DESCRIPTIVE OSTROLOGY OF ALBURNUS AMIRKABIRI
(CYPRINIFORMES: CYPRINIDAE), A NEWLY DESCRIBED SPECIES FROM NAMAK LAKE BASIN, CENTRAL OF IRAN

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ABSTRACT
This study was conducted to provide a detailed description of the osteology features of Alburnus amirkabiri from the Qareh Chai River (Iran, Markazi Province). For this purpose, eight specimens of A. amirkabiri were collected from the Qareh Chai River by electrofishing and fixed in 4% buffered formalin after anesthesia. The specimens were cleared and stained for osteological examination and its detailed osteological characterizations and differences with available osteological data of other member of the genus Alburnus was provided.

Keywords: Cyprinidae, Alburnus amirkabiri, Osteology, Fish skeleton.

INTRODUCTION
The genus Alburnus (Rafinesque, 1820), a member of family Cyprinidae, is widespread in Europe and the northern parts of Southwest Asia (Bogutskaya et al., 2000; Kottelat and Freyhof, 2007; Jouladeh-Roudbar et al., 2015). Thirty-nine species of the genus are known (Coad, 2014) with 8 species recorded from Iranian interior waters, including A. alburnus (Linnaeus, 1758), A. chalcoides (Güldenstädt, 1772), A. filippii (Kessler, 1877), A. hohenackeri (Kessler, 1870), A. caeruleus (Heckel, 1843), A. atropatenae (Berg, 1925), A. muzzulensis (Heckel, 1843) and A. amirkabiri (Mousavi-Sabet et al., 2015). Members of this genus are characterized by an elongated and compressed body of small to moderate size, a terminal mouth, no barbels, moderated scales, short dorsal fin without a thickened ray, long anal fin and a fleshy keel between the bases of the pelvic.

Recently, Alburnus amirkabiri (Mousavi-Sabet et al., 2015) was described from Qareh-Chai River. There is no information available about biological features of this species, and its systematic position is unclear. Osteological characteristics are the useful features to study the taxonomy and phylogenetic relationships among fishes (Ramaswami, 1951; Howes, 1982; Bogutskaya, 1994; Mafakheri et al., 2014, 2015; Jalili et al., 2015a). Since, a complete overview of the osteological characteristic of A. amirkabiri is absent; therefore, this study was
Descriptive osteology of *Alburnus amirkabiri* conducted to provide a detailed description of its osteological features as a basis for further taxonomic research of this species.

Figure 1. Lateral view of *A. amirkabiri* from Qareh-Chai River.

**MATERIALS AND METHODS**

For this study, eight specimens of *A. amirkabiri* with a mean standard length of 147.68 ± 10.045 mm were collected from Qareh-Chai River (Fig. 1) by electrofishing and fixed in 4% buffered formalin. The specimens were cleared and stained with alizarin red S and alcian blue according to the protocol of Taylor and Vandyke (1985) for osteological examination. The cleared and stained specimens were studied under a stereomicroscope (Leica MC5); and different skeletal elements were dissected and scanned by a scanner equipped to a glycerol bath (Epson v700). Drawings of the skeletal elements were performed from obtained images using CorelDraw X6 software. The nomenclatures of the skeletal elements were followed (Howes, 1982; Rojo, 1991).

**RESULTS AND DISCUSSION**

The neurocranium is sub-triangular (Fig 2 a) and its anterior half is narrower and shallower than the posterior half (Fig 2 d). The ethmoid region consists of the paired lateral ethmoid, preethmoid-I and nasal and two unpaired supraethmoid and vomer. The supraethmoid bears a shallow notch at the middle and two pointed processes anterolaterally (Fig. 2a). Two tube-like nasal bones are located in the lateral part of the supraethmoid and the supraorbital canal runs through the nasal bones. The anterior part of the vomer is V-shaped, and its lateral part attached to the preethmoid-I. The posterior part of the vomer is pointed, and its ventral surface covered by the anterior part of the parasphenoid (Fig. 2b). The lateral ethmoid is concaved anteriorly and posteriorly, flattened ventrally and pointed lateroventrally. This bone is attached to the supraethmoid and frontal dorsally, vomer and parasphenoid ventrally and orbitosphenoid posteriorly (Fig. 2a and b). There is a large pore at the contact between the supra-ethmoid and frontal bones.

