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ORIGINAL ARTICLE

Inter-basin geometric morphometric comparison of the North Caucasian Bleak (*Alburnus hohenackeri*) populations in Iran

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Abstract
This study investigates inter-basin geometric morphometric variation in populations of the North Caucasian bleak (*Alburnus hohenackeri*) across four major hydrological basins in Iran (Caspian, Sistan, Harirud, and Tigris). A total of 849 specimens from 23 rivers were photographed laterally, and 13 anatomical landmarks were digitized and aligned using Procrustes superimposition. Multivariate analyses including PCA, CVA, Procrustes distances, Mahalanobis distances, and cluster analysis were conducted. PCA revealed that the first two components explained about 40% of the total shape variation, with major differences related to head morphology, body depth, mouth position, and caudal peduncle structure. Despite extensive overlap among populations, a relative separation of the Sistan population from the others was observed. CVA highlighted this pattern more clearly, showing a stronger distinction of Sistan, while Caspian and Tigris exhibited the greatest similarity and Harirud occupied an intermediate position. Pairwise Procrustes and Mahalanobis distances were all statistically significant ($p < 0.001$), with the greatest distances between Sistan and the other basins and the smallest between Caspian and Tigris. Cluster analysis confirmed this pattern by placing Sistan in a distinct branch, grouping Caspian and Tigris together, and positioning Harirud intermediately. These findings indicate that environmental heterogeneity and geographical isolation have driven divergent phenotypic adaptations among *A. hohenackeri* populations. The study highlights the effectiveness of geometric morphometrics for detecting inter-basin population structure and provides insights valuable for biodiversity conservation and freshwater fishery management.

Keywords: *Alburnus hohenackeri*, Geometric morphometry, Principal Component Analysis, Canonical Variate Analysis, Procrustes analysis

INTRODUCTION
The North Caucasian bleak (*Alburnus hohenackeri*), a member of the family Leuciscidae (Eagderi et al. 2022), is commonly found in the lower reaches of rivers and freshwater lakes, particularly among aquatic vegetation. This species can tolerate slightly brackish waters and is often abundant in river estuaries (Abdoli & Naderi 2009; Keivany et al. 2016; Esmaeili et al. 2017). Although native to the Caspian Sea basin, *A. hohenackeri* has been unintentionally introduced to several other aquatic systems in Iran, including the Hamun, and Sistan basins and the Zarivar Lake (Abdoli & Naderi 2009), also was reported in the Sirvan River, Tigris drainage (Mouludi-Saleh et al. 2022).

Among the many methods available to distinguish populations within a species, morphological analysis remains one of the most widely used. Morphological characteristics are essential for addressing questions related to evolution, behavioral ecology, conservation, and fisheries management (Nacua et al. 2010). These features also provide valuable insights into species distribution, ecological status, and feeding strategies (Su et al. 2019). Morphological differentiation among fish populations is often a consequence of natural selection and environmental factors (Gammanpila et al. 2017). Morphometric traits are highly plastic and can vary both within and between species due to environmental influences (Salehi et al. 2022). In many cases, such variations are primarily driven by ecological factors rather than genetic divergence.

Geometric morphometrics, a landmark-based method, has become a powerful tool in biological studies. It has been successfully applied in taxonomy,

