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Descriptive osteology of *Paracobitis iranica* Nalbant and Bianco, 1998 (Cypriniformes, Nemacheilidae) from Namak Lake Basin of Iran

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Abstract: This study was carried out to describe the osteological structure of Iranian crested loach, *Paracobitis iranica*, Nalbant and Bianco, 1998 an Iranian endemic loach species. For this purpose, ten specimens of *P. iranica* were collected from the Qom River. After clearing and staining, its osteological characteristics were examined and a detailed description of its skeletal structure was provided. The results revealed that *P. iranica* can be distinguished from other members of the family Nemacheilidae particularly *P. hircanica* (previously considered as *P. malapterura*) for lacking a sesamoid ossifications, a triangular-shaped lateral ethmoid, no connection between the sphenotic and epiotic, having six hypurals, having a bony bridge between parietal and pterotic, wider fontanel, absence of the basbrancial-4.

Keywords: Fish skeleton, Nemacheilidae, Loaches, Iran.

Introduction

Stone loaches, the family Nemacheilidae, are small benthic fishes (Golzarianpor et al., 2011) that found in fresh waters of Asia and its islands, Europe, and northeast Africa (Coad, 2016). They have a great diversity in Iranian freshwaters (Jouladeh-Roudbar et al., 2015; Azimi et al., 2015a, b), with more than 44 confirmed species, including 20 Iranian endemic species (Kamangar et al., 2014; Esmaeili et al., 2014; Freyhof et al., 2014; Freyhof et al., 2015; Mousavi-Sabetet al., 2015; Jouladeh-Roudbar et al., 2015). They are less known due to small size and low economic value (Kottelat, 1990; Coad, 2016) and their classification is still complicated; therefore, ichthyologists are trying to reveal their phylogenetic status (Prokofiev, 2010). Given, the osteological characteristics can play an important role in this regard (Sawada, 1982; Azimi et al., 2015a, b); since, osteology is a useful tool to study the taxonomy and phylogenetic relationships among fishes (Prokofiev, 2010; Sawada, 1982; Jalili et al., 2015).

The loaches with a high dorsal dermal-crest have been placed in the genus *Paracobitis* Bleeker, 1863 for many years, specifically those from Central Asia (Banarescu and Nalbant, 1964), Vietnam (Nguyen, 2005), the Middle East (Prokofiev, 2009) and China (Min et al., 2010). The genus *Paracobitis* was appointed by Bleeker (1863) for *Cobitis malapterura*. This genus is restricted to Near East and Middle Asia, and the species of *Paracobitis* from China should be assigned to the genera *Homatula* and *Schistura* (Nalbant and Bianco, 1998). The species of the genus *Paracobitis* are comparatively large-sized loaches inhabiting freshwaters of western Asia (Bănărescu and Nalbant, 1995; Nalbant and Bianco, 1998). There are thirteen valid species of the genus *Paracobitis* in the World, which eleven of them are reported from Iran (Kottelat, 2012; Coad, 2016; Mousavi-Sabet et al., 2014; Freyhof et al., 2014; Jouladeh-Roudbar et al., 2015). According to Freyhof et al (2014), Mousavi-Sabet et al. (2015), and Jouladeh-Roudbar et al. (2015), the valid *Paracobitis* species in Iran comprise *P. hircanica* Mousavi-Sabet, Sayyadzadeh, Esmaeili, Eagderi, Patimar & Freyhof, 2015, *P. atrakensis* Esmaeili, Mousavi-Sabet, Sayyadzadeh, Vatandoust & Freyhof, 2014, *P. basharensis* Freyhof, Esmaeili, Sayyadzadeh & Geiger, 2014, *P. longicauda* Kessler, 1872, *P. malapterura* (Valenciennes, 1846), *P. iranica* Nalbant and Bianco, 1998, *P. molavii* Freyhof, Esmaeili, Sayyadzadeh & Geiger, 2014, *P. persa* Freyhof, Esmaeili, Sayyadzadeh & Geiger, 2014, *P. rhadinaea* (Regan, 1906), P. smithi (Greenwood, 1976), and *P. vignai* Nalbant and Bianco, 1998.

Figure 1. *Paracobitis iranica* (a) from Qom River, (b) from Ghareh-chai River, and (c) *Paracobitis malapterura* from Kordan River.

Paracobitis iranica is an Iranian endemic species and described from the Qom River of the Namak Lake Basin and it also is reported from Ghareh-chai River (Coad, 2016; Jouladeh-Roudbar et al., 2015). Freyhof et al. (2014) considered *P. iranica* as a synonym of *P. malapterura* based on molecular data, which contradicts morphology (Jouladeh-Roudbar et al. 2015). Jouladeh-Roudbar et al. (2015) pointed out that the populations of both species are found in the Namak Lake Basin of Iran with distinct morphological differences (Fig. 1), therefore further study is required to find out its taxonomic position. Hence, the present study was performed to provide a detailed osteological characteristic of *P. iranica* from the Qom River. The results of this study will provided more evidences to solve its taxonomic situation and can also be used as a reference for further phylogenetic study of the members of this genus based on the osteological data.

