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Research article

Evaluation of body shape variation in species of genus *Garra* Hamilton, 1822 by geometric morphometry

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Abstract: In this study, body shape variation among four species of *Garra* Hamilton, 1822 distributed in Turkey was investigated using geometric morphometry method. In this context, 570 individuals from 22 populations of *G. rufa*, *G. turcica*, *G. orontesi* and *G. variabilis* were analysed. The specimens used in the study were collected during the field studies carried out between 2014 and 2022. The specimens were photographed from the left side view and the data were digitised by assigning 14 landmarks to the areas important for species identification. After generalised procrustes analysis (GPA), Principal Component Analysis (PCA) and Discriminant Function Analysis (DFA) and clustering analysis were used to determine the value of intraspecific similarities and differences. According to the data obtained as a result of the study; *G. turcica* and *G. variabilis* were the species with the highest intraspecific variation, while *G. rufa* was the species with the lowest intraspecific variation. For the species evaluated, the most variation was observed in the tip of the snout, eye position, caudal peduncle height, body height and the beginning of the operculum. It was observed that the range of phenotypic plasticity of the species was wide, this variation decreased in the intersection regions of the populations, and the level of plasticity increased in geographically distant populations.

Keywords: Phenotypic plasticity, morphological variation, adaptation, environmental variables, doctor fish

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Introduction

A total of 188 valid species belonging to the genus *Garra*, a member of the Cyprinidae family, have been identified (van der Laan, 2024). Of these, nine are distributed in Türkiye. Among these, *Garra rufa* was previously known to be distributed in a very wide area, including the Euphrates, Seyhan, Ceyhan, Eastern Mediterranean, and Orontes basins (Geldiay and Balık, 2007; Çiçek et al., 2015; 2023). Nevertheless, subsequent investigations have revealed that *Garra turcica* is present in the Eastern Mediterranean, Seyhan, and Ceyhan basins (Bayçelebi et al., 2018). It has been reported that the *G. turcica* specimens differs from the *G. rufa* in terms of features such as a blunt nose, a slimmer body, and 19 fixed diagnostic nucleotides in the COI barcode region. *G. turcica* is distributed in the rivers flowing into Iskenderun Bay (Bayçelebi et al., 2018).

Furthermore, individuals distributed in the Orontes basin were identified as a new species, designated as *Garra orontesi* (Bayçelebi et al., 2021).

Studies on the Orontes Basin population have demonstrated that this population exhibits distinct characteristics when compared to the *G. rufa* and *G. turcica* species. These characteristics include the presence of 17-21 spines on the first gill spine, 8½ dorsal fin rays, and a typical number of 2-3 scales between the pectoral and pelvic fin tips. In addition, the scales between the pectoral and pelvic fin tips, as well as the minimum K2P distance difference of 2.7% with *G. rufa* and 3.9% with geographically neighboring *G. turcica* in the COI barcode region, are further distinguishing characteristics.

Phenotypic plasticity refers to certain changes in an organism's behavior, morphology and physiology in response to a unique environment (Price et al., 2003). Phenotypic plasticity, which underpins the way organisms cope with environmental variation, encompasses any environmentally induced change (e.g. morphological, physiological, behavioral, phenological) that may or may not be permanent throughout an individual's lifetime (Kelly et al., 2012).

The aim of this study is to evaluate the intraspecific variation of *G. rufa* and *G. variabilis*, which are distributed over a wide area; *G. turcica*, which has been revalidated in recent years, and *G. orontesi*, which has been defined as a new species, were examined by geometric morphometry.

Material and Methods

Garra rufa, *G. variabilis*, *G. turcica* and *G. orontesi* samples (a total of 570 specimens) were obtained from 22 different localities from Ceyhan, Orontes, Eastern Mediterranean, and Euphrates-Tigris river basins by electrofishing between 2014 and 2022 (Table 1, Figure 1).

Table 1. Sampling locations and coordinates

	Species	Longitude	Latitute	Basin	Province	Habitat
1	*Garra turcica*	36.956530	35.633240	Ceyhan	Adana	Ceyhan River
2	*Garra turcica*	37.455190	36.041030	Ceyhan	Osmaniye	Kekiksuyu Stream
3	*Garra turcica*	37.301130	36.367600	Ceyhan	Osmaniye	Sabun Stream
4	*Garra turcica*	37.806670	36.789130	Ceyhan	Kahramanmaraş	Zeytin Stream
5	*Garra turcica*	38.199930	37.084050	Ceyhan	Kahramanmaraş	Hurman Stream
6	*Garra turcica*	37.027778	34.766389	Eastern Mediterranean	Mersin	Tarsus Stream
7	*Garra turcica*	36.857520	34.553680	Eastern Mediterranean	Mersin	Müftü Stream
8	*Garra turcica*	36.757500	34.523611	Eastern Mediterranean	Mersin	Mezitli Stream
9	*Garra turcica*	36.359160	35.922200	Ceyhan (Orontes)	Hatay	Çumruk Strean
10	*Garra orontesi*	36.374160	36.180270	Orontes	Hatay	Karasu Stream
11	*Garra orontesi*	36.088610	36.255000	Orontes	Hatay	Altınözü Spring Water
12	*Garra orontesi*	36.851667	36.686389	Orontes	Kilis	Karasu Stream
13	*Garra rufa*	37.509740	38.113160	Euphrates-Tigris	Adıyaman	Değirmen Stream
14	*Garra rufa*	37.327660	37.668700	Euphrates-Tigris	Gaziantep	Merzimen Stream
15	*Garra rufa*	38.032310	39.364540	Euphrates-Tigris	Diyarbakır	Kızıl Çubuk Stream
16	*Garra rufa*	38.228010	38.816810	Euphrates-Tigris	Malatya	Mollahan Stream
17	*Garra variabilis*	36.763889	37.254167	Euphrates-Tigris	Kilis	Konak Pond Output Water
18	*Garra variabilis*	37.327660	37.668700	Euphrates-Tigris	Gaziantep	Merzimen Stream
19	*Garra variabilis*	36.948380	37.990490	Euphrates-Tigris	Gaziantep	Nizip Stream
20	*Garra variabilis*	38.022639	40.489947	Euphrates-Tigris	Diyarbakır	Kuruçay
21	*Garra variabilis*	38.137778	40.905556	Euphrates-Tigris	Diyarbakır	Silvan Spring Water
22	*Garra variabilis*	37.808611	38.302222	Euphrates-Tigris	Adıyaman	Pirin Stream

