

Descriptive osteology of the endemic spined loach *Cobitis linea* from Iran

¹Pariya Jalili, ¹Soheil Eagderi, ²Hamed Mousavi-Sabet

¹ Department of Fisheries, Faculty of Natural Resources, University of Tehran, Karaj, Iran;

² Department of Fisheries, Faculty of Natural Resources, University of Guilan, Guilan, Iran. Corresponding author: S. Eagderi, soheil.eagderi@ut.ac.ir

Abstract. The osteological characteristics of *Cobitis linea* is described and compared with the other Iranian members of the genus *Cobitis*. For this purpose, the specimens were cleared and stained with alizarin red S and alcian blue for osteological examination. Then, a detailed description of the skeletal structure of this species was provided. Despite numerous similarities among the compared species of the genus *Cobitis*, several differences were observed that can be considered as distinctive osteological features for *C. linea*. *C. linea* can be distinguished from the other Iranian *Cobitis* by presence of the contact between ventral part of the mesocoracoid and scapula, separated hyporals and having a well-developed process at the middle part of the frontal.

Key Words: osteology, bone, *Cobitis linea*, Iranian loach.

Introduction. The family Cobitidae, spined loaches, has about 26 genera with about 177 species (Nelson 2006). The members of the genus *Cobitis* (Linnaeus, 1758) are distributed in a large part of Eurasia and northwestern Morocco, showing the widest distributional range of all cobitids genera (Sawada 1982). The members of this genus are characterized by an elongate body with 3 pairs of barbels, erectile spine below the eye, small dorsal and anal fins and truncated caudal fin (Coad 2015). The genus *Cobitis* has four confirmed species in Iran, including *Cobitis linea* (Heckel, 1847), *C. faridpaki* (Mousavi-Sabet et al 2011), *C. keyvani* (Mousavi-Sabet et al 2012) and *C. avicennae* (Mousavi-Sabet et al 2015).

C. linea is found in the Kor River basin and the upper Kul River drainage of the Hormuz basin (Bănărescu & Nalbant 1966; Bianco & Nalbant 1980). It is distinguished by the dark-brown lateral spots being reduced or absent, latero-caudal branch of the suborbital spine is reduced or absent and 14 branched caudal fin rays (Coad 2015). Bianco & Nalbant (1980) and Bohlen et al (2006), using the cytochrome *b* gene, placed this species in the subgenus *Bicanestrinia* Băcescu, 1962; whereas *C. linea* originally described in the genus *Acanthopsis* Agassiz, 1832 (Eschmeyer 1990).

Detailed osteological features of some *Cobitis* species including *C. faridpaki*, *C. keyvani* and *C. avicennae* (from the southern Caspian Sea and the Tigris River drainage basin) have been already described (Jalili et al 2014, 2015a, 2015b); but so far little attention has been paid to the *C. linea*. Therefore, the present study was conducted to provide a detailed description of the skeletal elements of *C. linea* and comparing it with those of other members of this genus in Iran.

Material and Method. Seven specimens of *C. linea* (41.44-76.60 mm SL, Figure 1) were collected during August 2014 by electrofishing from the Ghadamgah spring stream system in the Kor River basin (30°15'N-52°25' E), Fars Province, southern Iran and fixed in 4% buffered formalin. For osteological examination, the specimens were cleared and stained with alizarin red S and alcian blue according to Taylor & Van Dyke (1985). Then, the cleared and stained specimens were studied using a stereoscopic microscope (Leica MC5) and their skeletal elements were scanned by a scanner equipped with a glycerol

bath (Epson V700). The scanned images were illustrated by CorelDrawX6 software. Nomenclature and abbreviation of skeletal elements follow Rojo (1991) and Jalili et al (2014).

