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Original Article

Descriptive osteology of *Squalius orientalis* from Urmia Lake basin of Iran

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Abstract: Osteological features are important to study the taxonomy and phylogenetic relationship of fishes. Since there is no information is available about osteological features of *Squalius orientalis*, therefore this study was aimed to provide a detailed description of the osteological features of this species from the Urmia lake basin of Iran and comparing it with population of *S. orientalis* from the Caspian Sea basin. For this purpose, the specimens were collected from Zarineh River of the Urmia lake basin and cleared and stained with alizarin red S and alcian blue for osteological examinations. Finally, a detailed osteological features of this species was provided and compared with those of the Caspian Sea basin. Based on the results, having a longer pre-vomer, dorsally oriented parasphenoid alar, longer ventral blade-shaped process of the orbitosphenoid, concaved masticatory plate, pointed ascending process of the palatine, concaved posterior margin of the opercle, and a small posterior process of the cleithrum can differentiate *S. orientalis* of the Urmia Lake basin from those of the Caspian Sea basin. In addition, the observed osteological difference suggest that both studied populations belong to same taxon.

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Introduction

The genus *Squalius* Bonaparte, 1837 comprises medium-sized midwater fishes widely distributed in Europe and West Asia. The species of this genus were placed in *Leuciscus* Cuvier, 1816 (Coad, 2016; Turan et al., 2013), until morphological and molecular data showed that *Leuciscus* as earlier understood was paraphyletic (Bogutskaya, 1994; Zardoya and Doadrio, 1999; Coad, 2016). Approximately 45 species are recognized in this genus (Özuluğ and Freyhof, 2011) with 3 species, including *S. lepidus*, *S. orientalis* and *S. cephalus* reported from Iran (Jouladeh Roudbar et al., 2015a).

The members of the genus *Squalius* were characterised by numerous total vertebrae (commonly more than 40, up to 48); increased number of sensory cephalic pores (up to 12-20 in the supraorbital canal) in most species; often fused and very expanded fourth and fifth infraorbitals; and depressed neurocranium with a reduced interorbital septum. Other characters are a somewhat compressed body; moderate to large scales; a complete lateral line; no barbels; mouth terminal or subterminal; no notch in the upper jaw accommodating a tubercle on the lower jaw; thin lips with the lower one interrupted medially; a short dorsal fin without a thickened ray; a moderately long anal fin; long and hooked pharyngeal teeth in 2 rows (2,5-4,2, 2,5-5,2 or 3,5-5,3 modally) usually with hooked tips and spoon-shaped crowns; short gut; no keel on the belly; and short and relatively few gill rakers (Bogutskaya, 2002; Coad, 2016).

The populations of the genus *Squalius* in Iranian part of the Caspian Sea basin was considered as *S. orientalis* (Esmaeili et al., 2014; Jouladeh Roudbar et al., 2015a). In addition, this species was reported from the Namak Lake, Urmia Lake and Dasht-e Kavir basins of Iran (Jouladeh Roudbar et

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Figure 1. Lateral view of *Squalius orientalis* from the Zarineh River of the Urmia lake basin.

al., 2015a, b; Ghasemi et al., 2015). *Squalius orientalis* described from Abkhazia, Georgia and *S. turcicus* described from Aras River at Erzurum, Turkey (Coad, 2016). Both species were given as subspecies of *S. cephalus* in previous studies (Kuru 1975a, 1975b; Zengin et al., 2012). Both species have been listed as valid species by Turan et al. (2013). However, Coad (2016) put forward that *S. orientalis* and *S. turcicus* are synonym of *S. cephalus*. Therefore, this situation needs to be clarified. Since, the osteological characters are important diagnostic traits for interpreting systematics and phylogenetic relationships within Teleostei (Nybelin, 1973; Schultze and Arratia, 1988; Arratia, 1999; Keivany and Nelson, 2004, 2006). Hence, this study was conducted to provide a detailed descriptive osteology of *S. orientalis* from the Zarineh River, Urmia Lake basin and comparing it with those of *S. orientalis* from the Caspian Sea basin as well as to find out the taxonomic statue of those of the Urmia Lake basin. In addition, the results will provide a basis for further phylogenetic study of Iranian members of this genus based on osteological data.

