Descriptive osteology of *Barbus cyri* (Teleostei: Cyprinidae) from southern Caspian Sea basin

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Abstract: The present study was conducted to provide a detailed descriptive osteology of *Barbus cyri* from Southern Caspian Sea for the first time and comparing it with those of *B. lacerta* from the Tigris and Urmia lake basins. For this purpose, twelve specimens of *B. cyri* were collected from Safidrud River using electrofishing device and fixed in 10% buffered formalin. Then, the specimens were cleared and stained with alcian blue and alizarin red for osteological examination. A detailed description of the osteological features of *B. cyri* was provided. Based on the results, several differences were observed between the two species. *Barbus cyri* can be distinguished from *B. lacerta* by having three pharyngobranchial, a consumptive neural spine of second centrum and having 3rd and 4th vertebra with posterior position of neural spines.

Keywords: *Barbus*, Kura barbel, Cyprinidae, Caspian Sea, Skeleton, Bone.

Introduction
Over the years, the generic name *Barbus* (Cuvier and Cloquet 1816) has been used for about 800 nominal species of cyprinid fishes in Europe, Africa and Asia. As presently understood, however, it is split into several distinct genera (Coad 2015). This genus is characterized by a rounded or compressed body of moderate to very large size, large to very small scales (no scale sheath around the anal fin), the presence of barbels in most species, usually two pairs, often one pair and sometimes none (and individually variable within species), the last unbranched ray in the short dorsal fin (usually 7-8 branched rays but sometimes more) is thickened and spine-like and may bear teeth or be smooth and a short anal fin, usually with 5 branched rays (but some have 6) (Coad 2015).

There are 16 formerly recognized species of the genus *Barbus* in Iranian inland waters (Coad 2015); only two species including *B. cyri* (De Filippi 1865) and *B. lacerta* (Heckel, 1843) is now assigned to this genus. *Barbus lacerta* is found in the Tigris and Urmia Lake basins of Iran (Coad 2015). *Barbus cyri* has a wide distribution in the southern Caspian Sea basin (Berg 1949; Bianco & Banarescu 1982; Kiabi et al. 1999; Abdoli 2000; Esmaeili et al. 2014a, b; Motamedi et al. 2014). Berg (1948-1949) refers Caspian Sea basin specimens to *Barbus lacerta cyri*. But, this subspecies recognized as a full species by Naseka & Bogutskaya (2009).

Until now, the phylogenetic relationships of the members of this group is based on external morphological characters (i.e. corporal proportions, scale and ray number), and few internal ones (i.e. number of pharyngeal teeth and gill rakers) (Doadrio 1990). Since, osteological characters can provide valuable information in taxonomy and phylogenetic relationships in fishes (Keivany & Nelson 1998, 2004, 2006; Diogo & Bills 2006; Keivany 2014a, b, c, d), therefore, the present study was conducted to provide a detailed descriptive osteology of *B. cyri* from southern Caspian Sea for the first time and comparing it with those of *B. lacerta* from Tigris and Urmia lake basins. The results will provide a basis for further phylogenetic study of Iranian members of the
Barbus group using osteological data.

Materials and Methods
Twelve specimens of *B. cyri* were collected from Safidrud River (10.74±2.64mm; SL±SD) using electrofishing device and fixed in 10% buffered formalin, after anesthetizing in 1% clove oil (Fig. 1). The specimens were cleared and stained with alizarin red S and alcian blue according to the protocol of Taylor & van Dyke (1985) for osteological examination. The cleared and stained specimens were studied using a stereomicroscope (Leica MC5); and their skeletal elements were dissected and scanned by a scanner equipped with a glycerol bath (Epson V600). Drawing of the specimens were performed using CorelDraw X6 software. The terminology of skeletal elements follows Rojo (2010) and Howes (1982). The detailed osteological features of *B. lacerta* from Tigris and Urmia lake basins were provided by Jalili et al. (2015) and Razavipour (2013).

Results
The ethmoid region of the neurocranium consists of the preethmoid-I, lateral ethmoid, supraethmoid, vomer, kinethmoid and nasal bones (Fig. 2a, b). The supraethmoid consists of two vertical and horizontal sections; the horizontal section bears two small anterior processes and a shallow middle depression with a cartilaginous lateral margins (Fig. 2a). The vertical section of this bone is situated on the dorsal part of the vomer and the anterior part of the parasphenoid. The posterior part of the vomer is pointed and its anterior part has a V-shaped notch. The anterior part of the vomer is thicker than its posterior part. Two small semicircular preethmoid-I are present in the antero-lateral part of the vomer (Fig. 2c). The lateral ethmoid is located as a wall between the ethmoid and orbital regions; this bone is concaved dorsally and flattened ventrally and its ventral surface bears many small pores. A cylindrical kinethmoid exists between the maxillary bones (Fig. 3a).

