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Research Article

Morphological flexibility of *Oxynoemacheilus seyhanensis* in different habitats of Turkish inland waters: A case of error in describing a populations as distinct species

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Abstract: This study was conducted to investigate the morphological variation between seven populations of *Oxynoemacheilus seyhanensis* inhabiting three basins of Turkish inland waters. For this purpose, a total of 71 specimens were collected from Yildizeli, Taşköprü, Suşehri rivers (Kizilirmak basin), Büyükpotuklu, Pinarbaşi and Örenşehir rivers (Seyhan basin) and Akdağmadeni River (Yesilirmak basin). A total of 31 morphometric characteristics were measured. After standardization, data were analyzed using one-way ANOVA and Duncan, Kruskal-Wallis tests, principal component analysis (PCA), canonical variate analysis (CVA), non-parametric MANOVA and cluster analysis. The results showed significant differences in 26 traits between the studied populations of *O. seyhanensis* (P<0.05) revealing a high morphological flexibility of this deep-bodied species.

Keywords: Deep-bodied loach, Turkish inland waters, Kizilirmak basin, Morphometric.

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Introduction

Members of the family Nemacheilidae are small fishes inhabiting freshwaters of Asia, Europe, and northeast Africa (Çiçek et al. 2015, 2018a, b; Nelson et al. 2016; Sungur et al. 2017). With 51 species, nemacheilids have a great diversity in Turkish inland waters, which 34 of them are endemics (Çiçek et al. 2015, 2018b, 2020; Sungur et al. 2017; Kaya el. 2020; Yoğurtçuoğlu et al. 2020). Despite their high diversity and endemism in Turkey, knowledge about their morphological variation is limited; therefore, study of their phenotypic plasticity can help better understanding of their biological and ecological aspects.

Noemacheilus tigris seyhanensis was described by Banarescu (1968) from Zamanti River, Seyhan Basin, Turkey. Later, it was elevated to the species level as *Oxynoemacheilus seyhanensis* (Çiçek et al. 2015). Erk'akan & Kuru (1986) described *Orthrias angorae kosswigi* from the Yildiz Stream, Sivas Province, Kizilirmak Basin. This species also elevated to the species level as *O. kosswigi*, distributing in the Kizilirmak and Yeşilirmak basins. (Çiçek et al. 2015). Sungur (2020) treated *O. kosswigi* as junior synonym of *O. seyhanensis* based on comparing morphological and molecular data of specimens from their type localities.

Morphological characters are widely used to delimit fish populations to better management of their stocks (Turan 1999), to determine impact of environmental parameters of their habitats, inter and intra-species diversity, and ontogeny (Garrod &

Table 1. Morphometric characteristics of the studied populations of *Oxynoemacheilus seyhanensis*.

	Characters		Characters
1	Standard length (mm)	17	Caudal-fin length
2	Body depth maximal	18	Body width
3	Caudal peduncle depth	19	Caudal peduncle width maximum
4	Predorsal length	20	Head length
5	Postdorsal length	21	Snout length
6	Prepelvic length	22	Eye horizontal diameter
7	Preanal length	23	Postorbital distance
8	Caudal peduncle length	24	Head depth at nape
9	Dorsal-fin base length	25	Head width
10	Dorsal-fin depth	26	Inter Orbital
11	Anal-fin base length	27	Inter nasal
12	Anal-fin depth	28	Mouth width
13	Pectoral-fin length	29	Inner rostral barbel
14	Pelvic-fin length	30	Outer rostral barbel
15	Pectoral–pelvic-fin origin distance	31	Maxillary barbel
16	Pelvic–anal-fin origin distance		

Harwood 1984; Haddon & Wills 1995). In addition, study of morphological variations of fishes can indicate morphological flexibility, regional adaptations, and changes in ecological characteristics (Swain & Foote 1999). Based on the above-mentioned background, this study aimed to investigate the morphometric variations between seven populations of *O. seyhanensis* inhabiting Kizilirmak, Yeşilirmak and Seyhan basins. The results will help better understanding of morphological flexibility of this species to adapt different habitats that have been led to describing its different population as a distinct species.

