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Descriptive osteology of *Alburnoides holciki* (Teleostei: Cyprinidae) from Iran

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Abstract
Osteological structure of *Alburnoides holciki* Coad & Bogutskaya, 2012 from Hari River was described. Three specimens were cleared and stained for osteological examination and its detailed osteological characteristics and differences with available osteological data of other members of the family Cyprinidae, subfamily Leuciscinae, were provided. The genus *Alburnoides* had no unique osteological characteristics that can be used for genus identification. However, there are much variation in the osteological structures of the specimens, especially, the caudal skeleton.

Keywords: Fish bone, Cyprinidae, Harirud, Skeleton.

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Introduction

The genus *Alburnoides* is widely distributed from Europe to Asia Minor and Central Asia (Bogutskaya and Coad 2009; Coad and Bogutskaya 2009, 2012; Turan et al. 2014; Mousavi-Sabet et al. 2015 a, b; Schönhuth et al. 2018). Twelve *Alburnoides* species were considered to occur in Iranian Inland waters. *Alburnoides eichwaldii* (De Filippi, 1863) from Kura-Aras River system (Bogutskaya and Coad 2009), *A. namaki* Bogutskaya & Coad, 2009 from Namak Lake Basin, *A. nicolausi* Bogutskaya & Coad, 2009 and *A. idignensis* Bogutskaya & Coad, 2009 from the Tigris River drainage, *A. qanati* Coad & Bogutskaya, 2009 from Kor River Basin, *A. petrubanarescui* Coad & Bogutskaya, 2009 from Urmia lake Basin, *A. holciki* Coad & Bogutskaya, 2012 from the Hari River Basin, *A. tabarestanensis* Mousavi-Sabet et al. 2015a from the southern Caspian Sea basin, *A. parhami* from Atrak River drainage, *A. coadi* from Dasht-e Kavir Basin, *A. samiii* from Sefid River drainage (Mousavi-Sabet et al. 2015b), and *A. damghani* Jouladeh Roudbar et al. 2016a from Damghan River system. Recently, *Alburnoides* cf. *taeniatus* (Kessler, 1874) was reported from Hari River (Jouladeh-Roudbar et al. 2016b), but revision needs for its taxonomic status (Esmaeili et al. 2017). In addition, Eagderi et al. (2019) showed that *A. parhami*, *A. coadi* and *A. idignensis* are invalid species. They purposed *A. parhami* as a synonym of *A. holciki*, *A. coadi* as synonym of *A. namaki* and *A. idignensis* as synonym of *A. nicolausi* (Eagderi et al. 2019).

The species in the genus *Alburnoides* is distinguished based on having a combination of some morphological characters and different fin ray counts. Most description are based on morphological characters and molecular approaches (Jouladeh Roudbar et al. 2016a). Although there are many benefits to morphological descriptions, but morphological characters are sensitive to environmental changes. Also, molecular approaches are expensive and time consuming. So using different approaches are needed to distinguish *Alburnoides* spp. from Iranian inland waters.

Osteological characters are important for identification and classification of fish species, and understanding biological features of fishes such as swimming, feeding and respiration (Helfman et al. 2009; Keivany 2014a, b, c, d, 2017). In addition, the skeletal structures contain more biological information that researchers use to distinguish environmental conditions of fish habitats (Jalili and Eagderi 2015). Osteological characters can be utilized in ichthyological studies, especially fish systematics and potentially can resolve some complexities in

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Figure 1. Lateral view of *Alburnoides holciki* from Hari River drainage.

this context.

Since identification of *Alburnoides* species using morphological features show many intraspecific overlapping and due to morphological similarity of the members of the genus *Alburnoides*, using osteological data may help to better understanding of their taxonomic relationships. Therefore, the descriptive osteology provides a proper case study to understand the adaptation of skeletal features to a unique habitat and evolution of osteological features. Since there is no information available on the osteological features of the Iranian members of the genus *Alburnoides*. Therefore, this study was conducted to provide detailed osteological features of *A. holciki* from the freshwaters of Iran. The findings of this study also can be used as a basis for further phylogenetic studies of the members of this genus based on the osteological data.