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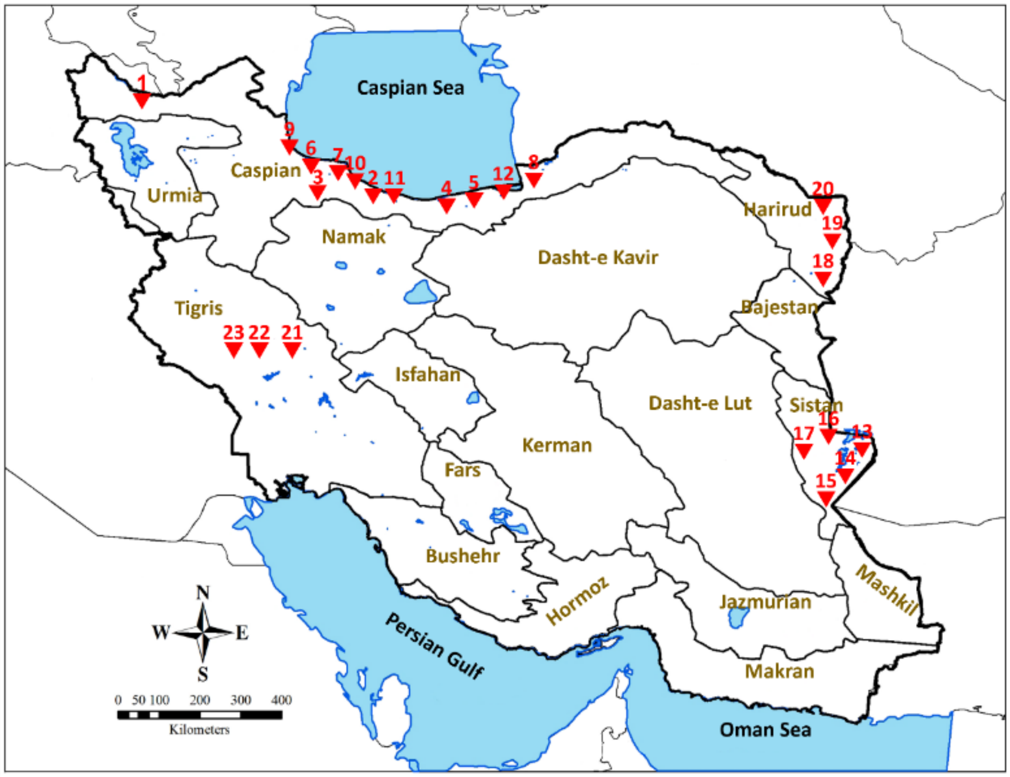

Fig.1. Map of the geographical location of the sampled areas of the studied *Alburnus hohenackeri* species (Keivany et al. 2016).

ecology, biogeography, phenotypic plasticity, and evolutionary biology to capture subtle shape variations (Nasri et al. 2019). Unlike traditional morphometric approaches that rely on linear measurements such as length and width, geometric morphometrics focuses on the spatial configuration of anatomical landmarks to extract shape data. This data can be visualized using deformation grids to better understand form changes across populations (Hosseini et al. 2022). In aquatic systems, morphology is closely tied to environmental pressures such as substrate type, water flow, vegetation, competition, and food availability (Gonzalez-Martinez et al. 2021). Thus, morphological studies are essential for identifying population structure and assessing biodiversity.

Given the ecological importance and environmental diversity of Iran's river basins, this study aims to assess inter-basin morphological variation in *A. hohenackeri* populations using geometric morphometric methods. By focusing exclusively on inter-basin comparisons, the research seeks to uncover shape divergences influenced by geography and habitat, thereby contributing to the understanding of population differentiation and its implications for conservation.

MATERIALS AND METHODS

A total of 849 specimens of *Alburnus hohenackeri* were collected from 23 rivers across four major river basins in Iran, Caspian, Sistan, Harirud, and Tigris, during the years 2009–2010. Sampling sites were selected based on the distribution of the species in different hydrological regions. Specimens were captured using electrofishing gear and seine nets. Immediately after capture, the fish were anaesthetized in 1% clove oil and fixed in 10% neutral buffered formalin. They were then transferred to the Ichthyology Museum of the Faculty of Natural Resources, Isfahan University of Technology, where they were preserved in 70% ethanol for further analysis.

Geographic coordinates of all sampling sites were recorded and are presented in Table 1, with their spatial distribution shown in Figure 1. For morphometric analysis, geometric morphometric methods were employed. Each specimen was

Table 1. Specifications of sampling sites.

