

# Comparative Osteology of Lotaks, *Cyprinion kais* and *C. macrostomum* (Cypriniformes, Cyprinidae), from Godarkhosh River, Western Iran<sup>1</sup>

M. Nasri, Y. Keivany, and S. Dorafshan

Department of Natural Resources (Fisheries Division), Isfahan University of Technology, Isfahan 84156-83111, Iran

e-mail: keivany@cc.iut.ac.ir

Received May 22, 2012

**Abstract**—Osteology of two cyprinid fishes, *Cyprinion kais* and *C. macrostomum*, from the Tigris–Euphrates basin was described and compared. Eight specimens of *C. kais* and ten specimens of *C. macrostomum* from Godarkhosh River (western Iran) were studied. The skeletal elements were prepared using clearing and softening methods and photographed. The differences between the two taxa include a deeper posterior position of the lower jaw with a much narrower labial surface, a longer last dorsal unbranched ray with weaker posterior serration, and a more embowed dentary, maxillary and premaxillary in *C. kais*. Based on these differences, the examined specimens of these two taxa could be easily distinguished.

**DOI:** 10.1134/S0032945213040103

**Keywords:** Cypriniformes, Cyprinidae, *Cyprinion*, fish skeleton, Ilam, kingfish, morphology, osteology, Tigris–Euphrates Basin

## INTRODUCTION

The genus *Cyprinion* comprises nine species, among which five are reported from Iran and three from Tigris–Euphrates basin (*C. kais* Heckel, 1843, *C. macrostomum* Heckel, 1843 and *C. tenuiradius* Heckel, 1849). The first two species are well distributed in inland waters of Iran, Iraq, Turkey, and Syria (Coad, 1995; 1996; 2012; Epler et al., 2001; Eschmeyer, 1998; Froese and Pauly, 2012). However, there is little information on these species. In Iran, *C. macrostomum* and *C. kais* are named Lotak-e Dahan bozorg (Big mouth Lotak) and Lotak-e Dahan koochak (Small mouth Lotak), respectively, and we propose “Lotak” for all species of *Cyprinion*, because there is no well-established common name for these taxa.

Durand et al. (2002) conducted phylogenetic and zoogeographical studies, using cytochrome *b*, on *C. macrostomum* and *C. kais* in the Middle East and denoted that the difference level between them was the least (0.4%) amongst the studied cyprinids. Patimar and Patimar and Nasri (2007) studied the age structure and growth of *C. macrostomum* in Ilam province, Iran. Nasri et al. (2010) studied the karyology of *C. kais* and Nasri et al. (“unpubl.”), that of *C. macrostomum* in Iran. However, little attention has been paid to the skeleton of fishes inhabited in Iran and there is only one osteological study on *Cyprinion* species (i.e., Howes, 1982), in which he only reviewed the anatomy and evolution of jaw structures in some *Cyprinion* spe-

cies. There are some uncertainties about the taxonomy and phylogenetic status of *Cyprinion* species and several authors considered the systematic status of Cyprininae species and genera with their phylogenetic links still doubtful (Bianco and Banareescu, 1982; Ekmekci and Banareescu, 1998; Howes, 1982).

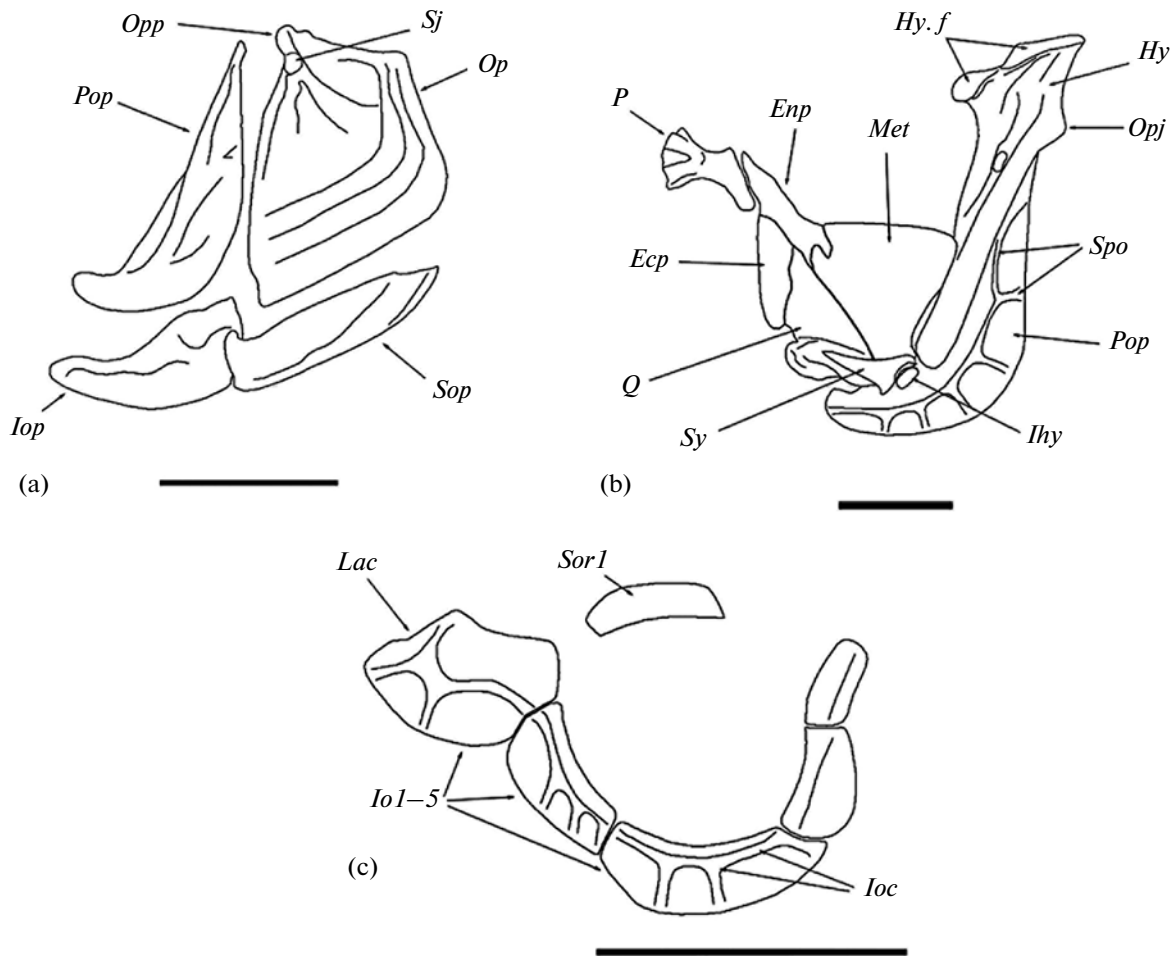
Some researchers considered *C. kais* Heckel, 1843 and *C. macrostomum* Heckel, 1843 as synonyms (Berg, 1949; Karaman, 1971), but Bianco and Banareescu (1982) denoted that they were wrongly considered as synonymous. Osteology is a useful tool to study the taxonomy and phylogenetic relationships among fishes (e.g., Bogutskaya, 1994; Howes, 1982; Ramaswami, 1951). In this study, the skeletal elements of the two species, *C. macrostomum* and *C. kais* are described and compared.