Material and Methods

Three specimens of *A. holciki* were collected using electrofishing from Hari River, then fixed in 10% buffered formalin after anesthesia by 1% clove oil solution (Fig. 1). The specimens were cleared and stained with Alcian blue and Alizarin red S according to the protocols of Taylor and van Dyke (1985) and Sone and Parenti (1995) with minor modifications. The cleared and stained specimens were studied using a stereomicroscope (SMP-120 model) and their skeletal elements were dissected and photographed by a digital camera. Drawing of the specimens were performed using CorelDraw X7 software. The terminology of skeletal elements was based on Rojo (1991) and Helfman et al. (2009).

Results

Infraorbital series: The circumorbital series include five infraorbitals (5^{th} is not shown in Fig. 2A) and one small supraorbital (Fig. 2B). The largest element of this series is roughly pentagonal in shape. Supraorbital is oval in shape locating at the anterolateral part of the frontal.

Neurocranium: In the ethmoid region, the supraethmoid is concaved in shape and its posterior part is serrated or concaved. The two elongated nasal capsules are present in the anterolateral part of the vomer. A small kinethmoid exists between the maxillary bones that sometimes is concave, convex and undulant. The sphenotic has an uncus view. The paired supraorbitals are triangular in shape and truncated in the corners. The paired lateral ethmoids are stream-lined in shape. The frontals are the largest bony element of the neurocranium roof, covering almost 60% of the head roof and its anterior edge sometimes attached to the posterior border of supraethmoid and lateral ethmoid. The two orbitosphenoids are fused connectingto the dorsal face of the parasphenoid. The pterosphenoids are bigger than orbitosphenoid that forms posterior wall of the orbit. These bones are attached to the orbitosphenoid anteriorly, dorsally to the frontal and attached to the sphenotic posteriorly. The parasphenoid is forked in shape in the anterior and posterior regions and its interior edges overlapped with vomer and posterior edge overlapped with basioccipital.

The occipital region is composed of the supraoccipital, exoccipitals and basioccipital. The supraoccipital is pentagonal 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 is a concaved masticatory plate. In the otic region, the parietal covers the upper and posterior portion of the neurocranium (Fig. 2B). The occipital region consists of paired exoccipital

