

## Research Article

# Descriptive Osteology of a Newly Described Stone Loach, *Oxynoemacheilus chomanicus* (Kamangar et al., 2014) (Cypriniformes, Nemacheilidae)

Parvin Mafakheri, Soheil Eagderi, and Amir Qadermarzi

Department of Fisheries, Faculty of Natural Resources, University of Tehran, P.O. Box 4314, Karaj, Iran

Correspondence should be addressed to Soheil Eagderi; [soheil.eagderi@ut.ac.ir](mailto:soheil.eagderi@ut.ac.ir)

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*Oxynoemacheilus chomanicus* is a newly described species of the family Nemacheilidae from the Choman River drainage of the Tigris basin. This study was conducted to provide the detailed osteological characteristics of this species and comparing them with those of other endemic species of the genus *Oxynoemacheilus* from inland water basins of Iran, namely, *kiabii*, *O. persa*, *O. brandtii*, *O. kermanshahensis*, and *O. bergianus*. For this purpose, nine specimens of *O. chomanicus* were collected, cleaned, and stained for osteological examination. Then, a detailed description of their skeletal structure was provided. The results showed that *O. chomanicus* can be distinguished from other studied species of the genus *Oxynoemacheilus* due to possessing an orbital shelf, number of the vertebrae, number of the hypurals, number of the unbranched rays in anal fin, features of the hemal and neural spines, and connection pattern of the parietal and frontal.

## 1. Introduction

Stone loaches, the family Nemacheilidae, are small benthic fishes [1] found in fresh waters of Asia and its islands, Europe, and northeast Africa [2]. This family have a great diversity in Iranian inland waters [1, 3, 4], with more than 40 reported species, including 24 endemic species [3–9]. They are less known due to small size and low economic value [10, 11] and their classification is still complicated; therefore, ichthyologists are trying to reveal their phylogenetic status [12]. Given that, the osteological characteristics can play an important role in this regard [13], since osteology is a useful tool to study the taxonomy and phylogenetic relationships among fishes [14, 15].

The genus *Oxynoemacheilus* is a species rich genus of the family Nemacheilidae known from Albania eastwards to central Iran [16] and has 11 reported species from Iran of which three include *O. chomanicus*, *O. kurdestanicus*, and *O. zagrodensis* recently described from the Choman River drainage of the Tigris basin [4]. The newly described species of *O. chomanicus* is found in the Choman River drainage

(Baneh, Kurdistan Province). Identification of this species is based on the morphological features that show many similarities to other members of this genus [4].

Regarding high diversity and morphological similarity of the members of the family Nemacheilidae, using combined data, including osteological and molecular data, can help to better understand their taxonomical relationship. Therefore, this study was conducted to provide detailed osteological characteristics of *O. chomanicus* and comparing them with those of other species of the genus *Oxynoemacheilus* from the inland water basins of Iran that their osteological data are available. The finding of this study can provide the osteological features of this species that can help to discrete this species from others and be used as a basis for further phylogenetic study of the members of this genus based on the osteological data.

## 2. Material and Methods

Nine specimens of *O. chomanicus* with standard length of  $38.6 \pm 1.3$  (mean  $\pm$  SD) were collected using electrofishing

device from the Choman River (Kurdistan Province, Iran). Then, specimens were anesthetized with 1% clove solution and fixed in 4% formaldehyde. For osteological examination, the specimens were cleared and stained using alcian blue and alizarin red based on Taylor and Van Dyke [17]. Pictures of the stained skeletal structures were obtained using an Epson V600 scanner equipped with a glycerol bath. The skeletal structures of each sample were observed and studied by an MS5 Leica stereomicroscope. The scanned images were illustrated by CorelDraw X6 software. Nomenclature and abbreviation of the skeletal elements follow Prokofiev [12, 18]. Detailed descriptions of the osteological features of *O. kiabii*, *O. bergianus*, *O. persa*, *O. brandtii*, and *O. kermanshahensis* were provided by Mafakheri et al. [19], Jalili and Eagderi [20], Mafakheri et al. [21], and Mafakheri [22], respectively.

### 3. Results

**3.1. Neurocranium.** The anterior part of the neurocranium is narrow with wider posterior part and its maximum width is about 60% of its length. The ethmoid region consists of the paired lateral ethmoids and unpaired supraethmoid-ethmoid and prevomer. The elongated supraethmoid-ethmoid bone is vertically fused to the prevomer and posteriorly sutured with the frontal (Figures 1(a) and 1(b)). The anterior part of the prevomer is heart-shaped (Figure 1(b)) and is posteriorly connected to the orbitosphenoid (Figure 1(c)). The lateral ethmoid is medially attached to the orbitosphenoid (Figure 1). The anterior part of the lateral ethmoid is projected and its posterior part is convex in shape (Figure 1).

