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Original Article

Description of skeletal structure and cranial myology of *Cobitis keyvani* (Cypriniformes: Cobitidae)

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Abstract: *Cobitis keyvani* is recently described from the sourhern Caspian Sea basin. Limited information is available about morphological features of *C. keyvani*, therefore this study was conducted to provide osteological characteristics and cranial myology of this species. For this purpose, nine specimens of *C. keyvani* were collected from the Talar River. The specimens were cleared and stained with alizarin red S and alcian blue for osteological examinations. The detailed skeletal structure and cranial muscles of *C. keyvani* were provided. Based on the results, *C. keyvani* can be distinguished from other members of the genus *Cobitis* by a contact between sphenotic and supraoccipital and a contact between pterosphenoid, parasphenoid, prootic and sphenotic in terms of osteological features.

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Introduction

The genus *Cobitis*, a member of family Cobitidae, is widespread from northern Africa, throughout Europe to eastern Siberia, southeast to Central Vietnam (Bohlen and Ráb, 2001; Vasil'eva and Vasil'ev, 2006; Kottelat and Freyhof, 2007). This genus has three valid species in Iran, including *Cobitis linea* (Heckel, 1849), *C. faridpaki* (Mousavi-Sabet, Vasil'eva, Vatandoust and Vasil'ev, 2011) and *C. keyvani* (Mousavi-Sabet, Yerli, Vatandoust, Ozeren and Moradkhani, 2012) (Mousavi-Sabet et al., 2012). However, *C. tenia* (Linnaeus, 1758) have been reported from Iran including southern Caspian Sea basin (Abdoli and Naderi, 2009); there are believes that *C. taenia* is a northern European species (Bohlen and Ráb, 2001) and its occurrence in the southern Caspian Sea basin is unlikely (see in Mousavi-Sabet et al., 2012).

This genus characterized by an elongated and compressed body, erectile spine below the eye, three pairs of short barbels, minute scales cover the body, small dorsal and anal fins, caudal fin rounded or truncated (Coad, 2014). Males also have bony extensions of their pectoral fin rays, known as lamina circularis.

Cobitis keyvani was recently described from Talar River (sourhern Caspian Sea basin) (Mousavi-sabet et al., 2012). This species is distinguished from *C. linea* by lack of second lamina canestrini at the base of the first pectoral fin ray, and from *C. faridpaki* by large, dark and obvious spots along the mid-flank (Coad, 2014). Since, limited information is available about morphological features of *C. keyvani*, therefore, this study was conducted to provide a detailed osteological characteristics and cranial myology of this species. The results will provide a basis for further phylogenetic study of Iranian member of the genus *Cobitis* using osteological and morphological data.

Material and methods

For osteological examination, nine specimens of

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Figure 1. Lateral view of *Cobitis keyvani* from Talar River.

C. Keyvani (Fig. 1) with mean standard length of 58.7 ± 0.6 mm (Mean ± SD) were collected from the Talar River (in the sourhern Caspian Sea basin, Iran). The caught specimens were anaesthetized using 1% clove solution and then fixed in 4% buffered formalin. For osteological examination, the specimens were cleared and stained with alizarin red S and Alcian blue according to the protocol of Taylor and Van Dyke (1972). Muscle fibers of the dissected specimens were stained according to Bock and Shear (1972). The stained specimens were studied using a stereomicroscope (Leica MC5), and different skeletal elements were dissected and scanned by a scanner equipped with a glycerol bath (Epson V700). The obtained images were drawn using CorelDrawX6 software. The terminology of skeletal elements follows Rojo (2010) and Sawada (1982) and Muscle terminology follows Winterbottom (1974). The scale bar of all drawings shows 1 mm.

Result and Discussion

Osteology of Cobitis keyvani

Neurocranium: In dorsal view, the posterior part of the neurocranium is oval-shaped and its anterior part is longer and narrower (Fig. 2). The ethmoid region consists of the lateral ethmoid, preethmoid II, supraethmoid-ethmoid, kinethmoid and pre-vomer (Figs. 2a, b, c). The supraethmoid-ethmoid is blade-shaped positioning on the middle of the pre-vomer and has a relatively deep depression on its anterior margin and a rounded and downward projection under this depression similar to *C. taenia* (Vasil'eva, 1984). The anterior part of the pre-vomer is wide; pointed posteriorly and overlaps with the anterior part of the parasphenoid. The two narrow preethmoid II are positioned at the anterior part of the prevomer. The preethmoid II bears two processes posteriorly *viz*. the dorsal and ventral processes; the dorsal process is articulated with the anterior part of the autopalatine and the ventral one with the anterior part of the pre-vomer. The preethmoid also bears a latero-external pointed process and an anterior facet which articulates with the maxilla. The lateral ethmoid is a long bone that has two posterior processes and one vertical process anteriorly which is connected to the orbitosphenoid (Fig. 2b). The lateral ethmoid is moveable and projected from skin. A free broad kineethmoid is located between two maxillae (Fig. 4a).

