Descriptive and comparative osteology of Bighead Lotak, *Cyprinion milesi* (Cyprinidae: Cypriniformes) from southeastern Iran

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Abstract

The descriptive osteology of *Cyprinion milesi* is hereby provided for the first time and compared with those of two other Iranian members of this genus, i.e. *C. macrostomum* and *C. kais*. For examine of osteological characteristics of this species, 12 collected specimens from Sarbaz River at Mokran basin, southeastern Iran were stained using Alizarin red S. The skeletal elements were separately photographed and described. Based on the results, differences in the osteological characters between *C. milesi* with *C. macrostomum* and *C. kais* include: the neurocranium in *C. macrostomum* and *C. kais* is sub triangular whereas in *C. milesi* is narrow and triangular, the lateral and posterior corners of supraethmoid in *C. milesi* unlike *C. macrostomum* and *C. kais* is elongated posteriorly connecting the lateral process of the parethmoid, the curvature of lower jaw in C. *milesi* is between the range of that of *C. macrostomum* and *C. kais*, the ceratohyal in *C. milesi* is two times longer than that of *C. macrostomum* and *C. kais*, the horizontal arm of the preopercle and the length of opercle in *C. milesi* are longer than that of *C. macrostomum* and *C. kais*, the number of dorsal fin pterygiophores in *C. milesi* is lower than those of *C. macrostomum* and *C kais*, the origination of dorsal fin in *C. milesi* is anterior than that of *C. macrostomum*, in *C. milesi*, the supraneurals are separated from each other whereas in *C. macrostomum* and *C. kais*, they are articulated with each other and the last unbranched dorsal fin ray in *C. milesi* is more slender in shape than that of *C. macrostomum* and *C. kais* and its serration is covering one-third of its length, the neural complex is T-shape in *C. milesi* but it is axe-shape in other studied *Cyprinion*.

Key words

Inland water Fishes of Iran, Osteology, Cyprinidae, Ichthyology, Biodiversity.

Introduction

The members of the genus *Cyprinion*, belonged to Cyprinidae, distribute in south and east Asia in Nepal, India, Pakistan, Afghanistan, Iran, Turkey, Iraq, Arabian peninsula and Syria (Mirza, 1969; Froese & Pauly, 2014; Coad, 2014). Howes (1982) pointed out that *Semiplotus* (Bleeker, 1859) from Nepal to Vietnam, *Scaphiodonichthys* (Vinciguerra, 1890) from Indochina and *Cyprinion* Heckel, 1843 are synonymous. That was disapproved by Krupp (1983). Formerly, *Cyprinion* and *Capoeta* constituted the genus *Scaphiodon* (Heckel 1843)

(Coad, 2014), but the genus *Cyprinion* separated from *Semiplotus* by having barbels, fewer number of dorsal fin rays and more unbranched rays in anal fin (Banarescu & Herzig-Straschil, 1995; Banarescu, 1992).

The genus *Cyprinion* contains five species in Iran distinguishing due to morphological characters, especially mouth form and dorsal fin rays (Coad, 2014; Abdoli, 2000; Kafuku, 1969). Osteological characters can provide valuable information for various purposes such as phylogenetic analysis (Diogo & Bills, 2006), archaeolo-
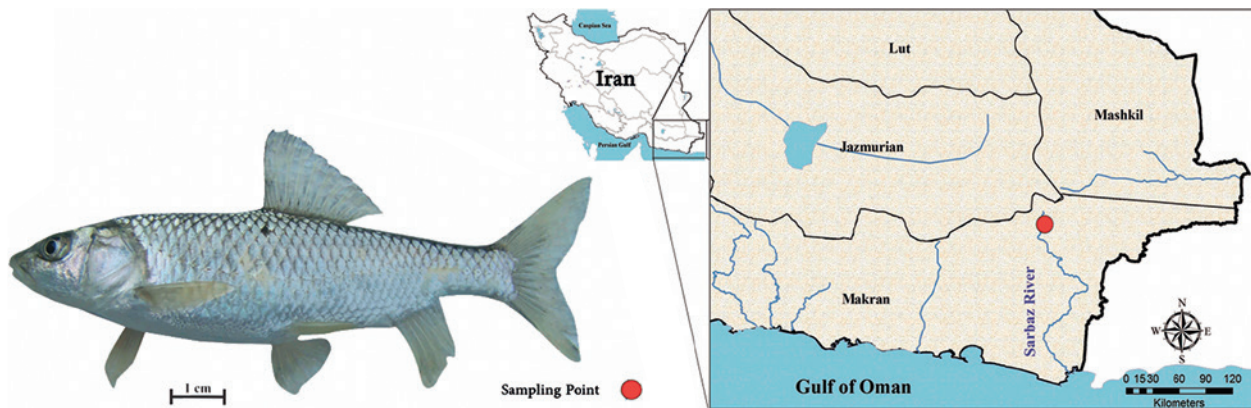
Fig. 1. Lateral view of *Cyprinion milesi* (Left) and map of sampling site from Sarbaz River (Right).

gy (CARNEVALE *et al.*, 2011; HILTON, 2003), ontogeny and developmental study (FIAZ *et al.*, 2012; BRITZ & CONWAY, 2009; BRITZ, 1996) of fishes, particularly in the genus *Cyprinion* due to its few morphological distinguishable characters in terms of taxonomy.

A few osteological works on genus *Cyprinion* is available. HOWES (1982) studied the anatomy and evolution of the jaw in the genus *Cyprinion*. The diversity of pharyngeal teeth of *C. macrostomum* populations from Karkheh river basin studied by NASRI *et al.* (2008). Osteological comparison of *C. macrostomum* and *C. kais* from western Iran revealed some differences between them (NASRI *et al.*, 2013b). Given wide distribution of this genus in Asia and morphological diversity of the member of the genus *Cyprinion* (NASRI & EAGDERI, 2013; NASRI *et al.*, 2013a; COAD, 2014), it is expected to understand their within and between species diversity using osteological characters to better understanding of their taxonomic status. Hence, this study was aimed to provide detailed osteological features of another species of this genus, i.e. *C. milesi*. The results of this study can offer further osteological information to investigate the phylogenetic relationship of this genus and answering some questions about the zoogeography of the member of this genus.