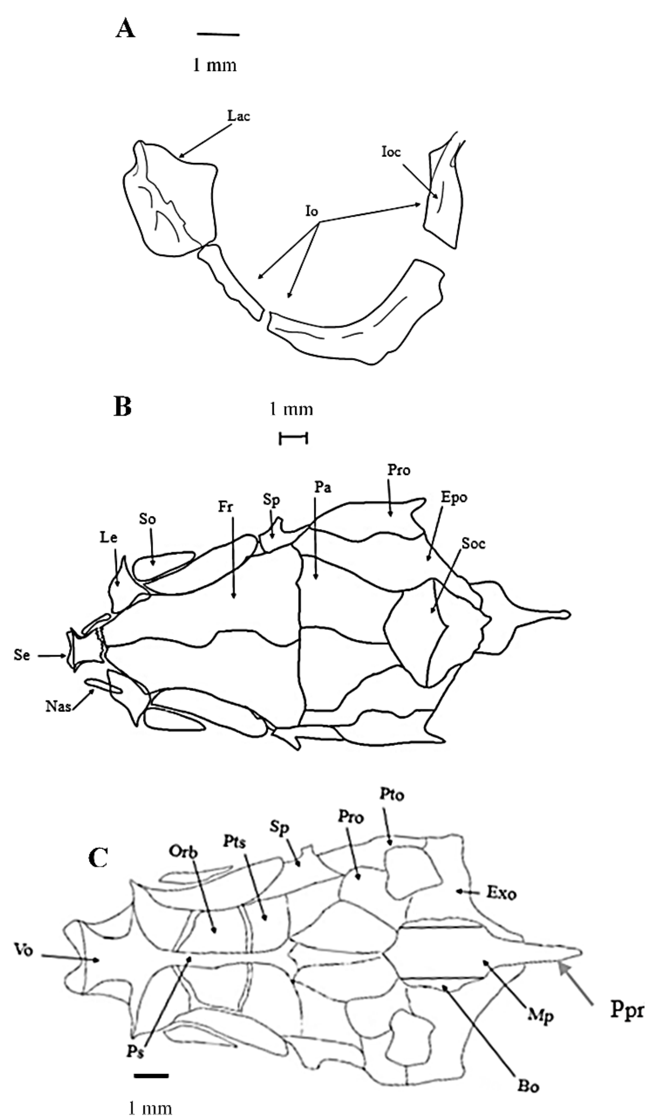


Figure 2. Lateral view of the Infraorbital series (A. Io: Infraorbital; Ioc: Infraorbital sensory canal; Lac: Lachrymal) and Circumorbital series, (B) dorsal and (C) ventral views of the neurocranium in *Alburnoides holciki* (Bo: Basioccipital; Epo: Epiotic; Exo: Exoccipital; Fr: Frontal; Le: lateral ethmoid; Mp: masticatory plate; Nas: Nasal; Orb: orbitosphenoid; Pa: Parietal; Ppr: Pharyngeal process; Pro: Prootic; Ps: Parasphenoid; Pto: Pterotic; Pts: pterosphenoid; Se: Supraethmoid; Soc: Supraoccipital; So: Supraorbital; Sp: Sphenotic; Vo: Vomer).

and both unpaired supraoccipital and basioccipital. The paired exoccipitals are connected to the posterior part of the supraoccipital. Spinal column and Weberian apparatus attached by a pharyngeal process in the dorsal part of the basioccipital (Fig. 2C).

Weberian apparatus: The weberian apparatus made of the first four centra with their related ossicles, including claustrum, scaphium, intercalarium and tripus. The scaphium (J-shaped in the lateral view) and claustrum are located on the first centrum. The thin intercalarium is located on the second centrum. There are many pores on the surface of the third supraneural (Fig. 3). Pore sizes and counts are different in three studies specimens. It may be due to different levels of ossification in fishes that highly related to fish size.

Opercular series and hyomandibular arch: The anterior element, the preopercle is roughly L-shaped. The ventral part of the preopercle overlaps the dorsal part of the interopercle. The subopercle is located below the opercle overlapping the branchiostegal rays. So, the anterior part of the subopercle is located on the interopercle (Fig. 4).

The hyomandibular is long and almost Y-shaped and its posterior part bears two protuberances; one for connection to the opercle by the opercular joint and spine and socket joint, while its anterior part bears one protuberance for attachment to the otic capsule. The ventral part of the hyomandibular is connected to a long and serrated symplectic which is enclosed by a bony complex including ectopterygoid, endopterygoid, metapterygoid, and quadrate. The palatine has three shapes in the specimens, including triangular and curved, forked and concaved from lateral view and thin and tripartite. The palatine is almost concaved in one side. The ectopterygoid is L-shaped.

Branchial arch: The basibranchials are thin rectangle and concaved from the lateral view. The third basibrachial is longer than basibranchial 1-2. The ceratobranchials are five pairs with the slender strip shape and the last one have different shape possessing the pharyngobranchial teeth with dental formula of 2.5-4.2 (Fig. 5). The pharyngeal teeth were in two rows and in two forms, serrated and unserrated.

Upper and Lower jaws: The upper and lower jaw are the anterior-most elements and thin L-shaped in front view bearing narrow vertical and curved horizontal processes. This bone possesses an anterior descending protuberance. The premaxillaries are ahead or below the maxillary. A mid-lateral ascending and anterior descending processes exist in the maxillary. The three paired bones, including the dentary, angular and