Materials and Methods

Ten specimens of *P. iranica* (Fig. 1) with standard lengths of 50.9±3.7 mm (Mean ± SD) were collected by electrofishing from the Qom River (Namak Lake basin, Iran. The collected specimens were anesthetized in 1% clove solution and then fixed in 5% buffered formalin. For osteological examination, the specimens were cleared and stained with alizarin red S and alcian blue based on Taylor and Van Dyke (1985). Then, the skeletal structures were dissected and photographed using scanner (Epson v600) equipped with a glycerol bath. The skeletal structure of specimens were observed and studied by a Leica stereomicroscope (MS5, Germany). The obtained images were illustrated using CorelDrawX6 software. Nomenclature and abbreviation of the skeletal elements were done according to Prokofiev (2009, 2010) and Azimi et al. (2015a).

Results

Neurocranium:

The posterior part of the skull is wider. The ethmoid region consists of the pre-ethmoid-II, lateral ethmoid, supraethmoid, prevomer, and kinethmoid (Fig. 2a, b, c). There is a notch in the anterior edge of the supraethmoid. The posterior part of the prevomer is wider and its anterior part is V-shaped (Fig. 2a). The prevomer is connected

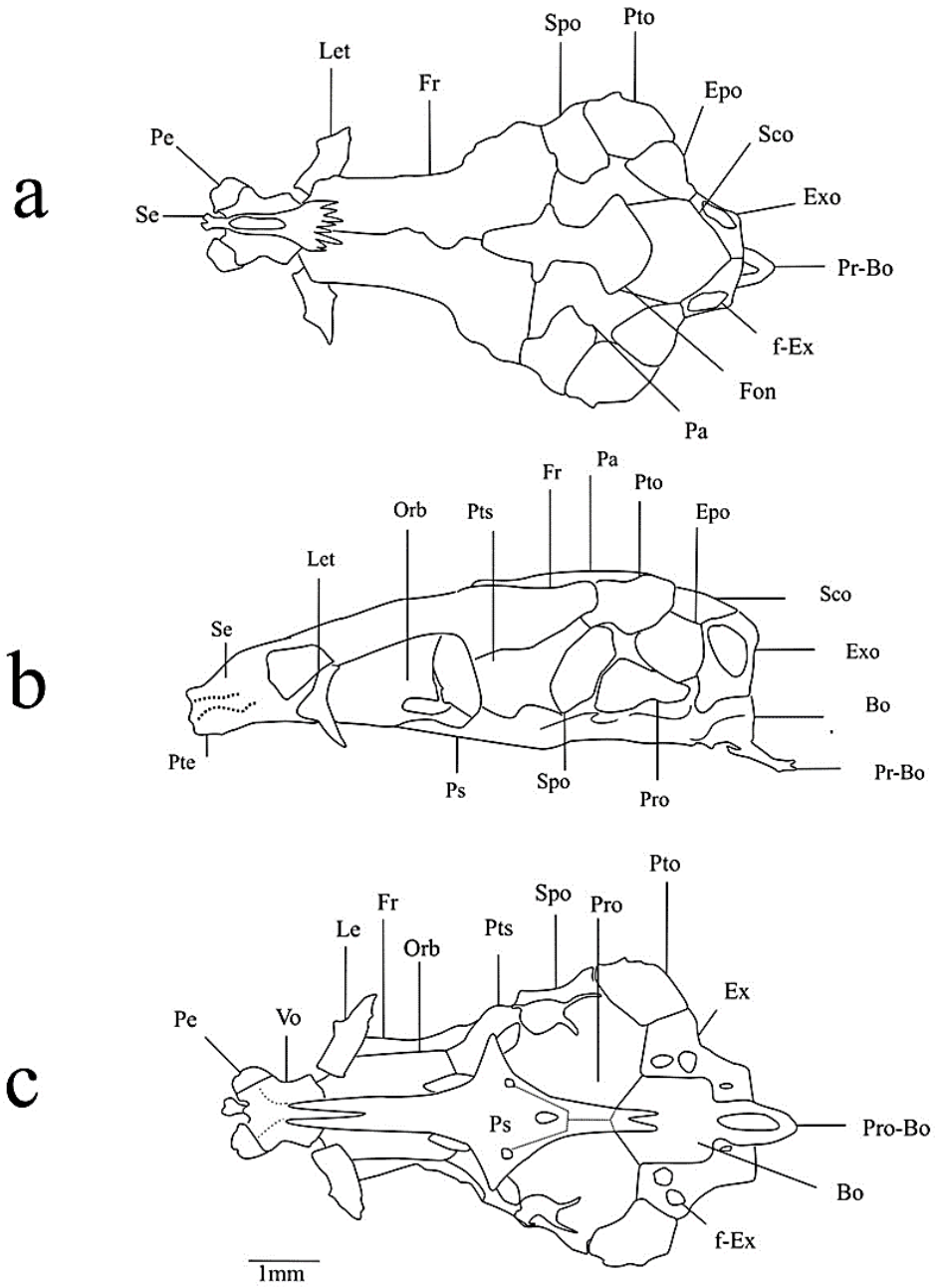

Figure 2. The neurocranium of *Paracobitis iranica*. (a) Dorsal, (b) lateral, and (c) ventral views. fon: fontanel, fr-Exo: foramen exoccipital, pr-Bo: basioccipital process, Epo: epiotic, Exo: exoccipital, Fr: frontal, Let: lateral ethmoid, Orb: orbitosphenoid, Pa: parietal, Pe: prevomer, Pro: prootic, Ps: parasphenoid, Pto: pterotic, Pts: pterosphenoid, Se: supraethmoid, Soc: supraoccipital, and Spo: sphenotic.