Materials and methods

Twelve specimens of *C. milesi* (92±13 mm; SL±SD) were collected from Sarbaz River near Sarbaz town in Sistan and Baluchistan Province (Fig. 1) using electrofishing. The specimens were fixed in buffered formaldehyde 10% after anesthetizing in 1% clove-oil solution. For osteological examination, the specimens were cleared and stained with alizarin red S and alcian blue according to the protocol of (TAYLOR, 1967). The skeletal structures were studied under a stereomicroscope (Leica M5). Then, they photographed using a digital camera (6six mega pixels) and a scanner using 3400 megapixel equipped with a glycerol bath. The skeletal elements were drawn based on 2D digital pictures using CorelDraw X7 software. The skeletal nomenclatures follow (HOWES, 1982; ROJO, 1991).

Results

Neurocranium

The neurocranium is elongated and triangular, and its width at the pterotic region is three times of the supraethmoid region. The neurocranium is well-ossified and the frontal, parietal, supraorbital, nasal and supraethmoid bones cover the neurocranium dorsally. Furthermore, some parts of the pterotic, epiotic, supraoccipital, sphenoid, lateral ethmoid, preethmoid and mesethmoid are visible in dorsal view (Fig. 2A). Frontals covers more than 50% of the head roof. The two rainspout-shaped nasals position in both sides of the supraethmoid, supporting the nostrils. Their lengths are about half of the supraethmoid's length. The nasal is connected to junction of the supraethmoid and lateral process of parethmoid and encloses the terminal head of the supraorbital lateral line canal. Supra-cranial lateral line canal is enclosed by the pterotic, parietal, sphenoid, frontal, lateral process of the parethmoid, nasal and supraethmoid (Fig. 2A). This canal is divided into two branches at the anterior of frontal to support the upper and lateral faces of the ethmoid region.

Ethmoid region

This region is comprised of the paired parethmoids, unpaired cartilaginous mesethmoid and supraethmoid. The lateral process of the parethmoid forms the anterior wall of the orbit and separate orbit region from the ethmoid region. This bone bears a lateral projection orienting downward (Fig. 2A, B). The ethmoid region is divided into two lateral parts that are covered by the supraethmoid and prevomer dorsally and ventrally, respectively. The lateral and posterior corners of the supraethmoid are elongated posteriorly connecting to the lateral process of
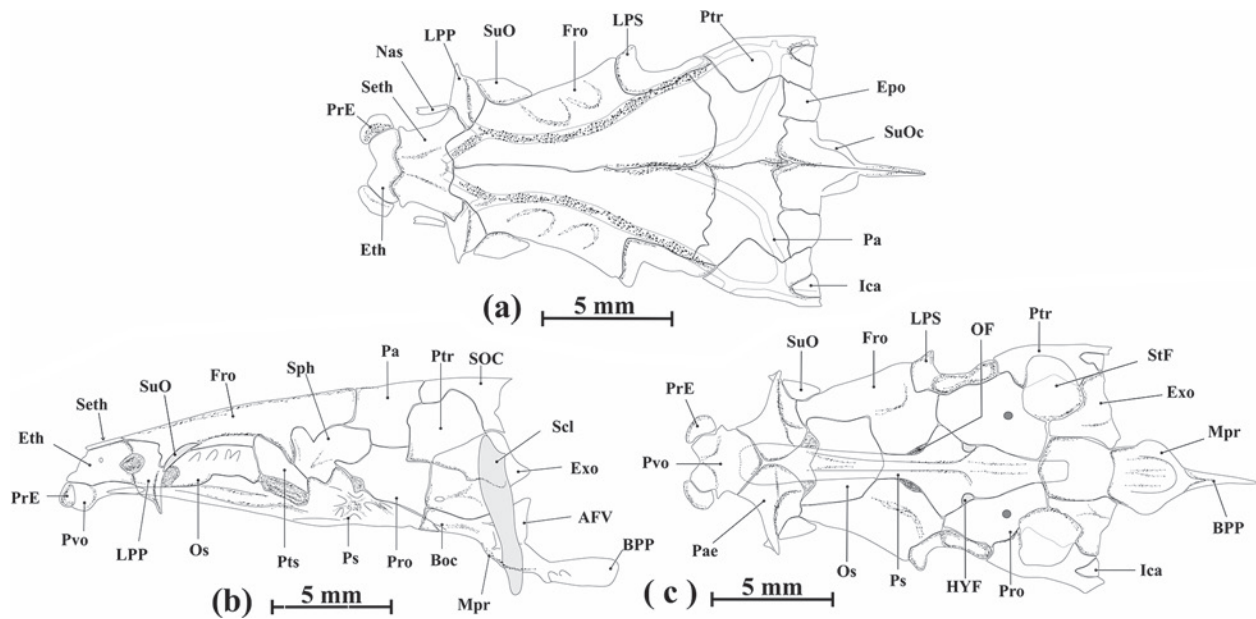
Fig. 2. Dorsal (A), lateral (B) and ventral (C) views of the neurocranium in C. milesi. (AFV – Articulation facet for the first vertebra; Boc – Basioccipital; BPP – Basioccipital Posterior Projection; Bs – Basisphenoid; Epo – Epiotic; Eth – Ethmoid; Exo – Exoccipital; Fro – Frontal; Hyf – foramen for hypophysial artery; HYF – Hyomandibular Joint Face; Ica – Intercalar; LEt – Lateral Ethmoid (Parethmoids); LPP – Lateral Process of parethmoid; LPS – Lateral Process of Sphenotic; MPr – Masticatory Process; Nas – Nasal; OF – Orbital Foramen; OpF – Optic Foramen; Os – Orbitosphenoid; Pa – Parietal; Pae – Parethmoid; Peth – preethmoid; PoT – Post temporal; Pro – Prootic; Ps – Parasphenoid; Ptr – Pterotic; Pts – Pterosphenoid; Pvo – Prevomer, SCl – Supra Cleithrum; Seth – Supraethmoid; Sph – Sphenoid; StF – Subtemporal Foramen; SOC – supraoccipital crest; SuC – Supraoccipital crest; SuO – Supra Orbital).

the parethmoid. The preethmoid ventrolaterally positions at the anterior corners of the ethmoid and forms a joint face for palatine and maxilla. The ethmoid possesses a smooth notch on its anterior edge enclosing kinethmoid. The toothless prevomer positions at the anteroventral of neurocranium and forms the palate of mouth. The prevomer is heart-shaped bearing two lateral projections that their length and width are equal (Fig. 2C).