The orbital region is composed of the frontal, orbitosphenoid, pterosphenoid, parasphenoid, lacrimal and sclerotic bones. The frontals have long and narrow anterior part. The frontals are separated posteriorly by the fontanle. The orbitosphenoid is dorsally connected to the anterior part of the frontal and ventrally to the parasphenoid. The pterosphenoid is ventrally connected to the parasphenoid and dorsally to the posterior part of the frontal. Sawada (1982) mentions the pterosphenoid is attached only to the parasphenoid in *Cobitis*, *Niwaella*, *Sabajenewia*, *Lepidocephalus* and *Acanthopsoides*, but in *C. keyvani* this bone meets the sphenotic, prootic and parasphenoid. The parasphenoid extends from the ethmoid region to the occipital region. The middle part of this bone is wide and connected to the pterosphenoid. Two lateral processes present in the

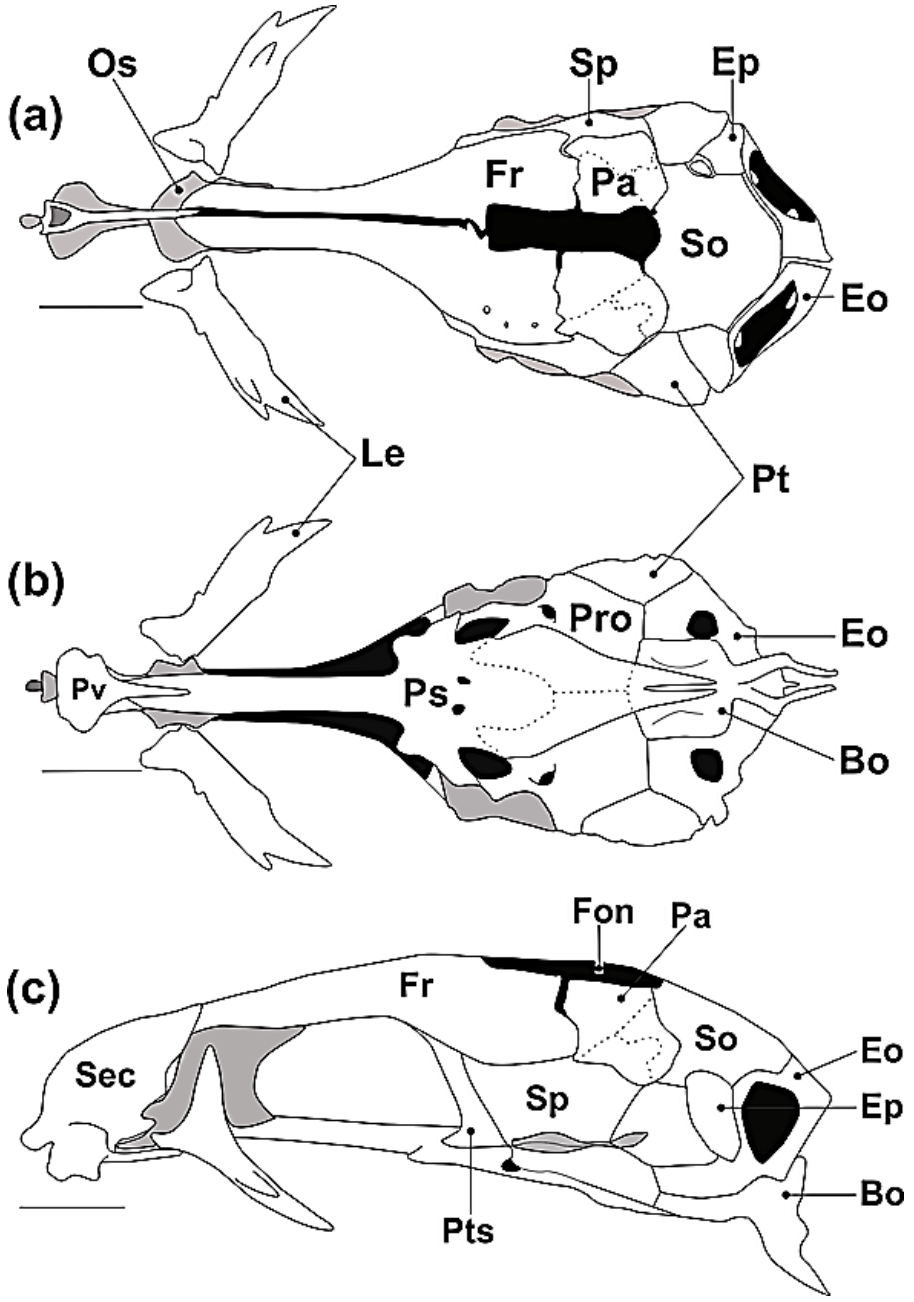

Figure 2. The neurocranium of *Cobitis keyvani*. (a) Dorsal, (b) ventral and (c) lateral views. Eo: exoccipital; Bo: basioccipital; Ep: epiotic; Fr: frontal; Le: lateral ethmoid; Os: orbitosphenoid; Pa: parietal; Pv: prevomer; Pro: prootic; Ps: parasphenoid; Pt: pterotic; So: supraoccipital; Sp: Sphenotic. Eo: exoccipital; Bo: basioccipital; Ep: epioti; Fr: frontal; Fon: fontanel; Pa: parietal; Pts: pterosphenoid; Sec: supraethmoid-ethmoid; So: supraoccipital; Sp: Sphenotic.

middle part of the parasphenoid directing posteriorly (Fig. 2b). The parasphenoid is bifurcate posteriorly (Fig. 2b). A long lacrimal bone is situated at the anterior part of the lateral ethmoid.