Materials and Methods

Ten specimens of *S. orientalis* (Fig. 1), with a mean standard length of 97.2±13.5 (Mean±SD) mm, were collected from the Zarineh River (Near the Shahin-Dezh town, western Azarbaijan Province, Iran) during summer 2013 by electrofishing device. The collected specimens were anaesthetized using 1% clove oil solution and fixed in 10% buffered formaldehyde. For osteological examination, the specimens were cleared and stained with alizarin red S and alcian blue according to Taylor and Van Dyke (1985) (Fig. 2). The cleared and stained specimens were studied under a stereomicroscope (Leica MC5); and different skeletal elements were dissected and scanned by a scanner equipped to a glycerol bath (Epson V600). Drawings of the skeletal elements were performed from obtained images using CorelDraw X6 software. Nomenclature and abbreviation of the skeletal elements were followed Rojo (1991) and Jalili *et al.* (2014 a, b and c). The detailed osteological features of *S. orientalis* from the Caspian Sea basin was provided by Jalili (2015).

Results

The anterior half of the neurocarnium is narrower and shallow. In the lateral view, three and four foramens present at the anterior and posterior half of the neurocranium, respectively. Six bony elements, including the supraethmoid-ethmoid, lateral ethmoid, pre-vomer, nasal, kine-ethmoid and pre-ethmoid-I form the ethmoid region. The supraethmoid-ethmoid bears the horizontal and vertical parts; the horizontal part is wide with two developed posterior processes and the vertical poart has a triangular shape. The pre-vomer is V-shaped anteriorly and serrated posteriorly. The pre-ethmoid-I can be bony or cartilaginous and is connected to the prevomer antero-laterally. Two long and narrow nasal bones were positioned at the lateral side of the supraethmoid-ethmoid (Fig. 3c). A small kine-ethmoid is situated at the anterior part of the pre-

Figure 2. The lateral view of the cleared and stained *Squalius orientalis* from the Zarineh River of the Urmia lake basin (Scale bare=2 mm).

