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Original Article

Body shape comparison of *Cyprinion macrostomum* (Heckel, 1843) and *Cyprinion watsoni* (Day, 1872) using geometric morphometrics method

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Abstract: This study investigated shape differences in two species of *Cyprinion macrostomum* from Tigris basin and *Cyprinion watsoni* from Hormuz basin using discriminate function analysis. Coordinates of 17 external landmark points on 2D pictures were used for the analysis. There were significant differences of the two species. *C. macrostomum* have longer head length, snout length, preventral distance, head height, body height and length of pectoral fin bases than those of *C. watsoni*. The caudal peduncle length, caudal peduncle depth and anal fin base length in *C. watsoni* are longer than those of *C. macrostomum*, the pectoral fin in *C. macrostomum* was originated more posteriorly than that *C. watsoni*. Based on the geometric morphometrics differences, the two species can be well recognized and differentiated.

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Introduction

Species identification is the basic component of biodiversity conservation and fisheries management (Ibañez et al., 2007). Many biological aspects of an organism such as feeding efficiency, locomotion performance, vulnerability to predators, and reproductive success can be studied using body shape analysis (Guill et al., 2003). Fishes can adopt to the environment conditions in various ways to enhance their viability (Nacua et al., 2010). Hence, quantifying phenotypic differences among species may help to understand its natural history across a species' geographic range, which have implications for both theoretical and applied works in ecological and fishery science.

The traditional morphometrics is time consuming having lots of errors and low accuracy. Genetic methods are costly and not readily available in the field (e.g., Hutchinson et al., 2001; Keyvanshokooh and Kalbassi, 2006; Ghasemi et al., 2007). In geometric morphometrics, data is obtained from the coordinates of landmark-points (Rohlf and Marcus, 1993; Adams et al., 2004), which are morphological meaningful points of specimens (Richtsmeier et al., 2002). Geometric morphometrics techniques have been used in many aspects of ichthyology including identification of fishery stocks (Cadrin, 2000; Mohadasi et al., 2013), studying the body shape variation within and between fish populations (Nacua et al., 2010; Heidari et al., 2013), analysis of head shape variation (Cavalcanti, 2004), scale shape analysis to identifying species, genera, and local populations (Ibañez et al., 2007), and body shape variation due to rearing temperature (Sfakianakis et al., 2011).

Five species of the genus *Cyprinion* have been reported from Iran and there are some complexity in their taxonomy (Bianco and Banarescu, 1982; Howes, 1982; Banarescu and Herzig-Straschil, 1995; Abdoli, 2000; Coad, 2013). Some works were

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Table 1. The geographical information of sampling sites.

Species	Number	River	Basin	Province/Town	Latitude	Longitude
Cyprinion macrostomum	24	Kashkan	Tigris	Lorestan, Pole-Dokhtar	33°09'28"N	47°42'50"E
Cyprinion watsoni	40	Goodar	Hormuz	Hormuzgan, Bastak	27°19'27"N	54°27'46"E

performed on the morphological and biological aspects of this genus in Iran, Turkey, Iraq and Pakistan (Kafuku, 1969; Bianco and Banarescu, 1982; Banarescu and Herzig-Straschil, 1995; Yilmaz et al., 2005; Patimar and Nasri, 2007; Nasri, 2008; Nasri et al., 2008; Yüksel and Gaffaroğlu, 2008) but all of them are based on traditional or descriptive methods. Meanwhile, all members of the genus *Cyprinion* in Iran have relatively similar appearance (i.e. body shape) requiring subtle meristic data to distinguish them. Even within these characters, there is not a considerable degree of variation. Geometric morphometrics methods has not been used to assess the body shape variation among the genus *Cyprinion*, therefore, this study was conducted to compare the body shape of two confirmed *Cyprinion* species i.e. *C. watsoni* and *C. macrostomum* with visualization techniques afforded by the geometric morphometrics. The results of the present study can help to find morphological distinctions that may also be used to differentiate these two closely related species and better understanding of body shape pattern among the members of the genus *Cyprinion*.

Materials and methods

In total 64 specimens including, 24 *C. macrostomum* from the Kashkan River (Tigris basin) and 40 *C. watsoni* from the Goodar River (Hormuz basin) were collected using electrofishing (Table 1). The specimens were fixed into 4% buffered formaldehid after anesthetizing in 1gL^{-1} clove solution and were transported to the laboratory for further examinations.

In the laboratory, the specimens were identified using the mouth form, dorsal fin ray characters and the number of gill rakers according to Abdoli (2000) and Coad (2013). Then, the left side of each specimen were photographed using digital Kodak (6 mega pixels) camera. Seventeen landmark-points were defined and digitized on 2D images using the tpsDig2 software version 2.16 (Rohlf, 2010) (Fig. 1). The adequacy of tangent shape for statistical analysis where investigated using the tpsSmall (Rohlf, 2003). The non-shape information were removed from landmark configurations using General Procrustes Analysis (GPA), the covariance matrices were generated and the shape differences between the two species analyzed using discriminant function Analysis (DFA) in MorphoJ 1.02j (Klingenberg, 2011). The patterns of body shape differences were illustrated in the wireframe in relation to each other for the quantification and visualization purposes.