The orbital region comprises the frontal, parasphenoid, ptersphenoid, orbitosphenoid and circumorbital bones. This region is the largest part of the neurocranium. The frontals are trapezoid in shape and connected to the orbitosphenoid and ptersphenoid ventrally. Two orbitosphenoid bones fused to each other and form a blade process along the ventral border. The ventral part of this process attached to the parasphenoid (Fig. 2d). The petersphenoid is a concaved bone and has some pores in its middle part and located between the orbitosphenoid and sphenotic bones. The parasphenoid is elongated and possesses two wings in its middle part bend dorsally connecting to the prootic (Fig. 2b).
The circumorbital series consists of five infra-orbital and one supra-orbital elements (Fig. 2 c). The first infra-orbital is lachrymal and oval shaped possessing a pointed process in the posterodorsal part. The 3rd infra-orbital is elongated, and its posterior part is wider than the anterior part (Fig. 2 c). The 5th infra-orbital is the smallest element. The supra-orbital is crescent-shaped and situated in the lateral part of the frontal (Fig. 2 a and c). The number of infraorbital bones is different in *A. chalcoides* from 4 to 7 as a result of fusions or separations of bone elements (Musavi-Sabet *et al.*, 2014). This character demonstrates intraspecific variability.

The otic region is composed of the parietal, epiotic, sphenotic, pterotic and prootic. The rectangular parietal has a serrated margin posteriorly having some small pores (Fig. 2 a). The parietal is attached to the pterotic and sphenotic laterally and epiotic and supraoccipital dorsally; the supratemporal commissure runs along the posterior edge of the parietal bones. The sphenotic has an anterolateral process, which is connected to the posterolateral edge of the frontal (Fig. 2 a). The sphenotic is ventrally connected to the ptersphenotic and prootic and posteriorly to the pterotic. The pterotic possesses a dorsolateral process connecting to the exoccipital. The large prootic bones form the ventral surface of the otic region (Fig. 2 b); these bones are attached to each other ventrally and connected to the parabasisphenoid via a descending process. Two relatively large pores are observed in the anterior part of the prootic which has a protuberance in its dorsolateral part (Fig. 2 b). The neuromesencephalon is articulated with the hyomandibular by two articular processes. The ptersphenoid, sphenotic and prootic form the first facet. The second facet is longer than the first one and formed by the pterotic, sphenotic and prootic.

The occipital region consists of the supraoccipital, exoccipitals and basioccipital. In the middle part of the supraoccipital, a blade-shaped crest is present (Fig. 2 a). The exoccipital is concaved and has a large foramen in its middle part. The basioccipital has a pointed pharyngeal process and a concaved masticatory plate pointing laterally (Fig. 2 b).
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Figure 2. Neurocranial of *A. amirkabiri* from dorsal (a), ventral (b) and lateral (c) views (Boc-Basioccipital; BPP-Basioccipital Posterior Projection; Bs-Basisphenoid; Ep-Epithotic; Exo-Exoccipital; Fro-Frontal; Ica-Intercalar; IO1-Infrat orbital1 (lachrymal); IO2-Infrat orbital2 (Jugal); LeP-Lateral Ethmoid Process; Let-Lateral Ethmoid; LPS-Lateral Process of Sphenotic; Me-Mesethmoid; MPPP-Masticatory Plate of Pharyngeal Process; Nas-Nasal; OpF-Optic Foramen; Os-Orbitosphenoid; Par-Parietal; PoT-Post temporal; PrE-Pre Ethmoid; Pro-Prootic; Ps-Parasphenoid; Ptr-Pterotic; ScB-Sclerotic Bones; SCI-Supra Cleithrum; Seth-Supraethmoid; Soc-supraorbital sensory canal; StF-Subtemporal Foramen; SuC-Supraoccipital crest; SuO-Supra Orbital; V-Vomer).

In the branchiocranium, the upper jaw consists of the premaxillary and maxillary. The maxillary is a long bone and bears an ascending process in the middle and a descending process anteriorly. The lateral part of the maxillary is wide and bears a small notch. The premaxillary is located under the maxillary and has a rostral process tilted upwardly. A free
kinethmoid bone is situated between two maxillary in the front of the vomer. The dorsal part of the kinethmoid is wider than its ventral part (Fig.3a).