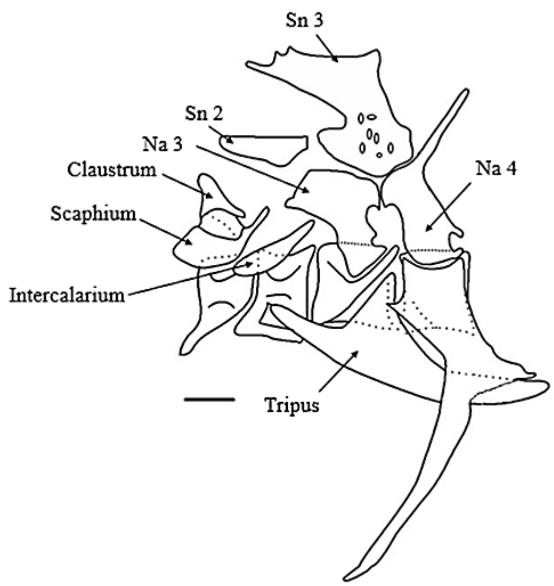


Figure 3. Weberian apparatus (Na: Neural arch; Sn: Supraneural).

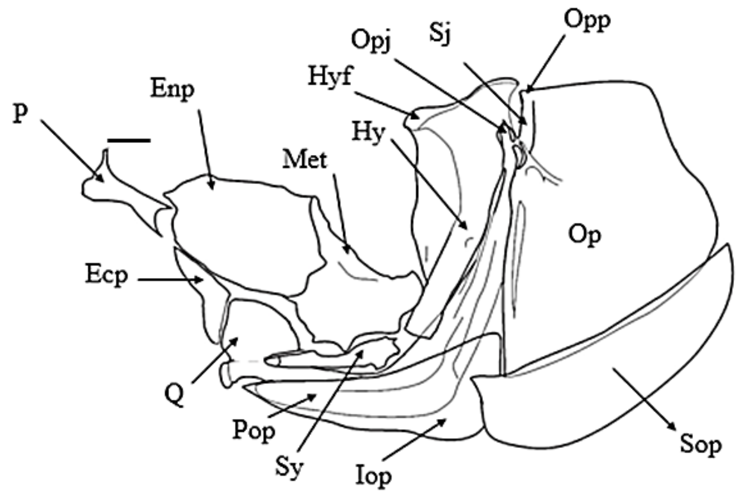


Figure 4. Lateral view of suspensorium and opercular series in *Alburnoides holciki* (Ecp: ectopterygoid; Enp: endopterygoid; Hy: hyomandibulare; Hyf: Hyomandibular joint face; Iop: interopercle; Met: metapterygoid; Op: Opercle; Opj: Opercular joint; Sy: Symplectic; Opp: Opercular prominent process; P: Palatine; Pop: Preopercle; Q: Quadrate; Sj: Spine and socket joint; Sop: subopercle).

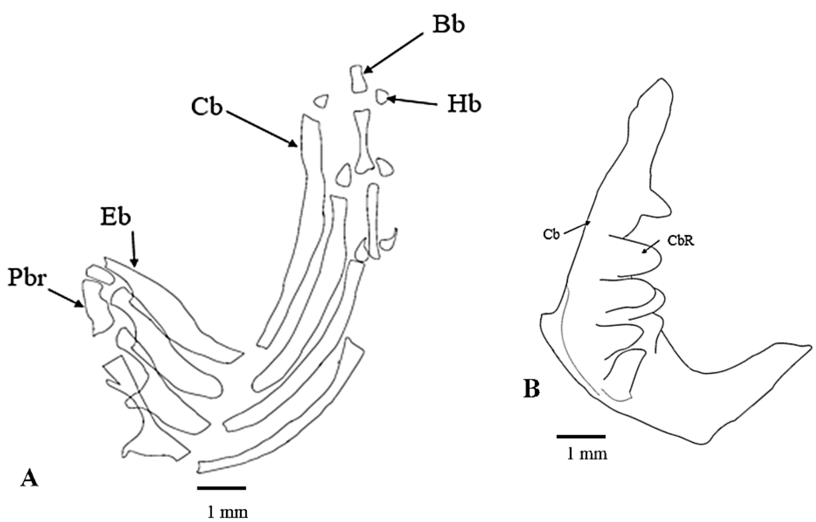


Figure 5. Dorsal view of the branchial apparatus (A) and pharyngeal teeth (B) in *Alburnoides holciki* (Bb: Basibranchial; Cb: Ceratobranchial; CbR: Ceratobranchial rakers (pharyngeal teeth); Eb: Epibranchial; Hb: Hypobranchial; Pbr: Pharyngobranchal).

retroarticular form the lower jaw (Fig. 6). The dentary is large forming the front of the lower jaw having a coronoid process in their corner before joining the angular.