Orbital Region

The supraorbital, orbitosphenoid and pterosphenoid bones form the orbital region (Fig. 2B, C). The supraorbital is elliptic-shaped and elongated, and positions beside the frontal and lateral process of parethmoid. The two orbitosphenoids attach to each other medially, connecting to the parasphenoid dorsally. The parethmoid, pterosphenoid and frontal are connected to the orbitosphenoid anteriorly, posteriorly and dorsally, respectively. The orbital foramen is located between the pterosphenoid, orbitosphenoid and parasphenoid.

Otic region

The parietal, pterotic, epiotic, intercalar, sphenotic and prootic create a chamber enclosing the otic capsules. The parietal covers the posterior portion of the neurocranium. *Cyprinion milesi* belongs to medioparietal fishes,

i.e. their parietals connect to each other and separated the frontal and occipital regions. The pterotics are positioned at the posterior corners of the neurocranium and along with the intercalar form the facet of the pectoral girdle. The hyomandibular is connected to the neurocranium via the hyomandibular articular facets forming by pterotic and sphenotic. The pterotic encloses a horizontal semicircular canal and the anterior part of the cephalic lateral line canal dividing it into two branches, including temporal and frontal commissure (Fig. 2A). The prootics form the ventral wall of the otic chamber and enclose the utriculus semicircular canal and the lapillus ear bone. The position of lapillus in relation to the prootic shown as dark circles in Fig. 2C. The subtemporal fossa is formed by exoccipital, prootic and pterotic with the major contribution of the pterotic.

Basicranial Region

There are three cartilaginous bones *viz.* the supraoccipital, exoccipital, basioccipital and one dermal bone, parasphenoid in this region (Fig. 2A-C). The supraoccipital is positioned at the dorsoposterior corner of the neurocranium. The supraoccipital forms the upper border of the foramen magnum. The supraoccipital crest is developed posteriorly and its tip touching the neural complex. The exoccipitals form lateral and ventral borders of the foramen magnum and part of the subtemporal foramen (Fig. 2C).
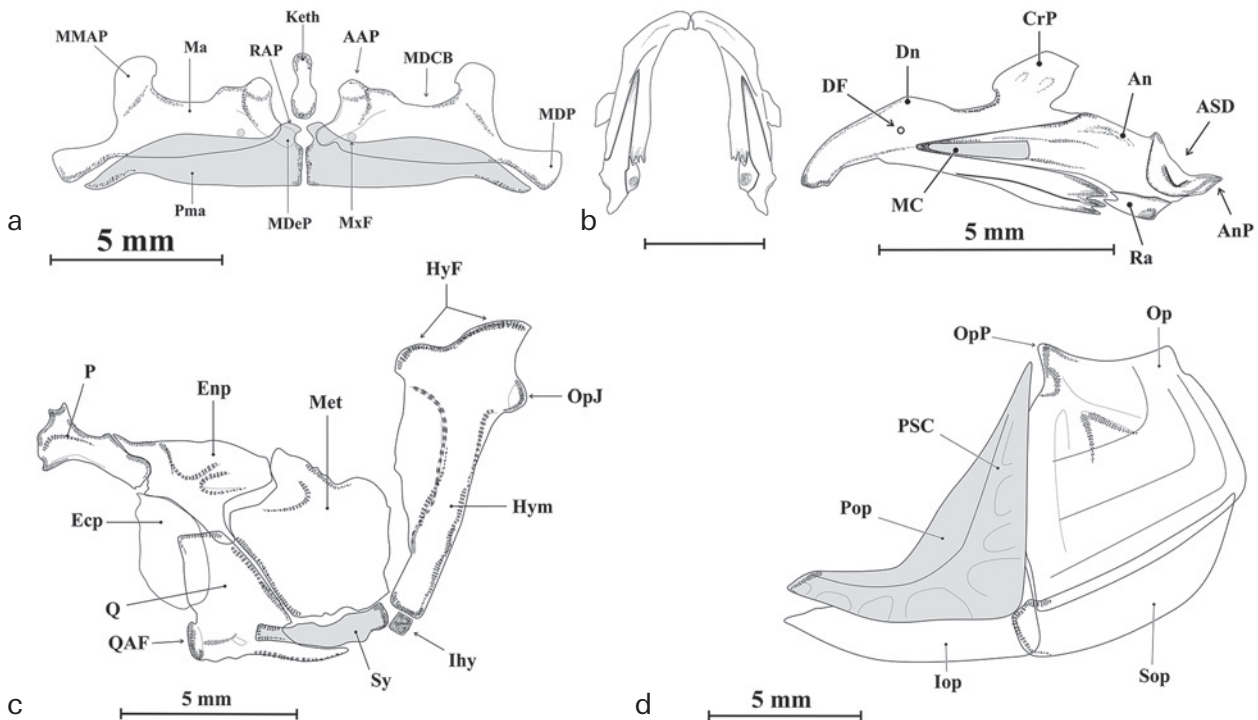
Fig. 3. Medial view of upper jaw (A), ventral and lateral view of lower Jaw (B), medial view of suspensorium (C) and medial view of opercular series (D) of *C. milesi*. (AAP – Anterior ascending Process; An – Angular; AnP – Angulo-articular Process; ASD – Articular Surface of Dentary; CrP – Coronoid Process; DF – Dentary Foramen; Dn – Dentary; Ecp – Ectopterygoid; Enp – Endopterygoid; HyF – Hyomandibular joint faces (condyles); Hym – Hyomandibular; Ihy – Interhyal; Iop – Interopercle; Keth – Kinethmoid; Ma – maxilla; MC – Meckel Cartilage; MDCB – maxillary dorsal concaved border; MDeP – Maxillary Descending Process; MDP – maxillary distal process; Met – Metapterygoid; MMAP – maxillary mid lateral ascending process; MxF – Maxillary Foramen; Op – Opercular; OpJ – Opercular Joint; OpP – Opercular Prominent Process; P – Palatine; Pma – Premaxilla; Pop – Preopercle; PSC – Preopercular Sensory Canal; Q – Quadrate; QAF – Quadrate Articular Face; Ra – Retroarticular; RAP – Rostral Ascending Process; Sop – Subopercle; Sy – Symplectic).

