The Effect of Body Shape Type on Differentiability of Traditional and Geometric Morphometric Methods: A Case Study of *Channa gachua* (Hamilton, 1822)

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ABSTRACT

Objective: The morphological differences of two populations of the Dwarf snakehead, *Channa gachua* (Hamilton, 1822) from Sarbaz (Makran basin) and Halil (Hamun-e Jaz Murian basin) rivers were studied using geometric and traditional morphometrics (GM and TM) methods to test the hypothesis that the type of body shape can produce different results.

Materials and Methods: A total of 16 landmark-points and 12 distance measurements were defined to analyse the body shape differences and the extracted data were analyzed using GM and TM methods.

Results: Our findings reject the hypothesis, and the results revealed that GM is more effective in detecting meticulous morphological differences.

Conclusion: In addition, the results suggest selecting a proper method i.e. GM or TM, based on the degree of accuracy needed i.e. if we need to find small shape differences within its signification, GM is a superior technique, or to show the type of differences, then we can use TM.

Keywords: Freshwater Fish, Morphology, Phenotypic Plasticity, Generalized Procrustes Analysis

INTRODUCTION

Comparative studies on the body shape in fishes are commonly used to understand many aspects such as resource management, evolution, behaviour, ecology and phenotype plasticity (1-6). In many works, which have investigated the morphological variation of the biological structures using traditional (TM) (7-9) and geometric morphometric (GM) (10-17) techniques, it has been suggested that GM is more effective to detect morphological disparities (4, 8). Additionally, it have been shown that both methods can lead to similar (12) or different results (9).

It bring to us this hypothesis that such differences may be related to the body shape type of the studied organisms. For instance, if a fish's body shape is simple, then we can expect similar results of both TM and GM methods; and if the body shape is complex, then we can expect a different result. Therefore, this study was conducted to compare the body shape of the dwarf snakehead, *Channa gachua*, using TM and GM methods.

Channa gachua is a species with large head scales and elongate dorsal and anal fins, and with the ability to breathe from air inhabiting tropical and sub-tropical water bodies (18). It is a fish species with a bottom rover

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Figure 1. Defined landmark-points to extract the body shape data of *Channa gachua*. (1) anterior-most point of the snout tip on the upper jaw, (2) center of the eye, (3) dorsal edge of the head perpendicular to the center of eye, (4) origin and (5) insertion point of the dorsal-fin base, (6) postero-dorsal end of the caudal peduncle at its connection to the caudal fin, (7) posterior end of the medial region of the caudal peduncle, (8) postero-ventral end of the caudal peduncle at its connection to the caudal fin, (9) insertion and (10) origin point of the anal-fin base, (11) most anterior point of the pectoral fin, (12) posterior edge of the opercle, (13) ventral end of the gill slit, (14) the line extends perpendicularly to the most basic part of the gill slit above the head, (15) The terminus of the oral clefts in the upper jaw and (16) ventral edge of the head perpendicular to the center of eye.

body shape pattern i.e. a simple body shape that expects the same results of both TM and GM methods. The biggest population of this species in the western Asia, is found in the Makran, Mashkid and Hamun-e Jaz Murian basins of Iran (18).

MATERIALS AND METHODS

In total, 40 specimens of *C. gachua* representing two different populations were collected from the Halil (n= 17, 28°40'29.43"N, 57°42'22.96"E, Hamun-e Jaz Murian basin) and Sarbaz (n= 23, 26°37'47"N, 61°15'31"E, Makran basin) rivers using an electrofishing device (Samus MP750). After anesthesia, the left sides of the fresh collected specimens were photographed using a copy-stand equipped with a digital camera (Kodak EasyShare Z650 with a 6 MP resolution) with fins erected by insect pins. Then, the sampled specimens were fixed in the buffered formalin and transferred to a laboratory.

For the GM method, a total of 16 homologous landmark-points were digitized using tpsDig2 software (version 2.16) (Figure 1) on 2D pictures. A Generalized Procrustes analysis (GPA) was used to remove non-shape data, including size, position and direction. The resulting data were analyzed using discriminant function analysis (DFA) and Hotelling's T-test to investigate the morphological distinction between the populations. Morphological disparities between the populations were visualized using deformation grids in MorphoJ software.

For the TM method, 12 distance measurements, including SL (standard length), SnL (snout length), ED (eye horizontal diameter), HL (head length), HW (maximum head width), HDO (head depth at posterior edge of the opercle), HDE (head depth at middle of the eye), BD (body depth at dorsal-fin origin), DFL (dorsal-fin length), AFL (anal-fin length) and CPL (caudal peduncle length) were taken using dial calipers to the nearest 0.1 mm. To remove the size from data, they were standardized using the Beacham formula as following (19):

$$M_{(t)} = M_{(0)} \left(\frac{L}{L_{(0)}} \right)^b$$

Where $M_{(t)}$ is standardized values of characters, $M_{(0)}$ = characters of the observed length L = standard length mean of all samples, $L_{(0)}$ = a standard length of each sample and b = regression coefficient between $\log L_{(0)}$ and $\log M_{(0)}$.