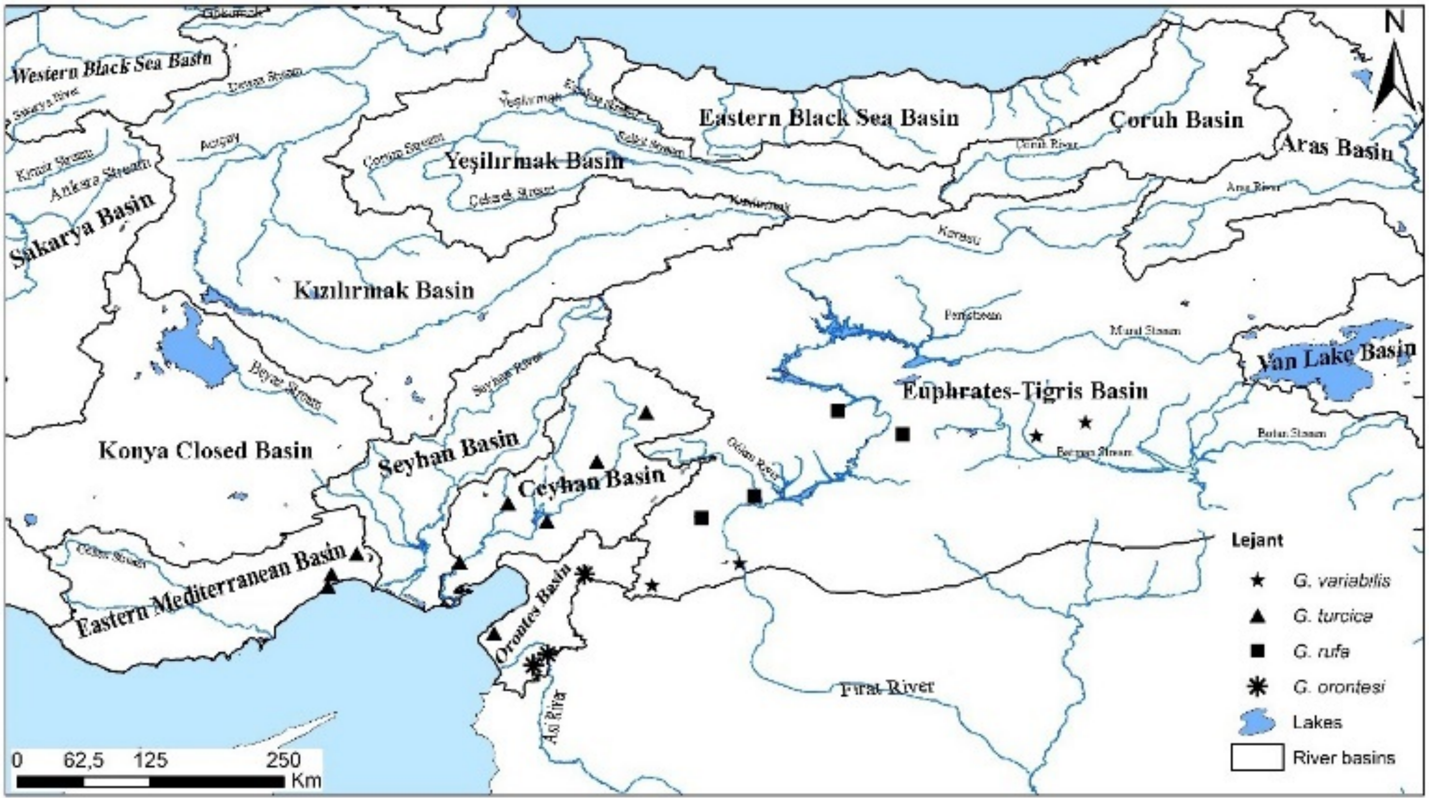

Figure 1. Sampling localities

Samples were fixed in 10% formalin and transferred to laboratory to keep for further examination. The left sides of specimens were photographed using a copy-stand equipped with a digital camera (Canon EOS 700D). To extract body shape data, 14 homologous landmark-points were digitized using tpsDig2 software (version 2.16) (Figure 2, 3) and data were superimposed to remove non-shape data, including size, position and direction using Generalized Procrustes Analysis (GPA). Principal Component Analysis (PCA) and Discriminant Factor Analysis (DFA) were used to assess phenotypic plasticity between populations for each species.

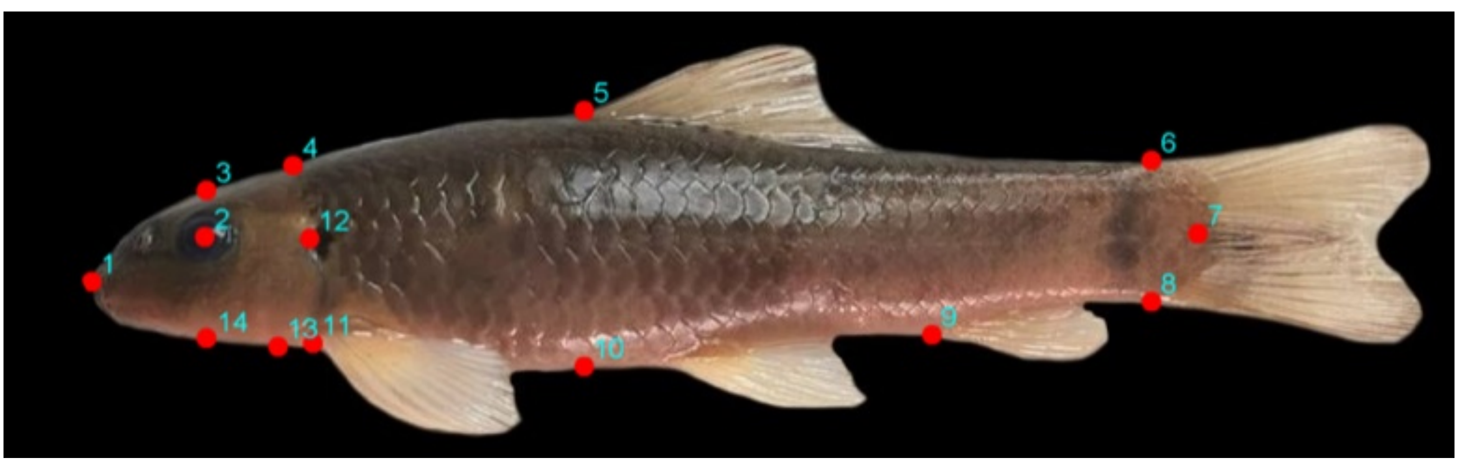

Figure 2. The 14 defined landmark-points for extracting the body shape data of *Garra* specimens

Figure 3. General body view of the studied species (A= *Garra orontesi*; Karasu Stream, Orontes Basin, Hatay; B= *Garra turcica*; Zeytin Stream, Ceyhan Basin, Kahramanmaraş; C= *Garra rufa*; Keysun Stream, Euphrates-Tigris Basin, Adıyaman; D= *Garra variabilis*; Konak Pond Outlet Water, Euphrates-Tigris Basin, Gaziantep).

Shape differences of the studied populations with consensus configuration of six groups were visualized using deformation grids presented along with DFA. The Mantel test was used to test for correlation between body shape and geographical coordinates. All the analysis were performed using PAST and MorphoJ software (version 1.01). To obtain a visual expression of the similarities and differences between populations, a cluster dendogram based on the calculated Euclidean distances was drawn with the PAST program (Hammer et al. 2001).

Results and Discussion

In this study, the intraspecific morphological variation of four *Garra* species (*G. rufa*, *G. variabilis*, *G. turcica* and *G. orontesi*) was determined by geometric morphometric analysis. For this purpose, 570 specimens belonging to 22 populations from Ceyhan, Asi, Eastern Mediterranean, and Euphrates-Tigris basins were analyzed. The data were subjected to DFA, PCA, and cluster analyses in order to determine the variation among the populations.

Garra orontesi

The results of the analysis the three populations of *G. orontesi* indicate that the first three principal components explain 62.71% of the total variance. The first (PC1), second (PC2) and third (PC3) principal components explain 36.54%, 15.85%, and 10.31% of the total variance, respectively. The PCA analysis revealed that the three populations exhibited minimal morphological variation, with a high degree of structural overlap. Station 12 exhibited the greatest degree of variation among the evaluated populations (Figure 4, Table 2).

Based on comparison between St. 12 and St. 11 populations, the results of the wireframe graph of body deformation curves represent that individuals of St. 12 exhibit a lower nose (1st landmark), a longer post orbital distance (3rd and 4th landmarks), a deeper body (5th and 10th landmarks), and a short-thick tail stalk (6th and 7th landmarks). When the body morphology of St. 10 and St. 12 populations, which are the most distant from each other, were compared, it was observed that the body shape completely overlapped, with the exception of the snout, which was shorter (1st landmark) in St. 12 population (Figure 5).

Table 2. Mahalanobis distance analysis of *Garra orontesi* populations (most closed * and most distant ** populations)

Station	10. St	11. St	12. St
10. St	-	5,22*	7,94
11. St		-	9,44**
12. St			-

Garra turcica

Sampled populations of *G. turcica* (St. 5-St. 9; St. 5-St. 8 and St. 9-St. 8) were compared among themselves. As a result of PCA analysis of populations, the first three principal components explained 79.3 (PC1: 43.45; PC2: 24.79; PC3: 8.79), 70.9 (PC1: 41.25; PC2: 19.44; PC3: 9.95) and 64.6 (PC1: 36.66; PC2: 18.14; PC3: 9.25) of the total variance, respectively for station pairs given above. The results of the PCA analysis indicated a separation between St. 5-St. 8 (Figure 6). However, this separation was relatively minor between St. 5-St. 9 and St. 9-St. 8 populations (Figure 7, 8). The analysis demonstrated that morphological variation increased at stations that were geographically distant from each other.