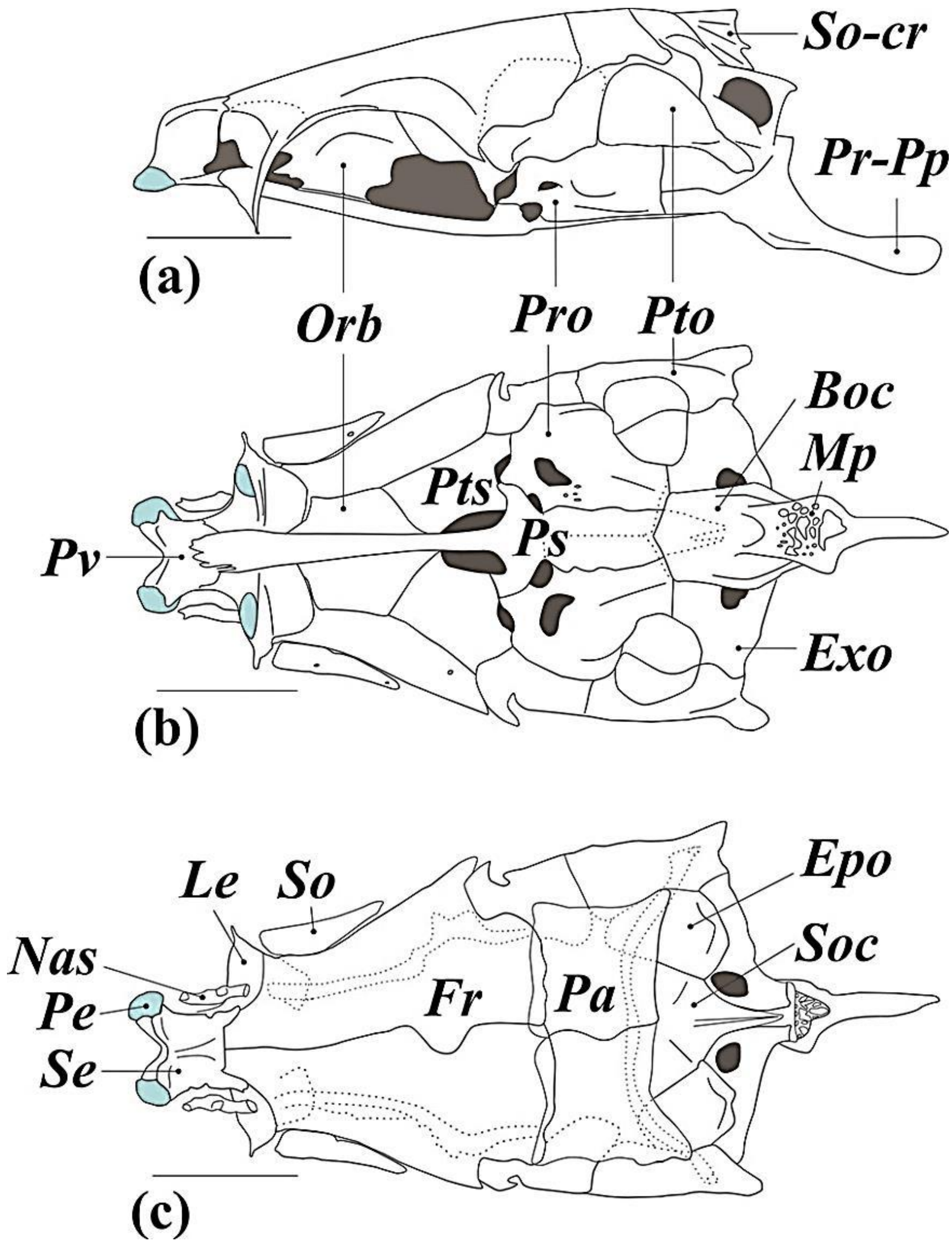

Figure 3. Lateral (a), ventral (b) and dorsal (c) views of the neurocranium of *Squalius orientalis* from the Zarineh River of the Urmia lake basin. Abbreviations: Boc: basioccipital; Epo: epiotic; Exo: exoccipital; Fr: frontal; Le: lateral ethmoid; Mp: masticatory palate; Nas: nasal; Orb: orbitosphenoid; Pa: parietal; Pe: preethmoid I; Pr-Pp: posterior pharyngeal process; Pro: prootic; Ps: parasphenoid; Pts: pterosphenoid; Pto: pterotic; So-cr: supraoccipital crest; So: supraorbital; Soc: supraoccipital; Sp: sphenotic; Se: supraethmoid; Pv: pre-vomer (Scale bar=3 mm).