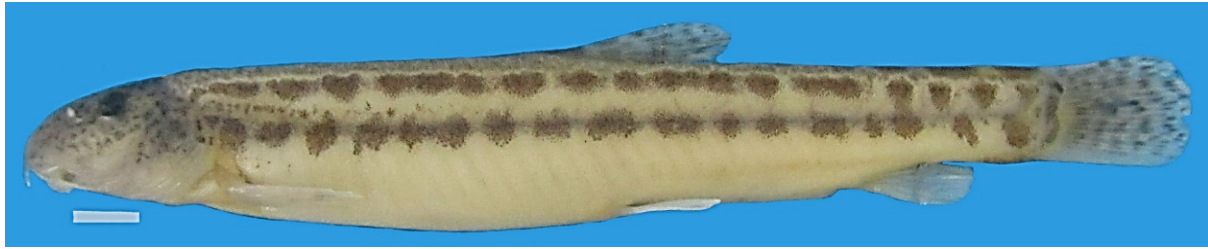


Figure 1. Lateral view of *Cobitis linea* (scale bar = 5 mm).

Results. The anterior half of the neurocranium is elongated and shallower than its posterior half (Figures 2a and 2c). The ethmoid region consists of the supraethmoid-ethmoid, lateral ethmoid, preethmoid II, kinethmoid and prevomer bones. The supraethmoid-ethmoid is fused to the antero-dorsal part of the prevomer. The supraethmoid-ethmoid-prevomer complex is dorsally connected to the frontal, posterior-medially to the orbitosphenoid and ventrally to the parasphenoid. The supraethmoid-ethmoid has an antero-dorsal depression and a short and pointed antero-ventral projection. The vomer is T-shape with thicker anterior part. The lateral ethmoid is a long bone with two antero-dorsal and antero-ventral processes; it also bears two latero-external processes and a latero-internal protuberance; the posterior part of this bone is projected from the skin as an erectile spine below the eye. The lateral ethmoid bears a moveable joint to the orbitosphenoid. The preethmoid II bears two dorsal articulated processes; the upper one is articulated with the anterior part of the palatine, and the ventral one is connected to the prevomer (Figure 2).

The orbital region comprises of the frontal, orbitosphenoid, ptersphenoid and parasphenoid. The frontal is narrow, and its medial part is wider; it bears a lateral process which covers the dorsal portion of the ptersphenoid (Figure 2b). The ventral part of the ptersphenoid is wider than its dorsal part. The orbitosphenoid along with the internal part of the lateral ethmoid participates in formation of the anterior wall of the orbit; the ventral parts of the orbitosphenoids are connected to each other. The parasphenoid bears two lateral processes in its middle part that are bended dorsally; the anterior part of this bone is narrower than its posterior. The posterior margin of parasphenoid is bifurcated via a deep groove whereas its anterior portion is pointed.

The otic region consists of the pterotic, prootic, epiotic, sphenotic and parietal. The pterotic is a relatively trapezoid in shape and its dorsal part is enclosed by the epiotic, sphenotic, parietal and supraoccipital; the ventral part of the pterotic is attached to the prootic and exoccipital. The epiotic is the smallest element with an oval-shape locating between the pterotic and exoccipital. The parietals are separated from each other; their anterior parts are dentated. The sphenotic is pentagon in shape and its posterodorsal part connected to the supraoccipital. The dorsal margin of the sphenotic weakly overlaps with the parietal and frontal. The prootic is a large bone situated under the dorsal part of the parasphenoid and its anterior margin is concaved and bears a foramen and some pores.

The occipital region is composed of the supraoccipital, exoccipital and basioccipital. The supraoccipital is large with a u-shaped anterior part. The exoccipital is dorsally connected to the supraoccipital and ventrally to the basioccipital and prootic; it bears a large dorsal foramen and three ventral pores that the middle one is largest one. The posterior part of the basioccipital has two pointed processes; the anterior edge of this bone is pointed (Figure 2c).

