Zamani-Faradonbe *et al.* (2015a) showed *Capoeta gracilis* specimens with a

smaller head, longer caudal peduncle and relatively deeper body living in fast-flowing water and lower depth, substrate index, Periphyton Cover Index (PeCI) and Potamal Cover Index (PoCI). They also increase in the depth and width of the river along with the increasing PeCI, PoCI; larger bed stones induce a larger head and shorter and deeper caudal peduncle forming a relatively deeper body shape.

The results of the relationship between habitat factors and body shape of Kura barbel (*Barbus cyri*) suggested depth, width, the average diameter of stream bed as effective factors. Also, the results of 2B-PLS revealed that individuals with a deeper body, large head, and deep caudal peduncle are significantly related to more depth, lower velocity, lower width, and to river beds with a bigger number of large stones; whereas individuals with a fusiform body shape, that is a lower depth, smaller head, deeper caudal peduncle, have a significant relationship with higher velocity, more depth, greater width and river beds with smaller stones (Zamani-Faradonbe *et al.*, 2015b).

The results of the present study showed significant body-shape differences among the populations of the species, *Garra rufa*, in Iranian inland waters; such information can be useful for further fisheries and stock managements or conservation programs in the region.

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