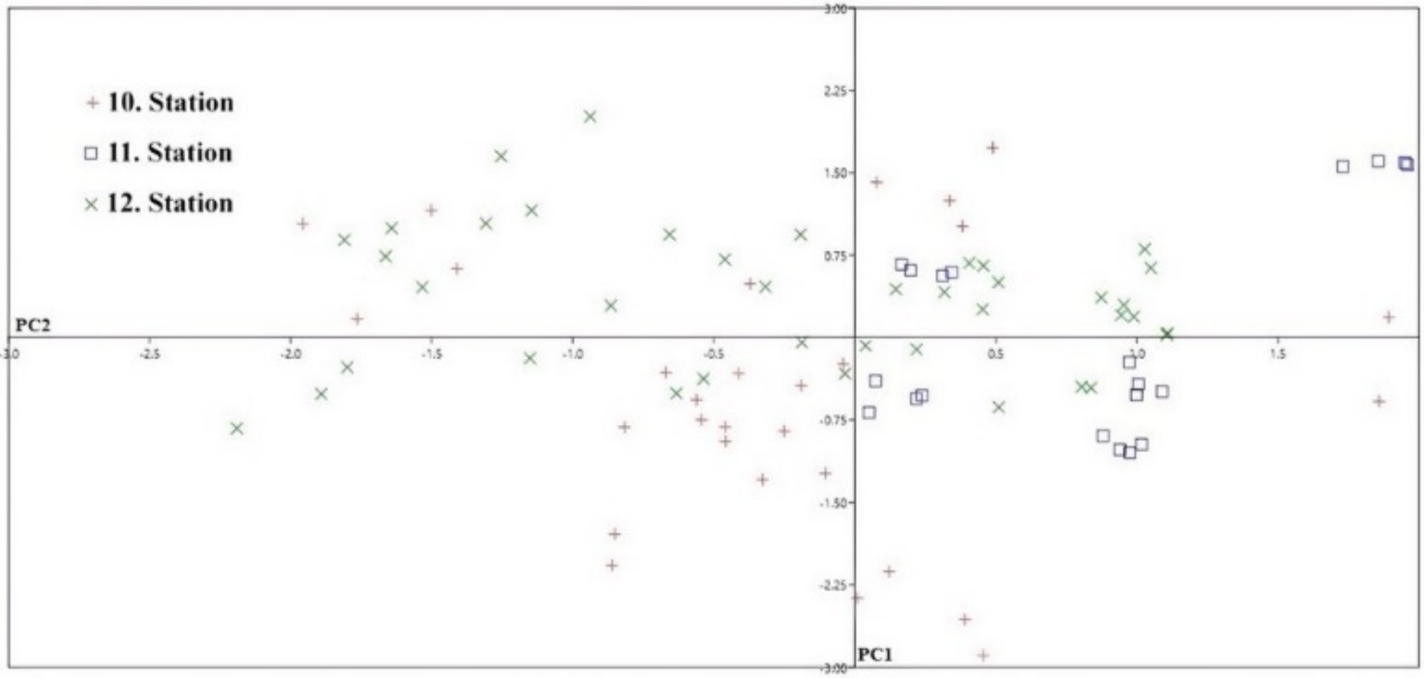

Figure 4. The following graph represents the principal components analysis of the body shape of *Garra orontesi* populations.

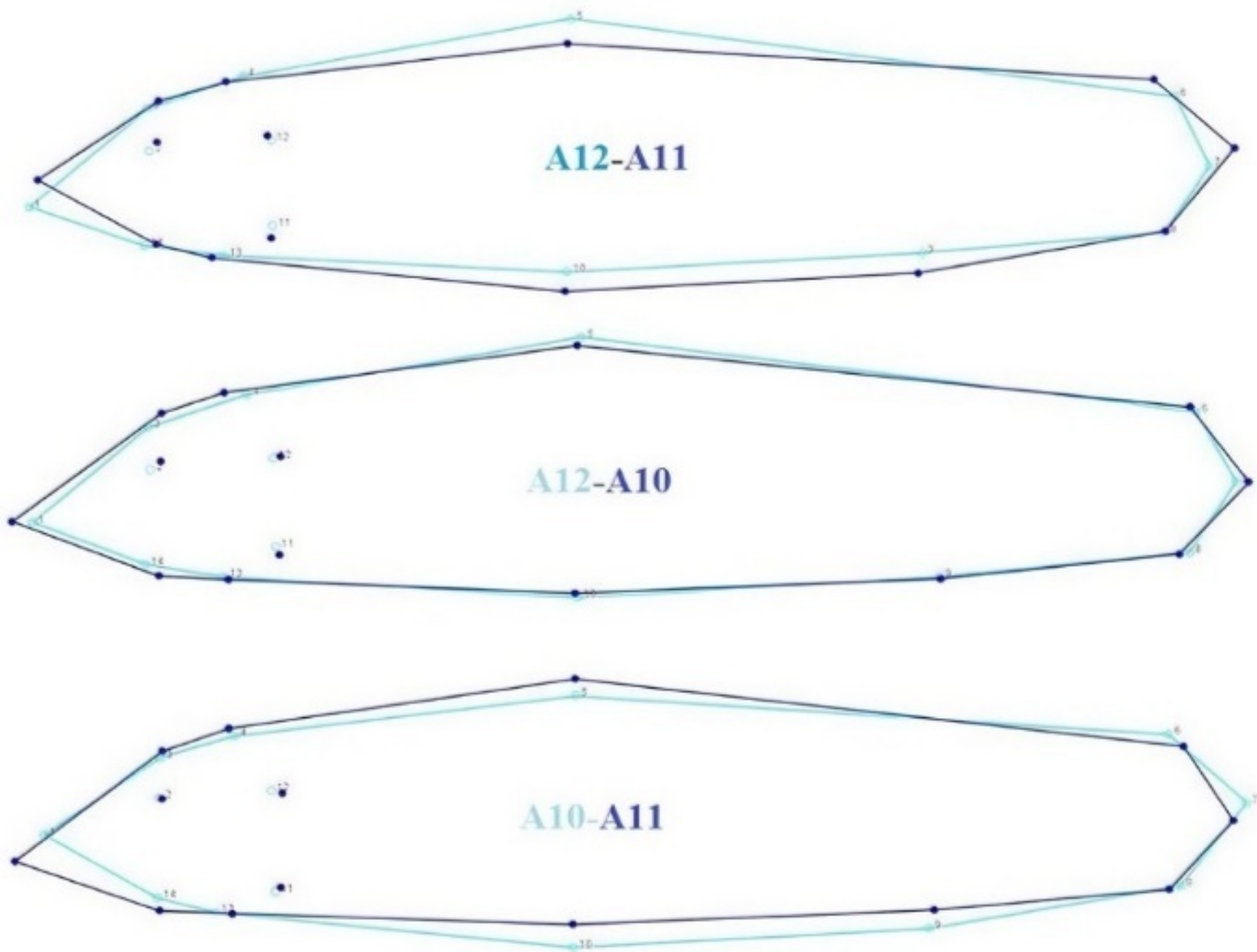

Figure 5. Discriminant function analysis wireframe plot of *Garra orontesi* populations