The orbital region includes the paired frontal, pterosphe- noid, and sclerotic, and the unpaired orbitosphenoid and parasphenoid. The frontal is the largest bony element of the neurocranium's roof. This bone is laterally connected to the orbitosphenoid, pterosphe- noid, and sphenotic and posteriorly to the parietal (Figures 1(a) and 1(b)). The frontal is connected to the lateral ethmoid via the orbital shelf (Figure 1(a)). The two frontals are connected medially but they are separated posteriorly and form the anterior part of the fontanelle (Figure 1(a)). The orbitosphenoid is posteriorly connected to the heavy bone of the pterosphe- noid. In the ventral plan, the parasphenoid is connected to the pre- vomer, orbitosphenoid, pterosphe- noid, prootic, and basioc- cipital (Figure 1(c)). The parasphenoid, orbitosphenoid, and pterosphe- noid enclose the orbital foramen (Figure 1(b)). The parasphenoid meets the prootic and pterosphe- noid via its lateral wings and form a large foramen in ventral plan of the neurocranium (Figure 1(c)). Two small bones of the sclerotic enclose the orbits.

The otic region consists of the parietals, epiotics, pterotics, prootics, and sphenotics (Figure 1). The parietal is located between the frontal and supraoccipital (Figure 1(a)). This bone is anterolaterally connected to the sphenotic and posterolaterally to the epiotic (Figures 1(a) and 1(b)). The pterotic is almost triangle in shape that it locates between the sphenotic and epiotic dorsally, and the prootic and exoccipital ventrally (Figures 1(a) and 1(c)). Connection of the epiotic and sphenotic avoids the relationship between the pterotic and parietal (Figure 1(b)). The pterosphe- noid, sphenotic, and

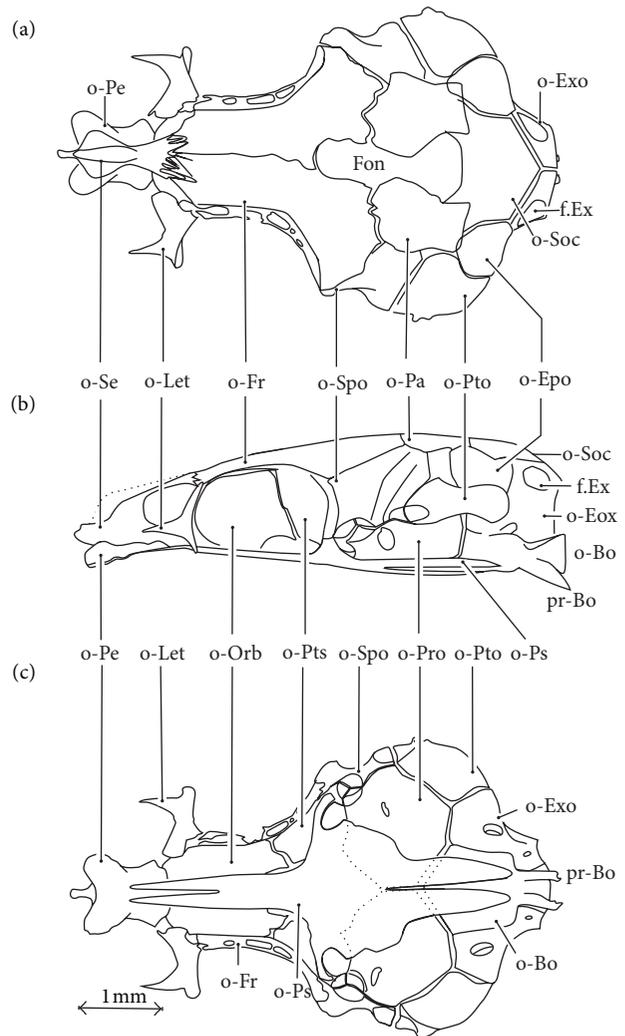


FIGURE 1: (a) Dorsal, (b) lateral, and (c) ventral views of the neurocranium of *Oxynoemacheilus chomanicus*. pr-Bo: basioccipital process; Bo: basioccipital; Epo: epiotic; Exo: exoccipital; fon: fontanelle; Fr: frontal; fr-Exo: foramen exoccipital; Let: lateral ethmoid; Orb: orbitosphenoid; Pa: parietal; Pe: prevomer; Pro: prootic; Ps: parasphenoid; Pto: pterotic; Pts: pterosphe- noid; Se: supraethmoid-ethmoid; Soc: supraoccipital; Spo: sphenotic.

prootic form the anterior articular facet for articulating the anterior condyle of the hyomandibular and the sphenotic and pterotic form the posterior articular facet for articulating the posterior condyle of the hyomandibular (Figures 2(b) and 2(c)).

