

DESCRIPTIVE OSTEOLOGY STUDY 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, markazi province, Iran. 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 members of the genus *Alburnus* were 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). Coad (2014) listed 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* (Güldenstaedt, 1772), *A. filippii* (Kessler, 1877), *A. hohenackeri* (Kessler, 1870), *A. caeruleus* (Heckel, 1843), *A. atropatenae* (Berg, 1925), *A. mossulensis* (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

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conducted to provide a detailed description of its osteological features as a basis for further taxonomic researches of this species.

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 in 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 Corel Draw X6 software. The nomenclatures of the skeletal elements were followed (HOWES, 1982; ROJO, 1991).

RESULTS AND DISCUSSION

The neurocranium was sub-triangular (Fig.2 a) and its anterior half was narrower and shallower than the posterior half (Fig.2 d). The ethmoid region consisted of a 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 were located in the lateral part of the supraethmoid and the supraorbital canal run 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 was pointed, and its ventral surface covered by the anterior part of the parasphenoid (Fig. 2b). The lateral ethmoid was concave anteriorly and posteriorly, flattened ventrally and pointed lateroventrally. This bone was attached to the supraethmoid and frontal dorsally, vomer and parasphenoid ventrally and orbitosphenoid posteriorly (Fig. 2a and b). There was a large pore at the contact between the supra-ethmoid and frontal bones.

The orbital region comprised the frontal, parasphenoid, ptersphenoid, orbitosphenoid and circumorbital bones. This region was the largest part of the neurocranium. The frontals were 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 was attached to the parasphenoid (Fig. 2d). The ptersphenoid was a concave bone and had some pores in its middle part located between the orbitosphenoid and sphenotic bones. The parasphenoid was elongated and possesses two wings in its middle part bend dorsally connecting to the prootic (Fig. 2b).

The circumorbital series was consisted of five infra-orbital and one supra-orbital elements (Fig.2 c). The first infra-orbital was lachrymal and oval shaped possessing a pointed process in the posterodorsal part. The 3rd infra-orbital is elongated, and its posterior part was wider than the anterior part (Fig.2 c). The 5th infra-orbital was the smallest element. The supra-orbital was crescent-shaped and situated in the lateral part of the frontal (Fig.2 a and c). The number of infraorbital bones was 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.

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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 parasphenoid 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 neurocranium is articulated with the hyomandibular by two articulatory facets. The ptersphenoid, sphenotic and prootic bones form the first facet. The second facet is longer than the first one and formed by the pterotic, sphenotic and prootic bones.

The occipital region consisted of the supraoccipital, exoccipitals and basioccipital bones. In the middle part of the supraoccipital, a blade-shaped crest was present (Fig.2 a). The exoccipital is concaved and had a large foramen in its middle part. The basioccipital had a pointed pharyngeal process and a concaved masticatory plate pointing laterally (Fig.2 b).



Fig.1: Lateral view of *A. amirkabiri*.