## MATERIALS AND METHODS

Eight specimens of *C. kais* with a mean standard length of  $7.2 \pm 0.6$  cm (*SD*) and 10 specimens of *C. macrostomum* with a mean standard length of  $7.3 \pm 1.3$  cm were collected in 2007–2008 from Godarkhosh River, Ilam province (Tigris–Euphrates basin) in western Iran by electrofishing. The specimens were anesthetized in 1% clove oil solution and fixed in 10% formalin and deposited at the Ichthyological Laboratory of Isfahan University of Technology. After a week, the specimens were transferred to 70% ethanol. The specimens were cleared and stained after Taylor and Vandyke (1985). Identification, nomenclature and

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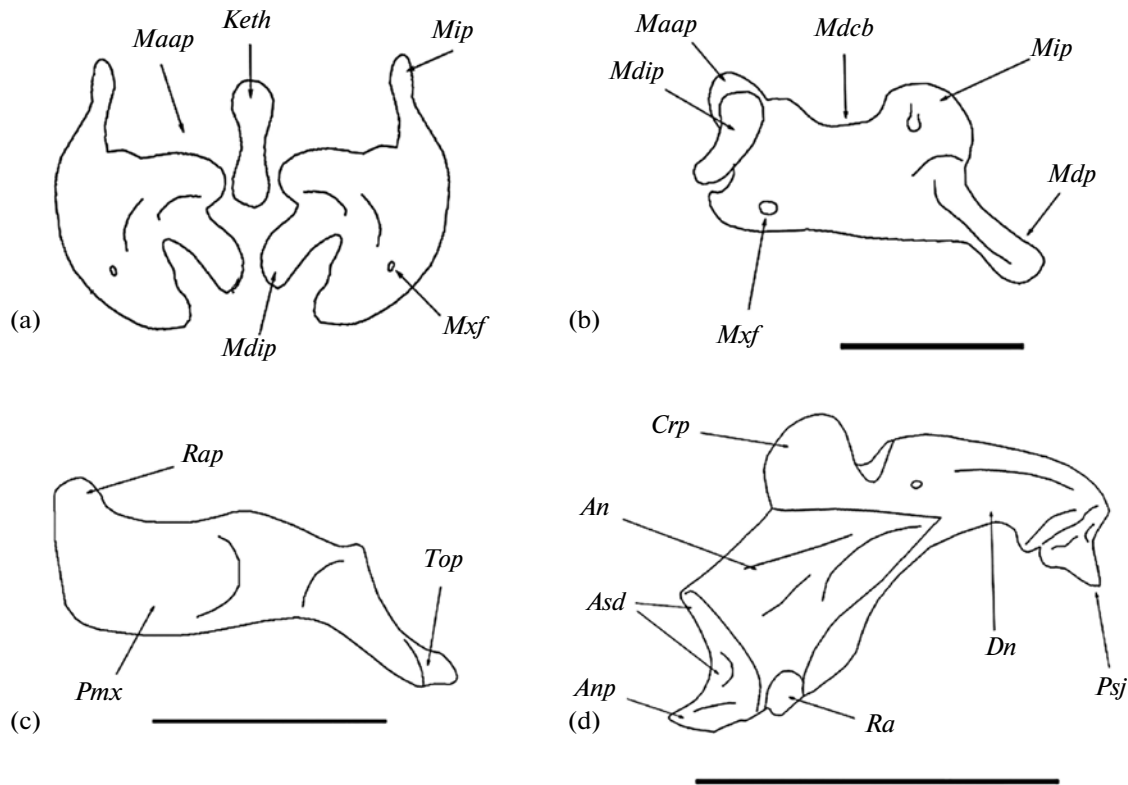
**Fig. 2.** Internal view of the right opercular series (a) and suspensorium (b) and lateral view of the left circumorbitals (c) of *C. macrostomum* (SL = 9.2 cm) (*Ecp*—ectopterygoid; *Enp*—entopterygoid; *Hy*—hyomandibular; *Hy.f*—hyomandibular joint face; *Ihy*—interhyal; *Iol-5*—infraorbitals; *Ioc*—infraorbital sensory canal; *Iop*—interopercular; *Lac*—lachrymal; *Met*—metapterygoid; *Op*—opercular; *Opj*—opercular joint; *Opp*—opercular prominent process; *P*—palatine; *Pop*—preopercular; *Q*—quadrate; *Sj*—spine and socket joint; *Sop*—subopercular; *Spo*—sensory canal of preopercular; *Sor1*—supraorbital 1; *Sy*—symplectic). Scale bar 5 mm.