The occipital region consists of both unpaired supraoc- cipital and basioccipital and the paired exoccipital (Figure 1). The supraoccipital forms the posterior part of the fontanelle. This bone is connected to the sphenotic by its anterolateral wings and posteriorly to the exoccipitals (Figure 1(a)). The exoccipital bears a large foramen and it is connected to the prootic anteroventrally (Figure 1(c)). The basioccipital is posteriorly narrower having a ring-shaped process (Figure 1(c)). This bone is situated between the exoccipitals and connected to the prootics anteriorly (Figure 1(c)). The basioccipital is

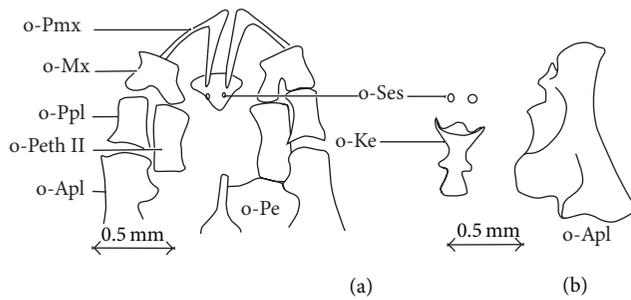


FIGURE 2: (a) Anterior part of the ethmoid region and (b) lateral plan of kinethmoid and autopalatine of *Oxynoemacheilus chomanicus*. Apl: autopalatine; ke: kinethmoid; Mx: maxilla; Pe: prevomer; peth II: preethmoid II; Pmx: premaxilla; ppl: prepalatine; ses: sesamoid.

posteriorly articulated to the first centrum. In a ligamentous space anterior to the ethmoid region, the paired preethmoid II, prepalatine, and sesamoid and unpaired kinethmoid are present (Figure 2). The prepalatine is located between the autopalatine and maxilla. The prepalatine is medially connected to the preethmoid II (Figure 2(a)). The rod-shaped preethmoid II is anteriorly connected to the maxilla and posteriorly to the prevomer. The cylindrically shaped kinethmoid is vertically located between jaws (Figure 2(a)).

**3.2. Jaws.** The upper jaw consists of the premaxilla and maxilla (Figure 3). The premaxilla has two processes, that is, pr. ascenden and pr. alveolar. The premaxilla is ligamentously connected to the kinethmoid. The maxilla is a large laminar bone which has a small anteroventral process connecting to the preethmoid II and prepalatine and an anteroventral process that is tilted downwardly reaching its counterpart (Figure 2(a)). The lower jaw is composed of the dental, articular, retroarticular, and coronomeckelian. The large dental has two processes, including narrow ramus dentalis and wide coronoid processes. The articular is overlapped with the dental and posteriorly articulated to the quadrate. The small bone of the coronomeckelian is attached to the medial face of the articular. The small bone of the retroarticular is articulated to the posteroventral part of the articular.

**3.3. Branchial Apparatus.** The branchial arch consists of the basibranchials, hypobranchials, ceratobranchials, epi-branchials, and infrapharyngobranchials (Figure 4). There are four basibranchials, which locate between the hypobranchials. The two first basibranchials are similar in shape. The hypobranchials are three pairs and almost square-shaped. In each side, there are five ceratobranchials of which the last one has different shape possessing pharyngeal teeth (Figure 4). The four paired epi-branchials situate between the rectangularly shaped ceratobranchials and the small infrapharyngobranchials. The infrapharyngobranchials are horizontally arranged on the epi-branchials.

**3.4. Hyoid Arch.** The hyoid arch is composed of the basihyal, dorsal hypohyal, ventral hypohyal, ceratohyal, epihyal, interhyal, urohyal, extra urohyal, and branchiostegals (Figure 5).