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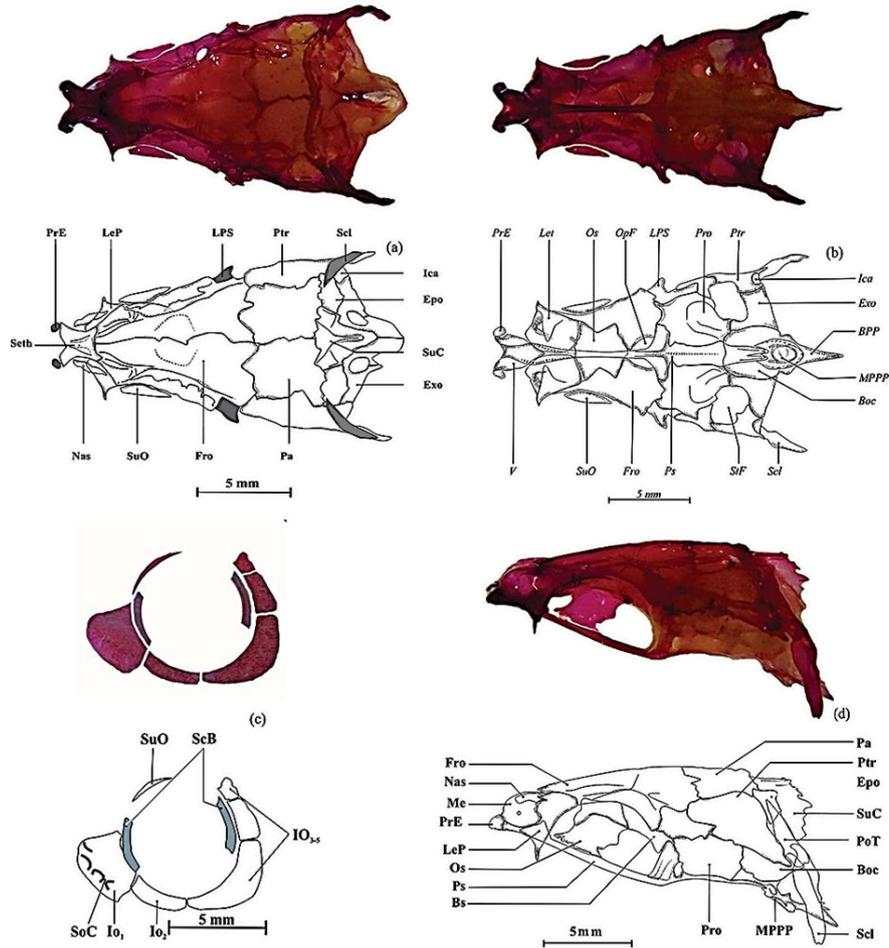


Figure 2. Neurocranium of *A. amirkabiri* from dorsal (a), ventral (b) and lateral (c) views (Boc-Basioccipital; BPP-Basioccipital Posterior Projection; Bs-Basisphenoid; Epo-Epiotic; Exo-Exoccipital; Fro Frontal; Ica-Intercalar; IO₁-Infra orbital₁ (lachrymal); IO₂-Infra orbital₂ (Jugal); LeP-Lateral 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; Pa-Parietal; PoT-Post temporal; PrE-Pre Ethmoid; Pro-Prootic; Ps-Parasphenoid; Ptr-Pterotic; ScB-Sclerotic Bones; SCL-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 upwards. A free

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kinethmoid bone was situated between two maxillary in the front of the vomer. The dorsal part of the kinethmoid was wider than its ventral part (Fig.3 a).

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

The suspensorium consistd of the hyomandibular, ectopterygoid, endopterygoid, metapterygoid, symplectic, quadrate and palatine (Fig.3 c). The Hyomandibular was triangular in shape. The quadrate had a posterior process and was anteriorly connected to the ectopterygoid, dorsally to the endopterygoid and posteriorly to the metapterygoid and symplectis (Fig.3 c). The symplectic was an elongated bone extending upto the ventral part of the hyomandibular. The palatine possesses an anterodorsal process and was anteriorly connected to the preethmoid-I and vomer and posteriorly to the endopteygoid. The endopterygoid was enclosed by the ectopterygoid, metapterygoid, quadrate and palatine. The posterior part of the endopterygoid was wider than the anterior part. The metapterygoid was almost the pentagon in shape. In *A. amirkabiri*, *A. chalcoides*, and *A. hohenackeri* entopterygoid and metapterigoid bones were larger and wider than the same bones of *A. atropatena* (Mousavi-Sabet *et al.*, 2015). The ectopterygoid was a small bone bearing an anterodorsally pointed process (Fig.3 c).

The opercular series composed of the opercle, preopercle, interopercle and subopercle (Fig.3 d). The opercle had 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 was wider than the posterior part. The preopercle was a thin and L-shaped bone, and its ventral border superimposes the posterior edge of the interopecle (Fig.3 d).