latero-medially and laterally to the pre-ethmoid-II and to the pre-palatine, respectively. The anterior part of the pre-ethmoid-II is connected to the anterior bulge of the maxilla. The lateral ethmoid is almost triangular in shape. This bone forms the sub-orbital spine (Fig. 2b). The unpaired kinethmoid is situated between the maxilla and premaxilla, and bears three small processes anteriorly and one process laterally.

The orbital region comprises the frontal, parasphenoid, pterosphenoid, and orbitosphenoid. The anterior part of the frontal is narrow. The frontal is connected to the parietal and sphenotic posteriorly (Fig. 2a). The fontanel is enclosed by the frontal anteriorly, by the parietal laterally, and by the superoccipital posteriorly. The anterior part of the fontanel is narrow and its middle part has two lateral notches (Fig. 2a). The parasphenoid is bifurcated at two ends and bear two alar processes laterally connecting to the pterosphenoids. It has two

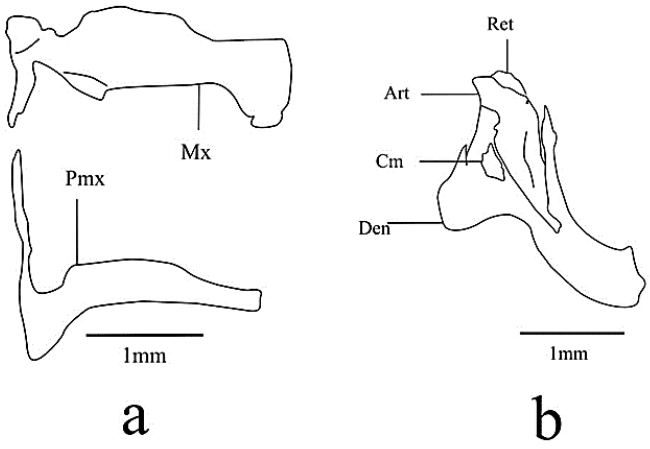

Figure 3. (a) Maxilla and premaxilla; and (b) dorsal view of mandible of *Paracobitis iranica*. Mx: maxilla, Pmx: Premaxilla, Art: articulare, Cm: coronomeckelian, Den: dentale, and Rar: retroarticulare.

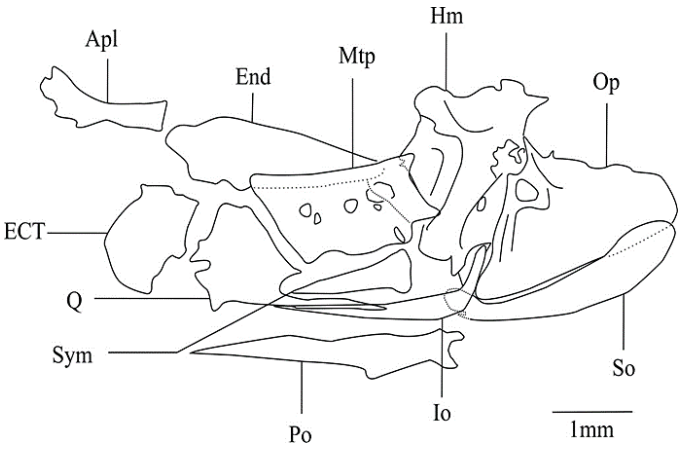

Figure 4. The suspensorium, palatine, and opercular series of *Paracobitis iranica*. Ect: ectopterygoid. End: endopterygoid, Hm: hyomandibular, Io: interopercle, Mtp: metapterygoid, Op: opercle, Po: praeopercle, Q: quadrate, So: subopercle, and Sym: symplectic

lateral small and one posterior larger foramina. The orbitosphenoid is connected to the lateral ethmoid ventrally, to the parasphenoid laterally, and to the pterosphenoid posteriorly.