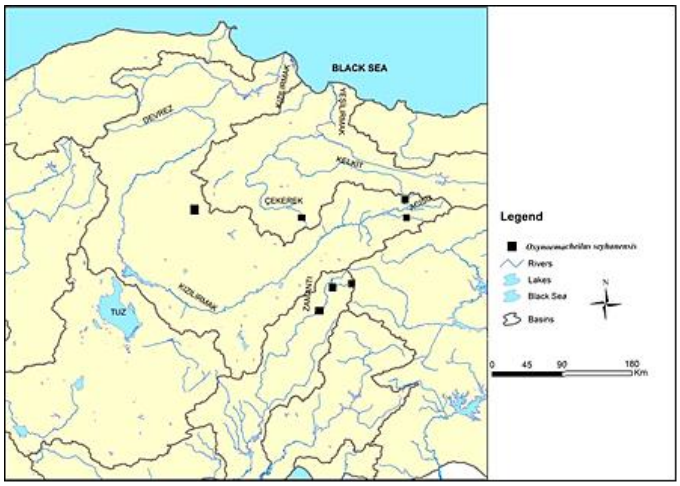

Fig.1. Distribution map for *Oxynoemacheilus seyhanensis in* Kizilirmak, Yesilirmak and Seyhan basins.

Materials and Methods

During 2019, a total of 71 specimens, including 9 from Yildizeli Stream, 15 from Taşköprü River, 10 from Suşehri River (Kizilirmak basin), 8 from Büyükpotuklu River, 10 from Pinarbaşi Stream and 9 from Örenşehir River (Seyhan basin) and 10 Akdağmadeni River (Yeşilirmak Basin) were collected using electrofishing device or seine nets (Figs. 1 and 2). After anesthesia, the specimens were fixed into 5% buffered formalin, transferred to the lab and 31 morphometric characteristics were measured using caliper with accuracy of 0.01 mm (Table 1) based on Armbruster (2012).

To remove size-dependent variations of the morphometric data, those of body and head characters were divided on standard (SL) and head (HL) lengths, respectively. The derived results were confirmed by testing significance of the correlation between transformed variables and standard length (Turan 1999). Then data were analyzed for normality distribution using the Kolmogorov-Smirnov. Parametric and non-parametric data were analyzed using the ANOVA + Duncan and Kruskal-Wallis tests, respectively in SPSS software (version 22). The significant morphometric data of the studied populations were compared using the principal component analysis (PCA) with P-value derived

Table 2. Mean±SD of the morphometric traits, and the results of one-way ANOVA analysis and Duncan test of the studied *Oxynoemacheilus seyhanensis* populations.