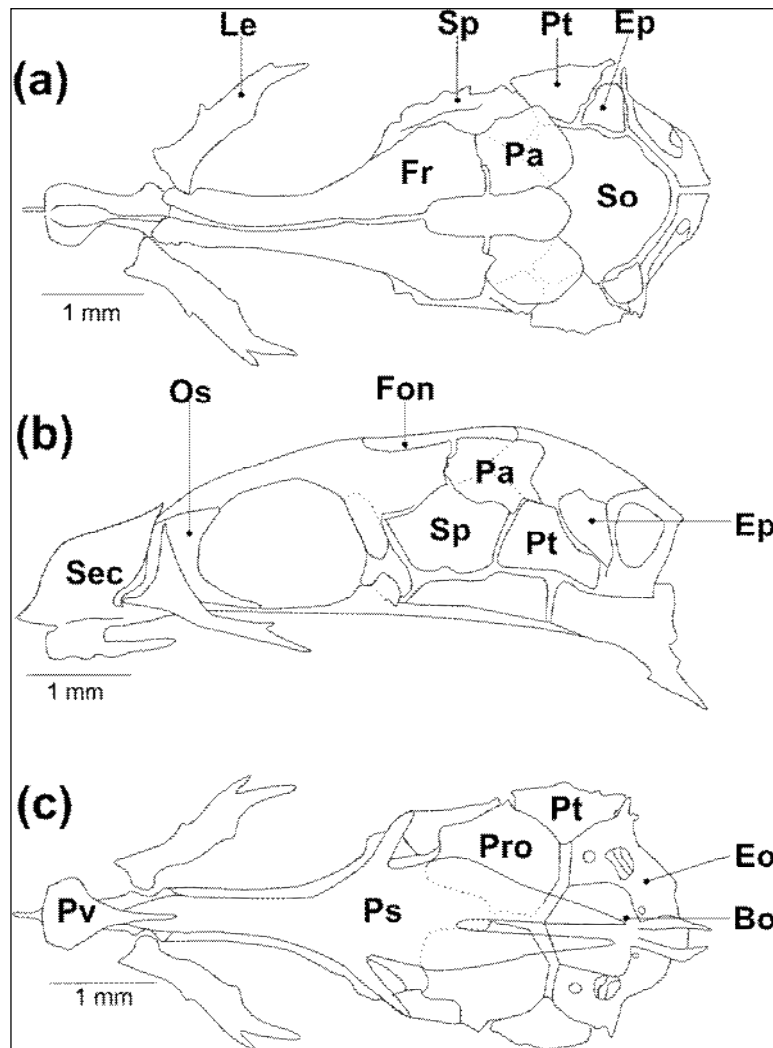


Figure 2. The neurocranium of *Cobitis linea*: dorsal (a), ventral (b), and lateral (c) views.

Abbreviations: Eo: exoccipital; Bo: basioccipital; Ep: epiotic; Fon: fontanel; Fr: frontal;

Le: lateral ethmoid; Os: orbitosphenoid; Pa: parietal; Pv: prevomer; Pro: prootic;

Ps: parasphenoid; Pt: pterotic; Sec: supraethmoid-ethmoid; So: supraoccipital; Sp: sphenotic.

In the branchiocranium, the upper jaw consists of the maxillae and premaxilla. The middle part of the maxillae is round in shape with a ventral process; the anterior portion of this bone bears a descending process. The premaxilla is L-shaped and the lateral end of its horizontal part bears an ascending process (Figure 3a).

The lower jaw is composed of the dentary, articular and retroarticular bones (Figure 3b). The coronoid process is blunt and orientated posteriorly; it is situated in the middle part of the dentary. The posterior part of the dentary overlaps with the anterior part of the articular. The articular is long and its posterior part bears an articular facet to articulate the quadrate. The retroarticular is the smallest bony element of lower jaw; it is situated under the posterior part of the articular.

The suspensorium consists of the hyomandibular, quadrate, symplectic, metapterygoid, ectopterygoid, endopterygoid and palatine. The hyomandibular is elongated ventrally and bears large anterior processes. The opercular articular condyle is situated at the middle part of the posterior rim of the hyomandibular.

