

Research Article

Geometric morphometric and meristic analysis of the deepwater goby, *Ponticola bathybius* (Kessler, 1877) (Teleostei: Gobiidae) in the Iranian waters of the Caspian Sea

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Abstract: Landmark-based geometric morphometric and meristic characters were used to explore morphological variation among three population samples of deepwater goby, *Ponticola bathybius*, (Family Gobiidae) along the southern Caspian Sea in Iran. Morphological differences were studied using the geometric morphometric method of thin-plate splines and multivariate analysis of partial warp scores on the left and dorsal sides of the specimens. Non parametric MANOVA discerned significant differences in shape variations among the population samples. Also, the three samples exhibited significant differences in meristic data. Mantel test results indicated that there was no relationship between the morphological data and geographic distances, suggesting spatially heterogeneous levels of morphological divergence.

Keywords: Perciformes, Morphological variations, Shape analysis, Landmark, Meristic.

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Introduction

Many fish species display considerable intraspecific morphological variation both among and within populations, which often is environmentally-induced (Turan 2006) or behaviorally influenced (Kocovsky et al. 2013). Population assessment models and assumptions often presume that populations comprise closed groups having homogenous life history features, which may lead to misleading results (Cadrin & Friedland 1999). Several morphological methods have been used to test for population-level discrimination; among these,

morphometrics, including geometric morphometric and meristic data, have been employed (Gholami et al. 2015).

Members of the family Gobiidae, one of the largest vertebrate families, generally are small benthic fishes whose paired fins are modified into an adhesive disk that enables them to rest on the bottom (Gill & Mooi 2012). Gobiids have little mobility and are highly benthic, as they can't able to switch the life history between benthic and pelagic habitats (Robinson & Parsons 2002) Members of the goby subfamily Benthophilinae are endemic to the Ponto-

Caspian region, where historical changes in salinity and water basins drove the speciation radiations and population patterns of this group (Stepien & Tumeo 2006; Neilson & Stepien 2009). Most benthophilins eat small macroinvertebrates, especially crustaceans. In the southern Caspian Sea benthophilins are in turn consumed by larger fishes, including sturgeons (Acipenseridae) and the Caspian seal (*Pusa caspica*), thereby playing an important ecological role in the food chain (Abdoli et al. 2012; Coad 2016).

The deepwater goby, *Ponticola bathybius* (Kessler, 1877), Gobiidae: Benthophilinae: Ponticolini (per the classification of Neilson & Stepien 2009), is a marine and brackish water species that is endemic to the Caspian Sea. *Ponticola bathybius* is the largest goby species in the Caspian Sea, reaching 315mm TL. It inhabits depths to 75 meters, living on sandy and shelly bottoms and occasionally on firm silt (Miller 2003). Little is known of the deepwater goby's ecological adaptations, life history, morphological variation, and population structure. Particularly, distributional and ecological data are especially depauperate from Iranian waters (Coad 2016). The present study thus aimed to shed light on the morphological shape variation of *P. bathybius* in the Iranian South Caspian Sea based on a variety of morphometric and meristic characters in order to discern whether there was a fine-scale population difference.

Materials and Methods

A total of 290 adult deepwater goby were collected using a long beach seine (mesh size 33mm) in April 2014 from three sites located at: (a) west coast (Bandar Anzali, 37°28'N, 49°27'E), (b) mid coast (Salmanshahr, 36°42'N, 51°11'E) and (c) east coast (Miankaleh, 36°51'N, 53°34'E) of the Iranian southern Caspian Sea (Fig. 1). In terms of water connectivity, the distance between (a) to (b) is about 175km, from (b) to (c) is about 212km, and from (a) to (c) is about 372km. To analyze the biological shape of the specimens, 17 points were used on the left side of the body and 10 landmarks on the dorsal