	Basin & Total Samples	River	Sample Count	Coordinates
1	Caspian (487)	Aras	13	45°08'52"E, 39°11'58"N
2		Shalmanrud	14	50°12'40.38"E, 37°09'19.5"N
3		Mezobon	16	50°3'16.14"E, 36°16'40.3"N
4		Noor	20	52°01'14.22"E, 36°34'41.32"N
5		Sorkhrud	26	52°26'35.3"E, 36°30'37.36"N
6		Goharrud	28	49°26'42.8"E, 37°30'36.4"N
7		Kaparbardrud 2	42	49°42'53.88"E, 37°26'18.9"N
8		Gorganrud 2	52	55°15'10.56"E, 37°18'26.2"N
9		Lamir	58	49°06'58.44"E, 37°37'23.76"N
10		Hajibekandeh	64	49°46'54.12"E, 37°26'49.98"N
11		Kaparbardrud 1	76	49°42'53.88"E, 37°26'18.9"N
12		Gorganrud 1	78	55°18'45.66"E, 37°17'50.7"N
13	Sistan (170)	Maleki	12	61°42'25.62"E, 31°5'18.77"N
14		Chah-Nimeh 1	24	61°40'27.42"E, 30°50'19.5"N
15		Sistan-e-Zapashneh	24	61°24'23.4"E, 31°3'37.8"N
16		Chah-Nimeh 2	20	61°40'27.12"E, 30°50'19.5"N
17		Hamzehabad	90	61°27'1.5"E, 30°51'19.56"N
18	Harirud (141)	Harirud	18	61°09'29.4"E, 36°17'15.72"N
19		Harirud Canal 2	50	61°10'22.32"E, 36°16'39.54"N
20		Harirud Canal 1	73	61°08'48.96"E, 36°28'5.52"N
21	Tigris (51)	Karun 2	15	48°46'52"E, 33°46'50"N
22		Karun 1	18	48°46'52"E, 33°46'50"N
23		Karkheh	18	47°00'54"E, 34°26'57"N

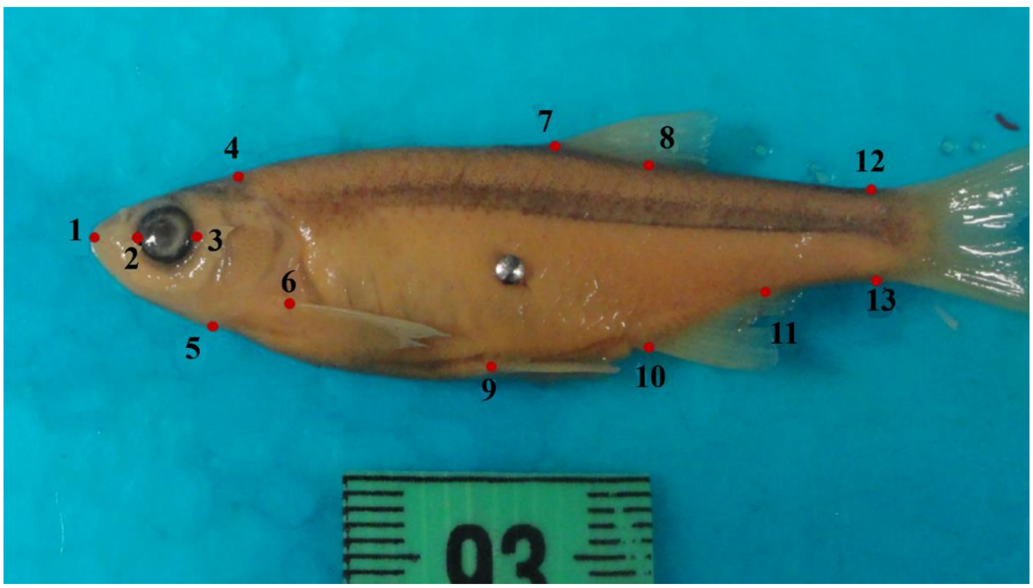

Fig.2. An *Alburnus hohenackeri* sample for landmark determination. Landmarks include (1) snout tip, (2) origin of the eye, (3) eye end, (4) head end, (5) under the operculum, (6) origin of the pectoral fin, (7) origin of the dorsal fin, (8) end of the dorsal fin, (9) origin of the pelvic fin, (10) origin of the anal fin, (11) end of the anal fin, (12) above the caudal peduncle, (13) below the caudal peduncle.

photographed from the left lateral side using a 14-megapixel Canon digital camera (Japan). Based on homologous anatomical features, 13 landmarks were digitized on each image using TPSdig2 software (version 2.10) (Fig. 2). These landmarks were selected to reflect overall body shape and were based on standard morphometric protocols.

Table 2. Variance values and eigenvalues of the first six main components of body shape analysis of *A. hohenackeri* populations in the basins of Iran.