Anterolaterally, the preethmoid envelops the head of the vomer (Figs. 1a, 1b). The massive mesethmoid is embraced by the supraethmoid dorsally, the vomer ventrally, and the lateral ethmoid laterally (Fig. 1b). Lateral ethmoid is slightly thin, elongated, and pointed ventrally.

The vomer, lateral ethmoid, parasphenoid, optic foramen, prootic, subtemporal foramen, pterotic, postroventral process of pterotic, exoccipital, basioccipital, masticatory plate and pharyngeal process make up the cranial floor (Fig. 1c). The prootic contacts the pterosphenoid ventrally and postrolaterally, the basioccipital posteriorly and the pterotic and frontal dorsally. The pterosphenoid interiorly contacts the orbitosphenoid. The posterior part of the basioccipital is well extended to form a plate that serves as a base for a horny plate (masticatory plate of pharyngeal process). The parasphenoid upwardly curved and the pterosphenoid interiorly sutured to the orbitosphe-

noid septum. Vomer is elongated posteriorly and its tension crossed the junction of the two lateral processes of the ethmoid. The vomer in ventral view is wide and anteriorly expanded and makes two anterolateral cylindrical uncus with shallow indentations that joined with anterior part of palatine. Subtemporal fossa made up of prootic, pterotic and exoccipital and not connected to posttemporal fossa.

**Opercular series.** The opercular series consists of the preopercular, opercular, subopercular and interopercular bones (Fig. 2a). The anterior edge of the preopercular covers the posterior border of hyomandibular and its posterior edge covers the anterior edge of opercular. The ventral corner of the opercular covers the upper corner of interopercular and its lower border covers the dorsal edge of subopercular. The opercular is connected to hyomandibular with a spine and socket joint. Subopercular is a knife-like bone that its anterior portion connected to inner face of posterior part of



**Fig. 3.** Anterior view (a) and medial view of maxillary (b), medial view of premaxillary (c) and dentary (d) in *C. macrostomum* ( $SL = 9.2$  cm) (*An*—angular; *Anp*—angulo-articular process; *Asd*—articular surface of dentary; *Crp*—coronoid process; *Dn*—dentary; *Keth*—kinethmoid; *Maap*—maxillary anterior ascending process; *Mdcb*—maxillary dorsal concaved border; *Mdip*—maxillary descending process; *Mdp*—maxillary distal process; *Mip*—maxillary mid-lateral ascending process; *Mxf*—maxillary foramen; *Pmx*—premaxilla; *Psj*—peg and socket joint; *Ra*—retroarticular; *Rap*—rostral ascending process; *Top*—tail of premaxilla). Scale bar 5 mm.

interopercular. The opercular has a prominent process that points toward the hyomandibular. The posterior edges of the opercular and subopercular cover the anterior margin of the cleithrum.