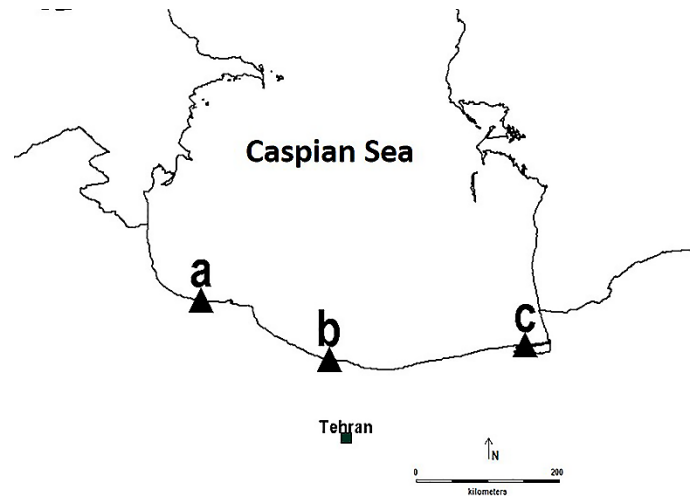


Fig.1. Sampling locations in the Iranian coastal waters of the Caspian Sea: (A) Bandar Anzali, (B) Salmanshahr and (C) Miankaleh.

head side and then photographed using a Sony SDC-HX 10V camera. Landmarks were marked on all specimens using the 2D digitization software TPS Dig2 (Rohlf 2010) (Fig. 2 A, B), and a TPS file was used to perform the calculations. A Generalized Procrustes Analysis (GPA) was performed to superimpose landmark coordinates as shape variables. This analysis reflected only true differences in shape, eliminating size and rotational/translational variations (see Dorado et al. 2012).

Additionally, nine meristic characters (first and second dorsal, pectoral, pelvic, anal, and caudal fin rays, mid line scales, number of scales above and below the mid line) were counted. Since the partial warp scores from each body side and meristic data were not normally distributed according to a multivariate normality test, a nonparametric MANOVA (NPMANOVA with 10000 permutations, with p-values Bonferroni-corrected) separately was used with (Mahalanobis 1936)) distances to test for possible differences among the three population samples of *P. bathybius*. Mann-Whitney U tests were used to delineate meristic characters that discriminated the population samples (Ferrito et al. 2007). An Unweighted Pair Group Method Analysis (UPGMA) was performed between the group consensus values for the morphometric and meristic data. Branch support values were calculated