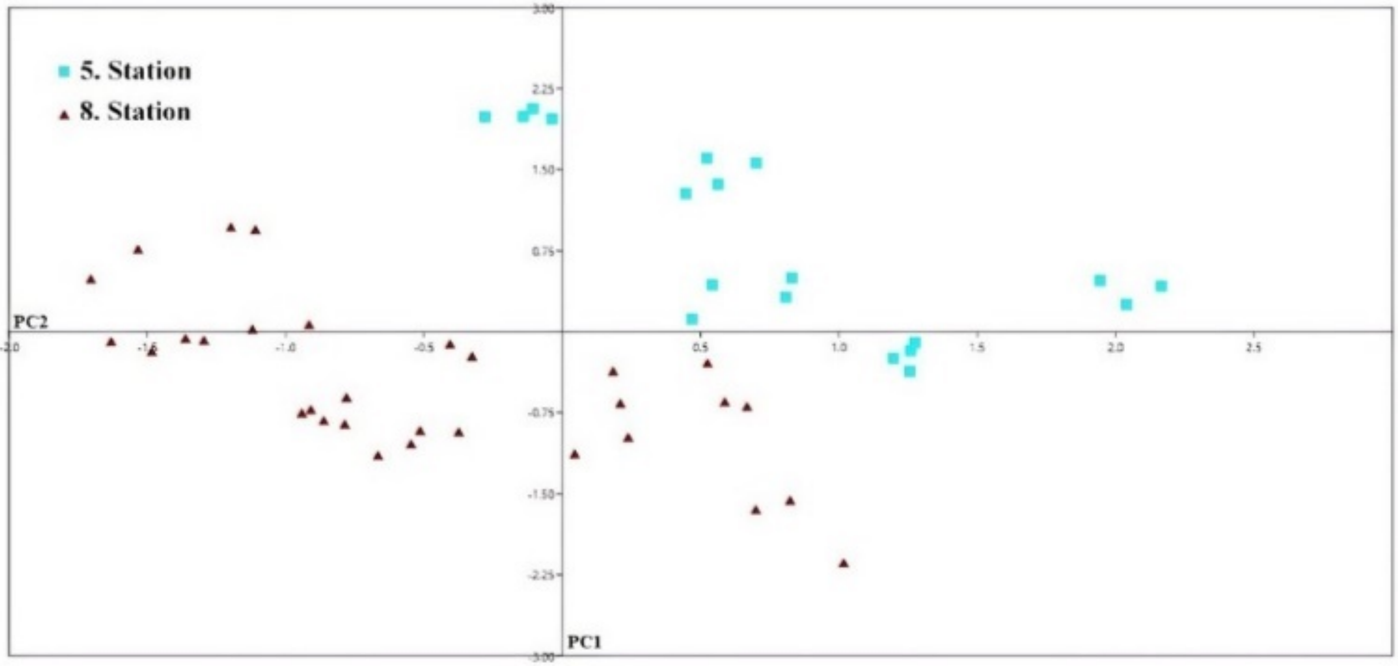

Figure 6. PCA plot of *Garra turcica* St. 5 and St. 8 populations

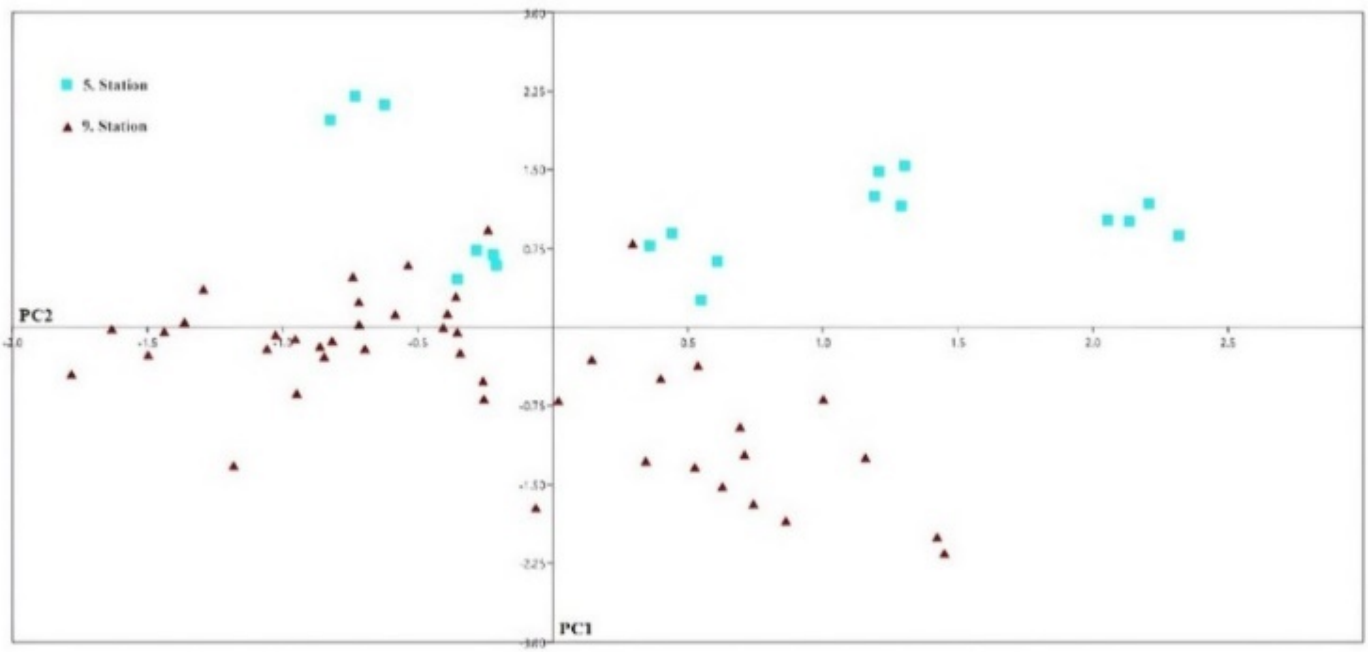

Figure 7. PCA plot of *Garra turcica* (St. 5 and St. 9 populations

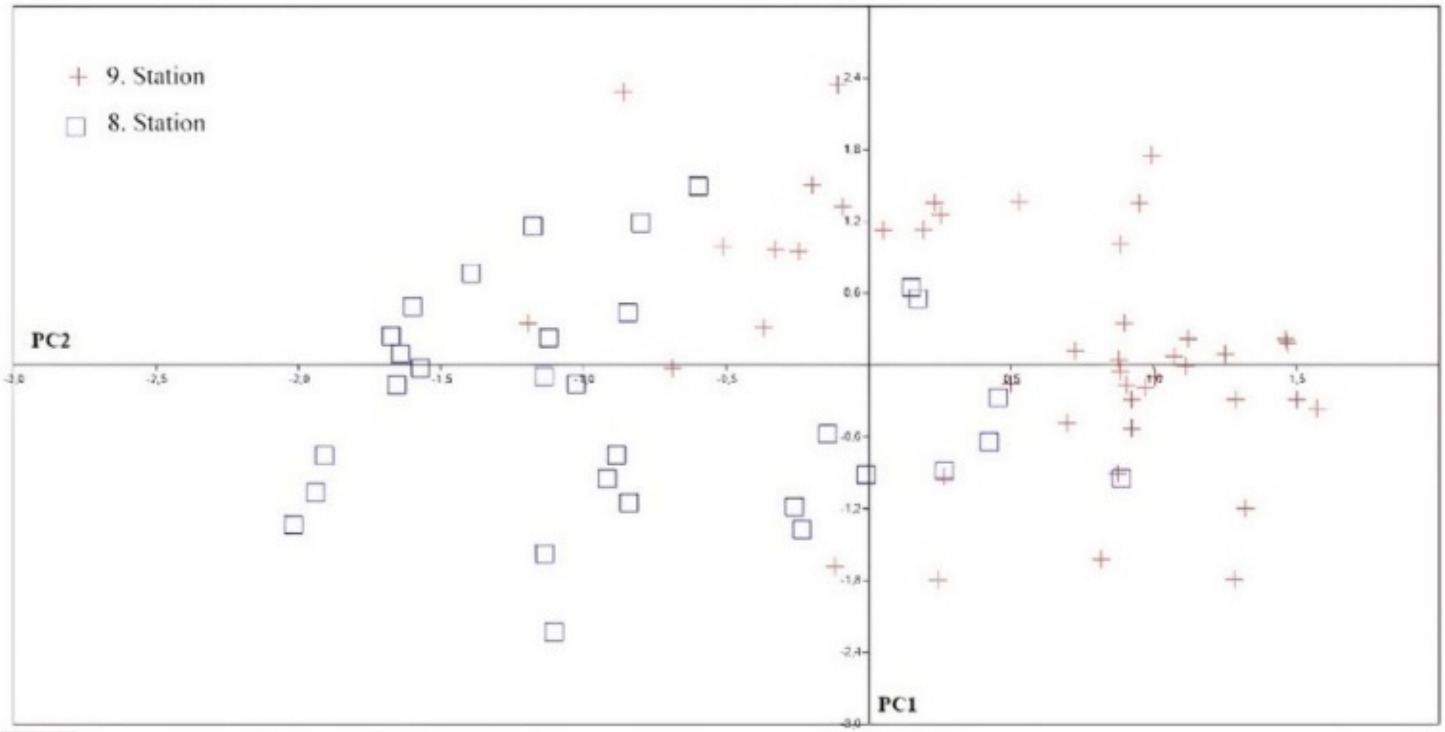

Figure 8. PCA plot of *Garra turcica* St. 9 and St. 8 populations

According to the analysis results of *G. turcica* populations, the first three principal components explain 66.97 of the total variance (PC1: 39.66; PC2: 15.74; PC3: 11.55) (Figure 9). As a result of the analysis, it was determined that different basin populations overlap and there is no clear distinction between the basins. The highest variation was observed at St. 6 and the narrowest variation was observed at St. 3.