The lower jaw comprises the dentary, angular and retroarticular (Fig.3b). A coronoid process in the posterior part of the dentary is developed and slightly bended dorsally. There are two pores at the middle part of the dentary and connected to the angular and retroarticular posteriorly. The retroarticular is a triangular element which its dorsal part is attached to the angular. In A. mirkabiri and other species of the genus Alburnus, including A. chalcoides and A. hohenackeri, the dentary is a deep bone with perpendicular coronoid process, whereas in A. atropatanae this bone is shallow, and its coronoid process is wide and oriented posteriorly (Musavi-Sabet et al., 2015; Jalili et al., 2015b).

The suspensorium consists of the hyomandibular, ectopterygoid, endopterygoid, metapterygoid, symplectic, quadrate and palatine (Fig.3c). The Hyomandibular is triangular in shape. The quadrate has a posterior process and is anteriorly connected to the ectopterygoid, dorsally to the endopterygoid and posteriorly to the metapterygoid and symplectic (Fig.3c). The symmetric is an elongated bone extending up to the ventral part of the hyomandibular. The palatine possesses an anterodorsal process and is anteriorly connected to the preethmoid-I and vomer and posteriorly to the endopterygoid. The endopterygoid is enclosed by the ectopterygoid, metapterygoid, quadrate and palatine. The posterior part of the endopterygoid is wider than the anterior part. The metapterygoid is almost the pentagon in shape. In A. amirkabiri, A. chalcoides, and A. hohenackeri endopterygoid and metapterygoid bones are larger and wider than the same bones of A. atropatanae (Mousavi-Sabet et al., 2015). The ectopterygoid is a small bone bearing an anterodorsally pointed process (Fig.3c).

The opercular series composed of the opercle, preopercle, interopercle and subopercle (Fig.3d). The opercle has an articulatory facet for connection to the hyomandibular anterodorsally and its ventral margin superimposes the dorsal edge of the subopercle. The anterior part of the subopercle is wider than the posterior part. The preopercle is a thin and L-shaped bone, and its ventral border superimposes the posterior edge of the interopercle (Fig.3d).
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The branchial apparatus includes four pairs of the ceratobranchial, four pairs of the epibranchial, three pairs of the hypobranchial, two pairs of the pharyngobranchial and three unpaired basibranchial bones (Fig. 4 a). The basibranchial-3 is longer than basibranchial 1-2. The hypobranchial-3 has an elongated and ventrally oriented process, whereas
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The hypobranchial 1 and 2 are round in shape. The pharyngobranchial-2 is semicircular in shape and larger than other one (Fig. 4 a).

The hyoid arch consists of the paired epihyals, hypohyals and ceratohyals, the unpaired urohyal and basihyal and three pairs of the branchiostegal rays (Fig. 4 b). The basihyal is a thin and long bone situating between the hypohyals posteriorly. The urohyal consist of the vertical and horizontal parts. The posterior margin of the horizontal part of the urohyal is sharply concaved and wider than its anterior part. The posterior margin of the vertical part of this bone is also concaved and bifurcated anteriorly. The interhyal is a small and rounded bone articulating with dorsal part of the epihyal. The posterior part of the ceratohyal is wider than its anterior part that is bifurcated and attached to the hypohyal. The pharyngeal teeth of *A. amirkabiri* are arranged in three rows with a formula of 2.4.1-1.5.2 (Fig. 4 c).

Figure 4. Dorsal view of branchial (a) and hyoid arch (b) and pharyngeal teeth (c) in *A. amirkabiri* (Bb – Basibranchial; Bhy – Basihyal; Brs – Branchiostegals; Cb – Ceratobranchial; Chy – Ceratohyal; Eb – Epibranchial; GR – Gill Rakers; Hb – Hypobranchial; Hhy – Hypohyal; Ihy – Interhyal; Pb – Pharyngobranchial; Uhy – Urohyal).
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The pectoral girdle consists of the cleithrum, supracleithrum, coracoid, mesocoracoid, scapula, posttemporal, supratemporal and radials of the pectoral fin (Fig. 5a). The cleithrum is L-shaped and its horizontal part is wider than the vertical part that is attached to the coracoid and scapula. The scapula possesses a large foramen on its middle face and a fossa on its posterior face which articulates with the first unbranched ray. The mesocoracoid is articulated dorsally to the cleithrum and ventrally to the coracoid and scapula (Fig. 5a). The coracoid bears a large foramen in the posterior part. The supracleithrum is a long bone and attaches to the epiotic dorsally and to the cleithrum ventrally; and its middle part is the widest part. The pectoral girdle has four radials that the first and forth ones are wider than others. The pectoral fin has 1 unbranched and 12 branched rays.