The large cylindrical ceratohyal intervenes between the hypohyals and the epihyal (Figure 5). The epihyal is triangular in shape. A small rod-shaped interhyal fits in a small groove on the posterodorsal part of the epihyal ventrally. The dorsal hypohyal and ventral hypohyal are connected firmly. Two small rod-shaped extra urohyals are located between the hypohyals and ligamentously connected to the urohyal and basihyal. The laminar bone of the urohyal bears a depression anteriorly and wings anterolaterally (Figure 5). The unpaired basihyal is T-shaped. There are three curved branchiostegals of which the first one is attached to the ceratohyal, the connection point of the second one is located between ceratohyal and epihyal, and the third one is connected to the epihyal.

**3.5. Suspensorium.** The suspensorium consists of the autopalatine, endopterygoid, ectopterygoid, metapterygoid, hyomandibular, quadrate, and symplectic (Figure 6). The autopalatine is a heavy bone articulating to the endopterygoid posteriorly (Figure 2(b)). There is a strong ligamentous attachment between the endopterygoid, ectopterygoid, metapterygoid, and quadrate. The triangle-shaped bone of the symplectic is inserted into the depression of the quadrate. A condyle for articulation to the articular presents in the anteroventral part of the quadrate. The hyomandibular bears two articular heads dorsally for the craniohyomandibular articulations.

**3.6. Opercular Series.** This series consists of the opercle, subopercle, interopercle, and preopercle (Figure 6). The opercle is the largest element of the opercular series and its dorsal margin is concaved. This bone has a large condyle, which is articulated to the hyomandibular. The opercle covers the paddle-shaped subopercle ventrally (Figure 7). The narrow curved preopercle almost locates at the rear of the interopercle (Figure 6). The interopercle is not completely ossified and connected to the subopercle posteriorly (Figure 6).

**3.7. Pectoral Girdle.** The pectoral girdle includes the cleithrum, coracoid, scapula, mesocoracoid, supratemporal, posttemporal, supracleithrum, and radials (Figure 7). The cleithrum is the largest bony elements of the pectoral girdle possessing the vertical and horizontal sections. Its vertical section is connected to the supracleithrum, whereas the horizontal one is connected to the scapula and coracoid. The coracoid is narrow anteriorly. The scapula is located between the cleithrum and coracoid. There is a large foramen on its middle face. The mesocoracoid is a small rod-shaped bone, which is medially located between the cleithrum and coracoid. The mesocoracoid indirectly connects these two bones (Figure 7). The supracleithrum bears a ridge on its lateral face. The elongated posttemporal is situated in the anterolateral depression of the supracleithrum. This bone is posterolaterally fused to the supratemporal. The supratemporal is a small tubular-shaped bone. There are four radials of which first two ones are narrow and elongated and the most medial one is the smallest and widest one.

**3.8. Pelvic Girdle.** The pelvic girdle consists of the paired pelvic bones and radials. The pelvic bones are horizontally

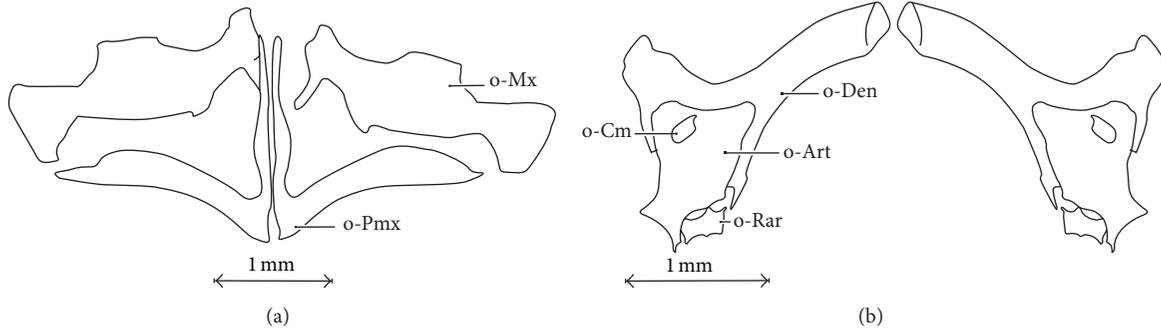


FIGURE 3: (a) Upper jaw and (b) medial view of lower jaw in *Oxynoemacheilus chomanicus* Art: articular; Cm: coronomeckelian; Den: dental; Mx: maxilla; Pmx: premaxillary; Rar: retroarticular.