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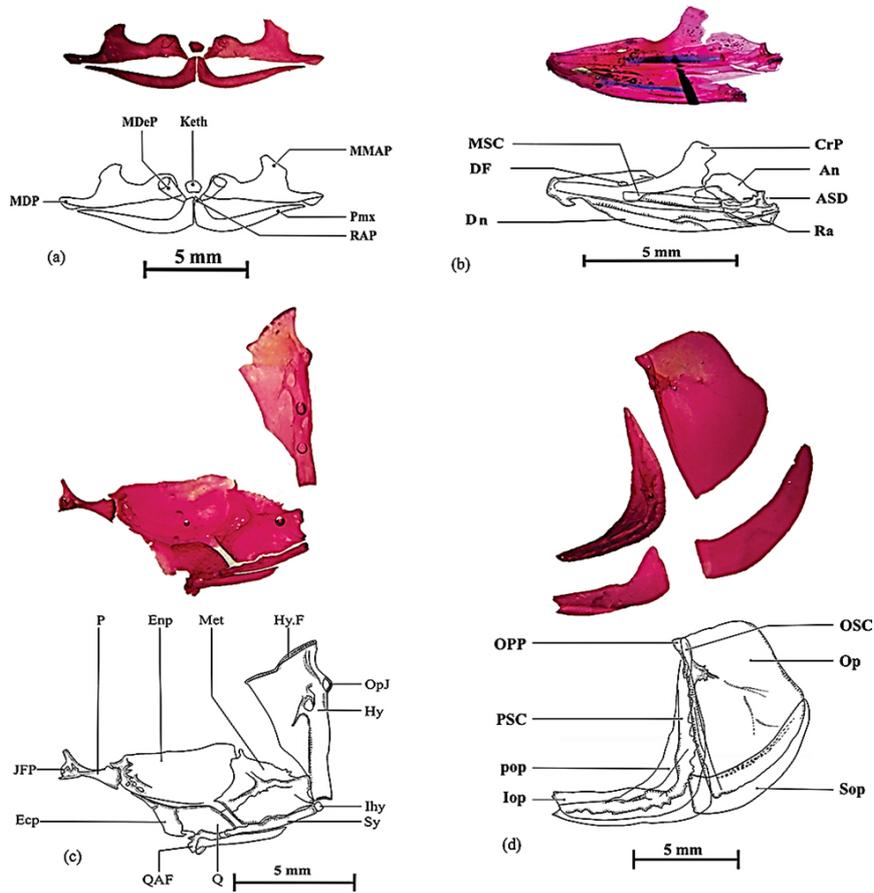


Figure 3. Anterior view of the upper jaw (a), lateral view of the lower jaw (b), suspensorium (c) and opercle series (d) in *A. amirkabiri* (An- Angular; ASD- Articular Surface of Dentary; CrP- Coronoid Process; DF- Dentary Foramen; Dn- Dentary; MSC- Mandibular Sensory Canal; Ra- Retroarticular; Ecp- Ectopterygoid; Enp- Entopterygoid; Hy- Hyomandibular; Hy.F- hyomandibular joint face; OpJ- Opercular Joint; Op- Opercular; OPP- Opercular Prominent Process; OSC- Opercular Sensory Canal; P- Palatine; Pop- Preopercular; PSC- Preopercular Sensory Canal; Pmx- premaxilla; Q- Quadrate; QAF- Quadrate Articular Face; RAP- Rostral Ascending Process Sop- Subopercular; Sy- Symplectic).

The branchial apparatus included 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 was longer than basibranchial 1-2. The hypobranchial-3 had an elongated and ventrally oriented process, whereas the

hypobranchial 1 and 2 were round in shape. The phrpharyngobranchial-2 was semicircular in shape and larger than other one (Fig.4 a).