The supraneural 1 (before neural complex), claustrum and scaphium from the weberian apparatus posteroventrally connecting to the exoccipital. The posterior projection of the basioccipital is elongated and positioned ventral to the weberian apparatus and adjoining the tripus (Fig. 2B). The vertebral column connects the neurocranium through basioccipital. The masticatory plate is the ventral expansion of basioccipital and provides stithy surface for the pharyngeal teeth (Fig. 2B, C). The parasphenoid situates ventral to the neurocranium and extended from the basioccipital to prevomer. The medial portion of the parasphenoid is expanded ventrolaterally forming a surface for ligamentous connection of the pharyngobranchials (Fig. 2B, C).

Branchiocranium

Upper Jaw

The upper jaw is comprised of the premaxilla, maxilla and kinethmoid (Fig. 3A). The premaxilla is toothless and has a curved fragile structure. The anterior part of the premaxilla has an ascending process that inflexed toward its counterpart. The ascending process of the premaxilla ligamentously connected to the kinethmoid. The dumbbell-shaped kinethmoid is positioned between the two maxillae. Premaxilla placed at the notch of maxillary descending process and when relaxed, it prevents retreating the premaxilla. Maxillae is larger than premaxilla, and its dorsal border is concave between the maxillary midlateral ascending and anterior ascending processes. The mid-lateral ascending process of the maxillae is positioned near its distal part and covers the palatine laterally.

Lower Jaw

Three distinctive bones present in the lower jaw *viz.* the dentary, angular and retroarticular that interconnected to each other (Fig. 3B). The head of dentary is curved toward its counterpart and symphyseally articulated with it. The curvature of the lower jaw in *C. milesi* is elongated and horseshoe-like in shape. The angular is a cuspid-shaped positioned posterior to the dentary. The lower jaw is connected to the quadrate through its posterior joint face. The mackel cartilage positions inside the dentary, and the dentary nerve passes through it (Fig. 3B). The retroarticular is a small bone connecting to the angular ventrally and interlocking to the dentary posteriorly. The distal portion of premaxilla and maxilla ligamentously connected to internal face of coronoid process at the dorsal edge of the dentary.
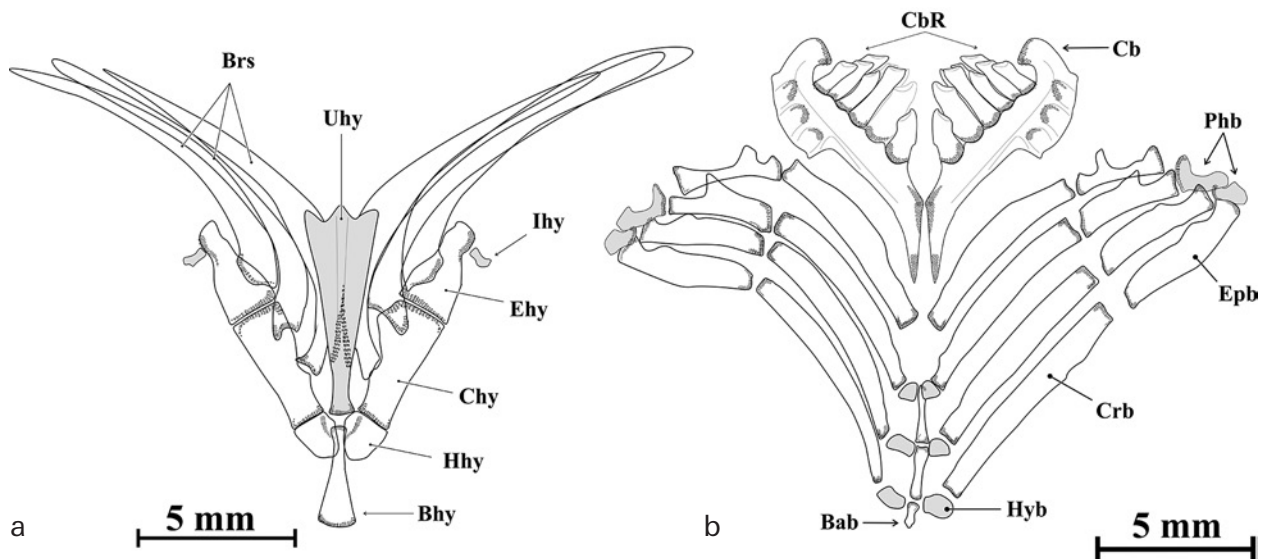
Fig. 4. Dorsal view of hyoid arch (Left) and dorsal view of branchial skeleton (Right) in *C. milesi*. (Bab – Basibranchial; Bhy – Basihyal; Brs – Branchiostegals; CbR – Ceratobranchial Rakers (Pharingeal teeth); Chy – Ceratohyal; Crb – Ceratobranchial; Ehy – Epihyal; Epb – Epibranchial; Hhy – Hypohyal; Hyb – Hypobranchial; Ihy – Interhyal; Phb – Pharyngobranchial; Uhy – Urohyal).

Suspensorium

The suspensorium consists 7 bones, including the palatine, endopterygoid, ectopterygoid, quadrate, symplectic, metapterygoid and hyomandibular (Fig. 3C). The suspensorium is articulated to the neurocranium via hyomandibular joint faces. The dentary is connected to the branchiocranium via quadrate. The head of palatine is larger than its posterior part and has a face to joint with preethmoid. The endopterygoid bears an anterodorsal projection that is posterodorsally connected to the upper edge of palatine. The ectopterygoid is elliptic-shaped and connected to the endopterygoid ventrally and to quadrate anteroventrally.

The hyoid arch and branchial skeleton are connected to the branchiocranium through the symplectic and interhyal. The symplectic medially attached to the quadrate. The quadrate covers the symplectic ventrally. The metapterygoid is positioned anteriorly, ventrally and posteriorly between the endopterygoid, quadrate and symplectic, respectively and connected to the hyomandibular by connective tissue. The hyomandibular is the largest element of the suspensorium. The opercular condyle presents at the posterodorsal edge of the hyomandibular.