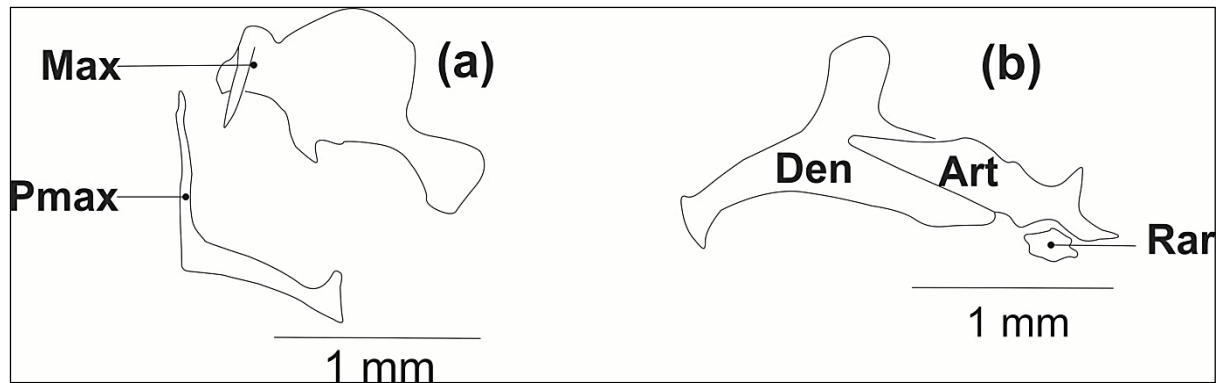


Figure 3. Internal view of the upper jaw (a) and lower jaw (b) (right side) of *Cobitis linea*. Abbreviations: Art: articulare; Den: dentary; Rar: retroarticulare; Max: maxillae; Pmax: premaxillae.

The metapterygoid bears two postero-dorsal processes and its middle portion is narrow. The endopterygoid is long and connected to the metapterygoid by the palatin. The palatin has a ventral blade-shape protubance and its antero-dorsal and postero-dorsal corners are pointed. The ectopterygoid is rather rhombic in shape and its dorsal half is shorter than its ventral half. The quadrate consists of the dorsal and ventral sections. The dorsal section possesses a postero-dorsal notch and the ventral one is thicker and pointed dorsally. The symplectic is a thin bone that its anterior part is situated inside posterior incisura of the quadrate (Figure 4).

The opercular series comprises the opercle, subopercle, interopercle and preopercle. The ventral part of the opercle is concaved and its antero-ventral corner is pointed and slightly covered by the anterior edge of the subopercle. The anterior part of the subopercle and interopercle bear two dorsal and ventral processes. The preopercle is knife-like in shape and extends to the postero-ventral part of the quadrate anteriorly.