The otic region includes five bones viz. the parietal, epiotic, sphenotic, pterotic, and prootic. The parietal is posteriorly connected to the supraoccipital and epiotic and ventrally to the prootic. The sphenotic bears an antero-lateral process and latero-ventrally forms a facet i.e. anterior hyomandibular articulatory facet. The pterotic is almost triangular in shape

The occipital region is composed of the supraoccipital, exoccipitals, and basioccipital. The posterior margin of the supraoccipital is pointed and it is connected to the exoccipitals ventrally. The exoccipital encloses the foramen magnum and bears a small foramen laterally (Fig. 2c). The basioccipital bears a posterior process with a large cavity centrally (Figs. 1c, 2c). There is an occipital condyle at the posterior part of the basioccipital for connecting to the vertebral centrum.

Branchiocranium:

The upper jaw consists of the maxilla and premaxila (Fig. 3a). The maxilla is a large laminar bone and its middle part is wider. The anterior part of the maxillae is narrow with an antero-ventral process that is tilted downward. The premaxila is a L-shaped bone, and the horizontal process, i.e. pr. alveolar is wider than vertical process, i.e. pr. ascended (Fig. 3a).

The lower jaw is composed of four bones, including the dentary, angular, retroarticular and coronomeckelian (Fig. 3b). In this complex, the dentary is the largest bony element. The dentary is posteriorly articulated to the quadrat. The coronoid process is dorsally positioned on the middle part of the dentary. The meckelian cartilage is present between the dentary and articular (Fig. 3b). The angular is triangular-shaped and situated at the postero-dorsal part of the retroarticular. The anterior part of the articular is narrower and inserted into the posterior part of the dentary. A small retroarticular is situated postero-lateral to the dentary. The retroarticular has several small pores.

The suspensorium is formed by the hyomandibular, ectopterygoid, endopterygoid, metapterygoid, symplectic, quadrate, and autopalatine. The hyomandibular is a wide bone and approximately trapezoid in shape. This bone has a pore on its middle part and several small pores dorsally. The hyomandibular dorsally possesses two articulatory condyles for articulating with the neurocranium and one articulatory condyle postero-laterally for articulating to the opercle. Anteriorly, the hyomandibular bears two processes. The quadrate bears a condyle antero-ventrally where it is articulated to the dentary. The quadrate has an incisures posteriorly where the triangular symplectic is located. The ectopterygoid, endopterygoid, metapterygoid, symplectic, and quadrate form a complex connecting to the anterior part of the neurocranium via autopalatine. The middle part of the endopterygoid is wide. This bone is connected to the ectopterygoid antero-ventrally and to the metapterygoid posteriorly. The

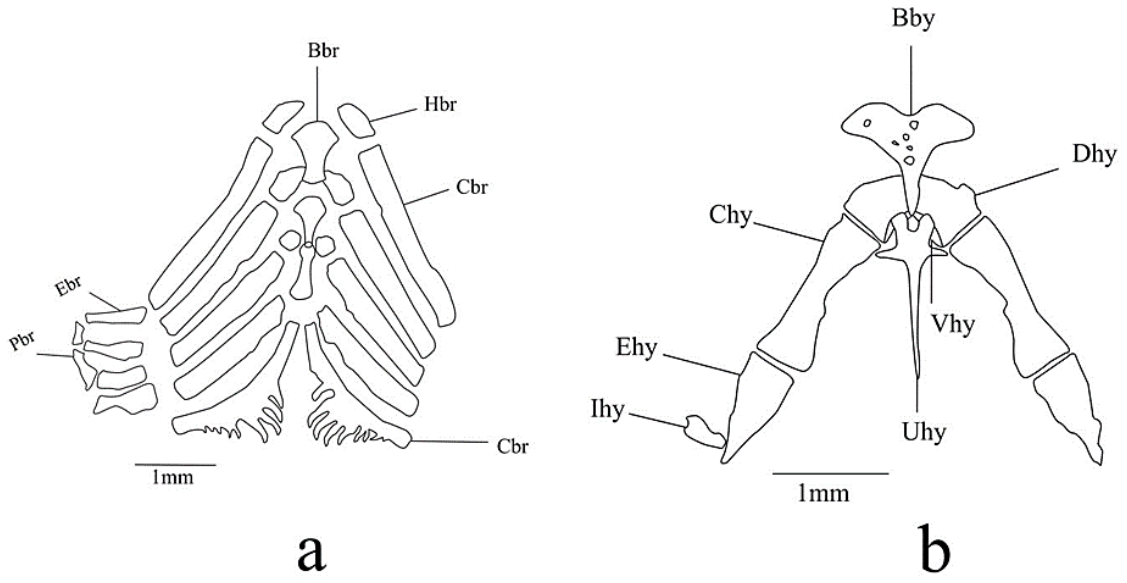

Figure 5. (a) Dorsal view of the branchial apparatus and (b) Dorsal view of hyoid arch of *Paracobitis iranica*. Bbr: basibranchial, Cbr: ceratobranchial, Ebr: epibranchial, Hbr: hypobranchial, Pbr: Pharyngobranchial, Bhy: basihyal, Br: branchiostegal, Chy: ceratohyal, Dhy and Vhy: dorsal and ventral hypohyal, Epi: epihyal, Ihy: interhyal, and Uhy: urohyal

metapterygoid bears several pores on its lateral face. The ectopterygoid situates anterior to the quadrate. The anterior part of the atopalatine is broad and connected to the prevomer (Fig. 4).