The otic region comprises of the parietal, epiotic, sphenotic, pterotic, and prootic (Fig. 2a, b). The parietal is almost square-shaped bone and its posterior edge sutured with supraoccipital. The sphenotic is trapezoid in shape and ventrally connected to the prootic and posteriorly to the pterotic. According to Sawada (1982) in the genus *Cobitis*, the contact between the sphenotic and supraoccipital is absent; while in *C. keyvani*, the posterodarsal margin of the sphenotic is attached to the anterolateral part of supraoccipital (Fig. 2a). There is no connection between the sphenotic and

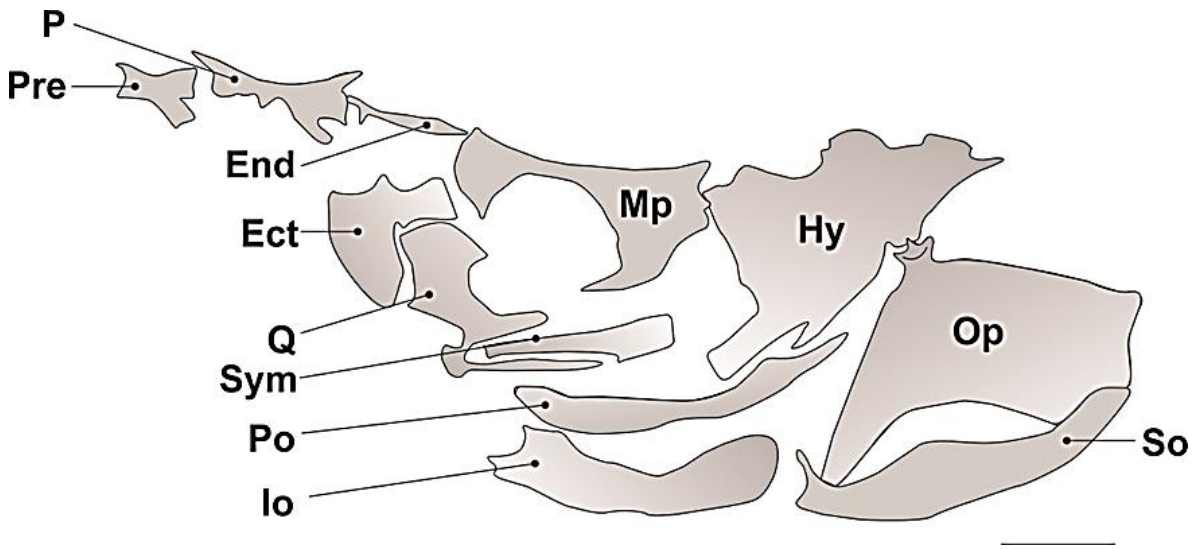

Figure 3. Lateral view of the neurocranium in *Cobitis keyvani* (left side). Ect: ectopterygoid; End: endopterygoid; Hy: hyomandibulare; Io: interopercle; Mp: metapterygoid; Op: opercle; P: palatine; Po: praeopercle; Pre: preethmoid; Q: quadrate; So: subopercle; Sym: symplecic.

epiotic (Fig. 2a). The ventral edge of the sphenotic forms the anterior facet of the neurocranium for articulating to the hyomandibular. The anterior part of the prootic is bifurcated which its medial branch is covered with the parasphenoid and the lateral branch attached to the pterosphenoid and sphenotic. This bone also has a large pore in its middle part. The pterotic is triangular in shape and enclosed by the epiotic, sphenotic and parietal bones. The epiotic is oval-shaped and attached to the supraoccipital posteriorly.