Hyoid arch: The anterior head of the basihyal is wider than its posterior head. The urohyal is like an arrow tail, and its anterior part is bipartite. The urohyal is connected to the cleithrum posteriorly and to the hypohyals and basihyals anteriorly. The internal branchiostegals and lateral branchiostegals are connected to the ceratohyal and lateral branchiostegals to the epihyal. The hyoid arch is connected to the suspensorium (at the rare of the preopercle) by a small rectangular interhyal (Fig. 7).

Pectoral girdle: An antero-medial downward process for overlapping or connecting to the lateral part of the coracoid exist in the ventral part of the cleithrum. The coracoid is wider in ventral part. This bone has an ascending process in dorsal part for connecting to the mesocoracoid. The medial part of the coracoid has a large pore in the surface which connects to the cleithrum. The scapula is located between the cleithrum, coracoid and mesocoracoid; the scapula has a small foramen and articulates to the first unbranched ray. The upper part of the

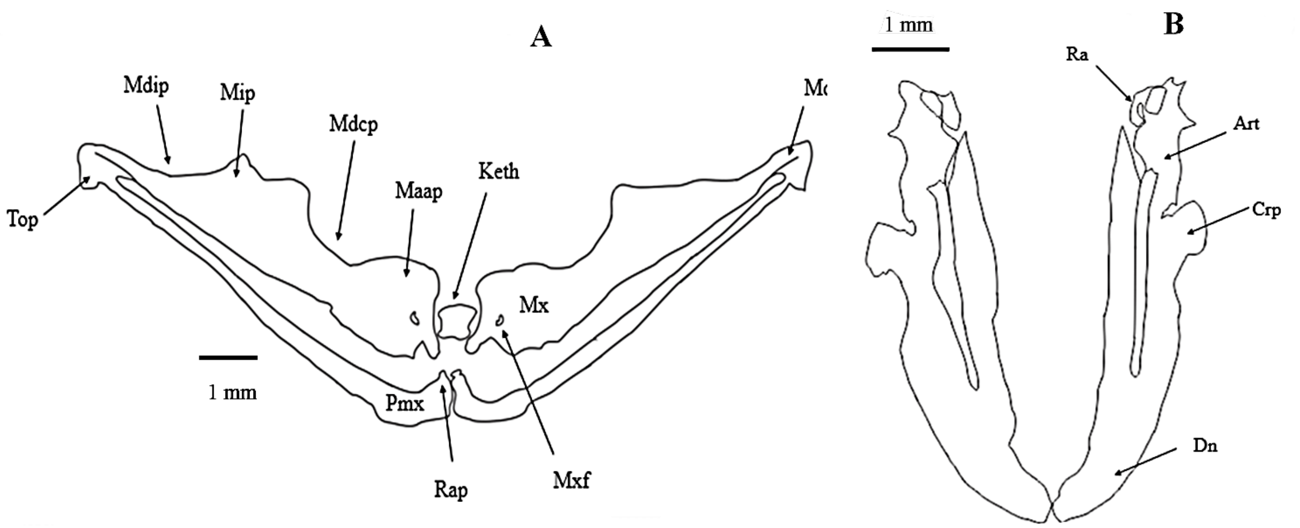


Figure 6. Ventral view of the upper (A) and lower jaw (B) in *Alburnoides holciki* (Art: Articular; Crp: Coronoid process; Dn: Dentary; Keth: Kinethmoid; Maap: Maxillary anterior assending process; Mdcp: Maxillary dorsal concaved border; Mdip: Maxillary dessending process; Mdp: maxillary distal process; Mip: Maxillary mid-lateral assending process; Mx: Maxillary; Mxf: maxillary foramen; Pmx: Premaxillary; Ra: Retroarticular; Rap: Rostral assending process; Top: Tail of premaxilla).