Components	Eigenvalue	Variance
1	0.000569	24.52
2	0.000364	15.70
3	0.000285	12.29
4	0.000222	9.56
5	0.000170	7.36
6	0.000158	6.84
Total		76.27

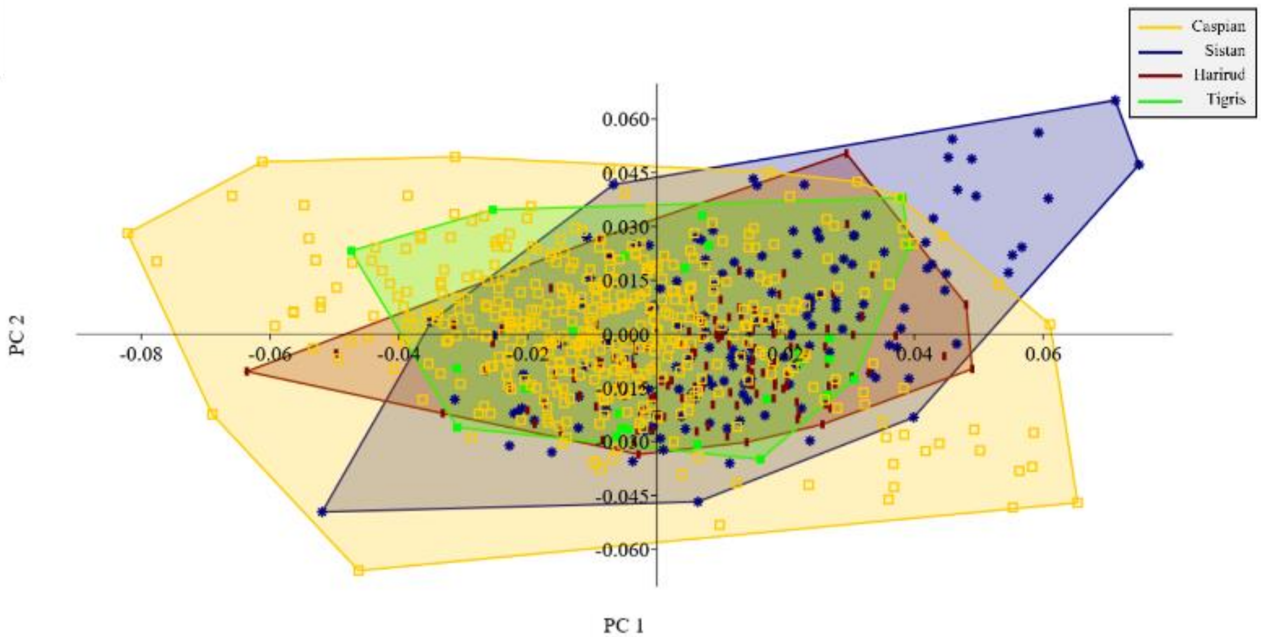

Fig.3. PCA diagram of the body shape *A. hohenackeri* populations in the Iranian basins.

The landmark data were processed and aligned using Generalized Procrustes Analysis to remove non-shape variation such as position, orientation, and scale (Bookstein 1991). Shape variables were then subjected to multivariate statistical analyses. Principal Component Analysis (PCA) and Canonical Variate Analysis (CVA) were performed to explore and visualize inter-basin shape variation. Mahalanobis and Procrustes distances, and cluster analyses were used to assess morphological differentiation among basins. All statistical analyses were conducted using PAST software (version 2.2).

Mean body shapes for each population were compared to the grand mean shape to evaluate deformation patterns across basins. An illustration of the landmark configuration and definition is shown in Figure 2.

RESULTS

The results of the PCA indicated that the first six components with eigenvalues above the cut-off collectively explained 76.27% of the total variance in body shape. (Table 2). The distribution of studied populations and the variation in body shape along the first and second principal component axes are illustrated in Figure 3.