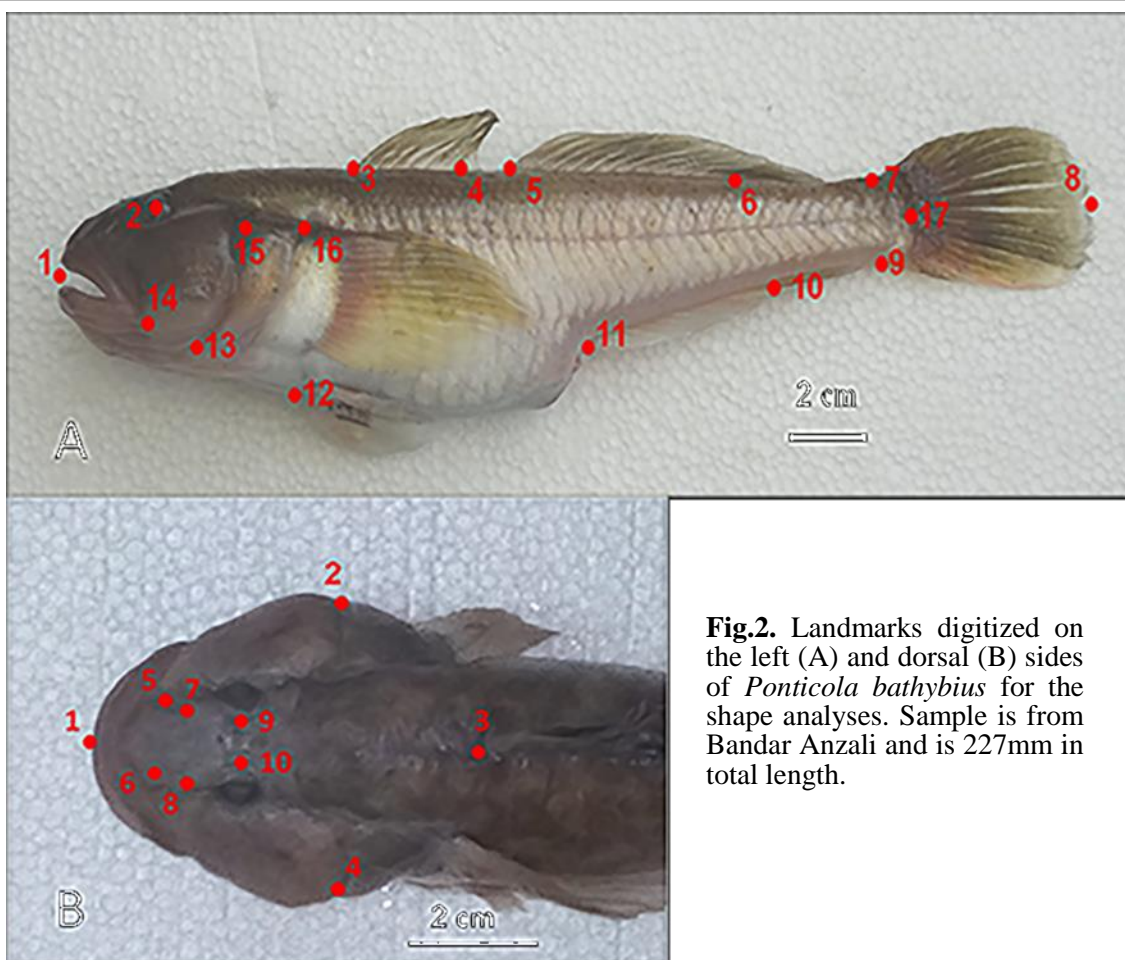


Fig.2. Landmarks digitized on the left (A) and dorsal (B) sides of *Ponticola bathybius* for the shape analyses. Sample is from Bandar Anzali and is 227mm in total length.

Table 1. Shape distances (Mahalanobis distances) between pairs of population samples, based on measurements for the left and dorsal sides.

Pairwise comparison	Mahalanobis distances between population samples		F - value	
	Left side	Dorsal side	Left side	Dorsal side
a vs. b	0.0003*	0.0003*	2.093	2.654
a vs. c	0.0003*	0.0036*	2.437	2.245
b vs. c	0.0003*	0.0099*	2.090	2.082

* $P < 0.0001$

by bootstrapping with 10000 randomizations. All statistical analyses were performed with PAST software. Shape variability among the population samples was shown based on a deformation grid using MorphoJ software (Tabatabaei 2011). Mantel's (1967) tests with 10000 permutations in XLSTAT v2010 (www.xlstat.com) were used to evaluate significance of correlations between the morphological matrices of morphometric or meristic distances among population samples and the geographic distance matrix (Hashemzadeh Segherloo

et al. 2012).

Results

Shape variations of the left and dorsal body sides significantly differentiated three population samples (df=2, F=2.605 for left side; df=2, F=2.720 for dorsal side; $P < 0.001$, Table 1). Differences in morphometric characters among the three population samples and their shape deformations based on the transformation grid are shown in Table 2 and Figure 3A, B, respectively. Also, NPMANOVA analyses of