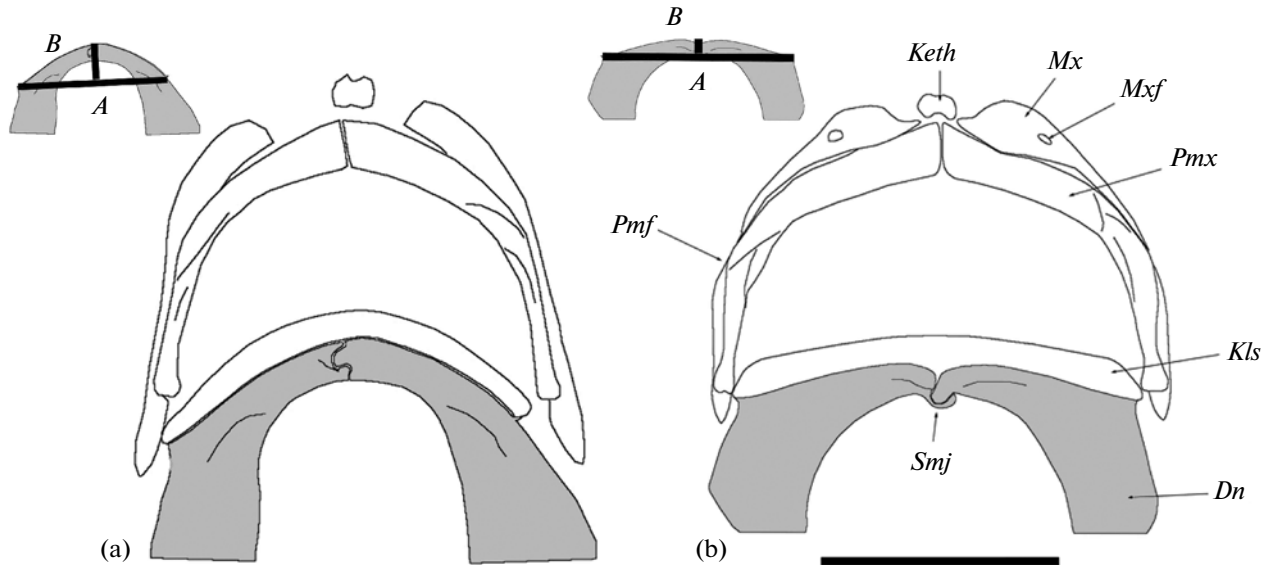
**Suspensorioum.** The suspensorium consists of the hyomandibular, ectopterygoid, endopterygoid, metapterygoid, quadrate and palatine (Fig. 2b). The suspensorium articulates anteriorly with the neurocranium via a joint between the palatine and the preethmoid and the vomer, and posteriorly via a joint between the hyomandibular and the prootic and pterotic. A crest of membrane bone extends to the dorsal half of the hyomandibular. The metapterygoid is a triangular element which is embraced by the hyomandibular posteriorly and the quadrate, ecto- and entopterygoid anteriorly. The entopterygoid is anteriorly connected to the palatine, quadrate, and ectopterygoid and posteriorly to the metapterygoid. Its posterior process covers the antero-medial border of preopercular. Autopalatine is posteriorly connected to the preethmoid and pre-vomer.

**Circumorbitals.** The infraorbital series consists of the lachrymal and four adjacent elements (Fig. 2c). The oval shaped lachrymal is the largest element

before infraorbital series and pointed ahead. In both species, infraorbital series are similar. Infraorbitals 2–5 are long and slender. A sensory canal passes across all the infraorbitals. There is only one supraorbital in both species.

**Jaws.** *Cyprinion macrostomum* and *C. kais* have a broad, subterminal mouth with cornified cutting edges called sector mouth (Howes, 1982). However, the mouth opening in *C. kais* is narrower than in *C. macrostomum*. The upper jaw consists of the premaxillary and maxillary. The premaxillary in the species of *Cyprinion*, as in other cyprinids, is a toothless bone and bears a long rostral process that continued upwardly and lied parallel to kinethmoid. The kinethmoid is connected to the maxillary with ligaments and continued dorso-posteriorly to anterior edge of supraethmoid (Fig. 3a).

Lower edge of the maxillary covers the upper edge of the premaxillary. A foramen pierces the maxillary anterodorsally (Figs. 3b, 3c). Its anterior an upper corner is thickened. The posteroventral portion of the maxilla is declined by  $45^\circ$ . The premaxillary process and the rostral process of the maxillary embraces the dorsoanterior part of the premaxillary. Behind the



**Fig. 4.** Ventral view of jaws in *C. kais* ( $SL = 8.6$  cm) (a) and *C. macrostomum* ( $SL = 9.2$  cm) (b) (A—jaw width; B—jaw radius; Dn—dentary; Keth—kinethmoid; Kls—keratinized lip surface; Mx—maxillary; Mxf—maxillary foramen; Pmf—premaxillary fold; Pmx—premaxilla; Smj—synarthritic mandibular joint). Scale bar 5 mm.

maxillary anterior ascending process, there is an antero-ventrally process connected to its partner from another maxillary. Connection of these processes make a notch that plays a backrest role for the premaxillary.

The lower jaw is a united bone made of the dentary, angular and the retroarticular (Fig. 3d). The retroarticular is a small element connected to the postero-ventral corner of the angular. The coronoid process is a massive and blunt, dorsally oriented process. The angular, which forms the articulation surface for the quadrate anterior edge of the dentary, is thickened and its surface covered by keratinized tissue. In both species the left dentary fits into a deep notch of its congener (peg and socket). In both species, medial peg being on the left dentary and inserting to a deep notch in right dentary (Fig. 3d). Angular is a gad like bone and latched with dentary in internal surface.