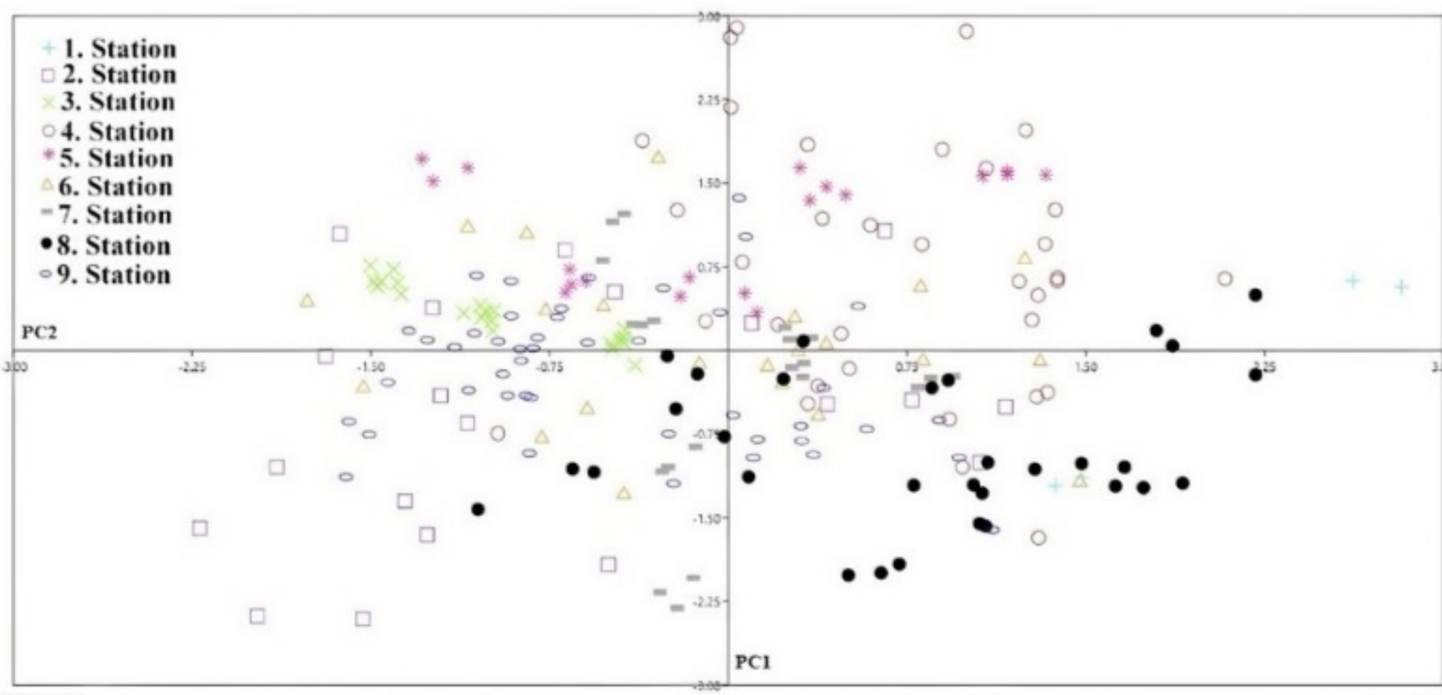

Figure 9. PCA plot of *Garra turcica* populations

A comparison of the wireframe graphs of St. 5 and St. 7 populations reveals that St. 5 individuals had shorter snout length (1st landmark), thinner heads (3rd and 14th landmarks), and longer caudal peduncles (7th landmark). The following characteristics were observed: shorter post-orbital distance (3rd and 4th landmarks), longer predorsal distance (4th and 5th landmarks), longer caudal peduncle (7th landmark), and thicker body (5th and 10th landmarks) (Table 3, Figure 10).

A comparison between St. 2 and St. 9 reveals that almost all landmarks are present in both populations, indicating a high degree of morphological overlap. However, the caudal peduncle (9th landmark) is thicker in St. 9 individuals.

A comparison of the body deformation curves of St. 1 and St. 5 populations reveals that St. 1 population exhibits a more terminal snout (1st landmark), a longer snout (1st and 4th landmarks), a shorter caudal peduncle (7th landmark), a more posterior anal fin beginning (9th landmark), and a deeper body (5th and 10th landmarks). Upon evaluation of all stations belonging to the *G. turcica* species, a considerable degree of variation was observed in the 1st, 5th, 7th, 9th and 10th landmarks.

Garra rufa

According to the principal components analysis, the first three principal components explain 69.21 of the total variance (PC1: 36.43, PC2: 20.56, PC3: 12.22). The analysis shows that there is no discernible division between the zones and that individuals from different groups overlap. All populations under study exhibit a high degree of morphological variety (Figure 11).

Table 3. Mahalanobis distances of *Garra turcica* populations calculated as a result of DFA (* distance of the closest populations, ** distance of the most distant populations)

Station	1. St	2. St	3. St	4. St	5. St	6. St	7. St	8. St	9. St
1. St	-	7,37	10,95	4,70	6,74	5,87	11,15	5,47	4,95
2. St		-	7,09	7,96	7,98	4,98	6,79	7,43	3,98*
3. St			-	10,63	16,48	7,75	14,99	13,26	9,42
4. St				-	8,11	9,46	7,80	6,60	5,08
5. St					-	10,90	18,64**	8,96	7,26
6. St						-	6,83	7,10	4,74
7. St							-	10,52	6,51
8. St								-	5,02
9. St									-

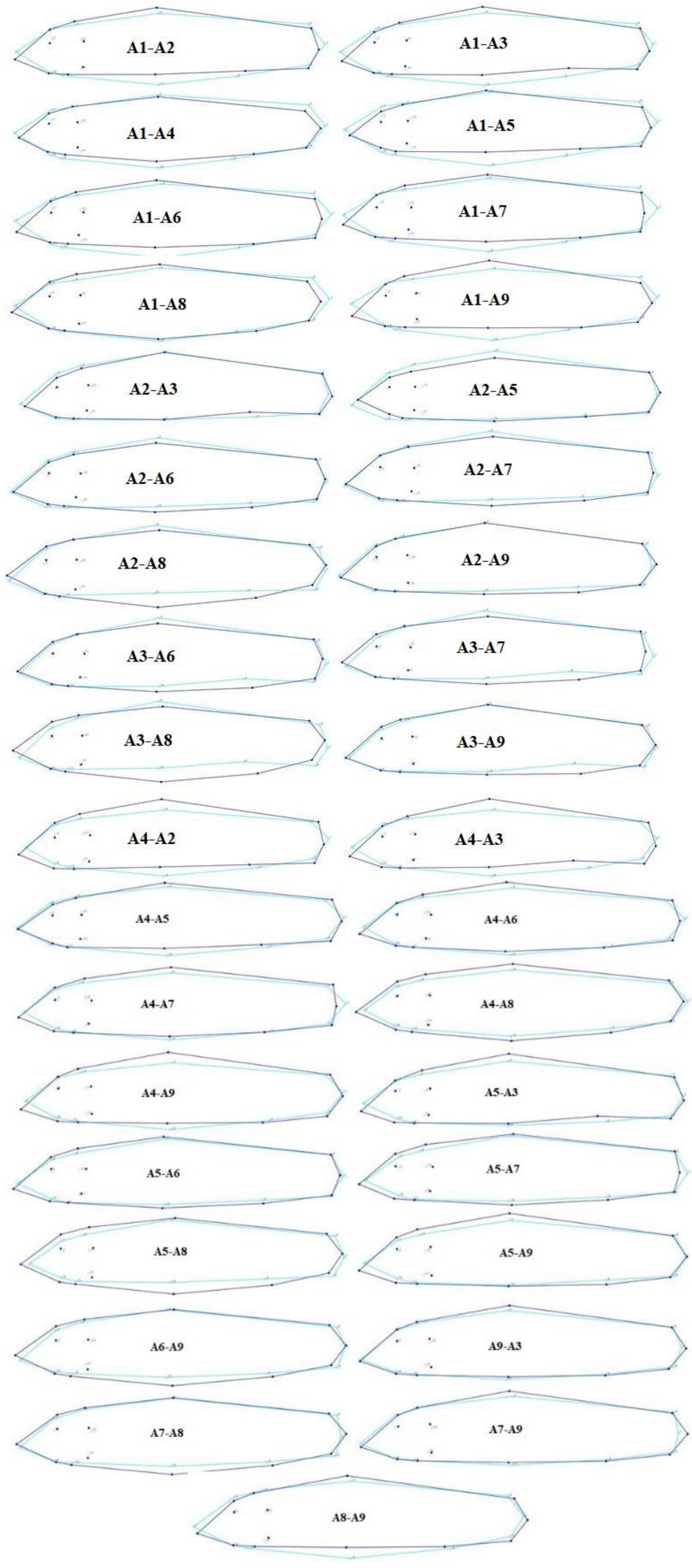

Figure 10. Wireframe graph showing body shape differences of *Garra turcica* populations as a result of DFA analysis.