The occipital region consists of the supraoccipital, exoccipitals and basioccipital. The supraoccipital is pentagon in shape, and its anterior part is concaved (Fig. 2a, b). This bone is posteriorly connected to the exoccipital. The exoccipital froramen is located on its dorsal face and two other pores are located on its ventral part. A pointed process presents in its lateroventral part of the exoccipital. The basioccipital possesses two posterior processes, which are not fused with each other (Fig. 2b). On the lateral part of the neurocranium, two facets present for articulating with the hyomandibular. The anterior facet is formed by the prootic and sphenotic, the posterior one forms by the sphenotic, prootic and pterotic bones.

Suspensorium: The suspensorium consists of the hyomandibular, ectopterygoid, endopterygoid, metapterygoid, symplectic, quadrate and palatine (Fig. 3). The dorsal part of the hyomandibular is wider than its ventral part. The anterior rim of the hyomandibular is almost triangular in shape, and its dorsal margin has a pointed process. Also, a large pore presents in its middle part of the hyomandibular. The opercular condyle is situated on the dorsolateral margin of the hyomandibular. A fossa is present on the dorsal part of the quadrate (Fig. 3). The symplectic is elongated and narrow and located at the posterior part of the quadrate. The metapterygoid is a flat bone overlapping the endopterygoid anterodorsally and concaved ventrally. The endopterygoid is thin and bears an anterior facet to articulate with the autopalatine. The autopalatine is slightly wide and has an abdominal facet which is articulated with the pre-vomer. This bone also has two anterior and posterior protuberances on its dorsal part which letter one is larger (Fig. 3).

Opercular series: The opercular series includes the opercle, preopercle, interopercle and subopercle (Fig. 3). The anterodorsal part of the opercle bears a process; and the hyomandibular condyle is positioned under this process. The anteroventral part of the opercle is pointed and overlapped by the subopercle. The subopercle has two anterior processes; its dorsal process is longer than ventral

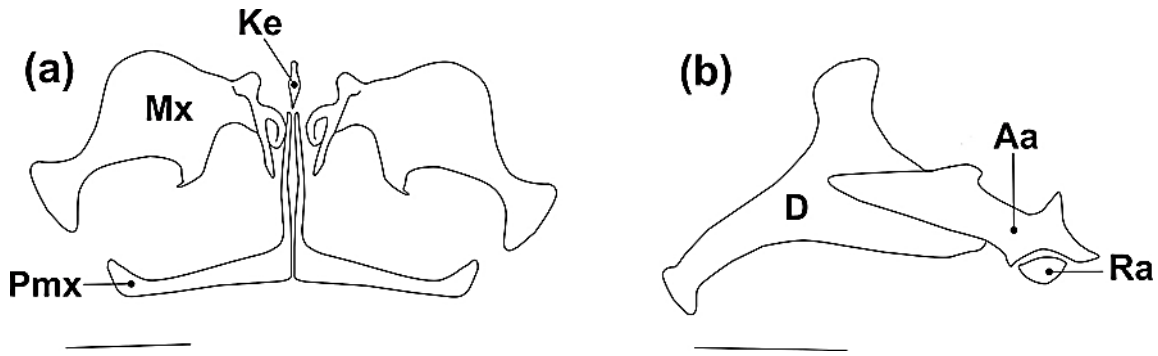

Figure 4. (a) Internal view of upper jaw and (b) lower jaw in *Cobitis keyvani*. Aa: Anguloarticular; D: dentary; Ke: kinethmoid; Mx: maxillae; Pmx: Premaxillae; Ra: retroarticulare.

one. The anterior part of the preopercle is pointed and covers the anterior part of the interopercle. The medial margin of interopercle is connected to the interhyal.

Jaws: The upper jaw consists of the maxilla and premaxilla. The premaxilla is L-shaped and thin and its horizontal part is wider than the vertical part. The horizontal part of this bone is directed dorsally (Fig. 4a). The maxilla bears a well-developed mediolateral downward process whereas in *C. taenia*, this process is small (Vasil'eva, 1984). This bone has an anterodarsal articular, which is connected to the preethmoid II. A long process presents in the anteroventral part of the maxillae (Fig. 4a).

The lower jaw is composed of the dentary, anguloarticular and retroarticular. The dentary possesses an anteroventral process that is wide and has the coronoid process in the middle part of its dorsal portion. In *C. keyvani*, the coronomeckelian is absent. A short triangular retroarticular is positioned at the dorsoventral side of anguloarticular. The anguloarticular has a facet posteriorly which is articulated with the quadrate.

