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Comparative Survey of Geometric Morphometrics and Traditional Methods in Male and Female of Anjak, *Schizocypris altidorsalis* (Bianco and Banarescu, 1982) from Sistan Basin, Iran

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Abstract

Morphological variation between male and female populations of Anjak, *Schizocypris altidorsalis* species in Sistan basin of Iran was studied using landmark-based geometric morphometric and Traditional morphometric. A total of 27 specimens (16 males and 13 females) were caught from one basin. A total number of 16 landmark-points were defined and digitized on 2D pictures, and 29 morphometric characters were measured. To eliminate non-shape data, including size, direction, and position, the extracted data were analyzed by the generalized procrustes analysis. The data of TM were analyzed by Hoteling t-test, and data of GM were analyzed and compared using multivariate principal component analysis (PCA) which showed male and female populations overlapped. Deformation shape grid and Wireframe based on the consensus body shape, very little separation the two populations based on the head size, body depth, and caudal peduncle length characteristics. Based on TM analysis, these characters could not differentiate between the female and male, except for two characters, length of pectoral fin and maximum body depth ($p < 0.00$). Comparative morphological studies revealed deformation of the grid and wireframe in more details. In the deformation grid, there are differences between the two populations due to folds and twists. Wireframe shows differences better in certain places, such as the head, caudal peduncle, and middle area of the body.

1. Introduction

Iran is located in the southern Palearctic biogeographic region bordering the Oriental and Ethiopian regions and is considered as exchange region for freshwater fishes [5], with 297 species in 109 genera and 30 families [9]. *Schizocypris altidorsalis*, belonging to the Cyprinidae family, is one of the economically valuable fishes. It is a rare native species of the genus *Schizocypris*. It belongs to the subfamily Schizothoracinae, which is found in the southeastern regions of Iran, Central Asia and China, India, Pakistan, and Afghanistan [1]. The Sistan area is located in the southeastern of Iran.

Anjak *(Schizocypris altidorsalis*), is a native benthopelagic and commercially important fish species in Hamun Lake and Hirmand River system in Sistan basin, Iran [11]. Together with snow trout (*Schizothorax zarudnyi*), Anjak make up the most favorite source of animal protein for Sistan residents [11]. As an endemic species in the Sistan region of Iran, *Schizocypris altidorsalis* Bianco and Banarescu, 1982 (Cyprinidae: Schizothoracinae) resides only in the Hamoun Lake, Helmand River, and its tributaries and presumably adjacent Afghanistan [4]. The genus *Schizocypris* of mediumsized snow trouts contains only two species that were found in Pakistan, Afghanistan, and Iran. *S. altidorsalis* is a benthopelagic species that has been reported from pools in dry river beds and still, reedy channels. The fish enter the Hamoun Lake from the upstream parts of the rivers, and return to more permanent rivers when water levels fall [4,12].

For the first time, in this paper, we simultaneously performed traditional morphometric and geometric morphometrics measurements for *S.altidorsalis*. The study of the body shape is a routine method for understanding many aspects of fish biology, such as resource management, evolution, behavior, and ecology [25,15]. For instance, geometric morphometric methods revealed some phenotypic plasticity evidences related to dam construction in *Capoeta gracilis* [13]. Intraspecific morphological variations in fishes are related to the adaptations of organisms to the habitat environmental conditions [2,6]. Hence, morphological studies are among the tools used to identify different fish populations and better understanding of habitat-associated morphological divergence [21]. Geometric morphometrics (GM), a quantitative approach to the analysis of shape, is widely applied to compare and determine shape variations of biological structures [27].

Some authors have implemented the geometric morphometric method (GM) [7, 19, 20, 29, 28, 17, 18, 10]. It has been suggested that GM is more effective in detecting morphological disparities [14, 23, 10]. Additionally, it has been shown that both methods can lead to similar [17] or different results [27, 10]. In the paper by Ganjali et al. 2021, the geometric morphometric method (GM) than the traditional method showed more differences and was more accurate.