The pelvic girdle includes the paired basipterygium, meta- pterygium and lateral- pterygium. The pelvic fin has 1 unbranched and 8 branched rays. The paired basipterygium is attached to each other in anterior and posterior parts (Fig. 5b). There is a deep hollow in the anterior part of the basipterygium. A free paired lateral- pterygium present in the lateral side of the basipterygium and 3 pair of the meta- pterygium are located behind the basipterygium and the latero- external one is largest and two other is connected to each other.

![Figure 5. Middle view of the pectoral girdle (a) and dorsal view of the pelvic girdle (b) in *A. amirkabiri* (Act- Actinost; Cle- Cleithrum; Co- Coracoid; DP- Distal Process; Mco- Mesocoracoid; MIP- Mid_lateral Process; PoP- Posterior Process; Sca- Scapula).](image-url)
The dorsal fin has 3 unbranched and 8-9 branched rays, 9 pterygiophores and one stay (Fig. 6a). The first pterygiophore is the largest one and supports the unbranched rays. In front of the dorsal fin, nine free supraneural bones present. The first supraneural is largest and supraneural 3-9 are thin and long.

The Anal fin possesses 3 unbranched and 11 branched rays, 12 pterygiophores and a small stay (Fig. 6b). The largest pterygiophore supports 2 unbranched rays. The dorsal fin originates at 15th vertebra in *A. amirkabiri* and at 14th vertebra in *A. atropateneae*, anal fin originates at 22nd and 21st vertebrae in *A. amirkabiri* and *A. atropateneae*, respectively (Mousavi- Sabet *et al.*, 2015).

Figure 6. Dorsal (a) and anal fines (b) skeleton in *A. amirkabiri* (AFS- Anal Fin Spine; C 15- Centrum 15; C 22- Centrum 22; DFS- Dorsal Fin Spine; Dpt- Distal Pterigiophore; MPt- Median Pterigiophore; NuC- Neural Complex; PPt- Proximal Pterigiophore; Sty- Stay; Sun- Supraneural).
In the axial skeleton, the number of the vertebrae is 40; the cranial and caudal parts of the vertebral column have 22 and 18 centra, respectively. The Weberian apparatus is formed by the first four anterior centra with four pair ossicles, including tripus, intercalarium, scaphium, and claustrum (Fig. 7 a). The first centrum has a small pleural rib and the pleural rib of the second centrum is long and is bended dorsally. The pleural rib of the 3rd centrum is absent and pleural rib of the 4th centrum is long and bifurcated. The number of vertebrae in A. atropatena, A. chalcoides and A. hohenackeri is 42, 42-45 and 37-41, respectively (Mousavi-Sabet et al., 2015; Jalili et al., 2015b).

The skeleton of the caudal fin is composed of the epural, parhypural, pleurostyle, uroneural and six hypurals bones (Fig. 7 b). The caudal fin of A. amirkabiri has 19 branched rays and various numbers of the procurrent rays.
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**LITERATURE CITED**


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وصف العظام للنوع *Alburnus amirkabiri* من نهر كاري شاي (ولاية مرکزي، إيران).

لذا الغرض، جمعت ثمان نماذج من النوع *A. amirkabiri* من نهر كاري شاي بواسطة الصيد الكهربائي ووضعت في 4% محلول فورمالين متعادل بعد التخدير.

النماذج نظفت وصبغت للفحص النسجي العظمي وتفاصيل الوصفات العظمية ومقارنة الاختلافات مع ما متوفر من بيانات العظام المثبتة لاعداد من الجنس

المفاتيح: الشبوطيات، عالم العظام، الهيكل العظمي *Alburnus amirkabiri* للأسماك.