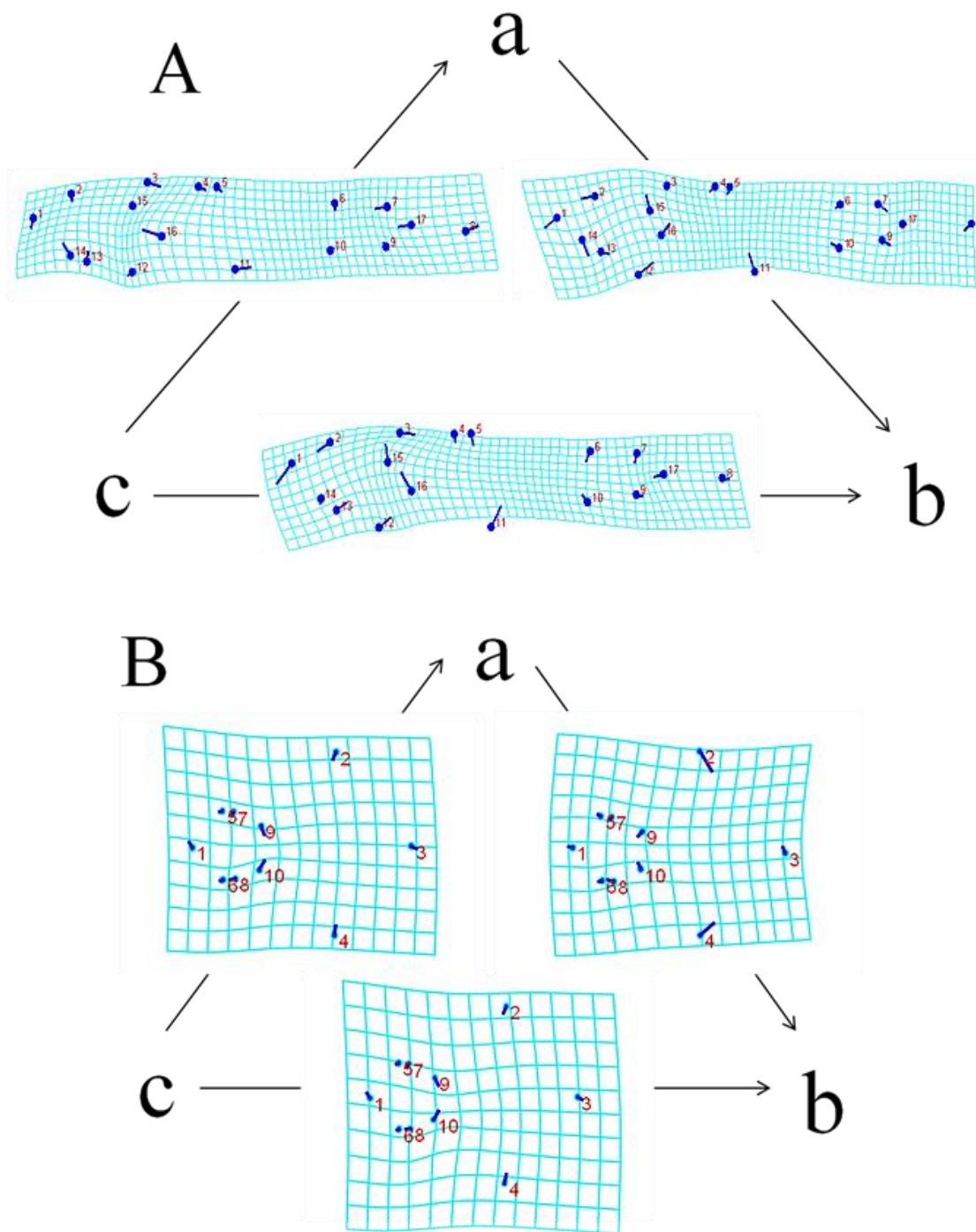


Fig.3. Shape deformation on the left side (A) and dorsal side (B) of the population samples based on a transformation grid.

the meristic data showed significant differences among population samples ($df=2$; $F=13.59$; $P<0.001$). Characters showing significant differences according to the Mann-Whitney U test results are

shown on Table 3. The UPGMA analysis for the morphometric and meristic data is plotted to show the pairwise similarities among population samples in Figure 4A, B and C. There was no correlation

Table 2. Pairwise comparisons between pairs of population samples from morphometric geometric data using MorphoJ software.