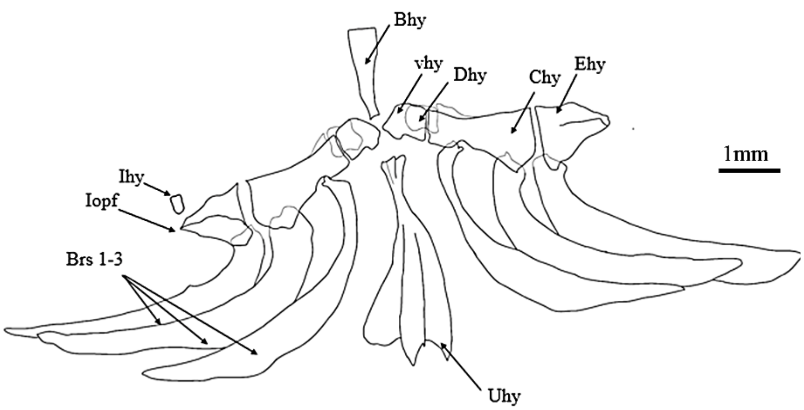
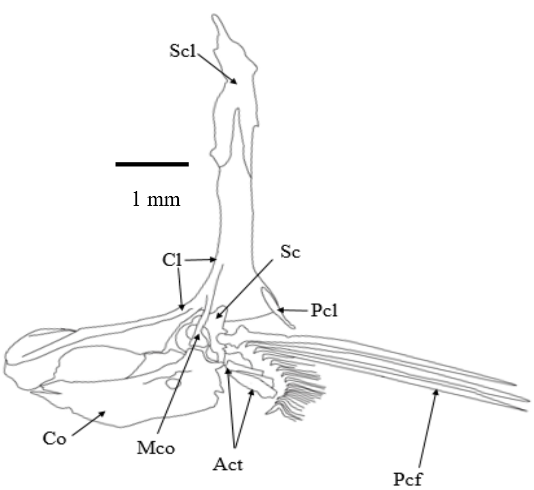


Figure 7. Dorsal view of the hyoid arch in *Alburnoides holciki* (Bhy: Basihyal; Brs 1-3: Branchiostegale 1-3; Chy: Ceratohyal; Dhy and Vhy: Dorsal and ventral hypohyal; Ehy: Epihyal; Ihy: Interhyal; Uhy- Urohyal; Iopf: Interopercular facet).



coracoid is attached to the mesocoracoid. The dorsal part of the mesocoracoid is attached to the medial face of the cleithrum. The pectoral fin bears four radials and the most internal radial is bifurcated in specimens (Fig. 8). The postcleithrum is slender shaped.

Pelvic girdle: The pelvic bones are horizontally located in the abdominal area. In the pelvic girdle, the basipterygium has U-shaped. The basipterygium has long posterior and mid-lateral processes. In the posterior-lateral side of the basipterygium bones, the two L-shaped

Figure 8. Lateral view of pectoral girdle in *Alburnoides holciki* (Act; Actinus; Cl: Cleithrum; Co: Coracoid; Mco: Mesocoracoid; Pcf: Pectoral fin rays; Pcl: Post Cleithrum; Ptt: Posttemporal; Sc: Scapula; Scl: supracleithrum).

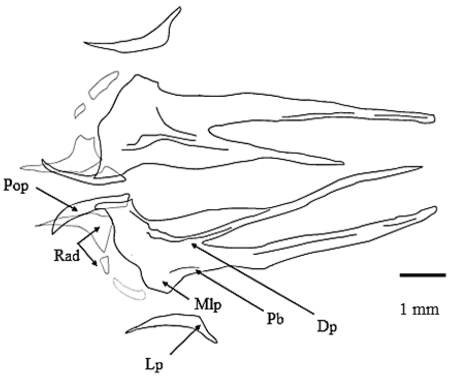


Figure 9. Ventral view of pelvic girdle in *Alburnoides holciki* (Dp: distal process; Lp: lateral pterygium; Mlp: mid-lateral Process; Pb: pelvic bone (basipterygium); Pop: posterior process; Rad: radials).