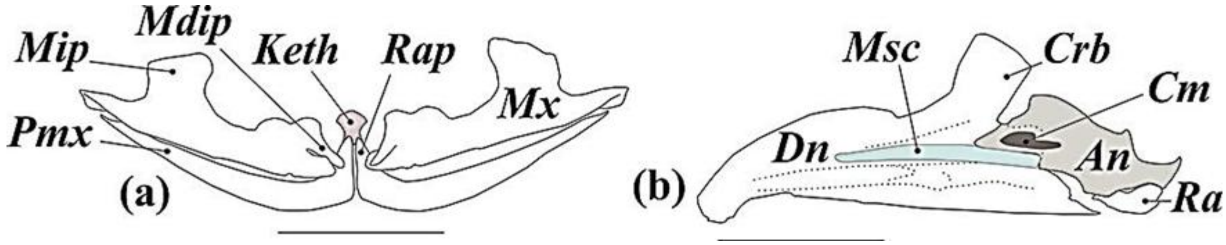

Figure 4. Upper (a) and lower (b) jaws of *Squalius orientalis* from the Zarineh River of the Urmia Lake basin. Abbreviations: An: Angular; Crb: coronoid process; Cm: coronomeckelian; Dn: dentary; Keth: kinethmoid; Ra: retroarticular; Mx: maxillary; Pmx: premaxillary; Mdip: maxillary descending process; Mip: maxillary mid-lateral ascending process; MSC: Mandibular Sensory Canal; Rap: rostral ascending process (Scale bar=3 mm).

vomer that its posterior margin is wider. The medial part of the lateral-ethmoid is the widest part of this bone and is connected to the orbitosphenoid, frontal and supraethmoid-ethmoid. There is a cartilaginous protuberance at the abdominal level of the lateral-ethmoid (Fig. 3).

The orbital regin comprises the frontal, parasphenoid, ptersphenoid, orbitosphenoid and circumorbital series. The anterior margin of the frontal is thin and its posterior part bears a postero-lateral processes. The supraorbital canal goes around the lateral margin of the frontal. Two trapizoid-shaped orbitosphenoids are connected and form a blade shape process connecting to the parasphenoid. There are two upward processes and two fossa in the middle portion of the parasphenoid; these two internal and external processes are attached to the ptersphenoid and pterotic, respectively. The anterior part of the parasphenoid is serrated and its posterior part is bifurcated (Fig. 3b). The lateral edge of the ptersphenoid is concaved and its abdominal is triangular in shape; there are a dorsal fossa and two protuberances on the middle part of this bone extending ventrally.

The otic region of the neurocranium includes the prootic, epiotic, sphenotic, parietal and pterotic bones. The posterior edge of the parietal encloses the supratemporal canal. The sphenotics is a small bone with a lateral process bended posteriorly. The pterotic is triangulare in shape on dorsal view. The epiotic has a process posteriorly. There is a rounded process with some pores at the posterior and anterior parts of the prootic. The prootic possess a blade shape process tilted ventrally connectiong to the parasphenoid (Fig. 3).

The occipital region consists of the supraoccipital, exoccipital and basioccipital. The posterior margin of the supraoccipital is pointed and its dorsal level has a developed crest. The exoccipital bears two dorsal and ventral processes. The basioccipital is trapizoid in shape and its posterior part is narrower. The posterior part of the basioccipital bears a pharyngeal process with a postero-ventral fossa; at the anterior portion of this process, there is a masticatory palate narrower than the width of the basioccipital (Fig. 3b).

In the upper jaw, the maxillae has a mid-dorsal ascending protuberance bending anteriorly; this bone also has an anterior descending process. The premaxillae is L-shaped and its horizontal part is larger than the ventral part (Fig. 4a). The lower jaw includes the dentary, angular, retroarticular and coronomeckelian (Fig. 4b). The coronoid process of the dentary is short and bended posteriorly; its posterior part is wider overlapping with the angular; at the postero-ventral edge of the angular, a triangular-shaped retroarticular is present. The tiny and long coronomeckelian presents at the medial face of the angular and dentary.

The opercle, preopercle, subopercle and interopercle form the opercular series (Fig. 5). The posterior margin of the opercle is concaved and bears a developed antero-dorsal process. The horizontal portion of the preopercle is longer than its vertical part. The subopercle has crescent shape with a wide anterior portion.