The opercular series consists of the opercle, peropercle, subopercle, and interopercle (Fig. 4). The opercle is the largest element of this series and possesses a condyle anteriorly. The paddle-shaped subopercular overlaps the postero-ventral part of the opercle. The narrow preopercular is pointed anteriorly and bears a notch posteriorly. The interopercular is pointed anteriorly and its posterior part curves dorsally.

The branchial apparatus includes five pairs of the ceratobranchial, four pairs of the epibranchiasls, three pairs of the hypobranchials and pharyngobranchials, and three unpaired basibranchials (Fig. 4a). The anterior part of the first and second basibranchials is wider. In each half, there are three hypobranchials and the first two hypobranchials are similar in shape and the last one is the smallest one. Five pairs of the ceratobranchials are located between the hypobranchials and epibranchials, and the last pair is modified as the tooth plats.

The hyoid arch consists of the paired interhyal, epihyals, ceratohyals, dorsal hypohyals, and ventral and, the unpaired urohyal and basihyal, and three pairs of the branchiostegals (Fig. 4b). The urohyal is T-shaped and its anterior edge bifurcated. The basihyal is also T-shaped and bears a small notch anteriorly. The dorsal and ventral hypohyals are connected firmly. The ceratohyals are the largest elements of the hyoid arch and have a cylindrical shape. There are three curved long branchiostegals; the first one is attached to the ceratohyal, the second one located between ceratohyal and epihyal, the third one attached to the epihyal. The interhyal is a small bone that its anterior part is narrow connecting to the epihyal (Fig. 4b).

Postcranium:

The pectoral girdle consists of the cleithrum, supracleithrum, coracoid, mesocoracoid, scapula, posttemporal, supratemporal and radials (Fig. 5a). The largest element of this complex is cleithrum that bears two vertical and horizontal sections. The horizontal section is longer than vertical one. The dorsal part of the supracleithrum is wider with a notch to connect the posttemporal that connects the pectoral girdle to the neurocranium. The coracoid is positioned posterior to the supracleithrum. The mesocoracoid is a narrow bone that its ventral part is wider connecting to the coracoid. The mesocoracoid is firmly connected to the cleithrum. The scapula is trapezoid-shaped. This bone connects to the coracoid ventrally. The pectoral girdle bears four radials that first one is the widest and fourth one is the longest and crescent-shaped. The pectoral fin has 1 unbranched and 9 branched rays.

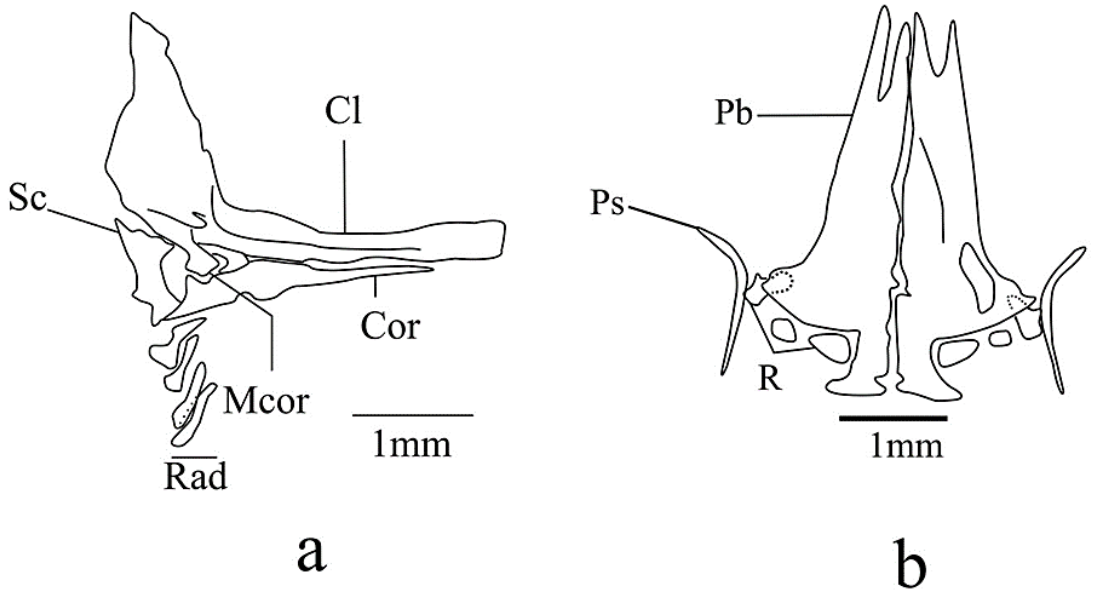

Figure 6. (a) Pelvic girdle and (b) Medial view of pectoral girdle of Paracobitis iranica. Cl: cleitherum, Cor: coracoid, Mcor: mesocoracoid, Rad: ossified pectoral radial, and Sc: scapula.