Branchial apparatus: The branchial apparatus includes five pairs of the ceratobranchial, four pairs of the epibranchial, three pairs of the hypobranchial, two pairs of the inphrapharyngobranchial and four unpaired basibranchial bones (Fig. 5a). The 4th basibranchial is small. There is a tooth-like process on the ventral surface of fifth ceratobranchial, and on its dorsal surface; there is a row of 7 to 9 pharyngeal teeth.

Hyoid arch: The hyoid arch comprises of the paired epihyals, hypohyals and ceratohyals, unpaired urohyal and basihyal, and three pair of the branchiostegal rays (Fig. 5b). The horizontal part of the urohyal has two lateral triangular processes and its vertical part is blade-shape situating on the dorsal part of horizontal portion (Fig. 5b). A small pore presents in the ventral margin of the vertical part of the urohyal. The basihyal is long with narrow middle part positioned between the hypohyals. The hypohyal consists of two parts *viz*. ventral and dorsal hypohyals that are connected medially. The anterior part of the ceratohyal is connected to the dorsal and ventral part of the hypohyals. The first branchiostegal ray is connected to the medial part of the ceratohyal. The second branchiostegal ray is connected to the joint of the ceratohyal and epihyal. The 3rd branchiostegal ray is attached to the epihyal. The dorsal part of the epihyal is pointed. The interhyal is a cylindrical bone positioning on the dorsal edge of the epihyal (Fig. 5b). The branchiostegal rays extend to the dorsal end of the subopercle (Fig. 7).

Pectoral girdle: The pectoral girdle consists of the cleithrum, supracleithrum, coracoid, mesocoracoid, scapula, posttemporal and radials (Fig. 5c). The cliethrum is L-shaped and has a blade-shape process on its posteroventral part. The dorsolateral part of the cleithrum is articulated with the ventral part of the supracleithrum. The supracleirthrum is long and dorsally connected to the posttemporal which attaches to the pterotic. The scapula has a large foramen in middle part and ventrally connected to the coracoid. The mesocoracoid is ventrally