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

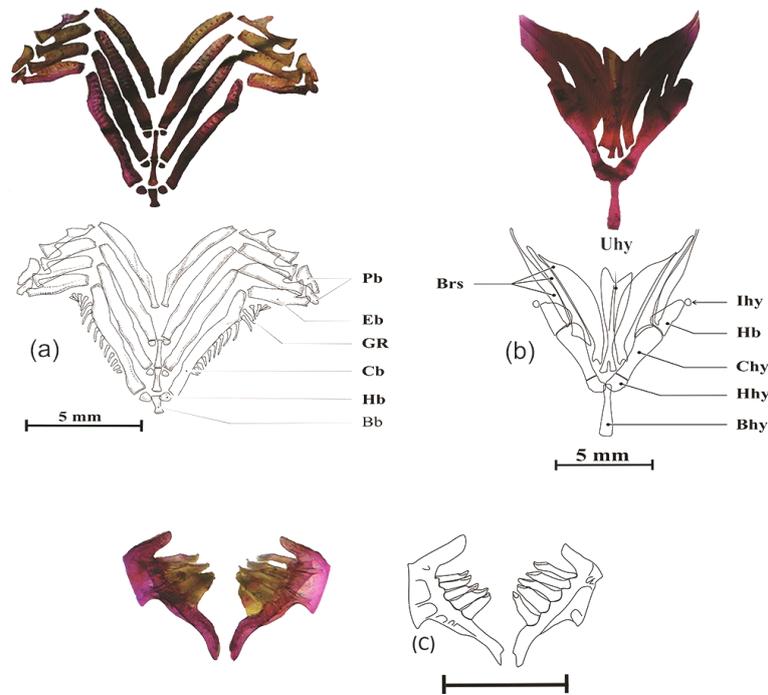


Figure 4. Dorsal view of branchial (a) and hyoid arch (b) and pharyngeal teeth (c) in *A. amirkabiri* (Bb – Basibranchial; Bhy- Basihyal; Brs- Branchiostegals; Cb – Ceratobranchial; Chy - Ceratohyal; Eb – Epibranchial; GR - Gill Rakers; Hb – Hypobranchial; Hhy- Hypohyal; Ihy- Interhyal; Pb – Pharyngobranchial; Uhy- Urohyal).

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The pectoral girdle consisted of the cleithrum, supracleithrum, coracoid, mesocoracoid, scapula, posttemporal, supratemporal and radials of the pectoral fin (Fig.5 a). The clietherum is L-shaped and its horizontal part was wider than the vertical part that was attached to the coracoid and scapula. The scapula possessed a large foramen on its middle face and a fossa on its posterior face which articulates with the first unbranched ray. The mesocoracoid was articulated dorsally to the cleithrum and ventrally to the coracoid and scapula (Fig.5 a). The coracoid bears a large foramen in the posterior part. The supracleithrum was 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 had four radials that the first and forth ones are wider than others. The pectoral fin had 1 unbranched and 12 branched rays.