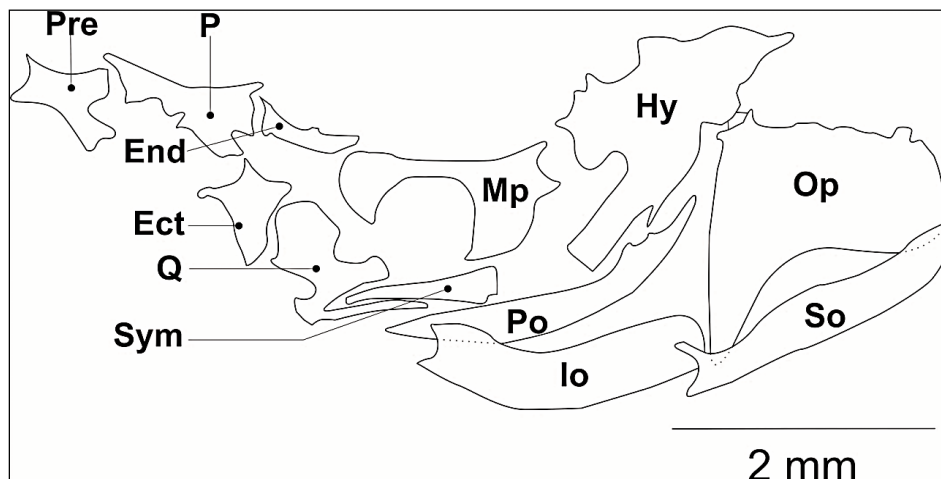


Figure 4. Lateral view of the neurocranium in *Cobitis linea* (left side). Abbreviations: Ect: ectopterygoid; End: endopterygoid; Hy: hyomandibulare; Io: interopercle; Mp: metapterygoid; Op: opercle; P: autopalatine; Po: preopercle; Pre: preethmoid II; Q: quadrate; So: subopercle; Sym: symplectic.

The branchial apparatus bears the five pairs of the ceratobranchial, four pairs of the epibranchial, three pairs of the hypobranchial, two pairs of the inpharyngobranchial and three unpaired basibranchial (Figure 5a). The anterior basibranchial is T-shaped and the posterior one is the longest element of this series and pointed anteriorly. The posterior pharyngobranchial is larger than the anterior one. The anterior element of the hypobranchial is large and the middle one bears an antero-lateral process. The fourth epibranchial is narrow medially and its dorsal margin is concaved. The fifth ceratobranchial is curved and modified into the pharyngial teeth bearing a row of the

pointed teeth with a dental formula of 11-13 in each side; the posterior part of this bone has a dental-like process.

The hyoid arch includes the epihyals, hypohyals, ceratohyals, urohyal, basihyal and three pair of the branchiostegal rays. The unpaired basihyal is situated between the hypohyals; its middle part is narrow. The hypohyal comprises two anterior and posterior parts; the anterior one is triangular-shape and the posterior one is quadrate-shape. The latero-external edges of the hypohyals are attached to the ceratohyals. The ventral part of the urohyal is wide medially and pointed dorsally; its posterior part is blunt and its maximal length is shorter than the length of the ventral portion. The ceratohyal is pointed anteriorly and its middle part is slender shaped with a ventral protuberance which is connected to the medial branchiostegal ray. The middle branchiostegal ray is attached to the contact between the ceratobranchial and the epihyal. The epihyal is a slightly triangular. The interhyal is cylindrical in shape and its ventral margin is covered by the posterior part of the epihyal (Figure 5b).

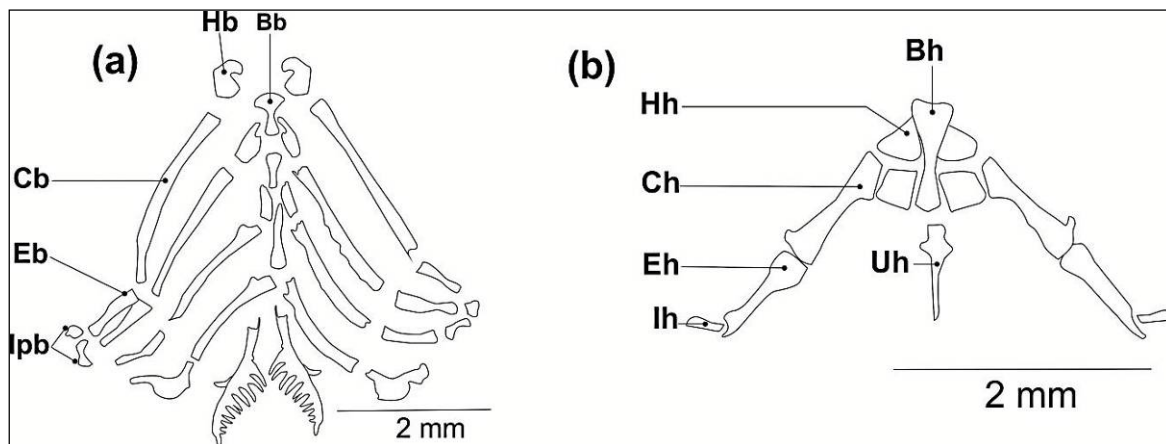


Figure 5. Dorsal view of the branchial apparatus (a), hyoid arches (b) in *Cobitis linea*. Abbreviations: Bb: basibranchial Bh: basihyal; Ch: ceratohyal; Cb: ceratobranchial; Eh: epihyal; Hh: dorsal and ventral hypohyal; Eb: epibranchial; Hb: hypobranchial; Ipb: infrapharyngobranchial; Ih: interhyal; Uh: urohyal.