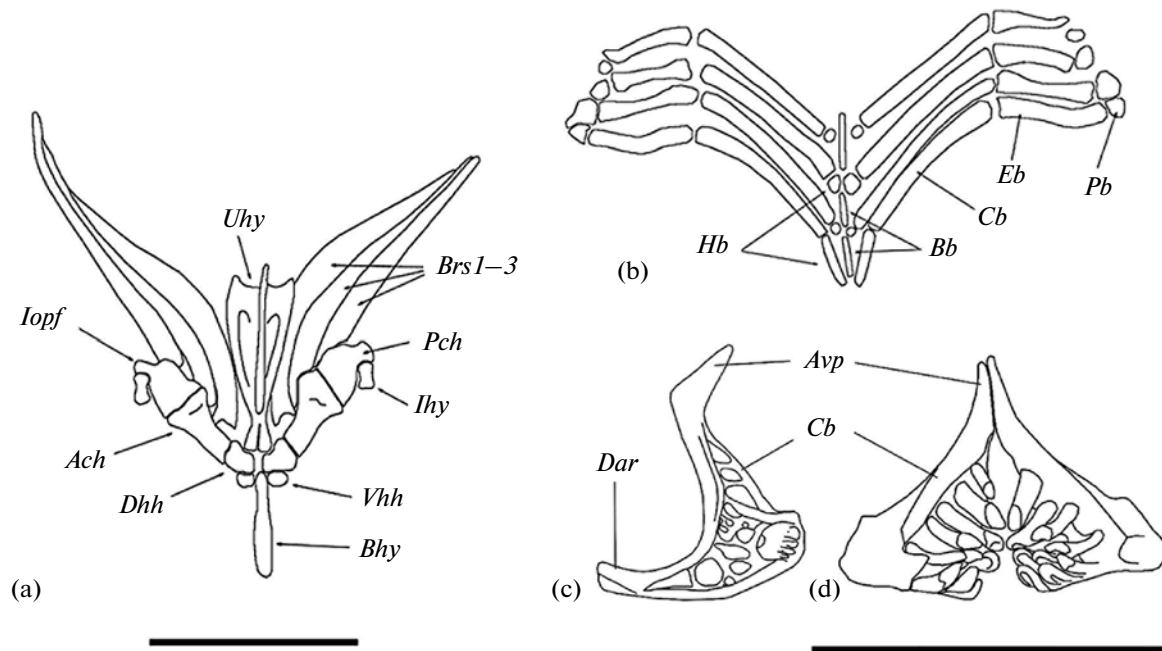
There are some differences between the two species in the shape of the jaws. The mouth opening in *C. kais* (Fig. 4a) is more curved than in *C. macrostomum* (Fig. 4b) because the maxillary and the dentary in *C. kais* are more curved than in *C. macrostomum*. In addition, dentaries curvature in *C. kais* is more than that of *C. macrostomum*. The ratio of  $B/A$  are 0.1 and 0.3 in *C. macrostomum* and *C. kais*, respectively (A: distance between the two lateral corner of dentaries, B: the distance between the junction of the two dentaries to line A). The premaxillary and maxillary are also narrower in *C. kais* than in *C. macrostomum* (Fig. 4).

**Hyoid and branchial arches.** The hyoid arch consists of the basihyal, ventral hypohyal, dorsal hypohyal, anterior ceratohyal, posterior ceratohyal, interhyal, urohyal and branchiostegals (Fig. 5a). The ante-

rior ceratohyal articulates with the dorsal and ventral hypohyals. A small stripe of cartilage separates the two ceratohyals. The posterior margin of the posterior ceratohyal bears an articulation surface for the interhyal. The urohyal is arrow tail shaped and anteriorly attached to the ventral hypohyals via a ligament. Its vertical and horizontal plates are triangular in lateral and dorsal views, respectively. There are three branchiostegal rays of which the first and the second are connected to the anterior and the third to the posterior ceratohyal (Fig. 5a). Hypohyal consists of the dorsal and ventral elements and the dorsal hypohyal is larger than the ventral hypohyal. The interhyal is an hour-glass shaped bone that connects the posterior ceratohyal to the suspensorium.

There are three medial basibranchials followed by four pairs of hypobranchials connected to the ceratobranchials 1–4 (Fig. 5b). There are four elongated ceratobranchials, four epibranchials and four pharyngobranchials. The basibranchials are thin and long rods. There are two rows of gill rakers in anterior border of ceratobranchials 1–4. The first hypobranchials are elongated but the others are shortened and spherical. There are four pharyngobranchials and five ceratobranchials in each side that the fifth one turned into the pharyngeal teeth. The branchial arches of the two species were similar.

The pharyngeal teeth are arranged in three rows with a dental formula that varies as 2.3.5–5.3.2, 2.3.5–4.3.2, 2.3.4–5.3.2, and 2.3.4–4.3.2 (in 308 examined specimens). Pharyngeal teeth of *C. kais* and *C. macrostomum* were similar (Figs. 5c, 5d). The tip of the teeth is shovel like. Except the first teeth of the second row, the head of the others are inclined toward the mouth



**Fig. 5.** Dorsal view of hyoid arches (a) and branchial apparatus (b) and lateral (c) and dorsal (d) views of pharyngeal teeth of *C. macrostomum* ( $SL = 9.2$  cm) (*Ach*—anterior ceratohyal; *Avp*—anterio-ventral process of ceratobranchial; *Bb*—basibranchial; *Bhy*—basihyal; *Brs 1–3*—branchiostegals 1–3; *Cb*—ceratobranchial; *Dar*—dorsal arm of ceratobranchial; *Dhh*—dorsal hypohyal; *Eb*—epibranchial; *Hb*—hypobranchial; *Ihy*—interhyal; *Iopf*—interopercular facet; *Pb*—pharyngobranchial; *Pch*—posterior ceratohyal; *Uhy*—urohyal; *Vhh*—ventral hypohyal). Scale bar 5 mm.

opening. The two ceratobranchials connected to each other via their antero-ventral processes.

**Dorsal fin skeleton.** The dorsal fin support is usually composed of 14–15 pterygiophores and a stay (Fig. 6). The dorsal fin has 3–4 unbranched rays (spine) and 10–16 (mostly 13–15) branched rays. The first pterygiophore of dorsal fin is wider than others and elongated to reach close to the ninth vertebral centrum. The first 2–3 unbranched rays are connected to the first pterygiophore. The last dorsal unbranched ray connected to the second pterygiophore and its posterior edge is serrated. All the dorsal rays are connected to the pterygiophores via a mobile joint that let them slightly move. There are seven supraneurals in front of the dorsal fin that connected to each other by connective tissue. In both species, the neural complex connected to the supraoccipital crest and the last supraneural connected to the first pterygiophore. The neural complex is an inverse isosceles triangle (axe shaped) bone that enclosed the neural spine of the first vertebra. The last unbranched ray of the dorsal fin is narrower and with finer serrations in *C. kais* (Fig. 6a) than in *C. macrostomum* (Fig. 6b).