Opercular series

The opercular series composes the preopercle, opercle, subopercle and interopercle (Fig. 3D). The two arms of the preopercle form a right angle to each other. This bone encloses the mandibular branch of the cephalic lateral line canal. The preopercle laterally covered the hyomandibular, and its horizontal arm is connected to the symplectic. The preopercle horizontal arm length is about 0.9 of its vertical arm. Its posterior and ventral laminar border covers the three other opercular bones. The opercle is the largest element of opercular series, and its length

is about 0.8 of its height. The opercle and subopercle are connected to the head of interopercle via connective tissue. The interopercle is positioned under the preopercle and connected to the lower jaw via connective tissue.

Hyoid Arch

The hyoid arch possesses two unpaired bones *viz*. the urohyal and basihyal. The basihyal is the anterior most element of the hyoid arch (Fig. 4A). The anterior head of basihyal is three times larger than its posterior head. The urohyal is like an arrow tail, and its posterior tips are blunt and equal in length. The urohyal is connected to the cleithrum posteriorly and to the hypohyals and basihyals anteriorly by connective tissue. There are three branchiostegals, one hypohyal, one ceratohyal, one epihyal and one interhyal in each half of the hyoid arch. The two thick hypohyals are positioned on both sides of the urohyal and basihyal junction. The two internal branchiostegals are connected to the ceratohyal and the lateral ones are connected to the epihyal by connective tissue. The third branchiostegal is wider than the others. The ceratohyal length is about 0.18 of neurocranium length. The hyoid arch is connected to the suspensorium by a short bar-shaped interhyal.

Branchial Skeleton

In the branchial, four anterior arches contain the branchial filaments and the fifth one transformed as the pharyngeal teeth. The branchial skeleton includes three unpaired basibranchials and three hypobranchials, four ceratobranchials, four epibranchials and two pharyngobranchials in each side that connected to each other via connective tissue (Fig. 4B). The branchial skeleton is connected to branchiocranium in three points, including (1) connection of the first basibranchial to basihyal, (2)
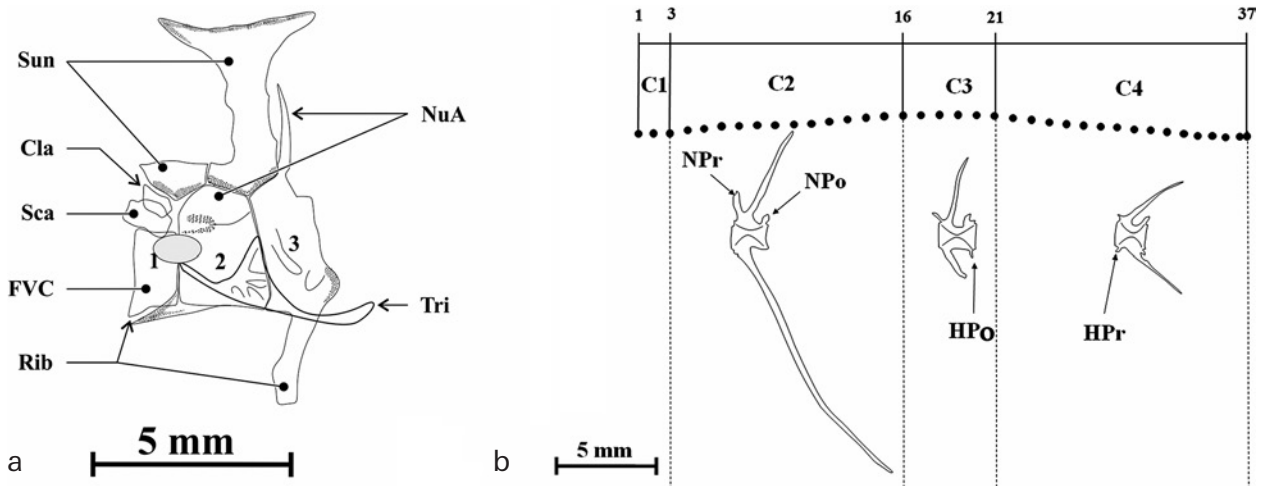
Fig. 5. Weberian apparatus (A) and schematic view of vertebral column displaying Weberian, Thoracic, Abdominal and caudal vertebrates (B) of *C. milesi*. Each filled circle represent a centrum. (C1 – Weber Region; C2 – Thoracic Region; C3 – Abdominal Region; C4 – Caudal Region; Cla – Claustrum; FVC – First Vertebra Centrum; HPo – Hemal Postzygapophyses; HPr – Hemal Prezygapophyses; NPo – Neural Postzygapophyses; NPr – Neural Prezygapophyses; NuA – Neural Arch; Rib – Ribs; Sca – Scaphium; Sun – Supraneural; Tri – Tripus).

connection of the pharyngobranchials to lateral portions of parasphenoid and (3) connection of the fifth cerato-branchial to the neurocranium. The pharyngeal teeth formula was as 2,3,5 – 5,3,2. The tips of teeth are shovel-like and covered by a white enamel. There are 16 gill rakers on the left first branchial arch.

Weberian apparatus

The supporting base for weberian apparatus is the three first vertebras and their related ossicles. These ossicles include the claustrum, scaphium, intercalarium (its position shown as a gray ellipsoid) and tripus (Fig. 5A). The tripus base positions on the second vertebrae and its posterior part passes underneath of the third vertebrae ribs and its tip deviated upward touching the gas bladder. The ligamentous connections between claustrum, scaphium, intercalarium and tripus make it possible to transmission and amplification of the seismic waves of gas bladder to the exoccipital. The first cervical vertebra is deformed and thinned, and its ribs are absent. The second verte-bra is mostly regressed and in the third vertebra, the ribs were shortened and thickened. The neural spine of the first vertebrae is absent, and it is atrophic in second ver-tebra. The neural spine of the third vertebra shortened and bent forwardly to the second supraneural. The first two supraneurals (neural complex) were interlocked to their related vertebrae. The second supraneural as the largest one is T-shaped and its posterior arm contacting the third supraneural supporting the ridge before the dor-sal fine.

Vertebral Column

There are 37 vertebrae of *C. milesi* including 3 weberian, 13 thoracic, 5 abdominal and 16 caudal (Fig. 5B). All ver-tebrae except the weberians' have neural prezygapophy-ses and neural postzygapophyses. The thoracic and ab-dominal vertebrae have larger zygapophyses and longer neural spines supporting the dorsal fin skeleton. The he-mal spines of the thoracic and abdominal vertebrae are absent, whereas those of caudal are attached to each other and their lengths are equal to their neural spines.