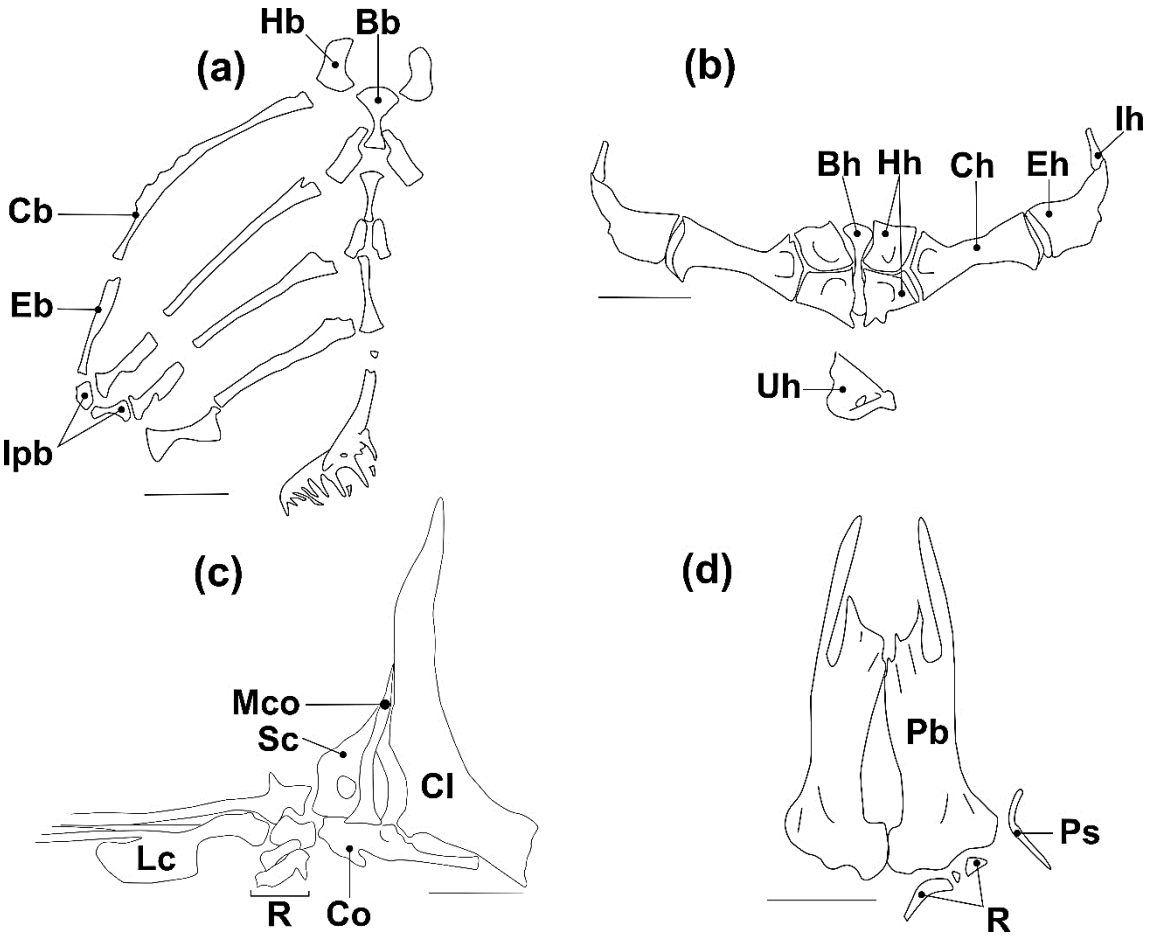

Figure 5. (a) Dorsal view of branchial apparatus, (b) hyoid arches, (c) medial view of pectoral girdle and (d) pelvic girdle of *Cobitis keyvani*. Bb: basibranchial Bh: basihyal; Ch: ceratohyale; Cb: ceratobranchial; Co: coracoid; Eh: epihyal; Hh: dorsal and ventral hypohyal; Eb: epibranchial; Hb: hypobranchial; Ipb: inphrapharyngobranchial; Ih: interhyal; Uh: urohyal; Cl: cleitherum; Lc: lamina circularis; Mco: mesocoracoid; R: radial; Sc: scapula; Pb: pelvic bone; Ps: pelvic splint.

connected to the coracoid and dorsally to the scapula and cliethrum (Fig. 5c). The pectoral fin bears three radials that first one is broader and second one has the ventral and dorsal processes. The third radial is widest one. The pectoral girdle has one unbranched and seven branched rays. In males, the second ray of pectoral girdle is broader and forms the lamina circularis (Fig. 5c).

Pelvic girdle: The pelvic girdle includes the paired pelvic bones, pelvic splint and radials. The posterior part of the pelvic bone is broader and its anterior part is bifurcate. Three radials present posterior to the pelvic bone. The second radial is the smallest one. The two pelvic splint bones are positioned in the lateral side of the pelvic bones. The pelvic fin has one unbranched and six branched rays (Fig. 5d).

Axial skeleton: The number of vertebrae is 42. The weberian apparatus and swim bladder capsule is formed by the four anterior centra (Fig. 6a). Also, four pair ossicles, including the tripus, intercalarium, scaphium and claustrum present in the weberian apparatus. The swim bladder capsule is formed from fourth centrum. In the lateral margin of the swim bladder capsule, there are two large pores that the posterior one is rounded and anterior one oval-shaped. The two pointed processes present in the latero-external portion of the swim bladder capsule. There are plentiful small pores on the wall of the swim bladder capsule. The cranial and caudal parts of vertebral column have 21 centra. In *C. keyvani*, similar to other members of Cobitinae including *Cobitis taenia taenia*, *C. taenia striata*, *C. koreensis*, *Acanthopsoides graciroides* and *Lepidocephalus guntea* (Sawada, 1982), the number of cranial

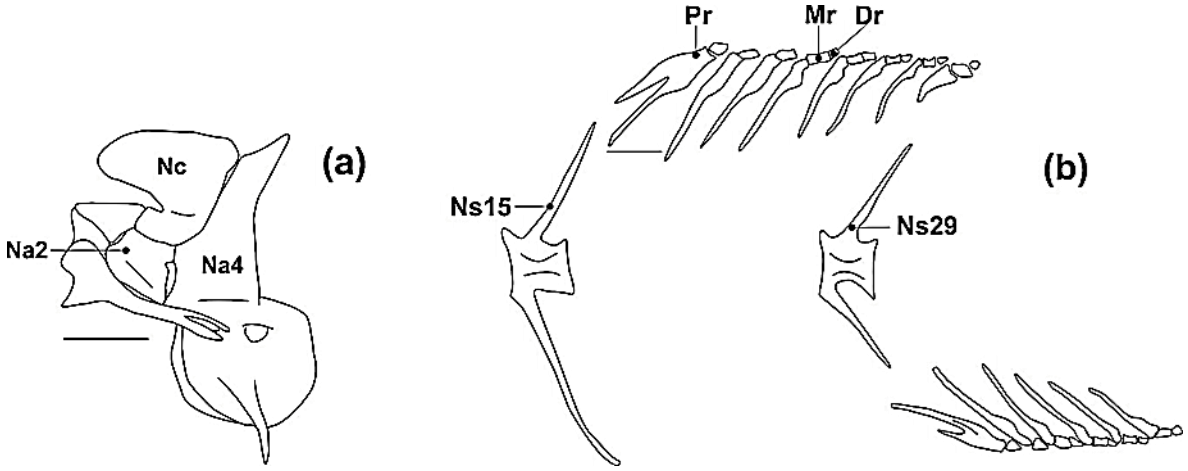

Figure 6. (a) Lateral view of the weberian apparatus and (b) dorsal and anal fins in *Cobitis keyvani*. Dr, distal radial; Mr, medial radial; Ns, neural spine; Pr, proximal radial; Na: neural arch; Nc: neural complex.