The pectoral girdle consists of six bones with the radials elements. The cleithrum is the largest one and crescent in shape and its ventral portion is wider. This bone is elongated dorsally and covered by the ventral portion of the supracleithrum. The supracleithrum is a narrow and long bone with a dorsal facet for articulating with the posttemporal. The posttemporal connects the pectoral girdle to the otic region of the neurocranium. The supratemporal is small and situated at the upper face of the anterior edge of the posttemporal; its dorsal part is connected to the lateral margin of the pterosphenoid. The coracoid bears a posterior notch in the lateral rim and its postero-dorsal part with the ventral margin of the scapula forms a facet for articulating with the mesocoracoid that connects these bones to the posterior edge of the cleithrum. The dorsal part of the scapula bears a pointed process. In addition, its postero-ventral margin is attached to the first unbranched ray. The pectoral girdle bears three separated radials that the medial one is the largest (Figure 6a).

The pelvic girdle possesses the paired pelvic bones, pelvic splint and radials. The posterior part of this girdle bears two extro-lateral and medio-lateral processes; the anterior margin of this bone bears three branches that the internal one is the shortest. The pelvic splint is narrow and long situating at the lateral side of the pelvic bone. There are three radials in each side; the middle radial is round in shape and smallest one; the medial one is long and tilted dorsally (Figure 6b).

The dorsal fin bears three unbranched and six branched rays, seven pterygiophores and one stay bone (Figure 7a). The anal fin has three unbranched and five branched rays, six pterygiophores and one stay bone (Figure 7b). The first pterygiophore of the dorsal and anal fins is next to the 17th and 31st vertebra,

respectively. The first pterygiophor of the dorsal fin is the largest one and bifurcated anteriorly (Figure 7a).

The caudal skeleton consists of five hypurals and unpaired epural, parhypural and pleurostyle bones (Figure 8a). The epural is broadened ventrally and attached to the posterior rim of the pleurostyle. The 5th hypural is the widest and triangular in shape. The neural and hemal spines of the second centrum are bifurcated. The caudal fin possesses two unbranched, 14 branched rays, 5 dorsal and 4-5 ventral procurrent rays. The five anterior centra and four pair ossicles, including the tripus, intercalarium, scaphium and claustrum, contribute to form the weberian apparatus (Figure 8b). The swim bladder capsule is formed by the 4th and sometimes 5th centra; it bears two lateral downward processes and two paired lateral foramens; there are many pores in the lateral and abdominal walls of the swim bladder capsule. The number of the vertebrae was 43 in the examined specimens.