Pectoral Girdle

The supporting bones of the pectoral fin include the pri-mary and secondary pectoral girdle. The primary pectoral girdle (chondral coracoid and scapula) is connected to the distal and internal edge of cleithrum and the secondary pectoral girdle includes cleithrum, supracleithrum and postcleithrum. (Fig. 6A). The scapula situates between the coracoid and cleithrum. The first unbranched pecto-ral fin ray is directly connected to the scapula, but others connected to the pectoral girdle mediated by actinosts. There are five actinosts in the pectoral girdle, and the first two ones are smaller and connected to the scapula but others are larger and connected to the posterior edge of the coracoid. The pectoral girdle is connected to the neu-rocranium (pterotic) via supracleithrum and connected to the branchiocranium (urohyal) via the distal arm of the cleithrum. The postcleithrum is connected to the internal face of cleithrum.

Dorsal Fin Skeleton

Seven supraneurals (including neural complex) present prior to the dorsal fin that not connected to each other (Fig. 6B). The dorsal fin has 10 pterygiophores and one stay. Each pterygiophore as a base of fin ray consists of three portions, the distal, medial and proximal processes. The first proximal pterygiophore of the dorsal fin is the largest one and its proximal tip overlapping with the neu-ral spine of the vertebra 9. There are four unbranched dorsal fin rays that the first three rays are attached to the first pterygiophore. The first and sometimes the two first
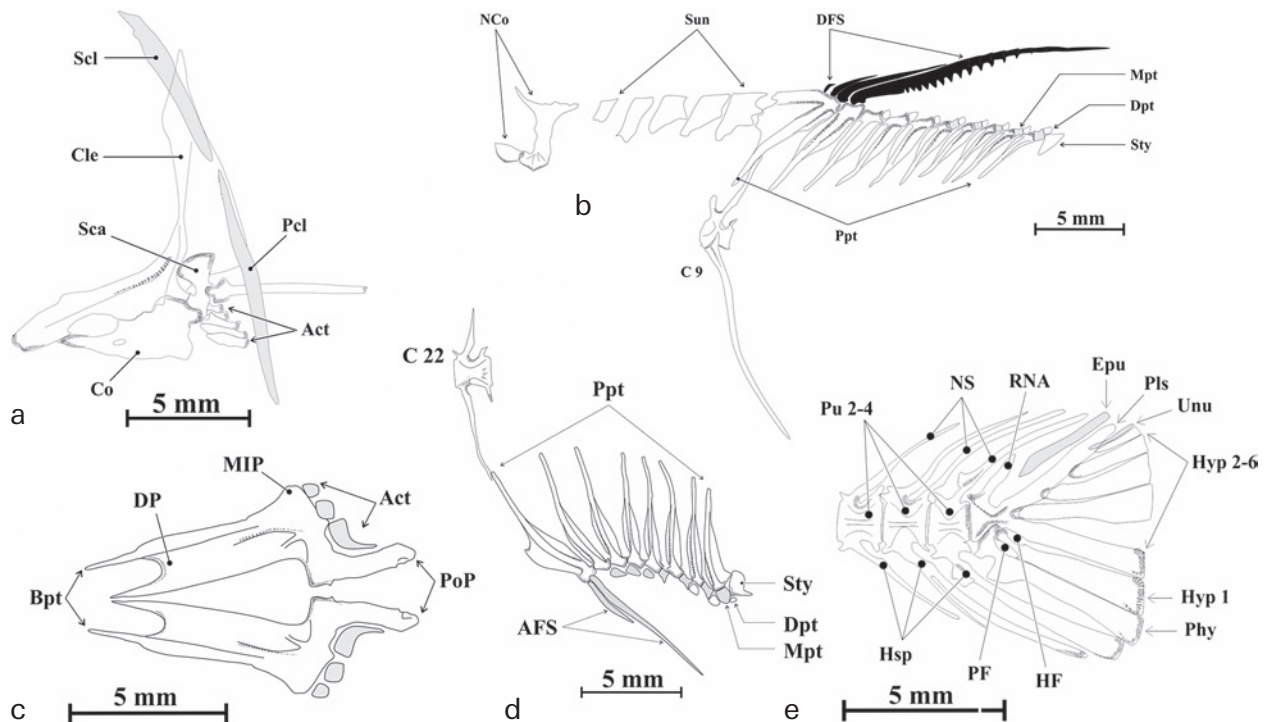
Fig. 6. Medial view of pectoral girdle (A), dorsal fin (B), dorsal view of pelvic fin (C), anal fin (D) and hypural plate (E) skeleton of *C. milesi.* (Act- Actinost; AFS – Anal Fin Spine; Bpt – Basipterygium; C 22 – Centrum 22; C 9 – Centrum 9; Cle – Cleithrum; Co – Coracoid; DFS – Dorsal Fin Spine; DP – Distal Process; Dpt – Distal Pterygiophore; Epu – Epural; HF – Hypural Foramen; Hsp – Hemal spine; Hyp – Hypural; MlP – Mid lateral Process; Mpt – Median Pterygiophore; NCo – Neural Complex; NS – Neural Spine; Pcl – Post Cleithrum; PF – Parhypural Foramen; Phy – Parhypural; Pls – Pleurostyle; Ppt – Proximal Pterygiophore; Pu – preural; RNA – Rudimentary neural arch; Sca – Scapula; Scl – Supra Cleithrum; Sty – Stay; Sun – Supraneural; Unu – Uroneural).

unbranched dorsal fin rays are covered by skin and cannot be seen in superficial observation.

Ventral Fin Skeleton

Ventral fin skeleton is enclosed by muscles and has no direct connection with skeletal elements. One basipterygium and three actinosts present in each side connecting medially (Fig. 6C). The internal actinost in each side is the largest, and its tail extended posteriorly parallel to the posterior process of the basipterygium. The anterior portion of the basipterygium is bifurcated, and its depth is about a quarter of its length and internal arm is shorter.

Anal Fin Skeleton

Eight pterygiophores and one stay present in anal fin. The two unbranched rays of the anal fin are connected to the first pterygiophore (Fig. 6D). The proximal tip of the first pterygiophore touches the tip of the hemal spine of the 22nd vertebra. Because the vertebrate 22 is a caudal vertebra, therefore, whole anal fin positions in the caudal region.