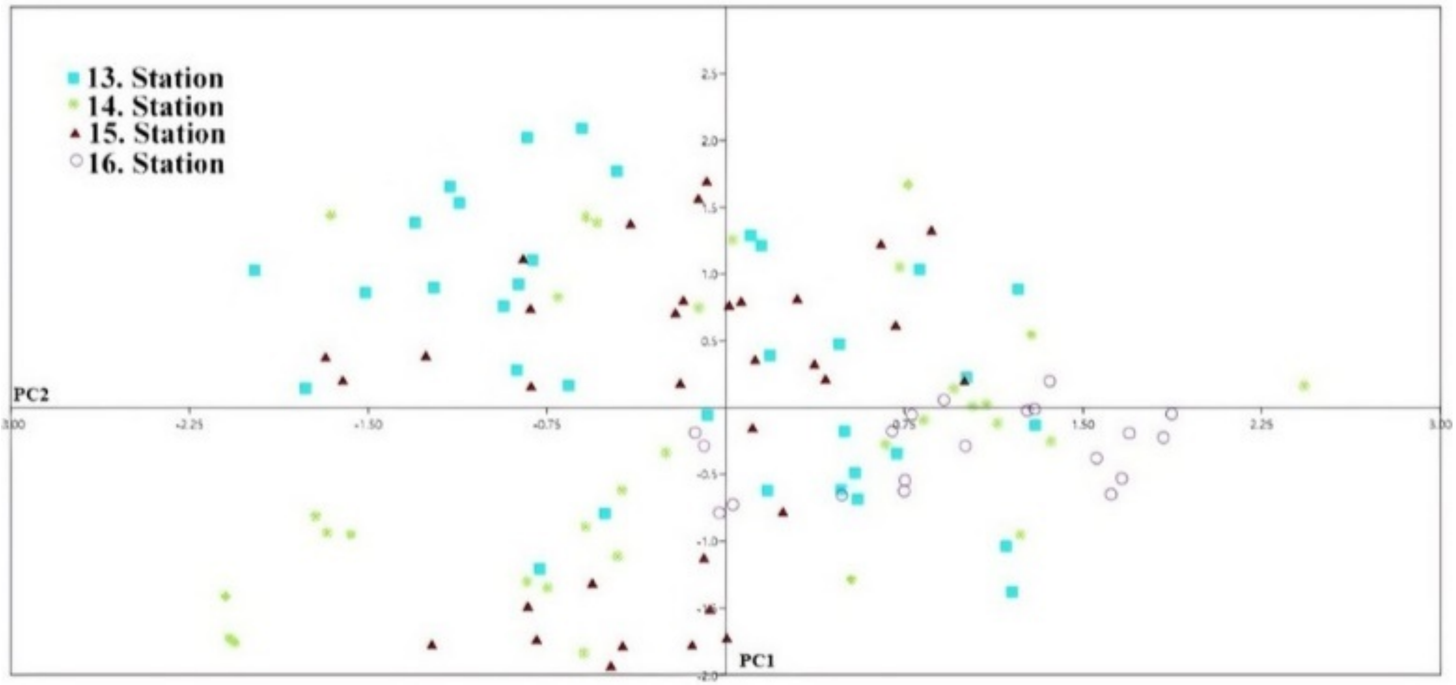

Figure 11. PCA plot of *Garra rufa* populations

Table 4. Mahalanobis distances of *Garra rufa* populations calculated as a result of DFA (* distance of the closest populations, ** distance of the furthest populations)

Station	13. St	14. St	15. St	16. St
13. St	-	3,96	3,74	6,95
14. St		-	2,76*	7,10
15. St			-	8,81**
16. St				-

The morphological variation of *G. rufa* among the evaluated stations is very low. In almost all populations the evaluated landmarks overlap. Only body width (9th and 10th landmarks) varies in some populations (Table 4, Figure 12).

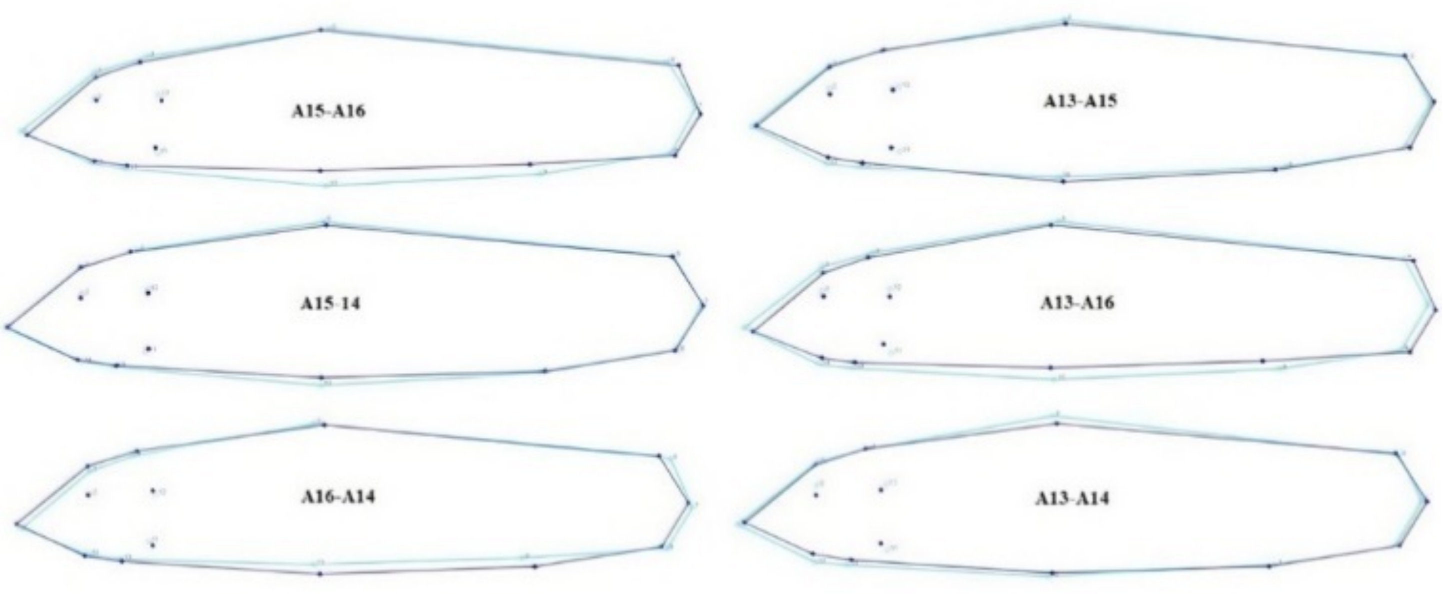

Figure 12. Wireframe graph showing body shape differences of *Garra rufa* populations as a result of DFA analysis.

Garra variabilis

As a result of the analysis, the first three principal components explained 66.83 of the total variance (PC1: 31.04; PC2: 25.34; PC3: 10.01). As a result of the PCA analysis, it was concluded that the individuals belonging to the populations were not grouped, all individuals overlapped, so the morphological variation in all populations evaluated was quite large (Figure 13).