The suspensorium includes the quadrate, symplectic, hyomandibular, endopterygoid,

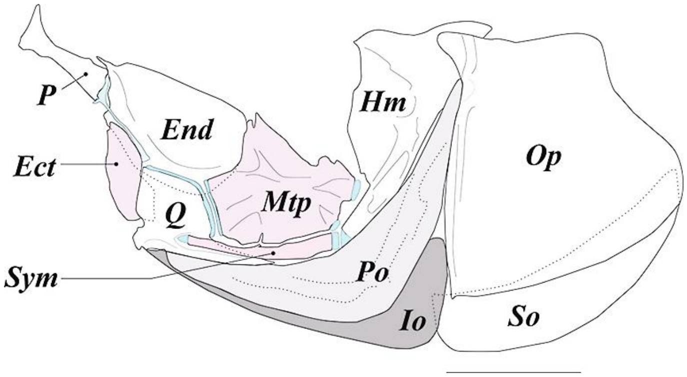

Figure 5. Lateral view of the suspensorioum and opercular series of *Squalius orientalis* from the Zarineh River of the Urmia lake basin. Abbreviations: Ect: ectopterygoid; End: endopterygoid; Hy: hyomandibulare; Iop: interopercle; Mtp: metapterygoid; Op: opercle; Opp: opercular prominent process; Opj: opercular joint; P: palatine; Pop: preopercle; Q: quadrate; Sop: subopercle; Sym: symplectic (Scale bar=3 mm).

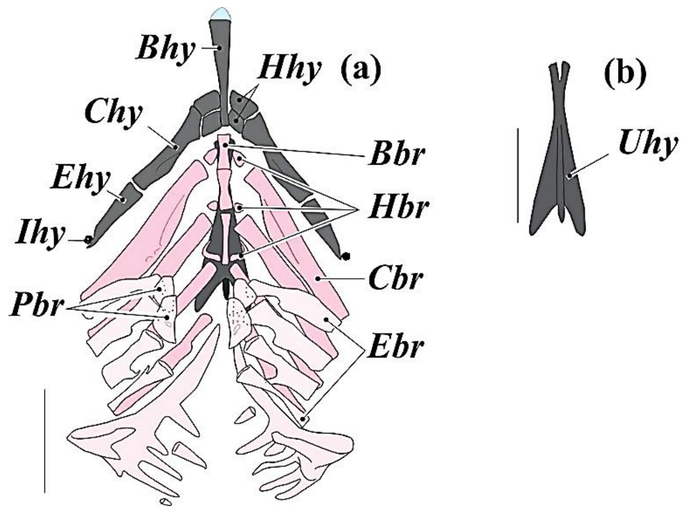

Figure 6 Dorsal view of the hyoid arch and branchial apparatus (a) and urohyal bone (b) of *Squalius orientalis* from the Zarineh River of the Urmia lake basin. Abbreviation: Bhy: basihyal; Chy: ceratohyal; Epy: epihyal; Hhy: dorsal and ventral hypohyal; Ihy: interhyal; Uhy: urohyal; Bbr: basibranchial; Cbr: ceratobranchial; Ebr: epibranchial; Hbr: hypobranchial; Pbr: infrapharyngo-branchial (Scale bar=3 mm).

ectopterygoid, metapterygoid and palatine (Fig. 5). The anterior margin of the hyomandibular is thin and round. The symplectic is positioned ventral to the hyomandibular and posterior to the quadrate. This bone has the blade-shaped vertical and thickened horizontal portions, respectively. The endopterygoid has an anterior triangular-shaped process and some mid-lateral protuberances; and its posterior part is broader. The ventral part of the endopterygoid is thicker than its dorsal portion. The middle part of the palatine is narrow; there are three small process anteriorly. The long ectopterygoid is positioned ventral to the endopterygoid (Fig. 5).

The branchial apparatus comprises three unpaired basibranchial, three paired hypobranchial, five paired ceratobranchial, four paired epibrancchial and two paired infrapharyngobranchial (Fig. 6a). The infrapharyngobranchials are semi-circular in shape and overlap each other. The epibranchial bears some pointed process posteriorly.

The hyoid series possess the unpaired basihyal and urohyal, and paired epihyal, ceratohyal, hypohyal and interhyal and three paired branchiostegal rays (Fig. 6a). The urohyal has a blade shape portion perpendicular to the ventral part; the posterior portion of its ventral part is wider (Fig. 6b). The anterior margin of the urohyal has a narrow groove ventrally. The anterior edge of the basihyal is wider. There is a protuberance at the anterior part of this bone. The hypohyal bears two dorsal and ventral parts.