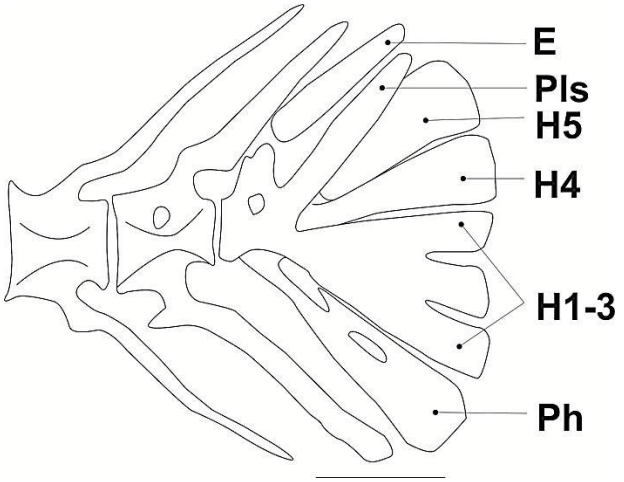

Figure 7. (a) Lateral view of the caudal fin in *Cobitis keyvani*. E: epural; H: hypurals; Ph: parhypurale; Pls: pleurostile.

vertebrae is about equal to those of caudal ones.

Unpaired fins: The dorsal fin has seven pterygiophores, one stay, three unbranched and seven branched rays (Fig. 6b). The first pterygiophore is the largest one and supports the unbranched rays. Four free supraneural bones present in the front of the dorsal fin.

The anal fin possesses three unbranched and five branched rays, six pterygiophores and a small stay (Fig. 6b). The first and largest pterygiophore supports the unbranched rays.

The skeleton of caudal fin consists of the epural, parhypural, pleurostyle, and six hypurals bones (Fig. 7). In *C. keivany*, the hyporal-1 is fused with the hypural-2 (sometimes the first three hypurals are fused). The caudal fin bears 17 branched rays and various numbers of the procurrent rays.

Finally, based on the osteological results, *C. keyvani* can be distinguished from other members of the genus *Cobitis* by a contact between sphenotic and supraoccipital and a contact between pterosphenoid, parasphenoid, prootic and sphenotic. Other features of *C. keyvani* are similar to other members of this genus reported by Vasil'eva (1984) and Sawada (1982).

Myology of Cobitis keyvani

Muscles of the cheek: The adductor mandibulae of *C. keyvani* comprises of A1, A2 and Aω sections, while in the studied cyprinids by Matthes (1963), Aω was absent, and the adductor mandibulae comprised A1, A2 and A3. In the studied species, the section A1 is the largest section of the adductor mandibulae and originates musculously from the dorsolateral margin of the hyomandibular. This section inserts as a wide tendon on the anterior portion of the maxillae. But in the studied cyprinids by Matthes (1963), A1 originates from the preoperculum and quadrate and runs forward to insert on the antero-external face of the maxillary. The section A2 originates from the ventral part of both hyomandibular and anterior rim of the preopercle and inserts tendinously onto the medial part of the coronoid process of the dentary. The posterior fibers of A2 are covered by A1. The sections Aω and A1 are attached anteriorly to a single tendon. The section Aω inserts on the posterodorsal edge of the anguiloarticular (Fig. 8).