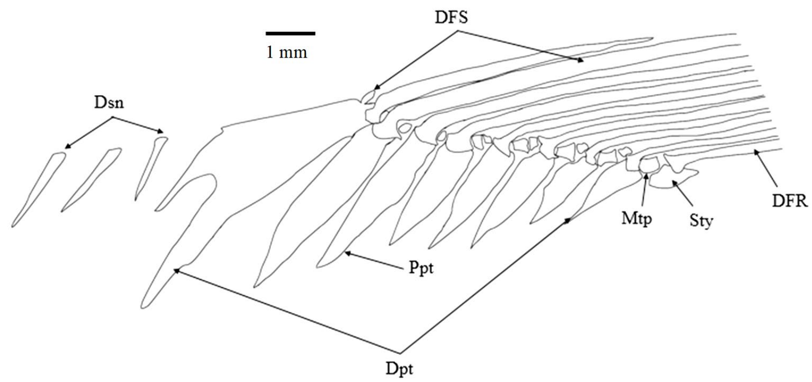


Figure 10. Lateral view of the dorsal fin skeleton in *Alburnoides holciki* (Dfs: Dorsal Fin Spin; Dfr: Dorsal Fin Ray; DPt: Dorsal Pterygiophore; Dpt: distal pterygiophore; Dsn: Dorsal Supraneurals; Mtp: medial pterygiophore; Ppt: proximal pterygiophore; Sty: stay).

lateral pterygia exist.

Dorsal Fin Skeleton: The dorsal fin bears 3 unbranched (first unbranched ray is smaller than the two others), 7½–9½ branched rays (the last two branched rays articulated on a single pterygiophore) and one stay. This semi-triangular shape bone is located after the last branched ray. The first pterygiophore is the largest, forked and supports 1th and 2th unbranched rays. In front of the dorsal fin, a different number of free supraneural bones are observed (more than seven) (Fig. 10).

Anal Fine Skeleton: The anal fin originates at hemal spine 19th-20th and continues to the last one. This fin has three unbranched and 11½–13½ branched rays. The first pterygiophore is the largest (with a wide base) and supports unbranched rays. The 1th and 2th unbranched rays are located at one pterygiophore. A polygonal and large stay bone is located at next to the last branched ray (Fig. 11).

Caudal Fin Skeleton: The caudal fin skeleton includes six hypurals (Fig. 12). The first hypural is larger than the other hypurals and attached to the parhyporal posteriorly. The long epural bone has a different shape in all specimens. The caudal fin shape was different between specimens in. There are 31-35 vertebras in the specimens (excluding the Weberian apparatus). The preurals have epihemal and epineural in the specimens. The caudal fin has 17 branched and 2-3 unbranched rays.

Discussion

Alburnoides holciki Coad and Bogutskaya, 2012 was described from Hari River basin in Afghanistan and Iran, and it bears pharyngeal teeth of -4.2 on the right 5th ceratobranchial and total vertebrate of 41 (including 4 Weberian vertebrae and last complex centrum) which is lower than we observed. They showed that dorsal fin rays were 3 unbranched and 8 ½ branched, but in our study, 7-9 branched dorsal-fin ray were observed. The branched pectoral fin rays were 13 and the anal-fin origin was under the 7th branched ray of the dorsal fin (Coad and Bogutskaya 2012). The anal fin branched rays were 15½ in our study, whereas in Coad and Bogutskaya (2012) reported as 13-16½.

There is no available information about the osteology of *Alburnoides* species, therefore, we compared certain aspects of *A. holciki* osteology with the other Cyprinidae family members such as *Chondrostoma regium, Cyprinion milesi, Garra rossica* (Nasri et al. 2016; Moezzi et al. 2019; Saemi-Komsari et al. 2020). The shape of the opercular series, lower jaw, hyoid arch and branchial skeleton in the Bighead Lotak, *C. milesi* and *C. regium* are similar to that of *A. holciki* and we found that these features could not separate *A. holciki* from other genera (Nasri et al. 2016; Moezzi et al. 2019). This is also true about the shape of the Weberian apparatus

