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Osteological description of the southern king fish, *Alburnus mossulensis* from I ranian part of the Tigris River drainage

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Abstract. *Alburnus mossulensis* Heckel, 1843 is one of the eight species of the genus *Alburnus* found in Iran. This study was conducted to provide a detailed osteological description of *A. mossulensis* and comparing it with those of other related members of this genus in Iran i.e. *A. atropatenae, A. chalcoides, A. filippii* and *A. amirkabiri*. For this purpose, five specimens of *A. mossulensis* were collected, cleared and stained for osteological examination. Then, a detailed description of its skeletal structure is provided. Based on the results, *A. mossulensis* can be recognized from the other osteologicaly studied members of this genus in Iran by having a straight dorsal and posterior parts of the opercle, L-shaped preopercle with right-angled, blunt posterior part of the vomer, possessing 11 supraneural, having 43 centra, developed zygapophys process, short and thick anterior part of the palatine and dorsally bended the coronoid process.

Key Words: Osteology, bone, inland water, freshwater fish, taxonomy.

Introduction. The members of the genus *Alburnus* (Rafinesque, 1820) with about 39 species are found in the Europe and the northern parts of southwest Asia (Bogutskaya et al 2000; Kottelat & Freyhof 2007; Mousavi-Sabet et al 2015). They are characterized by more or less elongated, compressed, moderately deep body shape, a terminal mouth, no barbels, scales of moderate size, short dorsal fin without spiny ray, a long anal fin and a fleshy keel between the pelvic fins (Saadati 1977; Coad 2015). There are eight confirmed species, including *Alburnus amirkabiri*, *A. atropatenae*, *A. caeruleus*, *A. chalcoides*, *A. filippii*, *A. hohenackeri*, *A. mossulensis* and *A. zagrosensis* in Iranian inland waters (Mousavi-Sabet et al 2014, 2015).

Alburnus mossulensis is found in the Tigris-Euphrates basin and adjacent basins, including the Persian Gulf, Lake Maharlu, Kor River and upper reaches of the Hormuz basins of Iran (Berg 1949; Bianco & Banarescu 1982; Abdoli 2000) and questionably from the Esfahan basin (Abdoli 2000). The morphometric and meristic features of *A. mossulensis* shows a high degree of the variability among its distribution area (Mousavi-Sabet et al 2015; Coad 2015). All identification characters of the members of this genus in Iran are based on their external morphology *i.e.* morphometric and meristic characteristics that are effected by environmental conditions of their habitat. Therefore, the osteological features can provide robust identification keys to study their taxonomy and phylogenetic relationships, since these features are useful in this regard among fishes by providing proper identification keys for their distinction (Rojo 1991; Nasri et al 2013; Mafakheri et al 2014). Hence, the present study was conducted to provide a detailed osteological description of *A. mossulensis* and comparing it with those of other species belong to the genus *Alburnus* in Iran *i.e. A. amirkabiri, A. atropatenae, A. chalcoides*, and *A. filippii*. The finding of this study can provide osteological data of *A.*

mossulensis as a basis for further researches on the other members of this genus and their phylogenetic relationship.

Material and Method. Five specimens of *A. mossulensis* with a mean standard length $(\pm SD)$ of 11.4 ± 3.2 cm were collected from Gamasiab River (Tigris River drainage, Kermanshah Province, Iran) (Figure 1) by electrofishing and fixed in 4% buffered formaldehyde. For osteological examination, the specimens were cleared and stained with alizarin red S and alcian blue according to Taylor & Van Dyke (1985). The 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). Drawings were made using CorelDrawX6 software. The nomenclature of the skeletal elements was followed Rojo (1991). Detailed descriptions of the osteological features of *A. atropatenae*, *A. chalcoides* and *A. filippii* were provided by Jalali Roshan (2013) and those of *A. amirkabiri* by Jalili et al (2015) and Mousavi-Sabet et al (2015).