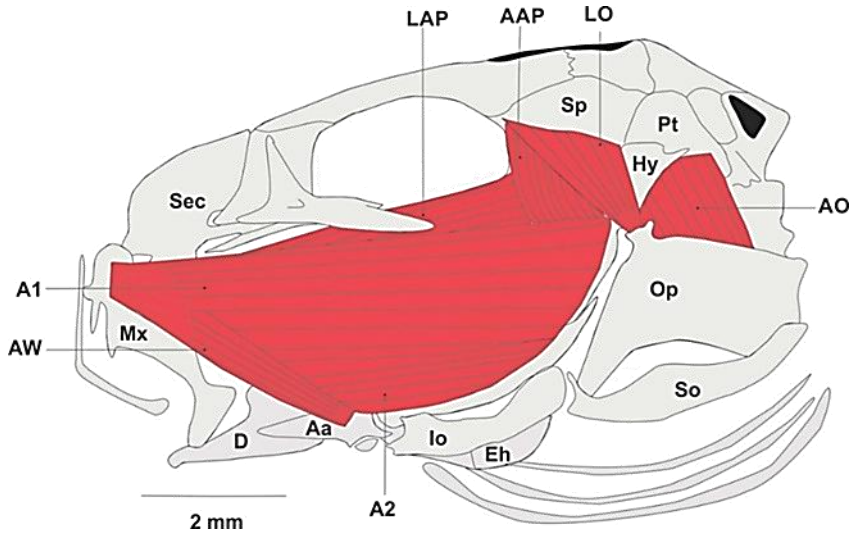

Figure 8. Lateral view of the head muscles in *Cobitis keyvani*. A1: subsection of the mandibulae Aw: subsection the adductor mandibulae; A2: subsection the adductor mandibulae; AO: adductor operculi; D: dentary; Hy: hyomandibular; LO: levator operculi; Mx: maxillae; Op: opercle; Pt: pterotic; So: subopecle; Sp: sphenotic.

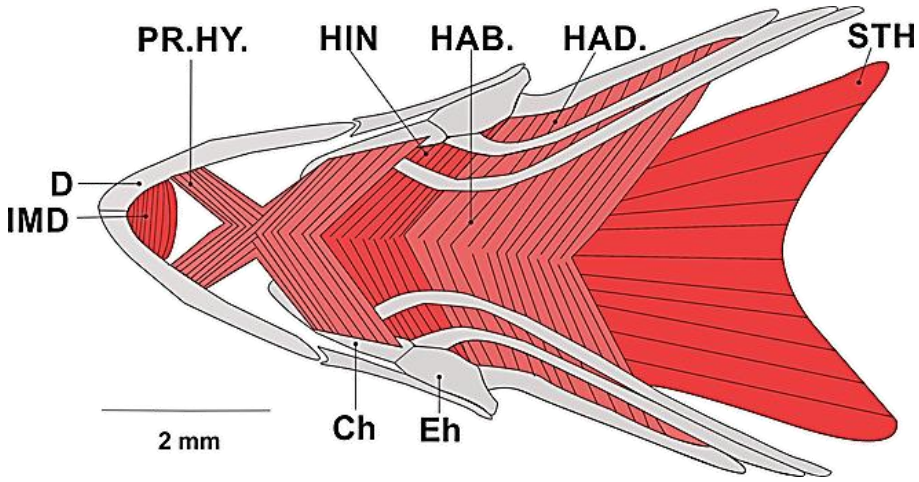

Figure 9. Ventral view of the head muscles in *Cobitis keyvani*. Ch: ceratohyal; D: dentary; Eh: epihyal; HAD.: hyohyoidei adductors; HIN: hyohyoides inferioris; HAB.: hyohyoidei; IMD: intermandibularis; PR.HY.: protractor hyoides; STH: sternohyoideus; Uhy: urohyal.

The adductor arcus palatinii occupies the lateral face of the hyomandibular. This muscle originates musculously from the pterspheno tic and inserts on the medial face of the hyomadibular. In other cypriniid fishes, it observes that the adductor arcus palatine consists of three parts and arises from the prootic and parasphenoid and insert on the inner dorsal surfaces of the palatine, pterygoid and hyomandibular bones (Matthes, 1963). The levator arcus palatini muscle originates musculously from the lateromedial margin of the parasphenoid and the ventral portion of the pterspheno tic and inserts musculously on the anterodorsal rim of the metapterygoid and the posterodorsal rim of the endopterygoid. The anterior fibers of levator arcus palatini is attached to the dorsal part of A2 muscle.

The levator opeculi muscle is conical in shape and originates with muscle fibers from the lateral face of the sphenotic. This muscle inserts as a tendon on the dorsal part of the opercular prominent process. The adductor opeculi originates musculously from the sphenotic and posterodorsal edge of the hyomandibuar and its fibers insert to the dorsal rim of opercle (Fig. 8).

Ventral muscles of the head: The intermandibularis muscle lies between the lower jaws. The protractor

hyoides muscle is anteriorly attached to the medial face of the dentary and posteriorly to the ventral face of the ceratohyal and epihyal. In the family Cyprinidae, the intermandibularis is sometimes absent or modified into a protractor hyoidei (Matthes, 1963). The hyohyoides inferioris connects two side of hyoid arch and its fibers are attached to the dorsal face of the hyoid arch similar to other cyprinid fishes (Matthes, 1963). The hyohyoideus abductor connects the left and right branchiostegal rays. The hyohyoidei addauctor has developed between the branchiostegal rays. The sternohyoideus is a large muscle and bears two bundles which are connected together. They originate tendinously from the anterior part of the urohyal and connect the hyoid arch to the cleithrum.

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