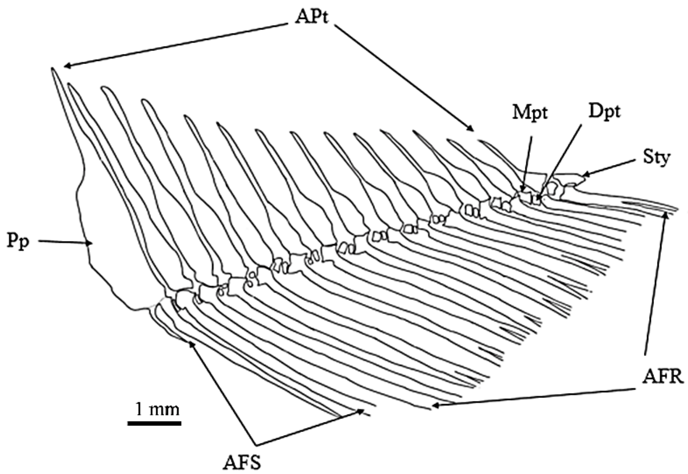


Figure 11. Lateral view of anal fin skeleton in *Alburnoides holciki* (Apt: Anal Pterygiophore; Dpt: distal pterygiophore; Mpt: medial pterygiophore; Pp: proximal pterygiophore; Sty: stay).

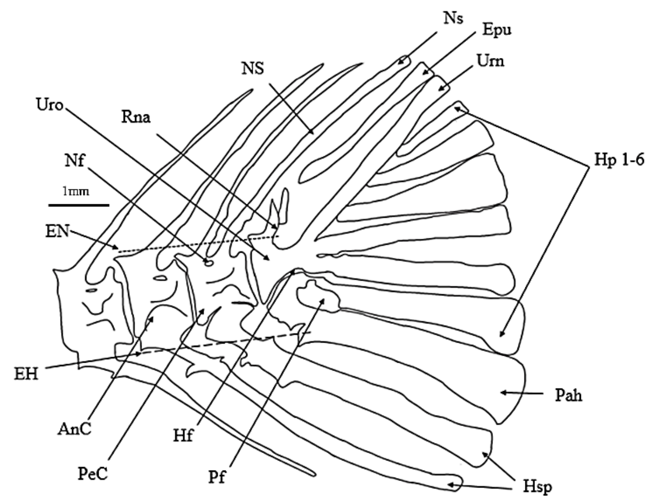


Figure 12. Lateral view of caudal fin skeleton in *Alburnoides holciki* (AnC: Antepenultimate Centrum; EH: Epihemal; EN: Epineural; Epu: Epural; Hp 1-6: Hypural Plates 1-6; Hsp: Hemal Spine; NS: Neural Spine; Pah: Parhypural; PeC: Antepenultimate Centrum; Rna: Rudimentary Neural Arch; Urn: Uroneural; Uro: Urostyle).

in *A. holciki* because it is morphologically very similar to *C. regium* (Heckel, 1843) (Moezzi et al. 2019). Moreover, there are ascending and descending processes in the maxillary in *C. regium*, *Garra* and *Cyprinion* (Alkhahem et al. 1990; Razavipour et al. 2014; Moezzi et al. 2019). The pelvic girdle in *G. rossica*, *B. cyri, C. regium, C. milesi* and the genus *Cyprinion* have the same structure with little variations in the size of different parts. The length of the post cleithrum in *C. milesi*, *B. cyri* and *G. rossica* is longer than that of *A. holciki* and *C. regium* (Nasri et al. 2016; Moezzi et al. 2019; Saemi-Komsari et al. 2020). However, some cases osteological traits can be distinguished different species from each other, e.g. *C. milesi* can be distinguished from *C. macrostomum* and *C. kais* based on shape of the neurocranium, the lateral and posterior corners of the supraethmoid, lower jaw, ceratohyal, horizontal arm of the preopercle and the length of opercle, number of the dorsal fin's pterygiophores, origination of the dorsal fin, supraneurals, the last unbranched dorsal fin ray, and he neural complex (Nasri et al. 2016).

The genus *Alburnoides* has no specific osteological characteristics that can be used for genus identification. Although all species of *Alburnoides* may be distinguishable based on osteological properties. Also, more osteological studies about members of these genera are proposed.

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