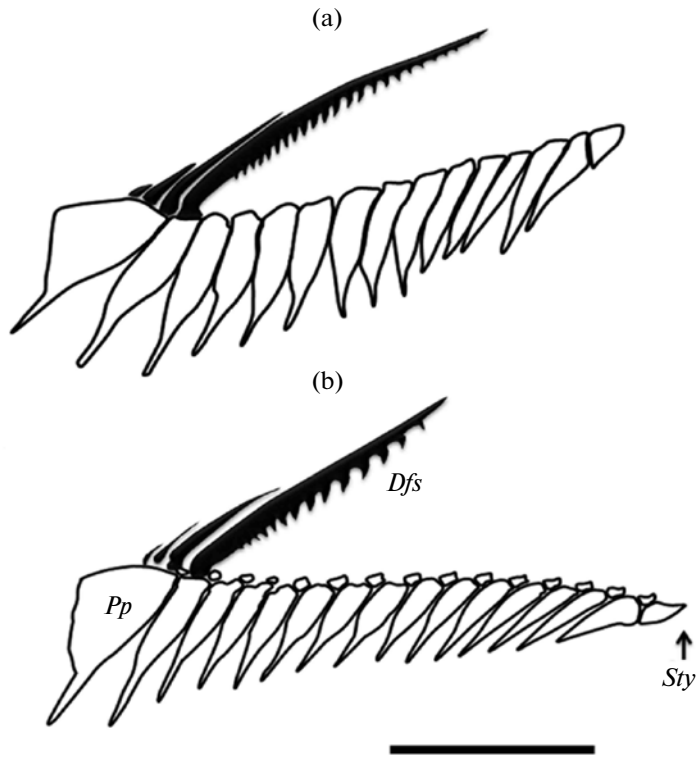
**Anal fin.** Anal fin support composed of eight pterygiophores and a stay (Fig. 7a). There are 1–2 unbranched rays in the anal fin. The unbranched fin rays and the first branched one, connected pally to the first pterygiophore. The terminal of the first anal pterygiophore is adjacent to the 23rd vertebra. The

specimens with two unbranched fin rays, commonly have a larger proximal pterygiophore.

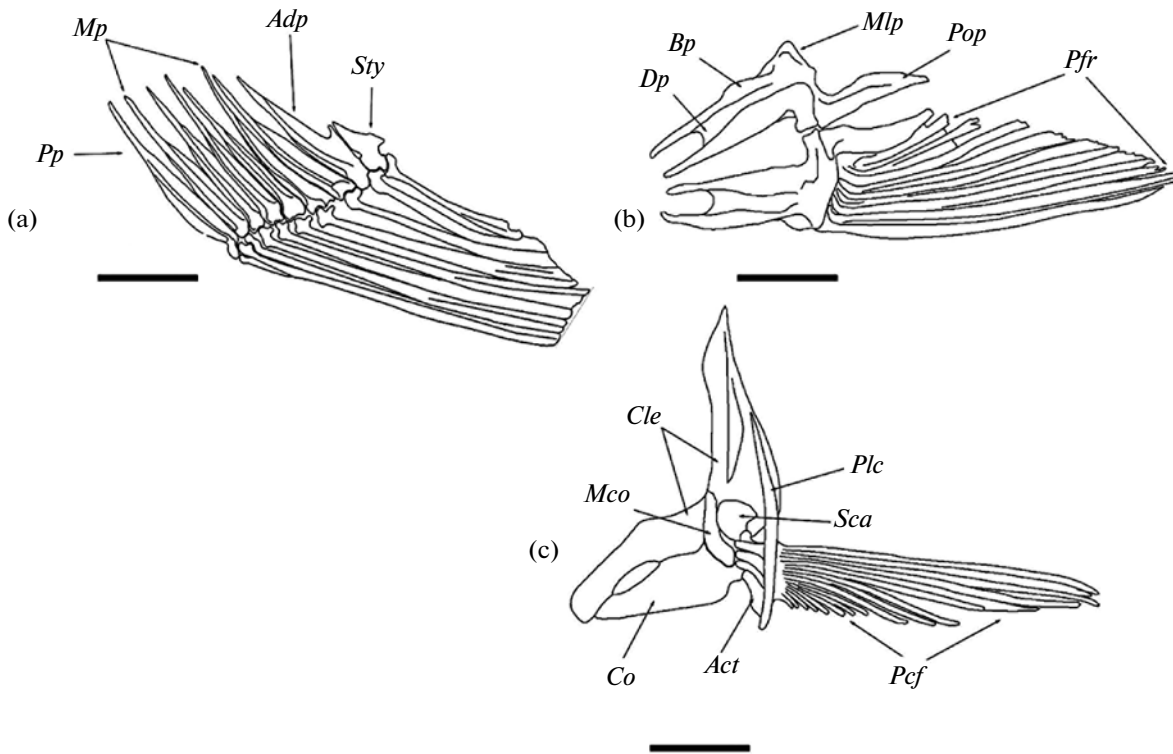
**Pelvic girdle.** The pelvic girdle is a simple structure that contained the pelvis, the mid lateral process and the posterior process (Fig. 7b). The pelvic girdle not connected to any other skeleton but enclosed by muscles. The distal process is bifurcated anteriorly. The posterior process is embowed and its tip is spine like. The two main bones of the pelvic girdle are deeply forked interiorly. There are no differences between the two species in their pelvic fin structure.