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

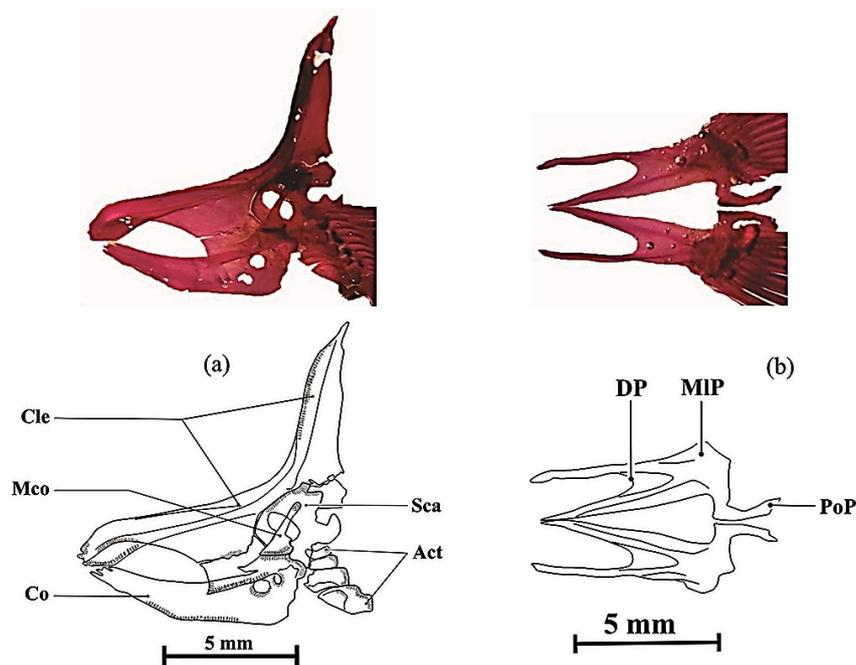


Figure 5. Middle view of the pectoral girdle (a) and dorsal view of the pelvic girdle (b) in *A. amirkabiri* (Act- Actinost; Cle- Cleithrum; Co- Coracoid; DP- Distal Process; Mco- Mesocoracoid; MIP- Mid_lateral Process; PoP- Posterior Process; Sca- Scapula).

The dorsal fin had 3 unbranched and 8-9 branched rays, 9 pterygiophores and one stay (Fig.6 a). The first pterygiophore was the largest one and supports the unbranched rays. In front of the dorsal fin, nine free supraneural bones present. The first supraneural was largest and supraneural 3-9 are thin and long.

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

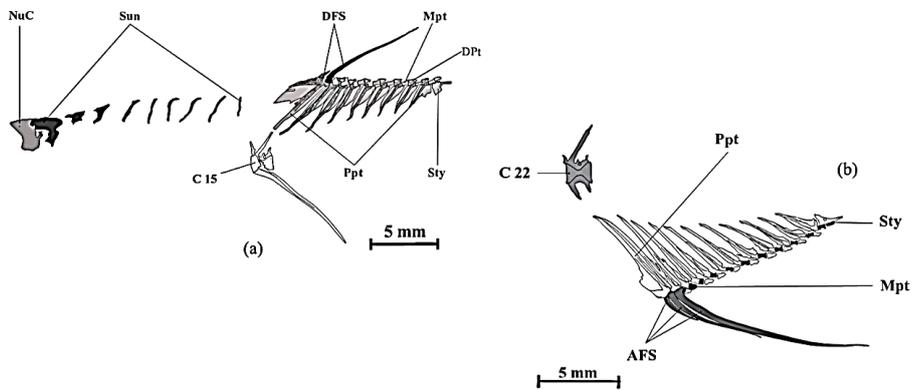


Figure 6. Dorsal (a) and anal fins (b) skeleton in *A. amirkabiri* (AFS- Anal Fin Spine; C 15- Centrum 15; C 22- Centrum 22; DFS- Dorsal Fin Spine; Dpt- Distal Pterygiophore; MPt- Median Pterygiophore; NuC- Neural Complex; PPt- Proximal Pterygiophore; Sty- Stay; Sun- Supraneural).