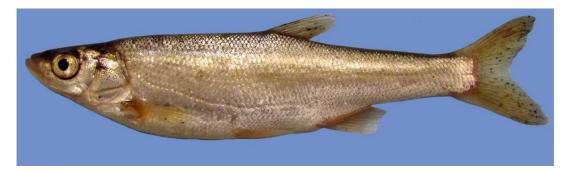


Figure 1. Lateral view of Alburnus mossulensis from Gamasiab River.

Results. The ethmoid region comprises the supra-ethmoid, lateral-ethmoid, preethmoid-I, nasal and vomer. The supra-ethmoid consists of two vertical and horizontal sections (Figure 2c). The horizontal section bears some dorsal pores and two anterolateral pointed processes; the lateral and dorsal margin of this bone is serrated. The vertical section of the supra-ethmoid is connected to both lateral-ethmoid and vomer. Two nasal bones are located in the lateral side of the supra-ethmoids; they have two lateral pores (Figure 2b). The supra-orbital canal runs through nasal bones. The lateral ethmoid is concaved anteriorly and posteriorly, flattened ventrally and pointed lateroventrally. This bone is attached to supra-ethmoid and frontal dorsally, vomer and parasphenoid ventrally and orbitosphenoid posteriorly. The lateral ethmoid bears an anterior process. The anterior part of the vomer is V-shaped and its posterior margin is blunt and connected to the parasphenoid (Figure 2a). The pre-ethmoid-I has a small semi-circular structure (Figure 2 a,b,c) and in some parts is cartilaginous.

The orbital region consists of the frontal, parasphenoid, ptersphenoid, orbitosphenoid and circumorbital bones. The frontal is large and has some small pores in its posterior portion. The middle part of the frontal bears a lateral process which is attached to the sphenotic process. Contact between the frontal and supra-ethmoid is serrated (Figure 2b). Two orbitosphenoids are fused forming a blade-shaped process along their ventral border. The pterosphenoid has a ventral pointed process which is connected to the parasphenoid wings; its dorsal part is connected to the posterior part of the frontal. The parasphenoid is anteriorly connected to the vomer and posteriorly to the middle part of the basioccipital. The dorsal part of this bone is bifurcated.

Circumorbital series comprises of 6-7 infra-orbital and supra-orbital elements (Figure 3c); the lachrymal is almost square in shape and has a dorsal protuberant. The 5th and 6th infra-orbitals are smaller than the others and attached to the lateral process of the sphenotic. The supra-orbital element is located in the lateral part of the frontal (Figure 2b).

The otic region comprises of the epiotic, parietal, pterotic, prootic and sphenotic. The parietal bears a dorso-lateral process and some small pores; the anterior margin of this bone is serrated (Figure 2a). The supratemporal commissure and supraorbital canals goes along the posterior and the lateral edge of the parietal, respectively. The sphenotic has an antero-lateral process which is connected to the postero-lateral edge of the frontal (Figure 2a); this bone is ventrally connected to the ptersphenotic and prootic. The dorsal part of the epiotic is covered by the parietal. The pterotic is broadened and covers the posterior part of the sphenotic. The prootic possesses a posterior protuberance and an anterior foramen; the prootic is attached to the parasphenoid via an ascending process.

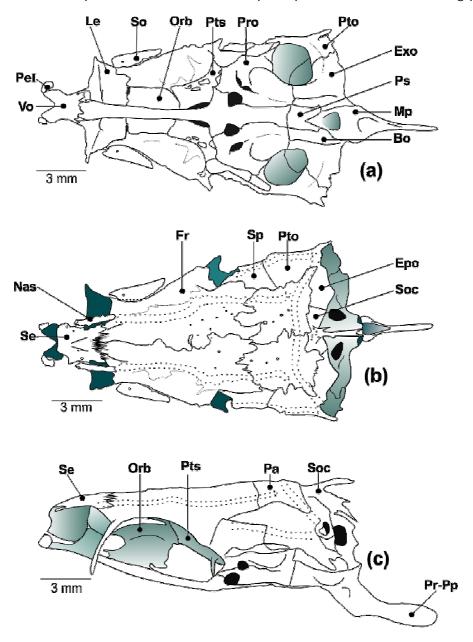


Figure 2. Neurocranium of *Alburnus mossulensis*, (a) ventral, (b) dorsal and (c) lateral view. Bo - basioccipital; Epo - epiotic; Exo - exoccipital; Fr - frontal; Le - lateral ethmoid; Mp - ventral masticatory plate; Nas - nasal; Orb - orbitosphenoid; Pa - parietal; Pe - preethmoid I; Pro - prootic; Pr-Pp - posterior pharyngeal process; Ps - parasphenoid; Pto - pterotic; Pts - pterosphenoid; Se - supraethmoid-ethmoid; Soc - supraoccipital; Spo - sphenotic; Vo - vomer.