The pectoral girdle consists of eight bones including cliethrum, supracleithum, postcliethrum, coracoid, mesocoracoid, scapula, posttemporal, supratemporal and four radials (Fig. 7a). The cliethrum is L-shaped and its horizontal part has a lateral protuberance and its vertical part has a developed process. In addition, the dorsal margin of the vertical part is thin connecting to the supracleithum. There is a tiny supratemporal anterior to the posttemporal that is connected to the pterotic. The dorsal margin of the coracoid is broad with a protuberance connecting to the mesocoracoid. It is long and connected to the coracoid and scapula. The scapula bears a large foramen. The pectoral girdle possesses four radials.

The paired pelvic bones, styloid and radials form the pelvic girdle (Fig. 7b). The anterior margin of the pelvic bone is bifurcated deeply and its posterior part has mid-lateral and posterior processes; the latter is weakly developed. There are three radials at the dorsal side of the pelvic bones. Two external radials are paired and the internal one is unpaired. Two long and thin styloid bone is located on the lateral side of

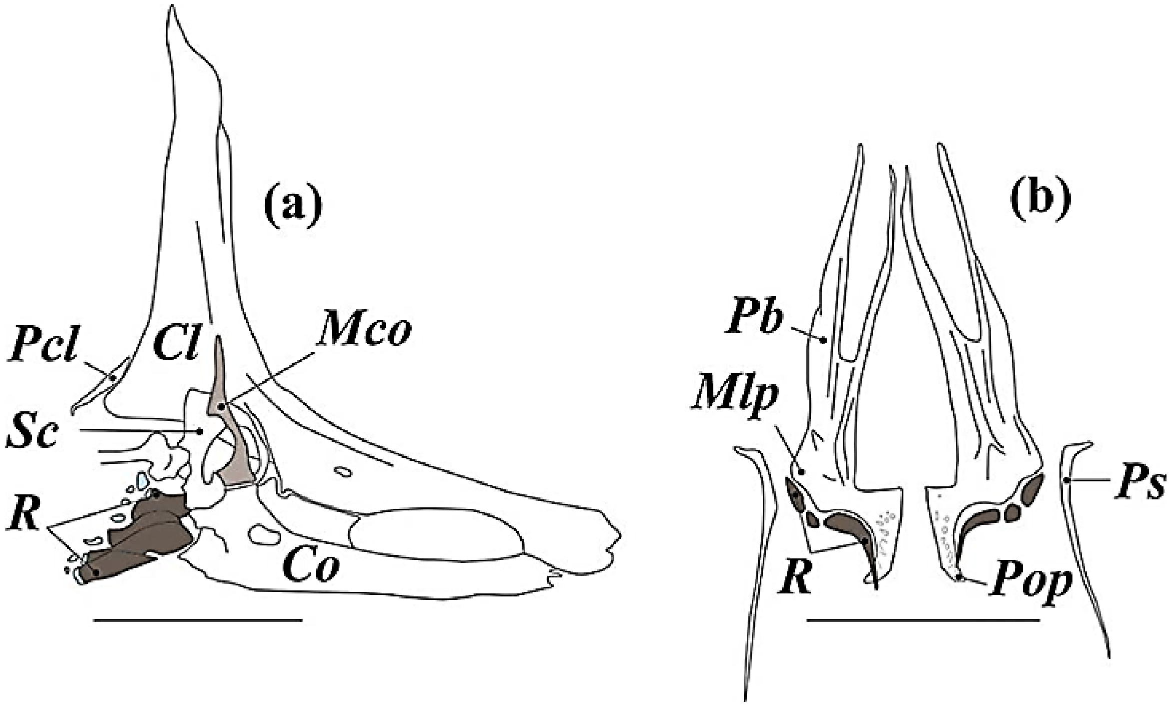

Figure 7. Medial view of the pectoral (a) and pelvic (b) girdles *Squalius orientalis* from the Zarineh River of the Urmia lake basin. Abbreviations: Cl: cleithrum; Co: coracoid; Mco: mesocoracoid; Mlp: mid-lateral process; Pb: pelvic bone; Pcl: postcleithrum; Pop: posterior process; Ps: pelvic splint; R: radials; Sc: scapula (Scale bar=3 mm).