Pairwise comparison	Morphometric Character changes
a vs. b	Increase in head length and mouth size; decrease in head height and width
c vs b	decrease in DF1 basal length, body height, head height and width, increase in caudal fin length, head length and body length
c vs. a	increase in caudal fin and total length, decrease in body height, head length and width, mouth size and anal fin basal length, Tip of the mouth downwards

Abbreviations: DF1: first Dorsal Fin; PF: Pectoral Fin; CF: Caudal Fin.

Table 3. Mann-Whitney U test for differences between the samples from 9 meristic values. Abbreviations: DFR1, First dorsal fin ray; DFR2, Second dorsal fin ray; CFR, Caudal fin ray; AFR, Anal fin ray; AbFR, Abdominal fin ray; PFR, Pectoral fin ray; Sc A, Scales above mid line; Sc B, Scales below mid line; Sc O, Scales on mid line.

Pairwise comparison	DFR1	DFR2	CFR	AFR	AbFR	PFR	Sc A	Sc B	Ac O
a vs. b	0.32	0.26	0.94	0.58	0.16	0.34	0.00*	0.00*	0.17
a vs. c	0.16	0.10	0.00*	0.52	0.16	0.05*	0.32	0.10	0.00*
b vs. c	0.56	0.014	0.00*	0.21	1.00	0.00*	0.00*	0.00*	0.00*

* $P < 0.05$

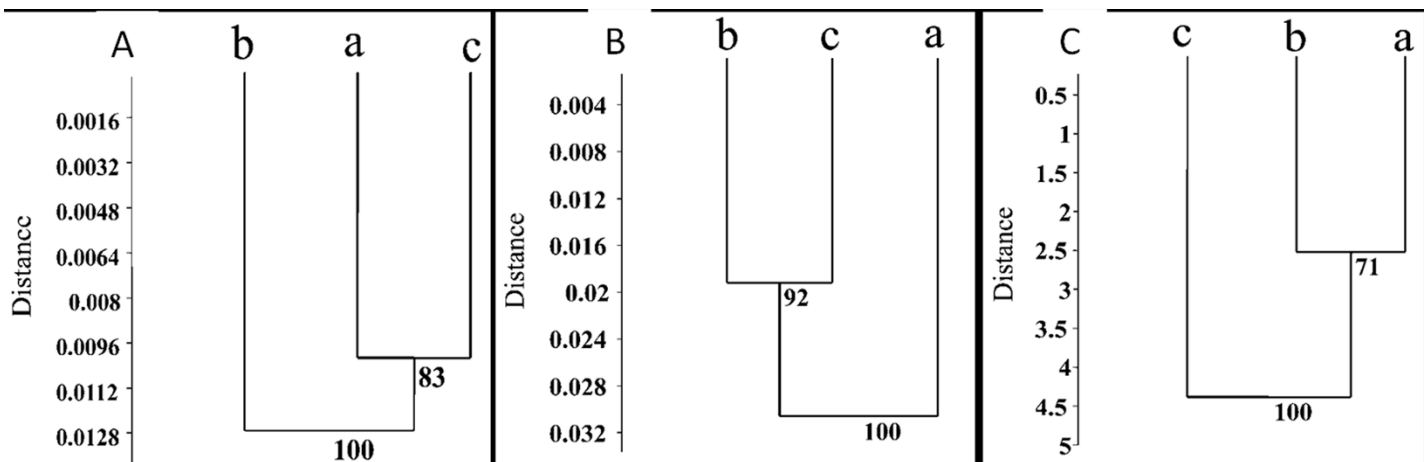


Fig.4. Dendrogram obtained from the UPGMA, showing the morphological distances among three population samples based on lateral side (A), dorsal side (B) and meristic (C) distances.

between geographic distances and the distance matrix for the morphometric and meristic data (Mantel's $r=0$, $P < 0.001$).