Analyses for GM method were performed using PAST and MorphoJ (version 1.01) softwares. Levin's test and DFA/ Hotelling's T-test in TM method were performed in SPSS V.19 and PAST software, respectively.

RESULTS AND DISCUSSION

Geometric Method

DFA and Hotelling's T-test showed that the two populations can be significantly differentiated in terms of the body shape ($P > 0.0001$) (Figure 2). The wireframe of the body shape showed differences in position of the snout and caudal peduncle length. In the Halil River population, the body was deeper and the eyes

Figure 2. Discriminant function analysis of *Channa gachua* populations from the Halil and Sarbaz rivers in GM method.

Table 1. Levin's test and mean ($\pm$ standard deviation) of each morphological character measured in *Channa gachua* from Sarbaz and Halil rivers populations (All measurements are shown in mm. Variables for which significant differences were obtained are highlighted in bold).

Characters	Sarbaz River (mean $\pm$ SD)	Halil River (mean $\pm$ SD)	F	P-value
SL	98.48 $\pm$ 0.00	98.48 $\pm$ 0.00	-	-
SnL	4.28 $\pm$ 0.94	4.48 $\pm$ 1.19	7.37	0.01
ED	4.4 $\pm$ 0.43	4.29 $\pm$ 0.98	12.92	0.001
HL	29.98 $\pm$ 2.03	30.1 $\pm$ 2.47	2.86	0.98
HW	21.09 $\pm$ 0.83	20.96 $\pm$ 0.99	1.61	0.212
HDO	19.14 $\pm$ 1.02	18.93 $\pm$ 1.1	0.401	0.53
HDE	10.05 $\pm$ 0.84	10.12 $\pm$ 0.91	1.31	0.294
BH	19.81 $\pm$ 1.27	19.49 $\pm$ 1.20	0.085	0.772
DFL	53.12 $\pm$ 1.38	53.04 $\pm$ 1.3	0.074	0.788
AFL	33.81 $\pm$ 1.79	33.24 $\pm$ 1.19	1.93	0.177
CPL	8.5 $\pm$ 0.90	8.3 $\pm$ 0.87	0.029	0.885

AFL = anal-fin length, BD = body depth at dorsal-fin origin, CPL = caudal peduncle length, DFL = dorsal-fin length, ED = eye horizontal diameter, HDE = head depth at middle of the eye, HDO = head depth at posterior edge of the opercle, HL = head length, HW = maximum head width, SnL = snout length, and SL = standard length.

and snout had a more ventral position than those of the Sarbaz River one (Figure 3).

Figure 3. Wireframe diagram consensus body shape graph of *Channa gachua* populations from the Halil and Sarbaz rivers in GM method.

Traditional Method

The results showed normality of the data. Levene's test showed significant differences in the snout length and eye diameter (Table 1) ($P < 0.05$). DFA/Hotelling's T-test showed no significant difference between the two studied populations ($P = 0.975$, $F = 0.334$, Hotelling's $t^2 = 5.64$) (Figure 4).

Figure 4. Discriminant function analysis of *Channa gachua* populations from the Halil and Sarbaz rivers in TM method.

Morphological variations in fishes are considered to be an important adaptive strategy for populations experiencing inconsistent environments such as rivers (20). In addition, fishes show a variety of body form associated with functions such as swimming and feeding to overcome different habitats and lifestyles (10, 21). The body shape can even display the lifestyles of a fish. Therefore, based on the diversity of the habitat features or lifestyles, the degree of a biological structure can show a higher plasticity (22), e.g. those fishes with a generalized body shape, are fusiform with a pointed head and forked tail. Therefore, fusiform body designs have a lot of variations in different parts of their body shape.

In the present study, the multivariate analysis did not show a significant difference in TM. However in TM, comparing each morphometric data revealed differences in SnL and ED similar to the results of the GM method. Our findings reject the hypothesis that differences may be related to the body shape type using GM or TM.

However, it is suggested to select a proper method viz GM or TM, prior to the analysis of the data based on the aim of the study and particularly degree of accuracy needed i.e. if we need to find small shape differences within its signification, then we can apply GM, or if our aim is to find traits which show differences, then we can use TM as well. Both methods reveal real differences but GM better signifies difference due to its higher detection ability.

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