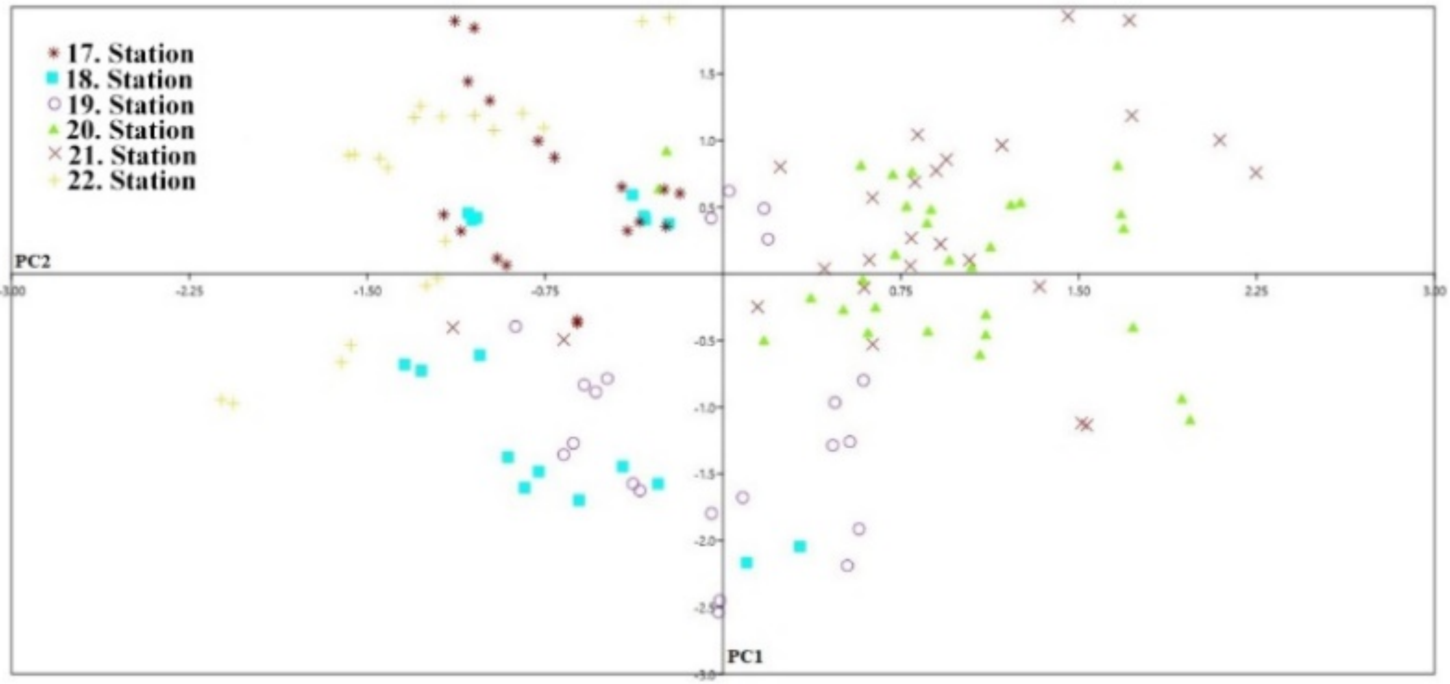

Figure 13. PCA plot of *Garra variabilis* populations

A comparison of the populations of St. 17 and the St. 22 reveals that the individuals of St. 17 have a longer snout (1st and 3rd landmarks), a narrower head (3rd and 1st landmarks), a longer postorbital distance (3rd and 4th landmarks), a shorter predorsal distance (5th and 6th landmarks), a pectoral fin that starts behind (11th landmark), and a thicker body (5th and 10th landmarks). The fourth landmarks indicate that the individuals have a longer postorbital distance (3rd and 4th landmarks), a shorter predorsal distance (5th and 6th landmarks), a pectoral fin that begins behind (11th landmark), and a thicker body (5th and 10th landmarks).

A comparison of St. 20 and St. 21 populations reveals minimal morphological differences between the individuals (Table 5). In Kuruçay individuals, the snout is shorter (3rd and 4th landmarks) and the body is thicker (5th and 10th landmarks).

In general, morphological variation is exceedingly high in all six stations of *G. variabilis*. The variation in body height (5th and 10th landmarks) and head landmarks (1st, 2nd, 3rd, 4th, 12th and 14th landmarks) is considerable (Figure 14). This variation is related to river typology, water flow rate, flow velocity and soil structure.

In their study on morphometric and meristic characters of *G. variabilis* species in four localities in the Tigris basin, Çiçek et al. (2016a) reported that the variation was very high. Similar findings were found in the data obtained in this study.

Table 5. Mahalanobis distances of *Garra variabilis* populations calculated as a result of DFA (* distance of the closest populations, ** distance of the furthest populations)

Station	17. St	18. St	19. St	20. St	21. St	22. St
17. St	-	8,10	7,13	10,82	10,56	12,61**
18. St		-	7,15	7,26	8,90	9,12
19. St			-	8,61	10,74	9,63
20. St				-	6,28*	8,44
21. St					-	11,88
22. St						-

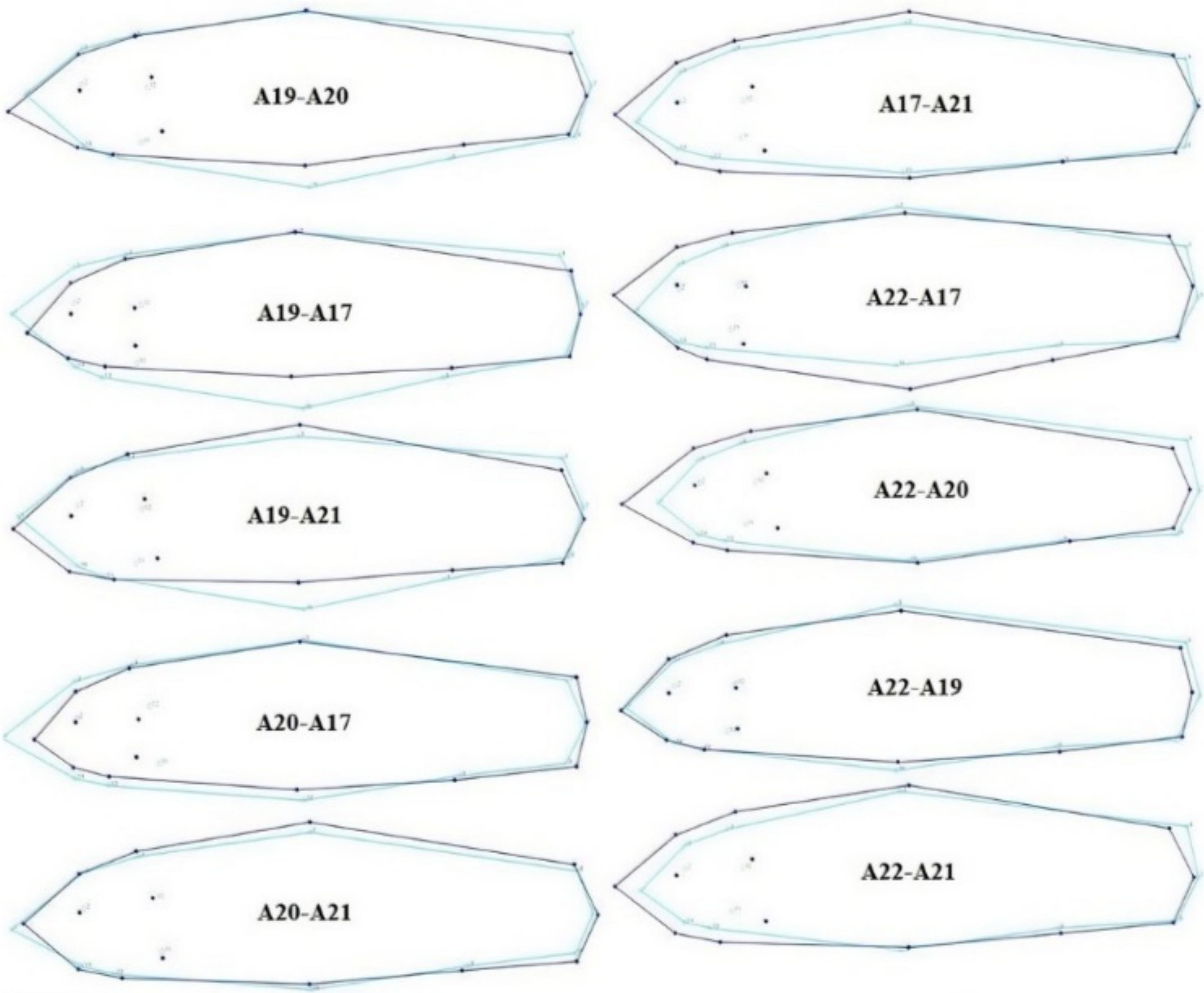

Figure 14. Wireframe graph showing body shape differences of *Garra rufa* populations as a result of DFA analysis.