The distribution of populations along PC1 and PC2 axes revealed considerable overlap among basins, although a relative distinction of the Sistan population from the others was evident (Figure 3). Shape changes were mainly expressed in the head region and body proportions:

PC1 axis: In the positive direction, the mouth (landmark 1) shifted forward and downward, the anterior margin of the eye (2) was displaced, and the dorsal (7 and 8) and anal fins (10 and 11) were shifted

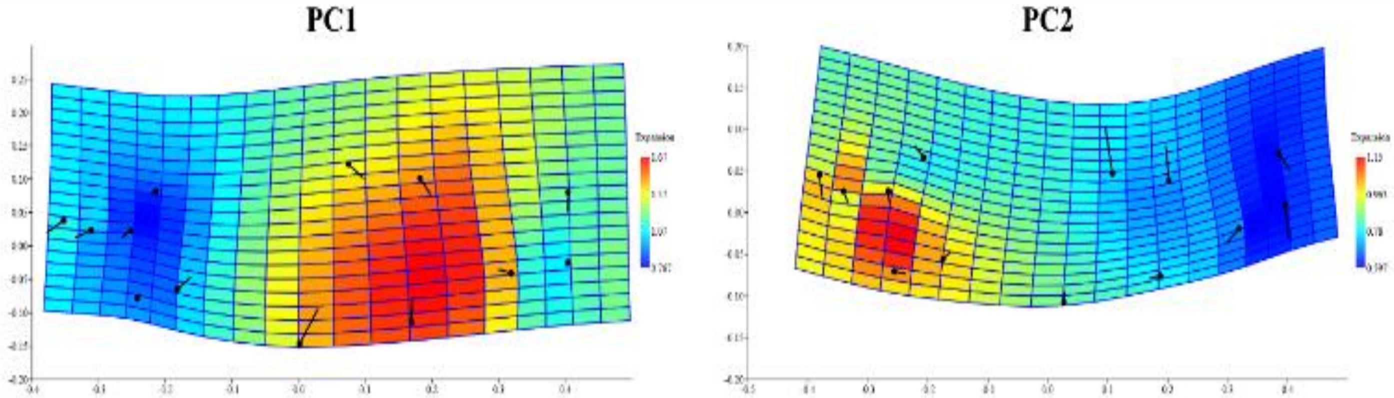

Fig.4. Body shape changes of *A. hohenackeri* populations in the Iranian basins in the direction of each of the PC1 and PC2 axes.

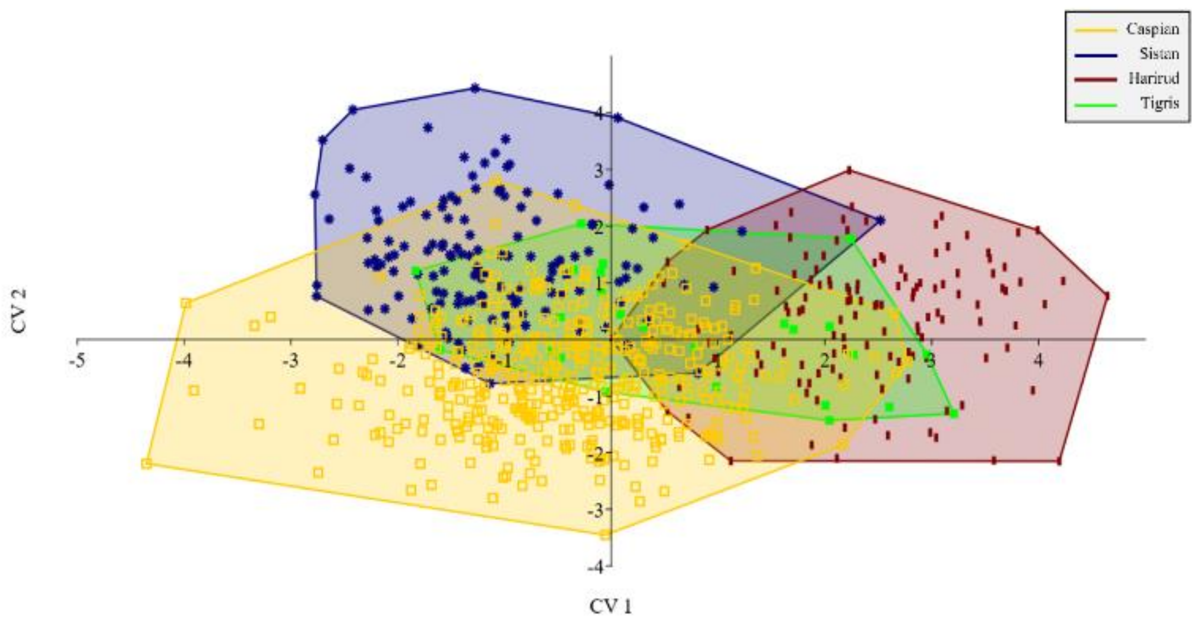

Fig.5. CVA diagram of the body shape *A. hohenackeri* populations in the Iranian basins.

posteriorly. At the same time, marked reductions in body depth (7, 8, 10, 11) and caudal peduncle depth (12 and 13) were observed, resulting in a slenderer body profile.