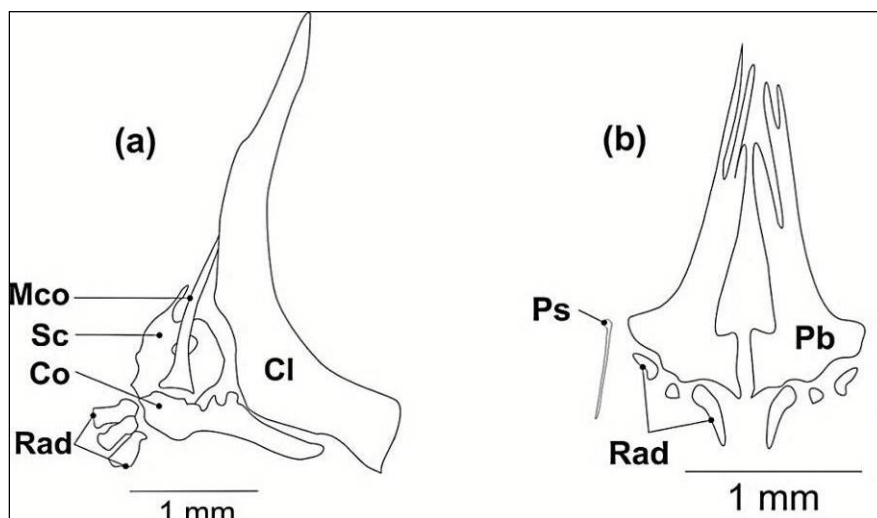


Figure 6. Medial view of pectoral girdle (left side) (a) and pelvic girdle (b) of *Cobitis linea*. Abbreviations: Cl: cleithrum; Co: coracoid; Mco: mesocoracoid; Rad: ossified radials; Sc: scapula; Pb: pelvic bone; Ps: pelvic splint; Rad: radials.

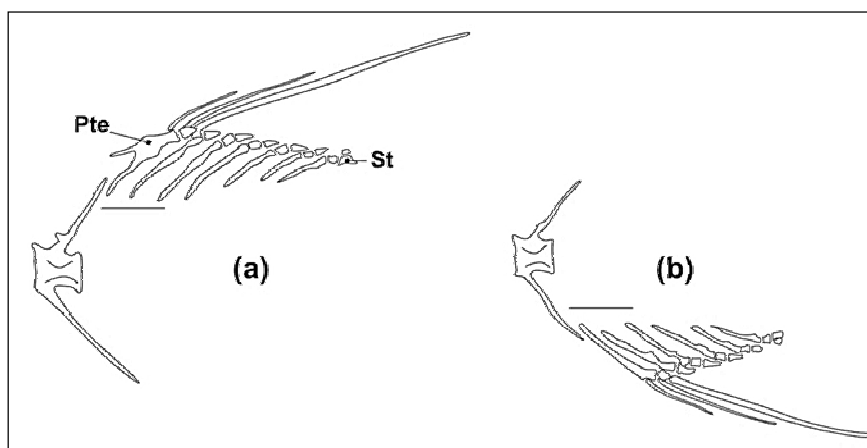


Figure 7. Lateral view of the dorsal fin (a) and anal fin (b) in *Cobitis linea*. Abbreviations: Pte: pterygiophore; St: stay. Scale bar 1 mm.

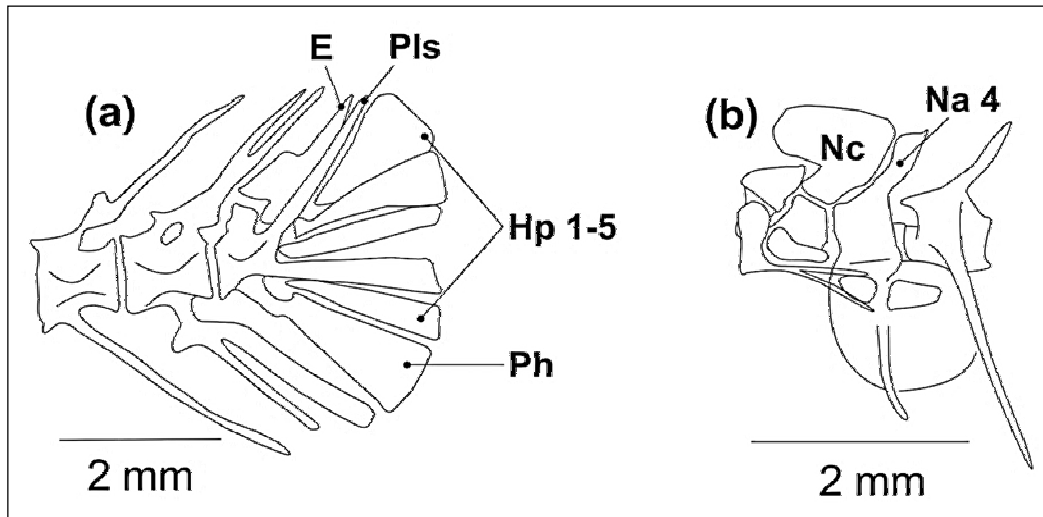


Figure 8. Lateral view of the caudal skeletons (a) and weberian apparatus (b) in *Cobitis linea*.
Abbreviation: E: epural; Hp1–5: hypural plates 1–5; Ph: parhypural; Pls: pleurostyle;
Nc: neural complex; Na: neural arch.