Hypural plate

The hypural plate comprises five vertebrae (Fig. 6E). The two last vertebrae are fused, and form the urostyle and three others and their neural and hemal spins supporting them. The parhypural and hypural 1–6 directly support

the caudal fin rays. The two uroneural ossicles position in both sides of the pleurostyle and are narrower and taller than the hypural 6. The epural is elongated and extended from the posterior rudimentary neural arch to the edge of hypural plate. The epural cooperates with the hemal and neural spines of preural 2–3 supporting the precorent rays. The hemal and neural spines of preural 4 have no direct role in bearing fin rays, but they support the hemal and neural spines of preural 3. The neural postzygapophyses of preural 3 is elongated, and its length equals to its former neural spine. The hemal spine of preural 3 is bifurcated medially.

Discussion

There is little information available about the osteology of Iranian fishes especially genus *Cyprinion*. Osteological characters can be utilized in ichthyological studies, especially fish systematics and potentially can resolve some complexities in this context. The five species of *Cyprinion*, reported from Iran are distinguishable based on mouth form and dorsal fin properties (Coad, 2014; Abdoli, 2000; Kafuku, 1969). Given wide distribution of this genus in Asia (Mirza, 1969; Froese & Pauly, 2014; Coad, 2014), diversity of geographical and climatic con-

dition in their distribution ranges especially in Iran's plateau, their ability to adapt to various environmental conditions and showing phenotype plasticity (Moosavi *et al.*, 2014; Nasri & Eagderi, 2013; Nasri *et al.*, 2013a) encouraged us to study some other aspects of its morphology, i.e. osteological characteristics as identifying features that can help to better understanding of their diversity.

In the two *Cyprinion* species from western Iran, the mouth of *C. kais* is more arched than that of *C. macrostomum* and there are apparent lobes in lower lips corners of *C. kais* (Nasri *et al.*, 2013b; Coad, 2014; Bianco & Banarescu, 1982). The mouth of *C. tenuiradius* resembles to that of *C. macrostomum*, but its last unbranched dorsal fin ray is smoothly serrated. *Cyprinion watsoni* can be distinguished based on lower numbers of dorsal fin rays. The key characters of *C. milesi* are oblique and longer mouth form in lateral view than that of other *Cyprinion* species, and thin last unbranched dorsal fin ray (Coad, 2014).

Neurocranium

The neurocranium in *C. macrostomum* and *C. kais* is sub triangular (Nasri *et al.*, 2013b), but that of *C. milesi* is narrow and triangular in shape. In *C. milesi*, the posterolateral corners of the supraethmoid are elongated posteriorly connecting to the lateral process of parethmoid whereas in *C. macrostomum* and *C. kais*, such a form has not been observed. In *C. milesi* and western member of *Cyprinion* of Iran, the preethmoid is present and forms a joint face for palatine and maxilla, but in some cyprinid fishes such as *Squalius* and *Scardinius*, preethmoid does not exist and in others, a separated face presents to attach the maxilla and palatine (Ramaswami, 1995). The prevomer of *Cyprinion* is overlapped by parasphenoid posteriorly similar to those of *Schizothorax* and *Orienus* (Ramaswami, 1995). The shape of prevomer varies in cyprinid fishes. In some fishes, it is blunt and non-pointed, but in some others is elongated and pointed (Ramaswami, 1995). The attachment of orbitosphenoids to each other in *C. milesi* and separation of the two orbits has been also reported in *C. macrostomum* and *C. kais* as a common feature in Cyprinidae (Rojo, 1991; Nasri *et al.*, 2013b; Ramaswami, 1995). The greater contribution of the pterotic in the subtemporal fossa is seen in *C. milesi* that is common in *Cyprinion* and *Semiplotus* (Howes, 1982; Nasri *et al.*, 2013b). Howes (1982) noted some variability in parietal length in *Cyprinion*, but in all of them, it extends posteromedially to form part of supraoccipital crest. In *C. milesi* like *C. macrostomum* and *C. kais*, the situation is similar.

Upper and lower Jaws

Howes (1982) denoted that the tip of the premaxilla in *Semiplotus* is compressed and shallowly bifurcated but no information provided about *Cyprinion*. The tip of the premaxilla in *C. milesi* is not bifurcated but shallow and

compressed similar to *C. macrostomum*, *C. kais* (Nasri *et al.*, 2013b), *C. mhalensis* and *C. acinaces* (Alkahem *et al.*, 1990). The anterior ascending process of the maxilla in *C. milesi* is shallow and thick similar to other Cyprinids (Howes, 1982; Nasri *et al.*, 2013b). Based on Alkahem *et al.* (1990), the dorsal crest of maxilla (i.e. its mid-lateral ascending process) in *C. acinaces* is thick and positioned more posteriorly than that of *C. mhalensis*. In *C. milesi,* the dorsal crest of the maxilla is not thick but positioned near the distal part of the maxilla. In *C. macrostomum* and *C. kais* (Nasri *et al.*, 2013b), the position of maxilla is similar to that of *C. milesi*. One of the key osteological character of the genus *Cyprinion* in Iran is the curvature of the lower jaw (Nasri *et al.*, 2013b; Coad, 2014). The curvature of the lower jaw in *C. milesi* is less than that of *C. kais* and more than that of *C. macrostomum*. Based on (Howes, 1982), the dentary of *C. kais* is the shortest and thickest among the member of *Cyprinion* and its congruent with differences between mouth form of *Cyprinion* (Nasri *et al.*, 2013b; Coad, 2014). Howes (1982) also pointed out that the lower jaw in *C. kais* is deeper posteriorly than that of other members of *Cyprinion*. This character refers to the main differences reported between Iranian *Cyprinion* (their mouth form). Basically, we can propose that the dentary of *C. milesi* is more elongated among Iranian *Cyprinion*.