The genus *Garra* exhibits considerable morphological variation, making it challenging to distinguish species based on their morphology. This is evidenced by the results of the analyses conducted within the scope of the study. The results of the hierarchical clustering analysis using Euclidean distance also corroborate this observation. The analysis yielded three main branches, as identified by UPGMA. In the first main branch, *G. variabilis* exhibited the greatest morphological divergence from the other species. In the other two branches, populations belonging to *G. rufa*, *G. orontesi*, and *G. turcica* were observed Figure 15).

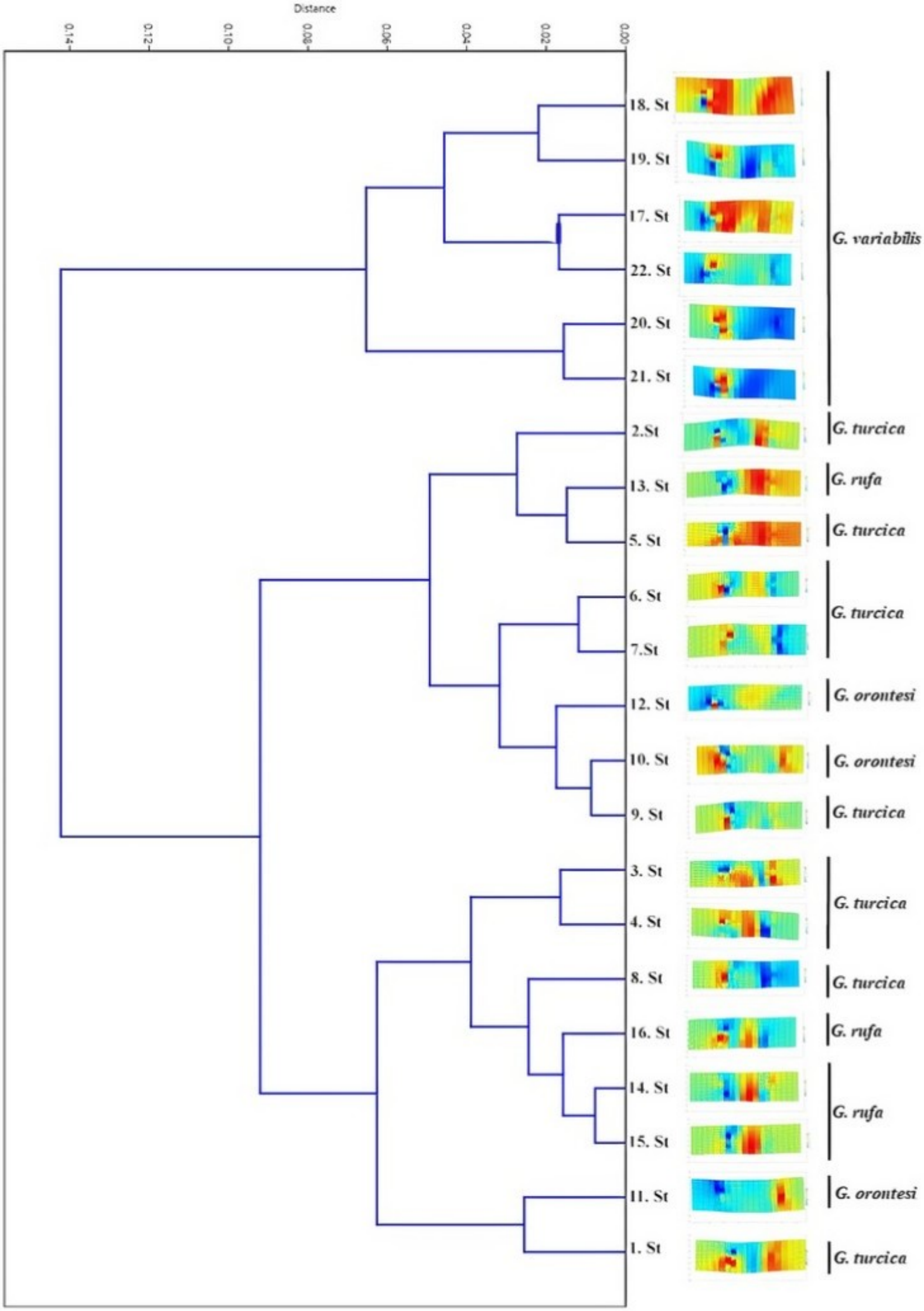

Figure 15. Intraspecific variation of *Garra* species based on Euclidean distance of geometric morphometric measurements by UPGMA (hierarchical cluster analysis).

Discussion

The distribution area of a species is not homogeneous due to differences in climate, geology, hydrology, hydromorphology, and physico-chemical characteristics. Consequently, sub-areas with different habitat characteristics can be found within the distribution area, which causes intraspecific morphological variation. Due to strong phenotypic plasticity, some species have very high morphological variation, whereas others may have lower morphological variation.

Moreover, these studies have numerous applications for the study of fish species and populations (Pianka, 1994; Watson and Balon, 2009), as well as for the investigation and comparison of fish species with distributed populations (Abbasi Ranjbar et al., 2020).

In this study, the intraspecific differentiation of the *Garra rufa, G. variabilis, G. turcica*, and *G. orontesi* species distributed in the Orontes, Ceyhan, Eastern Mediterranean, and Euphrates-Tigris basins was carried out by geometric morphometry. PCA analysis revealed that morphometric characters were not particularly effective in the intraspecific discrimination of the four *Garra* species, with high intraspecific variation levels. It is challenging to elucidate the underlying causes of morphological differences observed in fish populations. However, these differences in abiotic parameters, environmental factors, have been demonstrated to act as a locomotive which can induce phenotypic variations and affect fish performances (Mouludi-Saleh et al., 2020). Conversely, morphometric traits are influenced by environmental conditions and clearly demonstrate the habitat and phenotypic differences at the population levels (Jerry and Cairens, 1998). Consequently, the observed morphological differences in our study are attributed to the phenotypic plasticity of each species in response to their respective environments (Seçer et al., 2020).

Genetic characteristics of individuals influence variation in morphological traits, which may also be affected by environmental factors, including temperature, salinity, dissolved oxygen, radiation, water depth, current flow, food availability, and feeding (Antonucci et al., 2009; Fischer-Rosseau et al., 2009; Zami-faradonbeh et al., 2020; Seçer et al., 2022).

The geometric morphometry method yielded a body deformation curve that revealed the species with the highest intraspecific variation to be *G. turcica* and *G. variabilis*, while the species with the lowest intraspecific variation were *G. rufa* and *G. orontesi*. These results align with the findings of Çiçek et al. (2016a) and Çiçek et al. (2016b).

The morphologic characters exhibiting the most significant variation for *Garra turcica* were those pertaining to the head and mouth region, the snout tip, body height, and characters related to the caudal peduncle. For *G. variabilis*, the regions exhibiting the highest variation were determined to be the head and mouth region, the snout tip, nuchal height, body height, lateral line, and operculum origin regions. For the four *Garra* species, the most variation was found in the head and mouth region and the tip of the snout (1st, 2nd, 3rd, 4th landmarks), the height of the caudal peduncle (6th, 7th, 8th landmarks), body height, and the beginning of the operculum (5th, 10th, 13th landmarks). The variation in head and mouth shape can be considered reflective of differences in the selection of food items and direction of feeding (Langerhands et al. 2003). As a result of the study, it can be concluded that species of the *Garra* genus are adapted to different environmental conditions, and the morphological variation is wide. Physicochemical parameters and environmental changes (temperature, turbidity, salinity, depth, width, and velocity of water bodies) can lead to morphological differences and intraspecific variation (Seçer et al. 2022). As previously stated, the genus *Garra* exhibits greater morphological variation in the head and mouth region, body height, and the beginning of the operculum and caudal fin regions. This adaptation enables the species to occupy a wide range of habitats. However, the high degree of morphological variation suggests that this species possesses a high degree of plasticity, which may facilitate its distribution across different inland waters in Türkiye.

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Ethical Approval

The study was conducted by collecting the fish samples in dead conditions. An ethical approval is not required for the period during which the study was conducted.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Funding Statement

The authors don't declare any fund.

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