The orbital region comprises the frontals, parasphenoid, ptersphenoids, orbitosphenoids and circumorbital series. The frontal is a large bony element of the skull roof with a serrated anterior edge; it bears a mid-lateral pointed process. The supraorbital canal is enclosed by the lateral margin of the frontal. The two orbitosphenoids are fused via their ventral process that is also connected to the parasphenoid (Fig. 2b). The ptersphenoid is concaved in shape with two ventral processes for connecting to the parasphenoid. The ptersphenoid is dorsally attached to the frontal, anteriorly to the orbitosphenoid and posteriorly to the sphenotic. The anterior half of the parasphenoid is type-shape with a serrated anterior rim and its posterior half has a triangular structure that its posterior margin bifurcates via a deep groove (Fig. 2c).

In the circumorbital series, the number of the infraorbital bones was different in studied specimens.
There are 4-6 infraorbital and one supraorbital elements. The first circumorbital i.e. lacrymal, is the largest element of this series. The supraorbital is oval-shaped and located at the antero-lateral part of the frontal. The suborbital canal is enclosed by the infraorbital bony elements (Fig. 3c).

The otic portion of the neurocranium includes five bones viz. the parietal, epiotic, sphenotic,
pterotic and prootic. The postero-lateral margin of the parietal covers the dorsal part of the pterotic and epiotic. The epiotic is oval-shaped with a posterior process (Fig. 2a); it is situated between the pterotic and supraoccipital. The pterotic has a trapezoid shape (Fig. 2b) and its postero-lateral part is well-developed. The sphenotic possesses a lateral process which connects the middle process of the frontal (Fig. 2a). The ventral margin of the sphenotic is connected to the prootic. The two prootics are connected to each other ventrally and to the parasphenoid dorsally by a descending process. This bone has two pores on its anterior part.

The occipital region is composed of the supraoccipital, exoccipitals and basioccipital. The supraoccipital is pentagon in shape and has a blade-shaped crest. The exoccipital bears a large foramen on its middle part. In the dorsal part of the basioccipital, there are a pointed pharyngeal process and a concaved masticatory plate pointing laterally (Fig. 2c).

The upper jaw is made up of the maxillary and premaxillary. The two maxillaries are located at the dorsal side of the premaxillary; this bone possesses a mid-lateral ascending and an anterior descending processes; it has also a distal process and a fossa on its dorsal border. The premaxillary is wide and its anterior part has a rostral ascending process (Fig. 3a).

The lower jaw is composed of four bones including the dentary, angular, retroarticular and coronomeckelian. The coronoid process, situated on the dorso-middle part of the dentary, is oriented posteriorly. The middle part of the angular is wide and its posterior part bears an articulatory facet. The retroarticular is a small bone situating under the posterior part of the angular; the posterior part of the retroarticular is connected to the interopercle via the retroarticular-interopercle ligament. A small and elongated coronomeckelian is observed on the medial face of the angular (Fig. 3b).

The suspensorium is formed by the hyomandibular, ectopterygoid, endopterygoid,
metapterygoid, symplectic, quadrate and palatine (Fig. 4). The hyomandibular bone is almost triangular in shape and its posterior part bears two protuberances; the ventral part of the hyomandibular is connected to the interhyal. The ectopterygoid, endopterygoid, metapterygoid, symplectic and quadrate form a bony complex connecting to the anterior part of the neurocranium via the palatine. A long symplectic is enclosed by the metapterygoid posteriorly, by the quadrate anteriorly and by the preopercle posteriorly. The anterior part of the palatine bears three processes and a slightly deep depression for connecting to the vomer and preethmoid-I.

The opercular series is consists of four bones. The antero-dorsal part of the opercle has an opercular process and a socket joint that is connected to the hyomandibular. A L-shaped preopercle presents in the anterior side of the opercle; its vertical part is longer than that of its horizontal part. The ventral part of the preopercle overlaps the dorsal part of the interopercle. The subopercle has an antero-dorsal process pointed upwardly (Fig. 4).

The branchial apparatus includes five type bones including five pairs of the ceratobranchials, four pairs of the epibranchials, three pairs of the hypobranchials and the pharyngobranchial and three unpaired basibranchial. The fifth ceratobranchial is crescent in shape with a dental formula of 2.3.5-5.3.2 (Fig. 5a). The hyoid arch consists of the paired epiphyals, hypohyals and ceratohyals and the unpaired urohyal and basihyal, and three pairs of the branchiopterygoid rays. The urohyal has two vertical and horizontal sections. The posterior margin of its horizontal section is pointed and its middle part is wider. In addition, the anterior part of its horizontal section is bifurcated. A small interhyal locates at the dorsal part of the epiphyal. The posterior part of the ceratohyal is wider than its anterior part; its anterior part is bifurcated and attached to the dorsal and ventral hypohyals. The basihyal is T-shaped situating between the hypopals (Fig. 5b).