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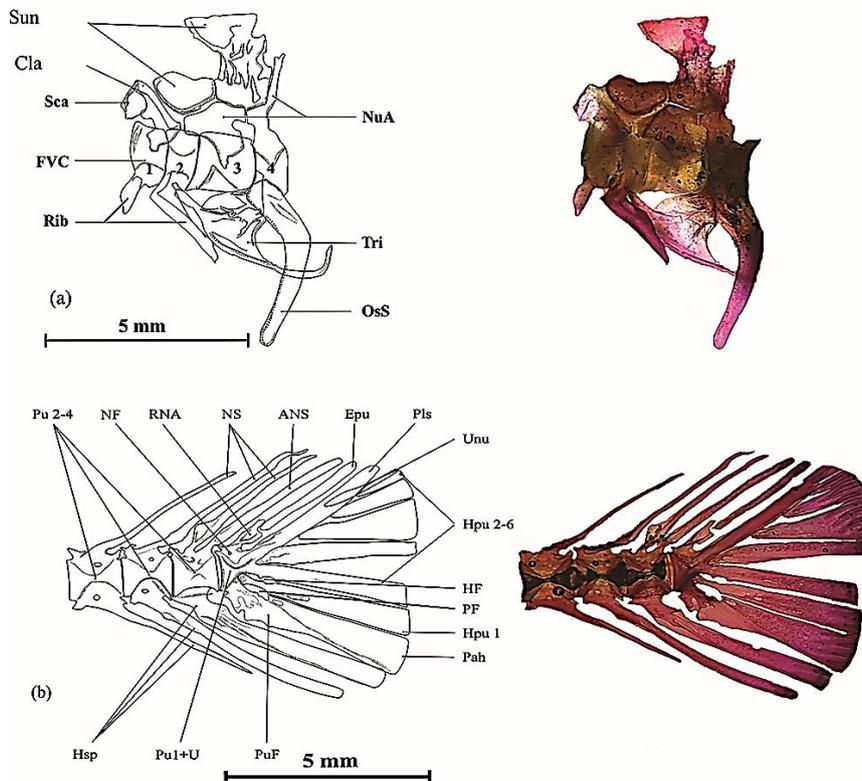


Figure 7. Lateral view of the Weberian apparatus (a) and caudal fin skeleton (b) in *A. amirkabiri* (ANS- Accessory Nural Spine; Cla- Clastrum; Epu- Epural; FVC- First Vertebra Centrum; HF- Hypural Foramen; Hpu- Hypural; HSp- Hemal spine; NF- Neural Foramen; NS- Neural Spine; NuA- Neural Arch; OsS- Suspensorium; Pah- Parhypural; PF- Parhypural Foramen; Pls- Pleurostyle; Pu- preural; Pu1+U- Preural 1+Urostyle; PuF- Preural Flange; Rib- Ribs; RNA- Rudimentary neural arch; Sca- Scaphium; Sun- supraneural; Tri- Tripus; Unu- Uroneural).

In the axial skeleton, the number of the vertebrae was 40; the cranial and caudal parts of the vertebral column have 22 and 18 centra, respectively. The Weberian apparatus was formed by the first four anterior centra with four pair ossicles, including tripus, intercalarium, scaphium, and claustrum (Fig.7 a). The first centrum had 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 was absent and pleural rib of the 4th centrum was long and bifurcated. The number of vertebrae in *A. atropatena*, *A. chalcoides* and *A. hohenackeri* was 42, 42-45 and 37-41, respectively (Mousavi-Sabet *et al.*, 2015; Jalili *et al.*, 2015b).

The skeleton of the caudal fin was 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 procurent rays.

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دراسة عظمية وصفية للنوع *Alburnus amirkabiri* (رتبة الشبوطيات: عائلة الشبوطيات)، الموصوف حديثاً من حوض بحيرة ناماك، وسط إيران

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الخلاصة

أجريت هذه الدراسة لاثبات وصف شكل العظام للنوع *Alburnus amirkabiri* من نهر كاري شاي ولاية مركزي، إيران. لهذا الغرض، جمعت ثمان نماذج من النوع A. *amirkabiri* من نهر كاري شاي بواسطة الصيد الكهربائي و وضعت في 4% محلول فورمالين متعادل بعد التخدير.

تم صبغ وتهيئة النماذج للفحص النسجي العظمي وتفاصيل الاوصاف العظمية و مقارنة الاختلافات مع ما متوفر من بيانات العظام المثبتة لاعداد من الجنس *Alburnus*.