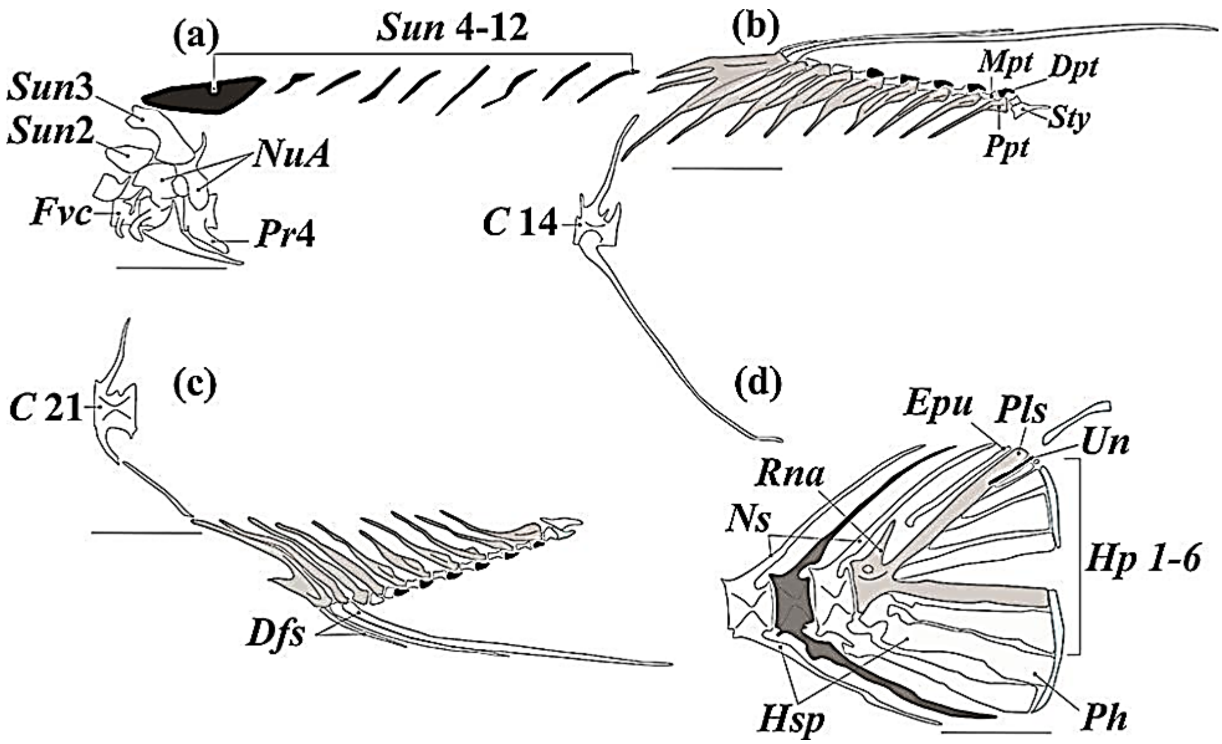

Figure 8. Lateral view of the Weberian apparatus (a), dorsal (b), anal (c) and caudal (d) fins in *Squalius orientalis* from the Zarineh River of the Urmia lake basin. Abbreviations: C14-21: centrum 14-21; Dfs: dorsal fin spine; Dpt: distal pterygiophore; Epu: epural; FVC: first vertebra centrum; Hp 1-6: hypural plates 1-6; Hsp: hemal spine; Mtp: medial pterygiophore; NuA: neural arch; Ns: neural spine; Pr 4: pleural rib 4; Ppt: proximal pterygiophore; Ph: parhypurale; Pls: pleurostyle; Rna: rudimentary neural arch; Sun: supraneural; Sty: stay (Scale bar=3 mm).

the pelvic bone.