Discussion. Based on the results, *C. linea* shows some differences in the neurocranium, branchiocranium, pectoral girdle and caudal skeleton compared to those of *C. faridpaki*, *C. keyvani* and *C. avicennae*. In *C. keyvani* and *C. avicennae*, the total length of the vomer is greater than the length of the supraethmoid, whereas in *C. faridpaki*, they have almost equal length. In some *Cobitis* species, such as *C. faridpaki* and *C. keyvani*, the parasphenoid has two rounded processes in its middle part that is not observed in *C. linea*.

In *C. linea*, the hypurals are separated versus the connected hypurals of other compared species. There are three basibranchial in *C. linea* and *C. avicennae*; while *C. faridpaki* and *C. keyvani* have four basibranchial that the 4th one is small or consumptive. According to Prokofiev (2009) the number of basibranchial is one of the most important parameter in loaches osteological characters.

The coronoid process in *C. linea* as *C. faridpaki* and *C. avicennae* is bended posteriorly; whereas, it is oriented dorsally in *C. keyvani*. The ventral part of the mesocoracoid is connected to the coracoid and scapula in *C. linea*, whereas in other species i.e. *C. faridpaki*, *C. keyvani* and *C. avicennae*, this bone is connected only to the coracoid.

Family Cobitidae is classified into five types based on 10 characters, including presence of the fronto-parietal fontanelle, contact between the frontal and parasphenoid, contact between the pterosphenoid and prootic, overlapping the parietal and pterotic, the presence of the supraorbital, contact between the sphenotic and epiotic, connection between sphenotic and supraoccipital and contact between pterotic and pterosphenoid (Sawada 1982). According to this classification, the genus *Cobitis* with genera *Niwaella*, *Sabanejewia*, *Acanthopsoides* and *Lepidocephalus* are formed as the same type, which is different from the others in having the frontal separated from the parasphenoid, and the pterosphenoid separated from the prootic (Sawada 1982).

Conclusions. We observed in *C. linea*, as in the other Iranian species, the pterosphenoid meets the parasphenoid, prootic and sphenotic. In addition, unlike *C. keyvani*, *C. faridpaki* and *C. avicennae*, the lateral process of the frontal is well-developed and extends to the parasphenoid in *C. linea*. In all Iranian species of the genus *Cobitis*, the following traits including separation of the parietal and pteroticis, separation of the sphenotic from the epiotic, absence of supraorbital, presence of contact between the sphenotic and supraoccipital that is covered by the parietal are found as synapomorphic characters. Finally, based on the results, *C. linea* can be distinguished from the other Iranian *Cobitis* species by presence of the contact between ventral part of the

mesocoracoid and scapula, separated hypurals and by having the well-developed process at the middle part of the frontal.

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Authors:

Pariya Jalili, Department of Fisheries, Faculty of Natural Resources, University of Tehran, P.O. Box: 31585-4314, Karaj, Iran, e-mail: pariya.jalili@yahoo.com

Soheil Eagderi, Department of Fisheries, Faculty of Natural Resources, University of Tehran, P.O. Box: 31585-4314, Karaj, Iran, e-mail: soheil.eagderi@ut.ac.ir

Hamed Mousavi-Sabet, Department of Fisheries, Faculty of Natural Resources, University of Guilan, Sowmeh Sara, P.O. Box: 1144, Guilan, Iran, e-mail: mousavi-sabet@guilan.ac.ir

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