Suspensorium and Opercular series

The suspensorium elements, especially the ectopterygoid and endopterygoid in *C. milesi* are well-ossified and expanded anteroposteriorly. This feature shows the elongation of the head in *C. milesi*. In opercular series, although some authors consider the preopercle as a part of opercular series, but this bone functionally related to the suspensorium (Rojo, 1991), but here we considered it as a part of opercular series for its relationship and cooperation with opercular bones. The opercle may derived from one of the branchiostegals (Rojo, 1991). Based on ontogenetical evidence, the subopercle has also developed from the branchiostegals positioning ventral to the opercle (Rojo, 1991). The horizontal arm of the preopercle and the length of opercle in *C. milesi* are longer than that of *C. macrostomum* and *C. kais* (0.9 versus 0.55 and 0.8 versus 0.65, respectively) (Nasri *et al.,* 2013).

Hyoid arch and branchial skeleton

The ceratohyal in *C. milesi* relatively is two times longer than that of *C. macrostomum* and *C. kais* (1.2 versus 0.6). Howes (1982) noted that the first branchiostegal ventrally connected to ceratohyal and considered this character as synapomorphy in *C. macrostomum* and *C. kais*. In *C. milesi* , the two first branchiostegals posteroventrally connected to the ceratohyal and the third one to the epihyal. In *C. milesi* similar to *C. macrostomum* and *Semiplotus* (Howes, 1982), the third branchiostegal is more deve-

loped. The pharyngeal teeth formula in *C. milesi* is 2,3,4 – 4,3,2 and gill rakers is 13 – 14 (Coad, 2014). The diversity in pharyngeal teeth and gill raker counts common in cyprinids, accordingly we can propose the pharyngeal teeth formula in *C. milesi* as 2,3,4 – 4,3,2 and 2,3,5 – 5,3,2 and the gill rakers' ranges as 13 – 16.

Weberian apparatus and vertebral column

In *C. milesi*, the neural complex is T-shape, but it is axe-shape in *C. mhalensis*, *C. acinaces* (Alkahem *et al.*, 1990), *C. macrostomum* and *C. kais* (Nasri *et al.*, 2013b). The anterior border of neural complex is concaved in cyprinids (Howes, 1982). Its anterior arm in *C. milesi* is close to the supraoccipital crest and its posterior border close to the third supraneural but not touching them. This situation is common in the members of genus *Cyprinion* (Howes, 1982; Alkahem *et al.*, 1990) but the form of neural complex varies among *Cyprinion* genera.

The vertebra in *C. milesi* is well-ossified and bear a relatively stout structure. The stoutness of neural spins in *Cyprinion* and *Garra* has been denoted in Arabian peninsula (Alkahem *et al.*, 1990). The presence of prezygapophyses and their relative long size in *C. acinaces* and *C. mhalensis* in all post weberian vertebrae except the caudal complex reported by Alkahem *et al.* (1990).

Fin Skeleton

The supraneurals and dorsal fin skeleton in the genus *Cyprinion* are important distinguishing characteristics because the length of dorsal fin and body height are closely related to these features (Howes, 1982). Alkahem *et al.* (1990) reported 5 – 6 supraneurals in *C. acinaces* and 7 – 8 in *C. mhalensis*. He also reported that among Southwestern Saudi Arabia cyprinids, only in *Cyprinion*, the supraneurals are connected to each other and in *C. acinaces*, the supraneurals are thicker than that of *C. mhalensis*. Seven supraneurals present in *C. macrostomum* and *C. kais* that are well-ossified and connected to each other (Nasri *et al.*, 2013b). The first supraneural in all studied *Cyprinion* is fused with its relative neural spin, and the last one articulates with the first dorsal fin pterygiophore (Nasri *et al.*, 2013b; Alkahem *et al.*, 1990; Howes, 1982), but in *C. kais* and *C. microphthalmum* the last supraneural as an synapomorphic character of this genus is not articulated with pterygiophore (Howes, 1982). In *C. milesi*, the supraneurals is separated from each other, and accordingly it can propose that there are variations in amount of the ossification of supraneurals in *Cyprinion*. The number of pterygiophores in *C. macrostomum* and *C. kais* were 14 – 15 (Nasri *et al.*, 2013b). The lower number of dorsal fin pterygiophores in *C. milesi* compared to *C. macrostomum* and *C. kais* confirms the lower numbers of its dorsal fin rays (Coad, 2014). The last unbranched dorsal fin ray in *C. milesi* is more slender in shape than that of *C. macrostomum*

and *C. kais* and its serration is covering one-third of its length. The tip of the first pterygiophore of dorsal fin in *C. macrostomum* touches the prezygapophyses of the 11th vertebra (Nasri *et al.*, 2013b). Therefore, the dorsal fin of *C. milesi* is originated anteriorly than that of *C. macrostomum*. It seems that the size of the first dorsal fin pterygiophore and 9th vertebra, the level of their overlapping, the position and overlapping of the first anal fin pterygiophore, and the hemal spine of its related vertebra can influence the body height in *Cyprinion*. The last pterygiophore (stay) supports no fin rays and can be considered as an atrophying pterygiophore. The change in preural 3 can provide a better supporting of the urostyle. We proposed that the extension of hemal and neural spines of preural 2 may strengthen of caudal fin for more effective maneuvering performance.

Conclusion

Finally *C. milesi* can be distinguished from *C. macrostomum* and *C. kais* based on the following osteological characters: (1) The neurocranium in *C. macrostomum* and *C. kais* is sub triangular versus narrow and triangular one of *C. milesi*, (2) the lateral and posterior corners of the supraethmoid in *C. milesi* unlike *C. macrostomum* and *C. kais* elongated posteriorly connecting the lateral process of the parethmoid, (3) the curvature of lower jaw in C. *milesi* is between the range of that one in *C. macrostomum* and *C. kais*, (4) the ceratohyal of *C. milesi* is two times longer than that of *C. macrostomum* and *C. kais*, (5) the horizontal arm of the preopercle and the length of opercle in *C. milesi* are longer than that of *C. macrostomum* and *C. kais*, (6) the number of dorsal fin's pterygiophores in *C. milesi* is lower than that of *C. macrostomum* and *C kais*, (7) origination of the dorsal fin is anterior in *C. milesi* than that of *C. macrostomum*, (8) in *C. milesi*, the supraneurals are separated from each other, whereas in *C. macrostomum* and *C. kais*, they are articulated with each other, (9) the last unbranched dorsal fin ray of *C. milesi* is more slender in shape than that of *C. macrostomum* and *C. kais* and its serration is covering one third of its length and (10) the neural complex is T-shape in *C. milesi* but it is axe-shape in other studied *Cyprinion*.

Acknowledgments

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