Characters	Yildizeli	Örenşehir	Büyükpotuklu	Akdağmadeni	Taşköprü	Suşehri	Pinarbaşi	*P*
Body depth maximal	19.54±1.11^{ab}	21.91±0.81^{c}	20.33±1.17^{b}	20.9±2.12^{bc}	20.42±1.40^{bc}	18.27±1.06^{a}	20.07±1.44^{b}	0.000
Predorsal length	51.25±0.99^{ab}	52.07±1.16^{b}	52.43±1.33^{b}	49.6±2.21^{a}	49.77±1.17^{b}	50.26±1.17^{a}	50.82±2.13^{ab}	0.004
Postdorsal length	35.13±1.47^{ab}	34.35±2.45^{a}	36.06±1.05^{ab}	35.8±3.23^{ab}	36.89±1.71^{b}	36.59±1.14^{b}	37.03±1.45^{b}	0.079
Prepelvic length	53.69±1.05^{a}	54.82±2.78^{a}	53.76±1.39^{a}	51.46±5.01^{a}	51.97±4.89^{a}	52.60±2.75^{a}	53.38±2.23^{a}	0.539
Preanal length	75.84±1.15^{b}	76.11±1.61^{b}	74.49±1.51^{ab}	74.44±1.49^{ab}	71.18±5.51^{a}	72.76±2.54^{ab}	74.20±1.42^{ab}	0.011
Dorsal-fin base length	14.45±0.8^{a}	15.94±1.08^{bc}	16.53±1.79^{c}	16.49±1.21^{c}	16.41±0.88^{c}	15.00±0.84^{ab}	15.77±1.5^{bc}	0.001
Dorsal-fin depth	20.31±0.72^{bc}	19.49±1.52^{b}	21.21±1.55^{c}	18.99±0.82^{ab}	18.89±1.34^{ab}	17.83±0.84^{a}	19.97±1.9^{b}c	0.000
Anal-fin depth	16.47±1.41^{bc}	14.73±1.36^{a}	17.22±1.9^{c}	14.96±1.35^{ab}	15.02±1.08^{ab}	14.27±1.36^{a}	15.50±2.35^{ab}	0.009
Pectoral-fin length	19.58±0.83^{ab}	17.87±1.18^{a}	20.61±1.28^{b}	17.83±2.68^{a}	18.31±1.38^{a}	18.38±1.02^{a}	19.07±2.17^{ab}	0.038
Pelvic-fin length	15.00±0.6^{b}	15.19±1.06^{b}	15.41±1.19^{b}	13.82±0.45^{a}	13.83±0.9^{a}	13.93±0.98^{a}	14.68±1.36^{ab}	0.001
Pectoral – pelvic-fin origin distance	27.03±2.35^{a}	29.95±2.07^{bc}	29.94±1.82^{bc}	28.52±1.55^{ab}	29.63±2.25^{b}	31.88±1.39^{c}	29.75±2.27^{bc}	0.000
Pelvic – anal-fin origin distance	20.80±1.05^{a}	21.26±3.97^{a}	20.52±1.37^{a}	21.62±1.37^{a}	21.4±1.5^{a}	22.16±1.07^{a}	21.52±2.02^{a}	0.620
Caudal-fin length	19.21±1.04^{b}	16.95±2.35^{a}	17.63±0.76^{ab}	16.89±1.59^{a}	16.75±1. 9^{a}	18.18±1.82^{ab}	18.26±1.17^{b}	0.001
Body width	14.66±2.18^{ab}	16.44±1.47^{c}	16.12±0.56^{bc}	16.14±1.04^{bc}	15.35±1.89^{abc}	14.27±0.82^{a}	15.57±1.12^{abc}	0.04
Caudal peduncle width maximum	6.64±0.22^{bc}	5.35±0.57^{a}	7.31±1.53^{c}	6.47±0.94^{abc}	5.75±1.9^{ab}	6.34±1.06^{abc}	6.5±1.56^{abc}	0.045
Inner rostral barbel	21.67±5.51^{a}	28.67±2.79^{b}	26.47±3.78^{ab}	28.14±4.19^{b}	27.52±6.77^{b}	24.67±2.5^{ab}	26.96±3.63^{ab}	0.052
Outer rostral barbel	25.98±6.65^{a}	36.19±2.15^{b}	35.05±5.67^{b}	32.07±2.17^{b}	31.82±3.45^{b}	33.40±3.92^{b}	34.83±5.082^{b}	0.000

Table 3. Mean±SD of the morphometric traits, and Kruskal-Walis analysis of the studied *Oxynoemacheilus seyhanensis* populations.

Traits	Yildizeli	Örenşehir	Büyükpotuklu	Akdağmadeni	Taşköprü	Suşehri	Pinarbaşi	*P*
Standard length	66.76±4.59	67.05±6.40	52.76±5.53	66.4±9.42	56.8±10.52	70.22±7.13	51.37±4.53	*
Caudal peduncle depth	13.89±0.68	12.87±0.18	12.68±0.09	14.06±1.63	12.36±0.66	12.87±1.03	11.65±1.26	0.000
Caudal peduncle length	16.64±0.83	18.86±1.33	18.70±1.82	16.87±1.97	19.84±4.93	21.21±5.78	18.3±1.39	0.004
Anal-fin base length	8.50±1.11	10.98±1.54	12.48±0.73	10.77±1.66	10.97±0.89	8.94±1.3	11.42±1.55	0.000
Head length	25.99±0.99	21.92±1.11	22.12±1.54	19.77±0.79	19.05±0.87	19.9±0.63	21.17±1.45	0.000
Snout length	40.01±1.5	50.74±1.74	47.99±3.98	50.3±2.5	49.99±3.51	48.55±3.16	48.46±3.85	0.000
Eye horizontal diameter	16.17±2.05	18.81±1.69	21.91±1.68	20.40±1.59	21.47±3.12	19.71±2.49	21.59±2.01	0.001
Postorbital distance	45.66±2.94	56.81±2.68	58.33±6.61	59.74±6.86	53.7±6.19	55.95±3.63	57.24±4.46	0.000
Head depth at nape	62.1±3.37	79.26±6.34	71.85±6.20	75.81±3.65	76.82±3.73	71.44±2.97	71.63±5.72	0.000
Head width	67.27±2.11	91.34±4.86	81.38±4.37	84.07±7.54	86.79±5.82	76.77±8.76	81.74±4.67	0.000
Inter Orbital	29.86±1.74	52.06±4.71	54.87±3.98	53.71±3.71	51.34±3.6	53.39±7.47	53.43±4.64	0.000
Inter nasal	25.1±2.47	28.69±2.79	30.48±1.96	32.21±3.31	31.72±3.35	30.94±2.00	31.25±2.46	0.000
Mouth width	37.82±1.97	59.43±4.3	51.84±1.94	50.09±3.23	49.77±4.6	47.71±3.11	50.48±3.12	0.000
Maxillary barbel	27.44±5.89	42.44±4.20	34.52±3.66	33.94±4.46	31.4±5.24	31.54±5.52	37.19±4.00	0.001