PC2 axis: Along the positive axis, the mouth (1) moved downward, the posterior head margin (4) and operculum (5) shifted position, and noticeable displacements occurred in the pectoral (6), pelvic (9), dorsal (7 and 8), and anal fins (10 and 11). These changes were accompanied by increases in body depth (7, 8, 10, 11) and caudal peduncle length (12 and 13), reflecting a deeper and more elongated body form (Fig. 4). Canonical Variate Analysis (CVA) demonstrated clearer patterns of morphological differentiation than PCA (Fig. 5). The Sistan population was distinctly separated, whereas the Tigris and Caspian populations exhibited the greatest similarity. The Harirud population occupied an intermediate position. Mahalanobis and Procrustes distances (Tables 3 and 4) further supported these findings. The largest Mahalanobis distance was observed between the Sistan and Caspian populations (1.856), while the smallest occurred between the Tigris and Caspian populations (0.951). Similarly, Procrustes distances revealed the greatest divergence between Sistan and Caspian (0.0312) and the smallest

Table 3. Mahalanobis distances of body shape resulting from CVA analysis in populations of *A. hohenackeri* from different basins of Iran.

	Caspian	Sistan	Harirud
Sistan	1.856		
Harirud	1.320	1.582	
Tigris	0.951	1.721	1.181

Table 4. Procrustes distances of body shape resulting from CVA analysis in populations of *A. hohenackeri* from different basins of Iran.

	Caspian	Sistan	Harirud
Sistan	0.0312		
Harirud	0.0270	0.0276	
Tigris	0.0210	0.0306	0.0233

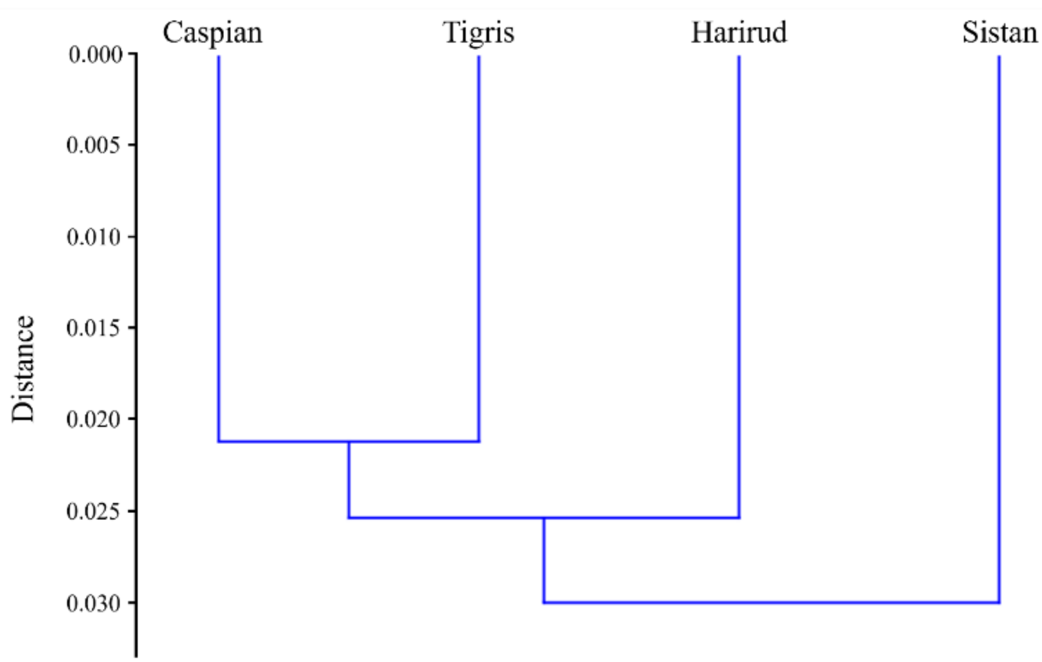

Fig.6. Cluster analysis of body shape for *A. hohenackeri* populations in different Iranian basins.

between Tigris and Caspian (0.0210), with the Harirud population consistently occupying an intermediate position.