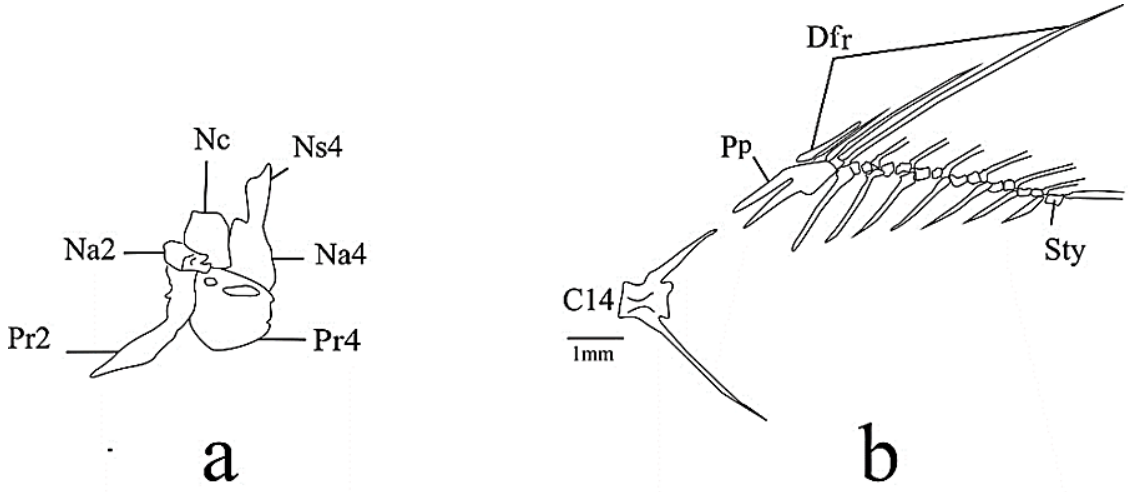

Figure 7. (a) Lateral view of the Weberian apparatus and (b) Dorsal fin of Paracobitis iranica; Dfr: dorsal fin rays, Dfs: dorsal fin spin, Pp: pterygiophore, Sty: stay, Na: neural arch, Nc: neural complex, Na: neural arch, Ns: neural spine, and Pr: pleural rib.

The pelvic girdle includes the paired pelvic bones, pelvic splint and radials (Fig. 6b). The pelvic bone is horizontally positioned in the ventral plan of the belly. It is bifurcated anteriorly and its posterior part is wider. The pelvic bone has a round process lateral-posteriorly. The pelvic girdle has three radials that second one is the smallest. The styloid is situated lateral to the anterior radial. The pelvic fin has 7 branched and 1 unbranched rays.

The dorsal fin bears 3 unbranched and 7 branched rays, 8 pterygiophores and one stay. The first pterygiophor is next to the 13th or 14th vertebra. The largest bone of the dorsal fin is the first pterygiophores that supports three unbranched rays. A triangular stay supports the last branched ray (Fig. 7b).

The anal fine originates at 26th centrum. This fin has 4 unbranched and 5 branched rays and is supported by 6 pterygiophores and 2 small stays (Fig. 8).

The caudal skeleton consists of the four centra along with the epural, parhypural, pleurostyle, uroneural and six hypurals (Fig. 9). This fin bears 18 branched rays. The number of the dorsal procurrent is 6 and the number of the ventral procurrent is 4.

Weberian apparatus and swim bladder capsule is

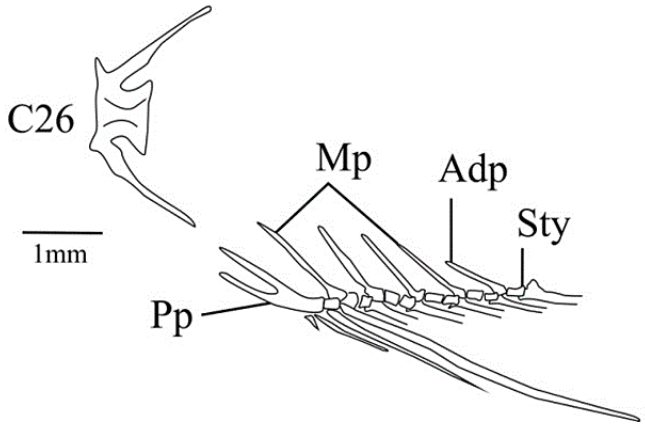

Figure 8. Anal fin of Paracobitis iranica. Adp: anal distal pterygiophore, Mp: mesial pterygiophore, Pp: pterygiophore, and Sty: stay.