2. Materials and Methods

2.1 *Sample collection and preparation*

For this study, 29 fish samples (16 males and 13 females) representing Anjak were obtained from Chahnimeh reservoirs, Sistan, Iran (30°50'21.7500"N, 61°41'39.8004"E) by fishing (Figure 1). The specimens were preserved in 4% buffered formaldehyde after anesthesia in 1% clove oil solution.

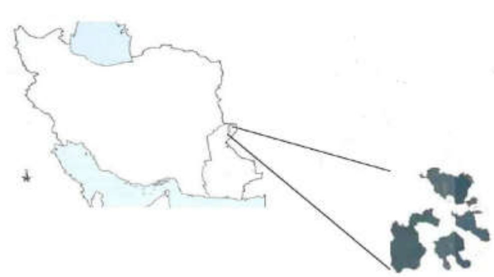

Figure 1. Study area from Chahnimeh reservoirs in Sistan Basin

2.2 *Traditional morphometric (TM)*

29 morphometric characters were measured with a digital caliper (0.05mm accuracy). The elimination of the size effects from the data set was done based on Elliott et al. 1995 using the formula of M adj= M (Ls/Lo)b, where M adj is the size adjusted measurement, M is the original measurement, Ls is overall mean of standard length for all fish from all samples in each analysis, Lo is the total length of fish parameter, and b was estimated for each character from the observed data as the slope of the regression of log M on log Lo, using all fish in all groups. Morphometric characters were analyzed for normality distribution using the Kolmogorov–Smirnov test and T- test in SPSS software version 22.

2.2.1 *Traditional morphometric characters*

Total Length, Fork Length, Standard Length, Head Length, Head depth, Head Width, Pre orbital distance, post orbital distance, Inter orbital distance, Eye diameter, Predorsal distance, Post-dorsal distance, Length of dorsal fin, Depth of dorsal fin, Length of Anal fin, Depth of Anal fin, Length of pectoral fin, Depth of pectoral fin, Length of ventral fin, Depth of ventral fin, Pre anal distance, Post anal distance, Distance between pectoral and ventral fin, Distance between Ventral and Anal fin, Length of Caudal fin, Depth of Caudal fin, Minimum body depth, Maximum body depth, Length of caudal peduncle, Mouth width.

2.3 *Geometric morphometric (GM)*

The purpose of geometric morphometry is to study the process of variability and body shape patterns Geometric morphometrics is suitable for comparing populations because the number of traits we consider is more than the traditional method. The left sides of fish specimens were photographed using a copy stand equipped with a digital camera (Canon G7). To extract data on the body shape, 16 homologous landmark points were defined and digitized using tpsDig2 software (version 2.31) (Figure 2) on 2D pictures.

2.3.1 *Geometric morphometric characters*

The following Geometric morphometric characters were measured (Figure 2): (1) anterior-most point of the snout tip on the upper jaw, (2) the beginning of the eye socket, (3) center of the eye, (4) the end of the eye socket, (5) the dorsal surface of the head is perpendicular to the center of the eye, (6) the end point of the gill operculum, (7) the origin point of the dorsal-fin base, (8) the insertion point of the dorsal-fin base, (9) the end of the caudal peduncle or the beginning of the procrand radius, (10) the middle part of the most end of the caudal peduncle, (11) the lower part of the most recessed of the caudal peduncle, (12) the origin point of the anal-fin base, (13) the insertion point of the anal- fin base, (14) most anterior point of the pectoral fin, (15) the most ventral point of the gill slit and (16) ventral surface of the head perpendicular to the center of the eye.