Cluster analysis based on Procrustes distances (Fig. 6), with a cophenetic correlation coefficient of 0.9086, confirmed these results. In this analysis, the Tigris and Caspian populations clustered together, the Harirud population formed an intermediate branch, and the Sistan population appeared as the most distinct group, forming a separate cluster.

DISCUSSION

This study, using geometric morphometric methods, demonstrated that *Alburnus hohenackeri* populations across four major Iranian basins (Caspian, Sistan, Harirud, and Tigris) exhibit a clear pattern of inter-basin morphological differentiation. The PCA results indicated considerable overlap among populations, although a relative separation of the Sistan population was observed compared to the others. Shape variation along the first principal component was primarily associated with changes in the head region and fin positions, while the second axis reflected shifts in the mid- and posterior body, including increased body depth and caudal peduncle length. These patterns are consistent with the findings of Langerhans et al. (2003), who highlighted the role of hydrodynamic pressures and environmental conditions in shaping morphological differences in freshwater fishes. Unlike PCA, CVA achieved a clearer separation among populations, revealing that Sistan was markedly distinct from the others, while Caspian and Tigris showed the greatest similarity, and Harirud occupied an intermediate position. This divergence between methods is expected, as PCA reflects total variance (including within-group variation), whereas CVA emphasizes maximizing between-group separation, thereby accentuating differences (Rohlf & Marcus 1993; Zelditch et al. 2012). In line with these observations, Cahyadi et al. (2021) demonstrated in native Indonesian fishes that while PCA showed substantial overlap, CVA successfully detected significant differentiation, underscoring the utility of CVA in geometric morphometric studies.

Cluster analysis further supported these findings. The high cophenetic correlation coefficient (0.9086) indicated a good fit of the dendrogram to the distance data. Clustering revealed that Caspian and Tigris grouped together, Harirud assumed a transitional position, and Sistan formed a distinct branch. Mahalanobis and Procrustes distances also reflected a similar pattern, with Sistan showing the greatest distances from other basins, and Caspian and Tigris exhibiting the least divergence. This convergence across multiple methods suggests that the observed inter-basin structure represents a consistent and biologically valid pattern (Mariu et al. 2023; Gholami et al. 2025).

These results are consistent with similar studies in Iran. For example, Salehi et al. (2022) reported that geometric morphometrics successfully revealed subtle population-level differences in Salmo trutta across the Caspian, Namak, and Urmia basins, with traits such as body depth and caudal peduncle length playing a key role. Mohadasi et al. (2013) found that *Alburnus chalcoides* populations from southern Caspian rivers exhibited differences concentrated in the abdominal and caudal regions, with the Anzali population being distinct due to its unique habitat conditions. Keivany et al. (2019) observed a similar pattern in *Capoeta capoeta*, where Harirud and Caspian populations were more similar, while Urmia was entirely distinct. Likewise, Razavi Pour et al. (2015) showed that *Capoeta damascina* body shape varied directly with environmental factors such as water temperature, quality, and flow, emphasizing the need to consider populations as independent management units.

Jalili et al. (2015) also highlighted high morphological plasticity in Alburnus filippii across rivers within the Aras basin, showing that even within a single basin, habitat differences can lead to distinct morphotypes. Similarly, Gholami et al. (2025) in the Sistan basin found that although PCA indicated high overlap, CVA and clustering analyses successfully identified significant inter-basin differentiation. These findings align well with the present study, which consistently identified Sistan as the most distinct basin.

From an ecological perspective, the pronounced divergence of Sistan likely results from geographic isolation, hydrological instability, and harsh local environmental pressures (Abdoli & Naderi 2009; Mariu et al. 2023). Conversely, the similarity between Caspian and Tigris populations may reflect convergent adaptation under comparable ecological conditions, while Harirud served as an intermediate. These outcomes are consistent with earlier reports on *A. hohenackeri* in Iran (Mouludi-Saleh & Keivany 2019; Gholami & Keivany 2025), which demonstrated that environmental pressures act as a major driver of morphological divergence. Additionally, results from Turan (2004) on *Trachurus mediterraneus* in the Mediterranean and Black Seas confirm that morphological differences can reliably distinguish populations and provide valuable insights for stock management.