formed by four fused anterior centra with their ossicles, including tripus, intercalarium, scaphium, and claustrum (Fig. 7a). The claustrum is oval-shaped and situated on the scaphium ventrally. The intercalarium is small and positioned between the scaphium and tripus. The swim bladder capsule has two pores on its lateral face that the posterior one is larger than rounded anterior one. The second and third centra of the trunk are fused. The ventral portion of the pleural rib of 2nd centrum is oriented anteriorly and its posterior part is narrow. The vertebral column of this species bears 42-44 vertebra, including 19-22 cranial and 24 or 21or 20 caudal centra.

Discussion

The present study provided a detailed skeletal description of the *P. iranica* from the Namak Lake Basin of Iran. The length of the occipital region of the neurocranium is less than one-third of its length in *P. iranica*, similar to the majority of loaches (Prokofiev, 2010). In addition, the maximum width of the neurocranium in *P. iranica*, similar to the majority of loaches, was observed at the level of the otic region (Prokofiev, 2010).

Paracobitis iranica has no seasamoid ossification, whereas this bony element was reported in *P. malapterurus* (Prokofiev, 2004a, 2009a), *Dzhunia amudarjensis* and *Oxynoemachelius angore* (Prokofiev, 2010). The lateral ethmoid of *P. hircanica* is L-shaped having a process which oriented anteriorly (Azimi et al., 2015a) that this process is absent in *P. iranica*.

The basbrancial-4 is absent in the *P. iranica* similar to other members of this genus (Prokofiev, 2009; Azimi et al., 2015a). However, this bone is found in

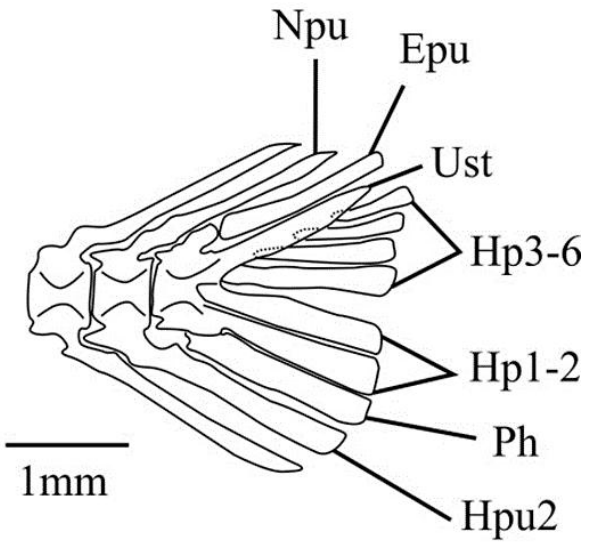

Figure 9. Caudal skeleton of Paracobitis iranica. Epu: epural, Hp1-6 hypural plates, Npu: neural processes of the preural centrum, Hpu2: hemal processes of the second preural centrum, and Ust: pleurostile.

Oxynoemachelius kiabii (Mafakher et al., 2014), *O. berganus* (Jalili and Eagderi, 2014), *O. kermanshahensis* (Mafakheri et al., 2015) and *Paraschistura nielseni* (Azimi et al., 2015b). The basihyal of *P. iranica* has a notch on its anterior edge that it is deeper in *P. hircanica* (Azimi et al., 2015a).

According to Porokofiev (2009), the hypural number in the members of the genus *Paracobitis* is six as observed in the present study regarding *P. iranica*, whereas five hypurals have been reported in *P. hircanica* (Azimi et al., 2015a). The neural processes of the preural-3 is trifurcated dorsally in *P. hircanica* (Azimi et al., 2015a), whereas that of P. iranica has no dorsal process.

In the anal fin of *P. iranica*, the number of unbranched and branched rays is 3 ½ and 5, respectively, whereas they are 3 and 5 ½ in *P. hircanica*, respectively. Furthermore, the number of unbranched and branched rays in *P. iranica* is 3 and 7, respectively, but they are 4 and 7 ½ in *P. hircanica*, respectively.

Based on the osteological features, *P. iranica* can be distinguished from other members of the family Nemacheilidae particularly *P. hircanica* (previously considered as *P. malapterura*) for lacking asesamoid ossifications, a triangular-shaped lateral ethmoid, no connection between the sphenotic and epiotic, having six hypurals, having a bony bridge between parietal and pterotic, wider fontanel, absence of the basbrancial-4.

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References

Azimi H., Mousavi-Sabet H., Eagderi S. 2015a. Osteology of Hircanian Crested Loach, *Paracobitis hircanica* (Pisces, Nemacheilidae). Our Nature 13(1): 8-18.