The pectoral girdle consists of the cleithrum, supracleithrum, coracoid, mesocoracoid, scapula, posttemporal, supratemporal and radials (Fig. 5a). The cleithrum bears two wide descending and ascending portions; its ventral part bears an antero-medial downward process connecting to the anterior part of the coracoid. Also, it has a dorsal process in its ascending part for positioning the supracleithrum. The posterior part of the coracoid is wider than its anterior part and bears an ascending process for connecting to the mesocoracoid. The medial part of the coracoid is bended ventrally and has a small pore. A semicircular scapula is located between cleithrum and coracoid bones; this bone bears a large foramen and articulated to the first unbranched ray. The ventral part of the mesocoracoid is V-shaped and attached to the coracoid and scapula; the dorsal part of the mesocoracoid is broadened and attached to the medial face of the cleithrum. The pectoral fin bears four radials; the lateral radial is the thickest and three others are long and flat.

Pelvic girdle includes the paired pelvic bone, pelvic splint and radials (Fig. 5b). The anterior part of the pelvic bone is U-shaped; this bone has a posterior long and a mid-lateral processes. The two L-shaped pelvic splint is located at the postero-lateral side of the pelvic bone. There are three radial bones in the pelvic girdle.

The Weberian apparatus is formed by four
anterior centra with their ossicles including the tripus, intercalarium, scaphium and claustrum (Fig. 6a). The tripus is positioned at the latero-ventral side of the second vertebra and its dorsal part extends to the posterior part of the pleural rib of the fourth centrum. The intercalarium is T-shaped and attached to the tripus and scaphium via the scaphia-intercalar and intercalo-tripodal ligaments. The clastrum is positioned on the dorsal part of the scaphium; they meet each other via a clastro-scaphal ligament. The ventral portion of the pleural rib of 2nd centrum oriented posteriorly. The two ventral processes of the fourth vertebra are fused to each other; the neural arch of this bone is short and connected to the neural arch and the supraneural of the third centrum. There are 42-44 vertebra including 19-22 cranial and 20-24 caudal centra.

The dorsal fin bears four unbranched and eight branched rays, nine pterygiophores and one stay (Fig. 6b). The dorsal margin of 4th unbranched ray is dentated. Two first pterygiophores are the largest and supports unbranched rays. The first pterygiophore is
next to the 15th or 16th vertebra. In front of the dorsal fin 9-10 free supraneural bones are observed. A triangular stay bone supports the last branched ray.

The anal fin originates at 28th centrum. This fin has three unbranched and five branched rays and is supported by six pterygiophores and two small stay bones (Fig. 6b).

The caudal skeleton consists of four centra with the epural, parhypural, pleurostyle, uroneural and six hypurals bones (Fig. 6c). The pleurstyle is fused to the last centrum. The neural arch of the second centrum is consumptive in some specimens. The first hyporal is the largest one and attached to the parhyporal. The epural is a long bone positioning at the dorsal part of the neural arch of the first vertebra.

Discussion
There are little differences in the caudal and anal structures, Weberian apparatus, branchial arch and the numbers of the vertebra and supraneural elements between Iranian members of the genus Barbus. *Barbus cyri* bears three pharyngobranchials, whereas *B. lacerta* bears two pharyngobranchial (Razavipour 2013). The number of the supraneural is variable in the specimens of this genus; in specimens of *B. lacerta* from the Tigris and Urmia lake basins, there are 7-8 and 7-10 supraneurals (Razavipour 2013), respectively, whereas *B. cyri* has 9 supraneurals.

*Barbus cyri* bears 42 vertebrae and its fourth vertebra has neural prezygapophyses and neural postzygapophyses, whereas in two other population of *B. lacerta*, 42 vertebra present, but their neural prezygapophyses of the fourth vertebra is absent (Razavipour 2013). Also, the neural arch of 4th centrum in Weberian apparatus is bended posteriorly in specimens of *B. lacerta* from the Urmia lake basin, but in *B. cyri* and *B. lacerta* of the Tigris basin, it is bended dorsally. In *B. lacerta*, the second centrum of the caudal skeleton bears two long neural spine and four centra form the hypural plate (Razavipour 2013), whereas in *B. cyri*, the second centrum of the caudal skeleton is consumptive and the hypural plate is formed by five vertebra. In addition, in *B. lacerta* of the Urmia lake basin, one stay is present in anal fin versus two stays of *B. cyri* and *B. lacerta* of the Tigris basin (Razavipour 2013).

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