Discussion

In this study, in order to distinguish inter-population variations in *P. bathybius*, multivariate analyses were performed separately for morphometric and meristic characters, since these variables differ statistically and biologically (Erguden et al. 2009). The results demonstrate significant differences among three population samples based on GM

method and meristic features. Morphological variation in fishes often is greatly influenced by their habitat conditions which may vary throughout their life history stages. Fish populations frequently display greater variances in morphological features, both among and within population groups and geographic areas, than do other vertebrates (Hossain et al. 2010). Long term adjustments may include shifts in their morphology. For example, changes in different environmental and ecological factors such as temperature, salinity, benthos type, food availability, predation pressure, light, water flow and

quality, and dissolved gasses - especially during ontogeny, can influence phenotypic differentiation, manifested by high plasticity in morphological characters (Ujjainia & Kohli 2011; Santos & Quilang 2012).

Morphometric expression is under simultaneous regulation by both genetic and environmental factors (Ujjainia & Kohli 2011). In contrast, variation in meristic characters is less dependent on environmental conditions, and is more influenced by genetic factors (Winfield & Nelson 2012). Therefore meristic characters are predicted to be set early in life and then remain fairly stable, but may be influenced by environmental conditions during larval development, such as the influence of temperature on the number of vertebrae (Begg & Waldman 1999).

The three sampling sites possess different substrate types, which may influence goby morphology. Notably, the Bandar Anzali sampling site (a) has a fine to mid gravel bottom, with a high slope and water velocity. The Salmanshahr location (b) has a mid to rough gravel bottom, with high slope and water velocity. In contrast to both, the Miankaleh site (c) has a muddy substrate, with a low slope and water velocity. In fact, a decrease in the diameter of sediment particles diameter is seen from west to east across the southern Caspian Sea (Khoshnavan et al. 2010).

In terms of topography, the three locations are characterized by sediment and terrain differences even at similar depths, including that bioturbation at Miankaleh (c) occurs at shallower depths (7m) than at two other sites (30 and 20 m for Bandar Anzali and Salmanshahr, respectively (Khoshnavan et al. 2010). The type of substrate can lead to changes in organism's community, as Deposit feeders prefer the muddy substrate whereas the suspension feeders prefer to predominant on sandy bottom (Castro & Huber 2008). Since the goby lives near shore along the coast during its ontogeny period, changes in food availability may influence morphological variation of deepwater goby.

Within the goby subfamily Benthophilinae,

Cerwenka et al. (2014) showed that two invasive gobies, the round goby *Neogobius melanostomus* and Kessler's goby *Neogobius kessleri*, exhibited a high degree of intraspecific variation in the upper Danube River. These features were correlated with fine-scaled geographic variations, such as substrate type (Cerwenka et al. 2014); similar patterns might occur in the deepwater goby. Shape variation may be explained by variations in feeding behavior and types of food available from habitat to habitat. Cullen et al. (2007) documented some differences in head shape between char (*Salvelinus alpinus*) from Coomasaharn Lough and other Irish populations, which reflected their planktivorous or benthivorous feeding habits.

Variations in water current velocity can induce phenotypic changes within populations of many fish species (Ohlberger et al. 2006), resulting in differences in body size and shape among habitats, which are adapted for efficient hydrodynamics. For example, shapes of fishes inhabiting waters of lower velocity tend to be more robust (having deeper bodies and deeper caudal peduncles, along with longer paired fins), whereas those from higher velocity waters trend to be more elongate and streamlined (Grnbaum et al. 2007). Immature par of Atlantic salmon exhibited differences in shape depending on water current velocity, with individuals from slower-moving waters having deeper bodies than those sampled in rapid-flowing waters (Paez et al. 2008). As we see fish inhabiting in Miankaleh locality with slow flow water are more elongate rather than two others. Additionally, body depth differences have been shown to be influenced by variations in swimming behavior that may be influenced by variable water depths (Rincón et al. 2007) and current speeds.