from NPMANOVA, canonical variate analysis (CVA) and cluster analysis (CA) in PAST V2.1 software.

Results

The morphometric characteristics except the caudal peduncle depth and length, anal-fin base length, head length, snout length, eye horizontal diameter, postorbital distance, head depth at nape, head width, inter orbital, inter nasal, mouth width and maxillary barbel length were parametric (P>0.05). The results of the parametric characteristics showed differences in all traits but the postdorsal and prepelvic lengths, pelvic–anal-fin origin distance and inner rostral barbel (P<0.05, Table 2). Kruskal–Wallis test of the studied populations showed significant differences in all non-parametric traits as well (P<0.05, Table 3).

The significant traits were used for PCA, CVA and cluster analyses. In PCA, only first component with 54.46% of the variance was higher than the Jolliffe cut of line (Jolliffe 2002), and the lengths of inter orbital and maxillary barbel were main discriminative characters. The PCA plot was drawn using two first PCs (PC1=54.46% and PC2 = 9.85%). According to the PCA plot (Fig. 3), the Yildizeli population was separated from others. CVA/ NPMANOVA also showed a separation and significant differences between the studied populations. The Yildizeli, Örenşehir and Pinarbaşi populations are well-separated from each other (Fig.

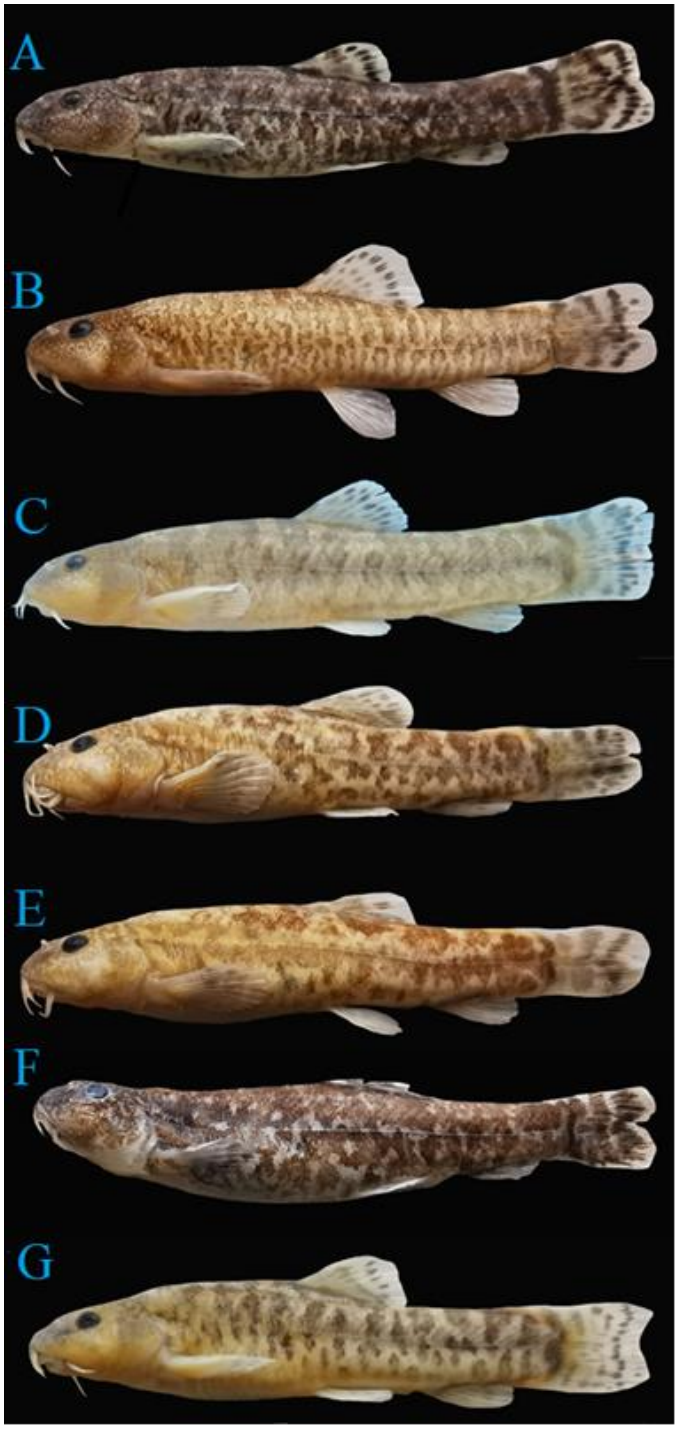

Fig.2. *Oxynoemacheilus seyhanensis*; A: Suşehri, 69.8mm SL; B: Yildizeli, 65.8mm SL; C: Taşköprü, 66.9mm SL; D: Pinarbaşi, 71.5mm SL; E: Örenşehir, 49.2mm SL; F: Büyükpotuklu, 62.6mm SL; G: Akdağmadeni, 78.5 mm SL

4). In the cluster analysis, two major clusters were recognized (Fig. 5), including the Yildizeli population in one clade and others in another clad.