The occipital region consists of the supraoccipital, exoccipitals and basioccipital. The supraoccipital is pentagon in shape and bears a blade-shaped crest that extends between two foramen of the exoccipital (Figure 2a). The basioccipital has a pointed pharyngeal process and concaved masticatory plate (Figure 2b). The exoccipital is enclosed by the

supraoccipital and epiotic dorsally and basioccipital and prootic ventrally; this bone has a large foramen in its middle part. The neurocranium is articulated with hyomandibular bone by two facets; the ptersphenoid, sphenotic and prootic form the first one and second one is longer formed by the pterotic, sphenotic and prootic.

The upper jaw consists of the premaxillary and maxillary. The maxillary is long and bears a descending process in its anterior part; the middle part of this bone is broadened. The dorsal part of the maxillary is narrow and bears a small groove. The premaxillary is located under the maxillary and has a rostral process that continued upwardly. A free kinethmoid bone is situated between two maxillary in front of the vomer (Figure 3a).

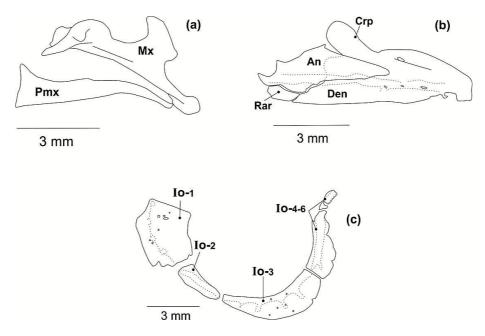


Figure 3. Anterior view of the upper jaw (a), lateral view of lower jaw (b) and lateral view of circumorbital series (c) in *Alburnus mossulensis*. An - angular; Crp - coronoid process; Den - dentary; Io-1-6 - Infraorbital 1-6; Mx - maxillae; Pmx - pre-maxillae; Rar - retroarticulare.

The lower jaw comprises dentary, angular and retroarticular (Figure 3b). The coronoid process of the dentary is finger-like and bended dorsally; there are two pores in the middle part of this bone; the posterior part of the dentary is connected to the angular and retroarticular. The retroarticular is triangular in shape and attached to the angular dorsally. The postero-dorsal part of the angular bears an articulatory facet which is articulated to the quadrate.

The suspensorium consists of the hyomandibular, ectopterygoid, endopterygoid, metapterygoid, symplectic, quadrate and palatine (Figure 4). The hyomandibular is broadened dorsally and narrowed ventrally; this bone bears three pores in the dorsal margin. The quadrate has a posterior process; it is anteriorly connected to the ectopterygoid, dorsally to the endopterygoid and posteriorly to the metapterygoid and symplectic (Figure 4). The symplectic is a long bone extending to the ventral part of the hyomandibular. The palatine is posteriorly connected to the endopterygoid and anteriorly to the vomer. The metapterygoid has a triangular protuberance on its dorsal rim and restricted by the endopterygoid, quadrate, symplectic and hyomandibular. There are two shallow grooves on the dorso-lateral margin of the metapterygoid. The anterior portion of the endopterygoid is narrower than its posterior part; the ventral margin of this bone is covered by the quadrate and ectopterygoid and its dorsal part is covered by the metapterygoid.

The opercular series composed of the opercle, preopercle, interopercle and subopercle (Figure 4). The ventral part of the opercle is wider and its posterior edge is

slightly concaved. There is a facet for articulating with the hyomandibular in the anterodorsal portion of the opercle. The anterior part of the subopercle is wide. The posterior margin of the interopercle is wider; the dorsal part of this bone overlaps with the preopercle. The preopercle is L-shaped and its vertical part is longer than the horizontal part.