**Pectoral girdle.** Pectoral girdle consists of the supracleithrum, cleithrum, postcleithrum, scapula, coracoid, mesocoracoid and four actinosts (Fig. 7c). Supracleithrum attaches to the pterotic dorsally and to the cleithrum ventrally. The postcleithrum articulates with the upper half of the cleithrum. The scapula anteriorly connects to the middle of the cleithrum and posteriorly to the first pectoral ray. The form of the pectoral girdle segments in the two species is similar. There are 10–15 branched rays in the pectoral fin.

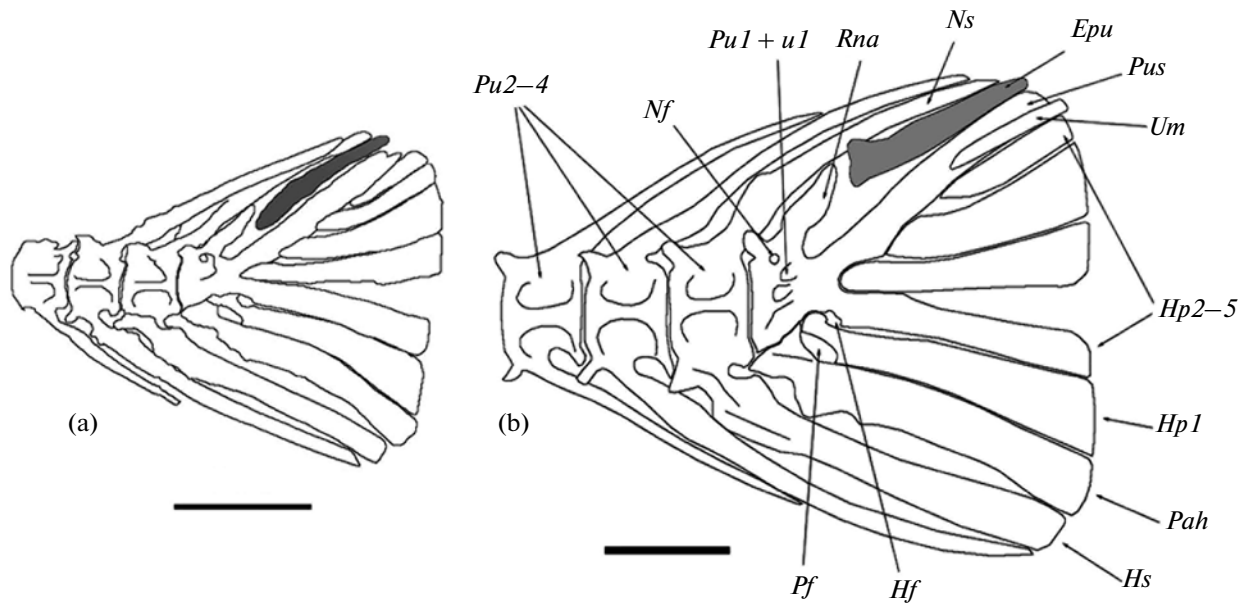
**Caudal skeleton.** In the caudal complex, posterior margin of elements fringed with cartilage. The pleurostyle and the hypural 2 are equal (Fig. 8). There are five hypurals connected to the urostyle. Epurals ventrally connected to the pleurostyle via connective tissue. The neural and hemal spines of the pleurostyle 2–4 are inclined backward to support the hypural plate. The neural foramens on the base of the rudimentary neural arch are similar in both species. In *C. kais*, the epural



**Fig. 6.** Lateral view of the dorsal fin spine in *Cyprinin kais* ( $SL = 8.6$  cm) (a) and *C. macrostomum* ( $SL = 9.2$  cm) (b) (*Dfs*—dorsal fin spine; *Pp*—pterygiophore; *Sty*—stay). Scale bar 5 mm.



**Fig. 7.** Lateral view of the anal fin (a), dorsal view of the pelvic fin (b) and internal view of the right pectoral fin (c) of *C. macrostomum* ( $SL = 9.2$  cm) (*Act*—actinost; *Adp*—anal distal pterygiophore; *Bp*—pelvic bone; *Cle*—cleithrum; *Co*—coracoid; *Dp*—distal process; *Mco*—mesocoracoid; *Mlp*—mid-lateral process; *Mp*—mesial pterygiophore; *Pef*—pectoral fin; *Plc*—postcleithrum; *Pfr*—pelvic fin rays; *Pop*—posterior process; *Pp*—pterygiophore; *Sca*—scapula; *Sty*—stay). Scale bar 5 mm.