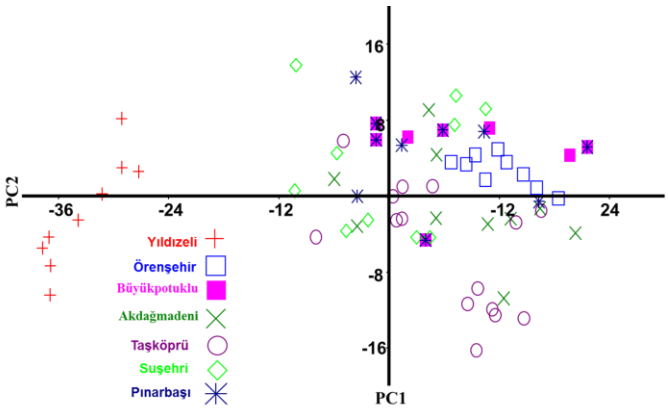

Fig.2. Principal components analysis graph of the morphometric traits of the studied *Oxynoemacheilus seyhanensis* populations.

Discussion

The study of the morphological characters is important to understand the evolutionary biology and diversity of an organisms (Dean et al. 2004). Several works have showed that a species inhabit diverse habitats can have different morphological features. In the present study, the results showed that *O. seyhanensis* populations from different rivers have significant differences in 26 morphometric traits indicating high diversity of their morphological characteristics i.e. they are morphological flexibilities for *O. seyhanensis* populations experienceing inconsistent environments such as rivers (Zamani Faradonbe et al. 2015; Mouludi-Saleh et al. 2019). In a species with a wide distribution area, we expect morphological differences between populations found in different environmental conditions (Marcil et al. 2006). Therefore, morphological differences as seen in the present study are related to the phenotypic plasticity (Jerry & Cairens 1998; Abbasi et al. 2020) of the members of this species.

The PCA and CVA results showed the Yildizeli population (type locality of the described synonym *O. kosswigi*) is separated from others and cluster analysis confirmed these results too. Inter orbital and maxillary barbel lengths were most discriminative characters for *O. seyhanensis* populations. Therefore,

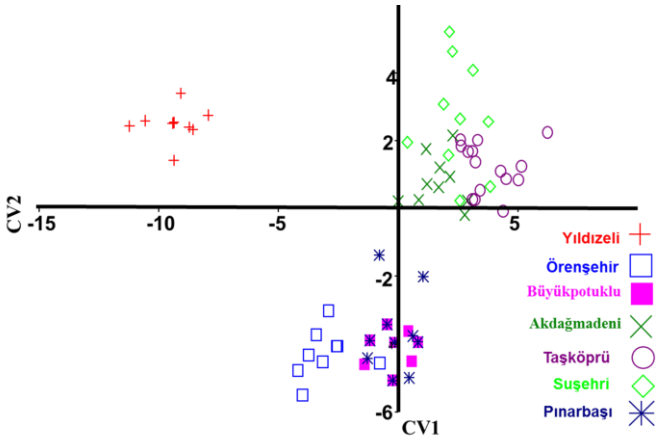

Fig.4. Canonical variate analysis graph of the morphometric traits of the studied *Oxynoemacheilus seyhanensis* populations.