Azimi H., Mousavi-Sabet H., Eagderi S. 2015a. Osteological characteristics of *Paraschistura nielseni* (Nalbant & Bianco, 1998) (Cypriniformes: Nemacheilidae). Iranian Journal of Ichthyology, 2(3): 155-164.

Bănărescu P., Nalbant T. 1964. Süßwasserfische der Türkei. 2. Teil Cobitidae. Mitteilungen aus dem hamburgischen Zoologischen Museum und Institut, 61: 159-201.

Bănărescu P., Nalbant T. 1995. A generical classification of Nemacheilinae with description of two new genera (Teleostei: Cypriniformes: Cobitidae). Travaux du Muséum d'Histoire Naturelle Grigore Antipa, Bucurešti, 35: 429-496.

Bleeker P. 1863. Sur les genres de la famille des Cobitioïdes. Nederlands Tijdschrift van Dierkunde, 1: 361-368.

Coad B.W. 2016. Fresh water fishes of Iran. Retrieved from http://www.briancoad.com. 2 January 2016.

Esmaeili H.R., Sayyadzadeh G., Özulug M., Geiger M., Freyhof J. 2014. Three new species of *Turcinoemacheilus* from Iran and Turkey (Teleostei: Nemacheilidae). Ichthyological Exploration of Freshwaters, 24(3): 257-273.

Freyhof J., Sayyadzadeh G., Esmaeili H.R., Geiger M. 2014. Review of the crested loaches of the genus *Paracobitis* from Iran and Iraq with the description of four new species (Teleostei: Nemacheilidae). Ichthyological Exploration of Freshwaters, 25: 11-38.

Freyhof J., Sayyadzadeh G., Esmaeili H.R., Geiger M. 2015. Review of the genus *Paraschistura* from Iran with description of six new species (Teleostei: Nemacheilidae). Ichthyological Exploration of Freshwaters, 26(1): 1-48.

Golzarianpor A., Abdoli A., Freyhof J. 2011. *Oxynoemacheilus kiabii*, a new loach from Karkheh River drainage, Iran (Teleostei: Nemacheilidae). Ichthyological Exploration of Freshwaters, 22: 201-208.

Jalili P., Eagderi S., Nikmehr N., Keivany Y. 2015. Descriptive osteology of *Barbus cyri* (Teleostei: Cyprinidae) from southern Caspian Sea basin Iran. Iranian Journal of Ichthyology, 2: 105-112.

Jouladeh-Roudbar A., Vatandoust S., Eagderi S., Jafari-Kenari S., Mousavi-Sabet H. 2015. Freshwater fishes of Iran; an updated checklist. AACL Bioflux, 8(6): 855-909.

Kamangar B., Prokofiev A., Ghaderi E., Nalbant T. 2014. Stone loaches of Choman River system, Kurdistan, Iran (Teleostei: Cypriniformes: Nemacheilidae). Zootaxa, 3755(1): 33-61.

Kottelat M. 1990. Indochinese nemacheilines. A revision of nemacheiline loaches (Pisces: Cypriniformes) of Thailand, Burma, Laos, Cambodia and southern Viet Nam. Verlag Dr. Friedrich Pfeil, München. 262 p.

Min R., Chen X.Y., Yang J.X. 2010. *Paracobitis nanpanjiangensis*, a new loach (Balitoridae: Nemacheilinae) from Yunnan, China. Environmental Biology of Fishes, 87: 199-204.

Mousavi-Sabet H., Sayyadzadeh G., Esmaeili H.R., Eagderi S., Patimar R., Freyhof J. 2015. *Paracobitis hircanica*, a new crested loach from the southern Caspian Sea basin (Teleostei: Nemacheilidae). Ichthyological Exploration of Freshwaters, 25: 339-346.

Nalbant T., Bianco P. 1998. The loaches of Iran and adjacent regions with description of six new species (Cobitoidea). Italian Journal of Zoology, 65: 109-123.

Nguyen V.H. 2005. Freshwater fishes of Vietnam. v. 2. 760 p.

Prokofiev A.M. 2004. Osteology and Relationships between Loaches of the Genus *Dzihunia* (Osteichthyes, Balitoridae). Zoologicheskij Zhurnal, 83: 826-838.

Prokofiev A.M. 2009. Problems of the classification and phylogeny of Nemacheiline loaches of the group lacking the preethmoid I (Cypriniformes: Balitoridae: Nemacheilinae). Journal of Ichthyology, 49: 874-898.

Prokofiev A.M. 2010. Morphological classification of loaches (Nemacheilinae). Journal of Ichthyology, 50: 827-913.

Sawada Y. 1982. Phylogeny and zoogeography of the superfamily Cobitoidea (Cyprinoidei, Cypriniformes). Memoirs of the Faculty of Fisheries of Hokkaido University, 28: 65-223.

Taylor W.R., Van Dyke G.C. 1985. Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. Cybium, 9: 107-119.