**Fig. 8.** Lateral view of the Caudal skeletons of *C. kais* ( $SL = 8.6$  cm) (a) and *C. macrostomum* ( $SL = 9.2$  cm) (b) (*Epu*—epural; *Hf*—hypural foramen; *Hp1–5*—hypural plates 1–5; *Hs*—hemal spine; *Nf*—neural foramen; *Ns*—neural spine; *Pah*—parhypural; *Pf*—parhypural foramen; *Pu2–4*—preurals 2–4; *Pu1 + u1*—compounded centrum; *Pus*—pleurostyle; *Rna*—rudimentary neural arch; *Um*—uroneural). Scale bar 5 mm.

is a bowed bone that does not have any process and its anterior head is under the rudimentary neural arch (Fig. 8a), but in *C. macrostomum*, the anterior head of the epural has an ascending process that dorsally touches the neural spine of the pleurostyle 2 (Fig. 8b).

## DISCUSSION

Generally, in cyprinids, there is little variation in the bones of the orbitotemporal region, including supraorbitals, frontals and supraorbital sensory canals (Ramaswami, 1951). In *Cyprinion*, as in some other cyprinids, the interorbital septum formed by ventromedial expansion of intralateral portions of orbitosphenoids (Ramaswami, 1951). In both species, as in some other cyprinids, the companionship of the ventral portions of orbitosphenoids to form an interorbital septum is evident. Howes (1978, 1980) noted that typically the wall of subtemporal fossa in cyprinids formed by exoccipital, prootic and a minor contribution from the pterotic with an exception in *Cyprinion*. We observed a substantial contribution of the pterotic in both species. In both species, the supraoccipital crest is high and the parietal extended postero-medially, forming part of the supraoccipital crest (Figs. 1a, 1b) as seen in *Semiplotus* (Howes, 1982). In some *Cyprinion* species (*C. kais* and *C. microphthalmum*), truncated basioccipital process associated with a marked reduction of the ventral plate (Howes, 1982) but we did not see any sign of ventral plate reduction in our specimens.

Ventrally directed supraethmoid, hypertrophied vomer and rostral curvature of the ethmoid bloc are

variable in cyprinids. In *C. macrostomum*, the curvature of the supraethmoid is high similar to that of *Onychostoma* and *Varicorhinus* (Howes, 1980). We failed to recognize the frontal-supraethmoid foramen, because the two frontals and supraethmoid latched to each other without any separations.

Alkahem et al., (1987) noted that the premaxillary in *C. mhalensis* was thin and narrow but thick and broad in *C. acinaces*, also its posterior process in *C. acinaces* was distally bifurcated but flat in *C. mhalensis*. The premaxillary posterior process in our species is uniformly thin. Howes (1982) in comparison of *Semiplotus* and *Cyprinion* denoted that the ascending process of premaxillary is usually missing or slightly developed, we found that the ascending process in *Cyprinion* was distinct, though, in comparison with *Semiplotus* is less developed (Fig. 4c). There are two thin and large anterior ascending processes on the maxillary in *C. mhalensis* but one anterior ascending process in *C. acinaces* (Alkahem et al., 1987). In our specimens, the maxillary has only one anterior ascending process. The maxillary posterior process in *C. acinaces* is expanded to form a plate-like structure but in *C. mhalensis* it is shortened and triangular. Also, they differ in presence of a large foramen in the upper part of the maxillary in *C. acinaces* and its absence in *C. mhalensis* (Alkahem et al., 1987). In this study, the maxillary posterior process in both species are somewhat similar to that of *C. mhalensis* but the maxillary foramen is clearly visible in both *C. macrostomum* and *C. kais*. The shape of the dentary, premaxillary and maxillary were different in the two species which is reflected in their mouth shapes (in *C. kais*, the mouth



opening is narrower). As noted by Howes (1982), the lower jaw of *C. kais* is deeper posteriorly than any other *Cyprinion* species and its labial surface is much narrower. He also noted that the lower jaw of *C. macrostomum* and *C. microphthalmum* resembles that of *Semiplotus*, not other *Cyprinion* species. He showed that *C. macrostomum* is different with other *Cyprinion* species in the curvature of the dentary, the complexity of its synarthritic joint and broadness of articular surface of the angulo articular.

It seems that the form of the lower jaw in *Cyprinion* genera is one of the most morphologically variable traits (e.g., Howes, 1982; Alkahem et al., 1987). The most important characters that distinguishes the *C. macrostomum* from *C. kais* is the form of mouth, which in *C. macrostomum*, its opening is very wide and almost straight (Banarescu and Herzig-Straschil, 1995). In *Cyprinion* species, the anguloarticular extends posteriorly only marginally beyond the dentary (Howes, 1982). He also noted that the degree of keratinization of lower lip is more developed in *C. macrostomum*, which is confirmed here.

A developed substantial third branchiostegal shelf was reported in *C. macrostomum* (Howes, 1982) and noted that this condition is more developed than in *C. kais*. In this study, this situation was not observed. Howes (1982) noted that interlocking of the last supra-neural with the first pterygiophore occurred only in *C. macrostomum*, we observed that condition in both species and we did not see any differences in this regard.

Finally Based on the observed differences between the two species (i.e. the deeper posterior position of the lower jaw with a much narrower labial surface, longer last dorsal unbranched ray with weaker posterior serration, and more embowed maxillary and pre-maxillary in *C. kais*), we conclude that these two taxa are morphologically distinct, though not well revealed by Cytochrome *b* analysis (Durand et al., 2002), probably due to their recent speciation.

#### ACKNOWLEDGMENTS

We would like to thank G. Abdali, Y. Nasri, J. Nasri, late H. Farokhi and H. Bagheri for their various help. This project was financially supported by Isfahan University of Technology.

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