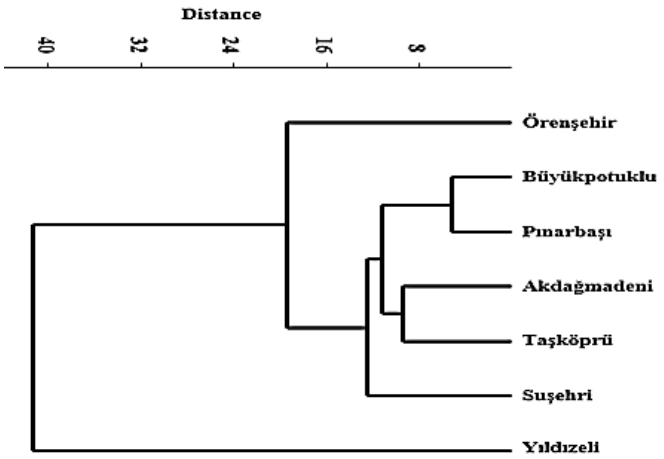

Fig.5. Cluster analysis of the morphometric traits of the studied *Oxynoemacheilus seyhanensis* populations.

these characters cannot be considered reliable for species delimitation in taxonomic studies of this deep-bodied stone loaches, because they can be intraspecific and even different in various ontogenic stages. In addition, as seen in Figure 1, a great diversity is observed in the body colouration of the studied population of the deep-bodied *O. seyhanensis*. Hence, using such characters as well as those of coloration to discriminate the members of this species have been led to erroneously describing treated *O. kosswigi* (Erk'akan & Kuru 1986; Sungur 2020). Therefore, inter orbital, maxillary barbel lengths and colour patterns to discriminate the deep-bodied stone loaches are not taxonomically reliable and are intraspecific in the members of this taxa.

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مقاله پژوهشی

انعطاف‌پذیری ریختی جویبارماهی سیهان *Oxynoemacheilus seyhanensis* در زیستگاه‌های مختلف آب‌های داخلی ترکیه: یک مورد خطا در توصیف یک جمعیت به عنوان یک گونه مجزا

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چکیده: این مطالعه به‌منظور بررسی تنوع ریختی هفت جمعیت از گونه *Oxynoemacheilus seyhanensis* ساکن در سه حوضه از آب‌های داخلی ترکیه انجام شد. برای منظور تعداد ۷۱ قطعه از رودخانه‌های یلدزلی، تاشکوپری، سوشهری (حوضه کیزل‌ایرماک)، بویوک‌پتوکلی، پینارباشی و اروانشیهر (حوضه سیهان) و اکداگمادنی (حوضه یشل‌ایرماک) جمع‌آوری شد. تعداد ۳۱ صفت ریخت‌سنجی اندازه‌گیری شد. بعد از استانداردسازی، داده‌ها با استفاده از آنالیزهای تجزیه واریانس یک‌طرفه و گروه‌بندی دانکن، کروسکال-والیس، تجزیه به مولفه‌های اصلی (PCA)، تجزیه همبستگی کانونی (CVA)، آنالیز MANOVA غیرنرمال و آنالیز خوشه‌ای مورد تحلیل قرار گرفتند. نتایج تفاوت معنی‌داری در ۲۶ صفت بین جمعیت‌های مورد مطالعه گونه *O. seyhanensis* را نشان داد ($P<۰/۰۵$)، که بیانگر انعطاف‌پذیری ریختی بالای این گونه با بدن مرتفع می‌باشد.

کلمات‌کلیدی: جویبارماهی بدن مرتفع، آب‌های داخلی ترکیه، حوضه کیزل ایرماک، ریخت‌سنجی.