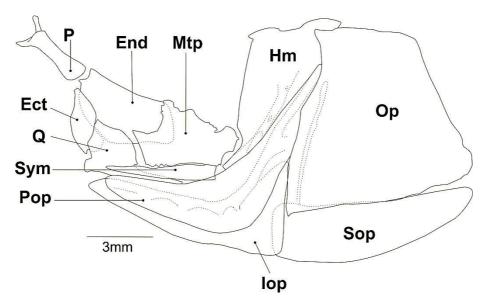


Figure 4. Lateral view of suspensurium in *Alburnus mossulensis*. Ect - ectopterygoid; End - endopterygoid; Hm - hyomandibular; Iop - interopercle; Mtp - metapterygoid; Op - opercle; P - palatine; Pop - praeopercle; Q - quadrate; Sop - subopercle; Sym - symplectic.

The skeleton of the branchial apparatus includes four pairs of the ceratobranchials and epibranchials, three pairs of hypobranchials, two pairs of pharyngobranchial and three unpaired basibranchial bones (Figure 5a). The pharyngobranchial-2 is semicircular in shape and larger than the others. The posterior hypobranchial is crescent in shape and larger than the others. The 2nd and 3rd epibranchial have the ventral processes; the anterior epibranchial is the widest one. The pharyngeal teeth bear a ventral pointed processes; it bears a posterior pointed process that is bended dorsally. The pharyngeal teeth are long and distributed in three rows with a dental formula of 5.2-2.5.

The skeleton of the hyoid arch consists of the paired epihyals, hypohyals and ceratohyals and unpaired urohyal and basihyal, and three pairs of the branchiostegal rays (Figure 5b). The basihyal is a thin and long bone situating between the hypohyals. The urohyal consist of the vertical and horizontal parts. The posterior margin of the horizontal part of this bone is concaved and wider than the anterior part. The dorsal part of the vertical part of this bone is pointed; the anterior part of the urohyal is bifurcated and connected to the ventral part of the hypohyals via two ligaments (Figure 5b). The interhyal is a small and rounded and ventrally attached to the dorsal part of the epihyal and dorsally to the ventral part of the hypomandibular. The posterior part of the ventral and ventral part is bifurcated and attached to the dorsal and ventral hypohyals.

Pectoral girdle consists of the cleithrum, postcliethrum, supracleithrum, coracoid, mesocoracoid, scapula, posttemporal, supratemporal and radials of the pectoral fin (Figure 6a). The cleithrum is L-shaped and its horizontal part laterally attached to the coracoid and posteriorly to the scapula; its vertical part bears a ventral protuberance that is connected to the postcleithrum. The coracoid is crescent in shape and its postero-dorsal part has a blade shaped process which is connected to the mesocoracoid. The middle part of the mesocoracoid is thin and its ventral edge is attached to the coracoid and scapula; the dorsal part of mesocoracoid is connected to the cleithrum and scapula.

There are four radials in the pectoral girdle where the first and forth one are wider. The pectoral fin has one unbranched and 12 branched rays.

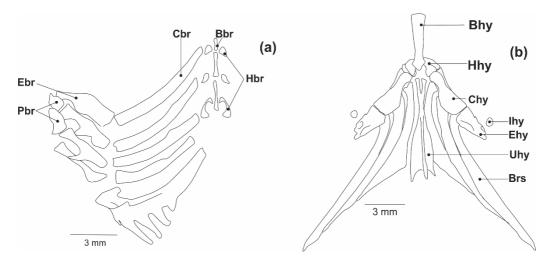


Figure 5. Dorsal view of branchial (a) and hyoid (b) arches in *Alburnus mossulensis*. Bhy - basihyal; Brs - branchiostegal rays; Chy - ceratohyal; Epy - epihyal; Hhy - dorsal and ventral hypohyal; Ihy - interhyal; Uhy - urohyal; Bbr - basibranchial; Cbr - ceratobranchial; Ebr - epibranchial; Hbr - hypobranchial; Pbr - inphrapharyngobranchial.