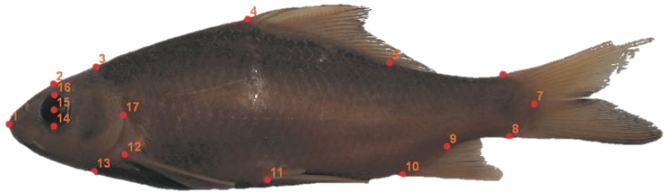

Figure 1. Seventeen defined landmarks on the left side of specimens. 1: the anterior-most point on the head; 2:the margin of head at the vertical nearest distance to the upper margin of the Orbital; 3:the junction of the head and trunk; 4: the front edge of dorsal fin base; 5:the posterior edge of dorsal fin base; 6: the upper edge of caudal fin base; 7: the most distant point of lateral line at the base of caudal fin; 8: the lower edge of caudal fin base; 9: the posterior edge of anal fin base; 10: the front edge of anal fin base; 11: the lateral edge of pelvic fin base; 12: the outer edge of pectoral fin base; 13: the lower corner of opercular opening; 14: the lower margin of orbital; 15: the center of orbital; 16: the upper margin of orbital; 17: the end of the head.

Results

The sum of digitization and orientation errors was 15% and the correlation between procrustes and tangent distances was 1, therefor tangent space approximation could be used for statistical analysis. The two species where separated based on discriminate function analysis with Mahalanobis distance 8.3239 and P-value <0.0001 (Fig. 2). Depicting the differences in body shape between

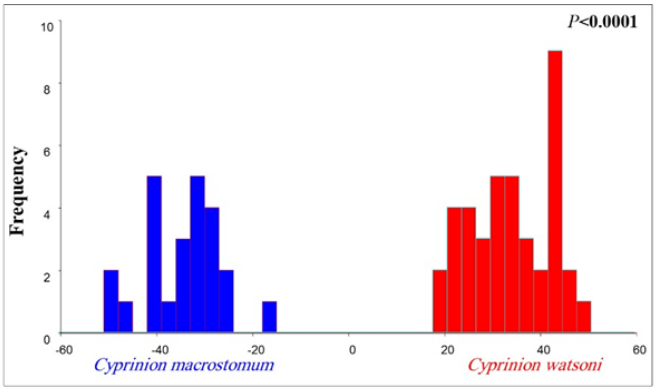

Figure 2. The histogram of discriminate analysis separating the two species based on geometrics.

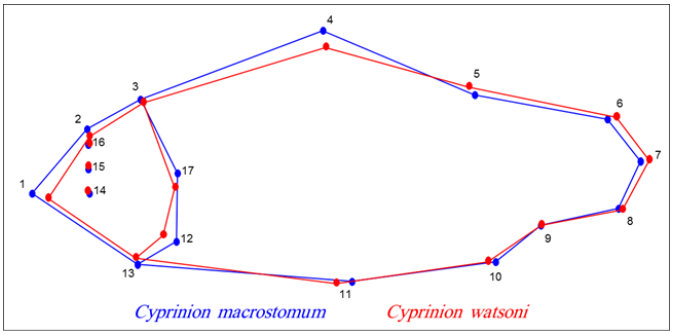

Figure 3. Visualization of the relative shape differences among species based on wireframes.

consensus body shapes of two species is presented in Figure 3. Discriminate function analysis indicated that *C. macrostomum* have longer head length, snout length, preventral distance, head height, body height and length of pectoral fin base to those of *C. watsoni*. The caudal peduncle length, depth of caudal peduncle and anal fin base length in *C. watsoni* are longer than those of *C. macrostomum*. The pectoral fin in *C. macrostomum* was originated more posteriorly than that of *C. watsoni* (Figs. 3 and 4).

Discussion

The results of this study showed that the body shape of *Cyprinion macrostomum* and *Cyprinion watsoni* are significantly different. This result was in agreement with previous works that used classical morphology of the genus *Cyprinion* (e.g, Banarescu and Herzig-Straschil, 1995; Abdoli, 2000; Nasri, 2008; Nasri et al., 2008a; Nasri et al., 2008b; Coad, 2013). The observed differences can be divided in two categories. First a higher head height of *C. macrostomum* and, second, a deeper body and longer dorsal fin base in *C. macrostomum*, plus a longer caudal length in *C. watsoni*. Whereas, based on previous findings, these two species could identified using the shape of mouth and dorsal fin ray that are more plastic characters.

Some authors considered the shape of species as a result of the environments and genetics (Costa and Cataudella, 2007; Costa et al., 2010). Environmental factors influence the shape of organisms via natural selection (Chan, 2001). The Kashkan River is a relatively large river with high productivity providing a lot of food resource, but the Goodar River is a small stream with low productivity that is sometimes dried in summer. Hence, some morphological differences between these two species may be related to phenotypic plasticity and responses to environmental conditions and depict different habitats. However, further studies are required to explain differences with different localities and all five species being included.

The key characters to distinguish these two species are the strength and serration of the last unbranched dorsal fin ray and branched dorsal fin rays in *C. macrostomum* (Coad, 2013). Fishes like *C. macrostomum* living in high current rivers need more efforts for survival. There is no previous comparative study on body shape among *Cyprinion* but Coad (2013) noted that the dorsal fin in

Figure 4. Visual differences between the two species (Left: *C. macrostomum*, Right: *C. watsoni*).

C. macrostomum has been originated from the front of the pelvic fins. The dorsal fin as a rudder has more fin rays in *C. macrostomum* than *C. watsoni*. The origination of dorsal fin than pelvic fins in *C. macrostomum* may be due to the high number of dorsal fin rays and its importance for adaptation to the environment.

In summary, this study was in agreement with the Coad (2013) but also in *C. watsoni* the dorsal fin origin is in front of the pelvic fins. Also our results have provided some morphological information to differentiate these two species more precisely.

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