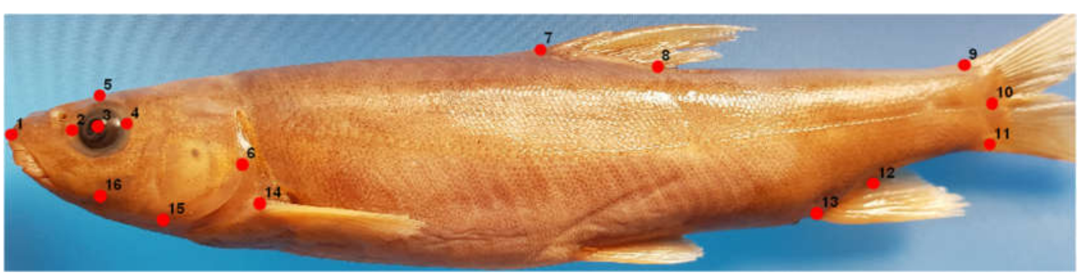

Figure 2. Defined landmark points to extract the body shape data of *S. altidorsalis*

Then, to remove non-shape variations (location, orientation and scale) from the landmark configurations, they were superimposed by GPA (General Procrustes Analysis) [25]. Finally, the principle component analysis (PCA) was also conducted.

3. Results and Discussion

3.1 *Traditional morphometric analysis*

Evaluate results and comparison of Traditional morphometric 29 specimens of Anjak (*Schizocypris altidorsalis*) shown in Table 1. In this study, number 13 female and 16 male numbers. In length of pectoral fin and maximum body depth p <0.00.

Table1. Statistical mean and results of T-test measuring traits of male and female Anjak, *S. altidorsalis* (Bianco and Banarescu, 1982) from Sistan Basin, Iran

Characters (mm)	Male(n=16)	Female(n=13)	P value
SL	125.53	125.53	-
HL	29.26±1.69	28.45±1.10	0.147
HD	14.93±2.74	16.89±7.52	0.342
Hwidth	12.55±0.81	1287±0.53	0.232
Pre orbit Dis	10.19±0.98	9.93±0.99	0.485
IntOD	9.85±0.52	9.94±0.48	0.636
Post Orbit D	14.57±1.22	13.74±1.04	0.636
EyeDiam	5.32±0.54	5.44±0.68	0.582
Pre DD	63.92±1.49	64.20±5.67	0.853
PosDD	76.52±5.21	77.75±3.38	0.47
LDfin	16.81±1.47	17.58±2.13	0.263
DDfin	30.05±1.36	30.02±2.46	0.963
LAfin	10.47±2.28	9.83±1.98	0.435
DAfin	24.05±3.14	24.05±2.84	0.998
LPecfin	19.51±1.87	20.86±0.90	0.026*
DPecfin	5.01±0.70	4.76±0.47	0.284
LVentfin	16.69±1.70	16.30±1.57	0.528
Dventfin	4.66±0.38	4.77±0.64	0.597
PreAD	97.06±2.43	97.09±2.86	0.969
PosAD	60.86±4.33	60.43±2.56	0.754
Dis P&V	39.49±1.85	39.73±2.71	0.781
Dis V&A	32.69±1.63	32.69±2.12	0.992
LcaudF	29.62±3.89	30.27±1.97	0.587
MaxBD	26.46±2.67	28.85±2.08	0.014*
MinBD	10.22±0.52	10.36±0.95	0.6
LCaudPed	15.35±2.07	15.36±1.55	0.989
MWidth	8.07±0.69	7.92±0.90	0.613

3.2 *Geometric morphometric analysis*

Based on the results, the first four components of PCA being above the Jolliffe cutoff line (PC1= 40. 9, PC2=23.81, PC3=9.66, PC4=8.63) Figure 3, were selected as the main factors separating female and male populations. As presented in Figure 4, the populations are grouped along the first two axes and the studied populations presented no in size but also overlapped. In deformation grid (Figure 5) and wire frame (Figure 6) changes are seen.

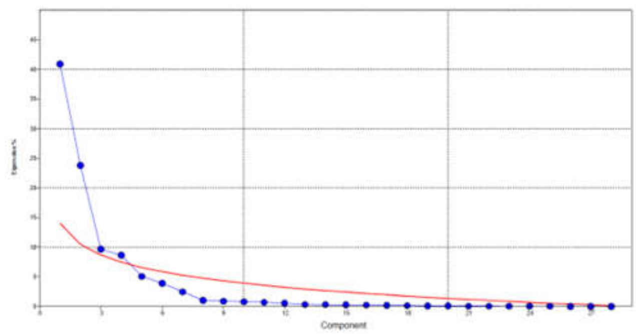

Figure 3. The screen plot diagram of PCA and the Jollief cutoff point (red line), showing the main (4) significant components Jolliffe line