Pelvic girdle includes the paired of the basipterygium, meta-pterygium and lateralpterygium (Figure 6b). The pelvic fin has one unbranched and eight branched rays. There is a deep hollow in the anterior part of the basipterygium; this bone has a dorsal process. A free paired lateral-pterygium is observed in the lateral side of the basipterygium and three pair of the meta-ptrygium is located behind the basipterygium, where the lateroexternal one is the largest one.

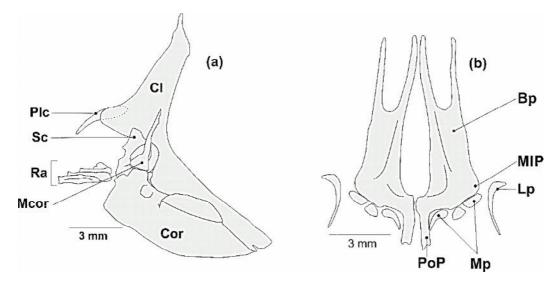


Figure 6. Medial view of the pectoral girdle (a) and ventral view of the pelvic gidle (b) in *Alburnus mossulensis*. Bp - basipterygium; CI - cleithrum; Cor - coracoid; Lp - lateralpterygium; Mcor - mesocoracoid; MIP - mid-lateral process of basipterygium; Mp - metapterygium; Plc - postcleithrum; PoP - posterior process of basipterygium; Ra - radials; Sc - scapula.

The dorsal fin has three unbranched and eight branched rays, nine pterygiophores and one stay (Figure 7b). The first pterygiophore is the largest and supports unbranched rays. In front of the dorsal fin, 11 free supraneural bones are observed (Figure 7a). The first supraneural is broadened and the supraneural 3-9 are thin and long. The anal fin

bears three unbranched and 11 branched rays, 12 pterygiophores and a small stay bone (Figure 7c). The largest pterygiophore supports 2 unbranched rays.

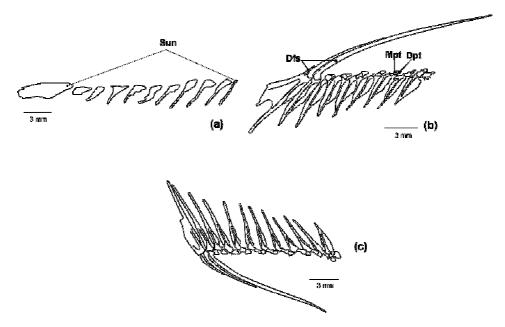


Figure 7. Lateral view of the supraneurals (a), dorsal fin (b) and anal fin (c) in *Alburnus mossulensis*. Dfs - dorsal fin spine; Dpt - distal petrigiophore; Mpt - median petrigiophore; Sun - supraneural.

In the axial skeleton, the number of the vertebrae is 43; the cranial and caudal parts of the vertebral column have 22 and 18 centra, respectively. The weberian apparatus is formed by the four anterior centra with four pair ossicles, including tripus, intercalarium, scaphium and claustrum (Figure 8a). The first centrum has a small pleural rib; the pleural rib of the second centrum is long and bended dorsally, pleural rib of the 3rd centrum is absent; and pleural rib of 4th centrum is long and bifurcated.

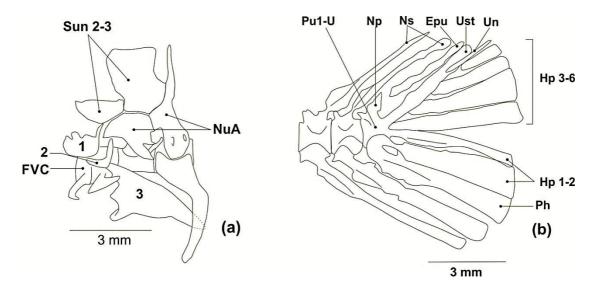


Figure 8. Lateral view of the weberian apparatus (a) and the caudal skeleton (b) in *Alburnus mossulensis*. Epu - epural; FVC - first vertebra centrum; Hp1–6 - hypural plates 1–6; Ns - neural spine; Np - neural process; NuA - neural arch; Ph - parhypural; Pu1-U - compouned centrum; Sun - supraneural; Ust - pleurostyle; Un – uroneural; 1 - Claustrum; 2 - interclarium; 3 - tripus.