From a conservation standpoint, these findings stress the importance of recognizing each basin as an independent management unit (Mondal 2024). Overlooking this diversity risks homogenization of populations and the loss of locally adapted phenotypes. In particular, the Sistan population should be considered a high conservation priority, and management or restoration plans must be designed at the basin-specific level. However, to validate the morphometric patterns identified here, complementary genetic analyses (e.g., mitochondrial markers and microsatellites) are essential to uncover the true extent of intraspecific diversity and population structuring (Turan 2004; Langerhans & Reznick 2010;).

Overalls, this study highlights both the adaptive potential of *A. hohenackeri* and the effectiveness of geometric morphometric methods in elucidating population structure and phenotypic plasticity. Integrative future research—combining morphometric, environmental, and genetic data—will further clarify population dynamics, support regional biodiversity conservation, and inform sustainable fisheries management in Iran's freshwater ecosystems.

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مقاله مروری

مقایسه ریخت‌شناسی هندسی مرواریدماهی قفقازی (*Alburnus hohenackeri*) در بین حوضه‌های آبی ایران

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چکیده: این مطالعه به بررسی تغییرات ریخت‌سنجی هندسی بین‌حوضه‌ای در جمعیت‌های مرواریدماهی قفقازی (*Alburnus hohenackeri*) در چهار حوضه اصلی ایران (خزر، سیستان، هریرود و تیگره) می‌پردازد. در مجموع ۸۴۹ نمونه از ۲۳ رودخانه به‌صورت جانبی عکسبرداری شدند و ۱۳ لندمارک آناتومیکی دیجیتال‌سازی و با استفاده از هم‌ترازی پروکراست تنظیم شدند. تحلیل‌های چندمتغیره شامل PCA، CVA، فاصله‌های پروکراست، فاصله‌های ماهالانوبیس و آنالیز خوشه‌ای انجام گرفت. نتایج PCA نشان داد که دو مؤلفه نخست حدود ۴۰ درصد از کل تغییرات شکل را توضیح می‌دهند، به‌طوری‌که عمده تفاوت‌ها به ریخت‌شناسی سر، عمق بدن، موقعیت دهان و ساختار ساقه‌ی دمی مربوط بود. علی‌رغم همپوشانی گسترده میان جمعیت‌ها، جدایی نسبی جمعیت سیستان از سایر جمعیت‌ها مشاهده شد. نتایج CVA این الگو را شفاف‌تر نشان داد و تمایز قوی‌تری برای سیستان آشکار کرد، در حالی‌که جمعیت‌های خزر و تیگره بیشترین شباهت را داشتند و هریرود موقعیت میانی را اشغال کرد. فاصله‌های زوجی پروکراست و ماهالانوبیس همگی از نظر آماری معنی‌دار بودند (*P*<۰/۰۰۱)، به‌طوری‌که بیشترین فاصله‌ها بین سیستان و سایر حوضه‌ها و کمترین فاصله بین خزر و تیگره مشاهده شد. آنالیز خوشه‌ای نیز این الگو را تأیید کرد و نشان داد که سیستان شاخه‌ای مستقل را تشکیل می‌دهد، در حالی‌که خزر و تیگره در یک گروه قرار گرفتند و هریرود جایگاه انتقالی داشت. این یافته‌ها نشان می‌دهد که ناهمگنی محیطی و جدایی جغرافیایی باعث شکل‌گیری سازگاری‌های فنوتیپی واگرا در جمعیت‌های *A. hohenackeri* شده است. این مطالعه کارایی بالای روش‌های مورفومتریک هندسی را در آشکارسازی ساختار جمعیتی بین‌حوضه‌ای نشان می‌دهد و دیدگاه‌های ارزشمندی برای حفاظت تنوع زیستی و مدیریت شیلات آب شیرین فراهم می‌کند.

کلمات‌کلیدی: *Alburnus hohenackeri*، مورفومتریک هندسی، تجزیه به مؤلفه‌های اصلی، تحلیل متغیرهای کانونیک، آنالیز پروکراست