There are 42 centra in the axial skeleton; the four anterior centra with tripus, intercalarium, scaphium and claustrum form the Weberian apparatus (Fig.

8a). The dorsal fin has 3 unbranched and 8½ branched rays, 9 pterygoiphore and one small stay bones (Fig. 8b). The dorsal portion of the stay bone is bifurcated and thinner. The anal fin has 3 unbranched, 9½ branched rays, 10 pterygoiphore and one stay bone (Fig. 8c). The first pterygoiphore of the dorsal and anal fins are located at the 14th and 21st centra, respectively. The caudal fin is formed by 6 hyporals, unpaired parhyporal, pleurstyle and paired uroneurals. The caudal fin has 10 dorsal and 9 ventral procurrent rays and 19 caudal rays (Fig. 8d).

Discussion

Intra-specific variation such as osteological characters are important in species delineation, as it is acknowledged that inadequate information on intra-specific geographic variation can lead to erroneous species descriptions (Ishihara, 1987; Irfan and Suneetha Gunawickrama, 2011). Osteological characters may also provide a major tool in examining variability within a species (Eastman, 1980), since different populations of the same species, which share external appearance, may vary in skeletal structures (Hilton and Bemis, 1999). The results of the present study revealed some differences in osteology among the two geographic populations of *S. orientalis* in Iran. The observed differences were in the structures of the neurocranium, opercle, palatine and pectoral girdle.

At the dorsal view of the neurocranium, the anterior portion of the pre-vomer is extended the supraethmoid-ethmoid in *S. orientalis* of the Urmia Lake basin population, whereas that of the Caspian Sea is almost at the level of the supraethmoid-ethmoid (Jalili, 2015). The parasphenoid alar in the Urmia Lake basin population is banded dorsally versus laterally oriented one of the Caspian Sea specimens (Jalili, 2015). Also the ventral blade shape process of the orbitosphenoid in the Urmia Lake basin specimens is longer than that of the Caspian Sea specimens (Jalili, 2015). The two later characters are led a deeper neurocranium in the Urmia Lake basin specimens.

According to Zhang (2005), the basioccipital is important character in cyprinids. In the examined population, the masticatory plate is concaved, whereas that of the Caspian Sea is flattened (Jalili, 2015). The anterior ascending process of the palatine in the Urmia Lake basin specimens were pointed and well-developed versus short and weakly developed one of the Caspian Sea population (Jalili, 2015). The posterior margin of the opercle is concaved in the Urmia Lake basin population versus straight one of the Caspian Sea population (Jalili, 2015).

Structure of the pectoral and pelvic girdles are important to identify the members of the subfamily Schizothoracine (Chen et al., 2001). In the pectoral girdle of the Urmia Lake basin specimens, the posterior process of the cleithrum is triangular in shape and small versus wide and well-developed one of the Caspian Sea specimens (Jalili, 2015).

The results revealed that osteological characters including having a longer pre-vomer, dorsally oriented parasphenoid alar, longer ventral blade-shaped process of the orbitosphenoid, concaved masticatory plate, pointed ascending process of the palatine, concaved posterior margin of the opercle, and a small posterior process of the cleithrum can differentiate *S. orientalis* of the Urmia Lake basin from that of the Caspian Sea basin. In addition, there is no variation in the bones forming the skull roof, postcranial and caudal fin skeletons of both populations. As conclusion, the results showed that bone arrangement seems to be a character for population differentiation in this species that can be caused under the influence of different environmental conditions. Additionally, the observed osteological differences suggest that both studied populations belong to same taxon.

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