The skeleton of the caudal fin composed of the epural, parhypural, pleurostyle, uroneural and six hypurals bones (Figure 8b). Caudal fin in studied species has 19 branched rays and various numbers of the procurrent rays. The two uroneurals are situated on the lateral side of the pleurostyle.

Discussion. Studied species shows numerous differences in the upper and lower jaws, neurocranium, suspensuriom and caudal skeleton compared to those of *A. amirkabiri*, *A. filippii*, *A. atropatenae* and *A. chalcoides*. In the jaws, the coronoid process is bended dorsally in *A. mossulensis*, similar to *A. filippii* and *A. amirkabiri* (Mousavi-Sabet et al 2015) versus posteriorly oriented one of *A. chalcoides* and *A. atropatenae*. The anteroventral process of the dentary is well-developed and posteriorly inclined in *A. chalcoides* and *A. amirkabiri* (Jalili et al 2015), whereas this process was not found in the other compared species. The medial process of the maxillae is pointed in *A. mossulensis*, *A. chalcoides* and *A. filippii* (Jalali Roshan 2013) while it is blunt in *A. amirkabiri* and *A. atropatenae* (Jalali Roshan 2013; Jalili et al 2015).

In the suspensorium, according to Mousavi-Sabet et al (2015) the dorsal rim of the entopetrygoid and metapetrygoid are reduced in *A. atropatenae*, the same state is observed in *A. chalcoides*, *A. filippii* and *A. amirkabiri* (Jalali Roshan 2013; Jalili et al 2015). The anterior process of the palatine of the *A. mossulensis* is short and thick versus longer and narrow one of the other compared with the other *Alburnus* species. In *A. mossulensis*, as in *A. amirkabiri* and *A. filippii* the anterior edge of the hyomandibular is protuberant (Jalali Roshan 2013; Jalili et al 2015), whereas in *A. atropatenae* and *A. chalcoides* it is straight (Jalali Roshan 2013; Jalili et al 2015), whereas in *A. atropatenae* and *A. chalcoides* it is straight (Jalali Roshan 2013). The dorsal and posterior edge of the opercle is straight in *A. mossulensis* versus concaved ones of *A. atropatenae* and *A. filippii* (Jalali Roshan 2013). In addition, in *A. amirkabiri* and *A. chalcoides*, the dorsal and posterior parts of the opercle are concaved and straight, respectively (Jalali Roshan 2013; Jalili et al 2015). In *A. mossulensis*, as in *A. chalcoides*, the antero-dorsal process of the opercle is well-developed and pointed (Jalali Roshan 2013), whereas in the other Alburnus species it is rounded and reduced. The preopercle of *A. mossulensis* is L-shaped with right-angled unlike versus other compared species.

In the caudal skeleton, the second centrum bears two neural-spines in *A. amirkabiri* and *A. atropatenae* (Jalali Roshan 2013; Jalili et al 2015). The zygapophys process of the parhypural is developed only in *A. mossulensis* and *A. atropatenae*.

In the neurocranium and axial skeleton, the posterior part of the vomer is blunt in *A. mossulensis* and *A. filippii* (Jalali Roshan 2013), whereas it is pointed in *A. amirkabiri*, *A. atropatenae* and *A. chalcoides*. *A. mossulensis* bears 11 supraneurals versus other compared species which have 9-10 supraneurals (Mousavi-Sabet et al 2015). The vertebral number in *A. mossulensis* and *A. filippii* is 43, whereas that of *A. amirkabiri*, *A. atropatenae* and *A. chalcoides* is 40-42.

Conclusions. Based on the obtained results, *A. mossulensis* can be recognized from the other osteologicaly studied members of the genus *Alburnus* in Iran by having a leveled dorsal and posterior parts of the opercle, an L-shaped preopercle with right-angled, blunt posterior part of the vomer, possessing 11 supraneural, having 43 centra, developed zygapophys process, short and thick anterior part of the palatine and dorsally bended the coronoid process.

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