There is a small fluctuation in mean salinity level from 12.6 to 13.5ppt, which increases from west to east across the Caspian Sea (Ibrayev et al. 2010), paralleling decreasing rain events from west to east (Gayoor et al. 2011). This salinity difference presumably does not influence morphology, as most members of this group of benthophiline gobiid fishes

are quite euryhaline, and can readily and rapidly physiologically adjust among a variety of salinity regimes (Karsiotis et al. 2012). Lima-Filho et al. (2012) showed morphological divergence in Atlantic populations of the frillfin goby (*Bathygobius soporator*), with its body height being the most variable feature, reflecting a gradual clinal increase with increased latitude, along with other ecological conditions. This may be related to their increased metabolism and growth in warmer waters, as has been discerned for many fishes (Ries & Perry 1995). Water temperature changes are higher in the Miankaleh locality compared to the other localities because of low substrate slope and water depth (Ibrayev et al. 2010). As deepwater goby has a spawning season period during early June to mid-July; the larvae are under warmer water than the other localities.

The Gobiidae family exhibits great diversity, often exhibiting extensive phenotype variation both among and within species (Nelson 2006). For example, Ghanbarifardi et al. (2014) classified Walton's mudskipper (*Periophthalmus waltoni*) in two separate population groups based on geometric morphometrics, with their morphology reflecting geographic separation between the Persian Gulf and the Gulf of Oman. Physiological parameters, such as salinity and temperature seasonality, were factors that were cited as influencing shape variation differences between the two population groups.

Khalijah Daud et al. (2005) indicated that there were two population groups of Boddart's goggle-eyed goby (*Boleophthalmus boddarti*) which significantly differed in body shape: those in north and those in middle and southern parts of peninsular Malaysia. Physical characteristics of their habitats, including water temperature and currents, were noted as possible factors that might influence these body shape differences. In contrast, no differences were found with their meristic data. Morphological shape data in this species thus appears to be more "plastic", enabling them to more rapidly adapt to habitats.

In the present study, morphological divergence

was discerned among *P. bathybius* population samples that are geographically connected, suggesting that they may display different microhabitat specificities. This could mean that morphological variations among population groups of *P. bathybius* in various geographic regions may arise in response to environmental and habitat differences, regarding to gobiid members are highly benthic with low mobility to move from habitat to habitat. Molecular genetic markers, such as nuclear DNA and microsatellite markers, should be applied to resolve the population genetic component underlying these phenotypic variations among geographic regions.

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مقاله پژوهشی

بررسی صفات شمارشی و ریخت‌شناسی هندسی گاوماهی عمقزی *Ponticola bathybius* (ماهیان استخوانی عالی: گاوماهیان) در آب‌های ایرانی دریای خزر

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چکیده: به منظور مطالعه تغییر صفات ریخت‌شناسی گاوماهی عمقزی میان سه نمونه جمعیتی در طول سواحل جنوبی ایرانی دریای خزر از صفات شمارشی و روش ریخت‌شناسی هندسی بر اساس نقطه‌نشان (لندمارک) بهره گرفته شد. تفاوت‌های ریخت‌شناسی با استفاده از روش ریخت‌شناسی هندسی بر اساس صفحه نازک توری و آنالیزهای چندمتغیره بر روی داده‌های *partial warp* قسمت چپ و پشتی نمونه‌ها بررسی شد. آزمون NPMANOVA تفاوت معنی‌داری را در تغییرات شکلی نمونه جمعیت‌ها نشان داد. همچنین سه نمونه جمعیتی تفاوت معنی‌داری را در صفات شمارشی نشان دادند. آزمون مانتل نشان داد که هیچگونه ارتباطی میان داده‌های ریخت‌شناسی و فاصله جغرافیایی وجود ندارد، که این سطوحی از ناهمگونی در ویژگی‌های زیستگاه را در ایجاد واگرایی ریخت‌شناسی پیشنهاد می‌کند.

کلمات کلیدی: سوفماهی شکلان، تفاوت‌های ریخت‌شناسی، آنالیز شکلی، لندمارک، صفات شمارشی.