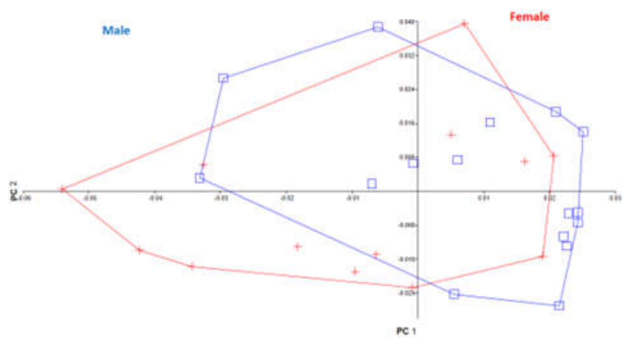

Figure 4. Principal component analysis diagram of male and female Anjak, *S. altidorsalis* in the Sistan Basin

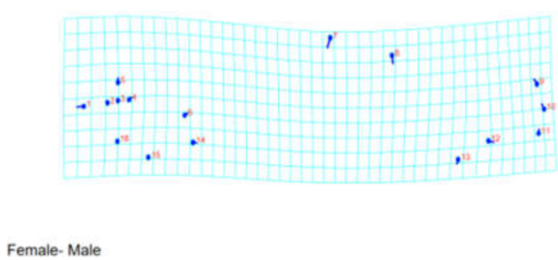

Figure 5. Comparison of the average body shape of male and female Anjak, *S. altidorsalis* in Sistan Basin using the deformation Grid network.

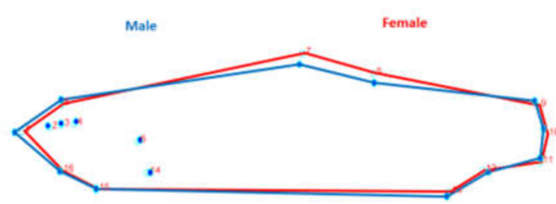

Figure 6. Comparison of the average body shape of male and female Anjak, *S. altidorsalis* in Sistan Basin using the deformation Wireframe network (Female: Red, Male: Blue)

Occurrence of prolonged droughts and Sistan region diminished spawning fish and uncontrolled fishing in this area Anjak the fear that goes with this species of value and that regional endemic economy fish disposable component of is considered at risk should be destroyed. This paper performed traditional morphometric and 2D landmark based geometric methodologies were used for the discrimination of one population of Anjak, *S. altidorsalis* collected from Sistan basin of Iran.

3.3 *Traditional morphometrics*

Based on TM analysis, these characters could not differentiate between female and male populations. Table 1 shows that the results were not significantly different p Value > 0 except two characters (length pectoral fin=0.026 and maximum body depth=0.014). It has been suggested that the morphological characteristics of fish are determined by an interaction between genetic and environmental factors [25-15].

3.4 *Geometric morphometrics*

Based on the results (deformation grid and wireframe) of male and female populations, the head, caudal peduncle, depth of body and tail (Figures 5 & 6) were more affected parts in terms of morphological changes, indicating that this species uses these areas to move and adapt in different environments. The PCA analysis along the first two axes (PC1 and PC2) of geometric-based data for the *S. altidorsalis* population showed that (Figure 4) overlapped. Therefore, due to the overlap, there is no difference based on this test (PCA) between the two populations of Anjak males and females.

4. Conclusion

Comparative morphological studies (TM & GM) revealed deformation grid and wireframe with more details. In the deformation grid, there are differences between the two populations due to folds and twists. Wireframe shows differences better in certain places, such as the head, caudal peduncle, and middle area of the body. In general, the geometric results are more accurate and better than Traditional morphometric. I hope that the drought problem will be solved in the future so that we can study more populations for phenotypic adaptation.

Conflicts of Interest

The author declares no conflict of interest associated with this manuscript.

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