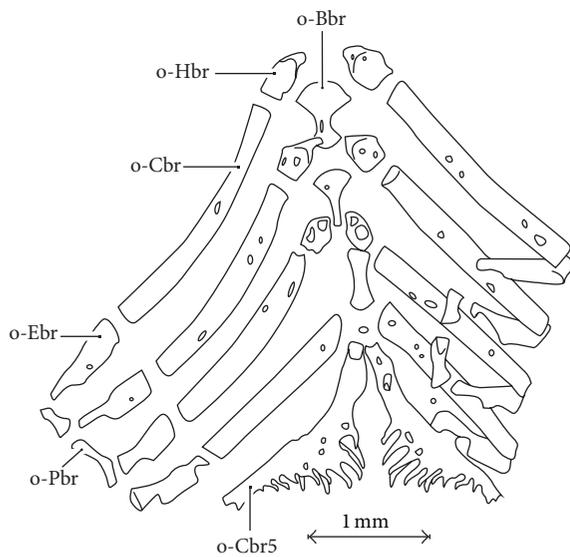


FIGURE 4: Branchial apparatus of *Oxynoemacheilus chomanicus*. Bbr: basibranchial; Cbr: ceratobranchial (cbr5: pharyngeal bones); Ebr: epibranchial; Hbr: hypobranchial; Pbr: infrapharyngobranchial.

located in belly area. The pelvic bones are firmly connected by a strong ligament medially. They have posterolateral processes, that is, pr. iliacus that along with the posterior process, that is, pr. Ischiadicus, forms a depression for positioning the rays and radials. The pelvic bone is anteriorly narrow (pubic process). There are three small radials. There are one unbranched ray, seven branched rays, and one small curved pelvic splint in the pelvic fin.

**3.9. Axial Skeleton and Unpaired Fins.** There are 37 centra of which first four centra form the Weberian apparatus and bony capsule. The hemal and neural spines are narrow. The dorsal fin has 9 pterygiophores and one stay and the first pterygiophore is the largest reaching the 11th or 12th vertebra. There are five unbranched and  $8_{1/2}$  branched rays in dorsal fin (Figure 8(a)). The number of the pterygiophores in anal fin is six with one stay in which first pterygiophore reaches the 24th centrum. The number of unbranched and branched rays in anal fin is 4 and  $5_{1/2}$ , respectively (Figure 8(b)).

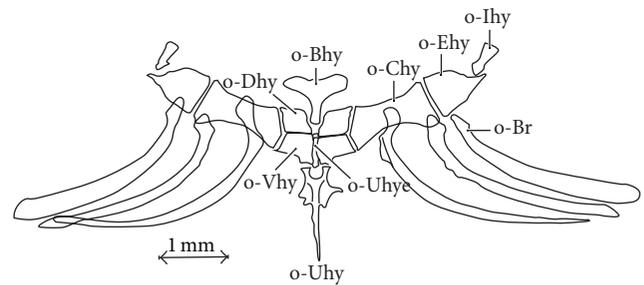


FIGURE 5: Hyoid arch of *Oxynoemacheilus chomanicus*. Bhy: basihyal; Br: branchiostegal; Chy: ceratohyal; Dhy and Vhy: dorsal and ventral hypohyal; Ehy: epihyal; Ihy: interhyal; Uhy: urohyal; Uhye: extra urohyal.

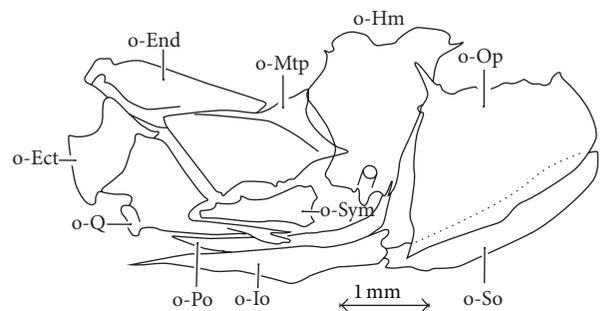


FIGURE 6: Medial view of the suspensorium of *Oxynoemacheilus chomanicus*. Apl: autopalatine; Ect: ectopterygoid; End: endopterygoid; Hm: hyomandibular; Io: interopercle; Mtp: metapterygoid; Op: opercle; Po: preopercle; Q: quadrate; So: subopercle; Sym: symplectic.

**3.10. Caudal Skeleton.** The caudal complex consists of the second preural centrum and its neural and hemal processes, first preural centrum, ural-1+ural-2, pleurostyle, hypurals, parhypural, epural, and principal caudal rays, and procurrent rays (Figure 9). The hemal process of the second preural is articulated to its centrum and its neural process is fused to its centrum. The last centrum is formed by the fused ural-1, ural-2, and preural-1. The wide rectangularly shaped parhypural along with the hypural-1 is articulated to the last centrum. The parhypural is dorsally connected to the hypural-1. There are

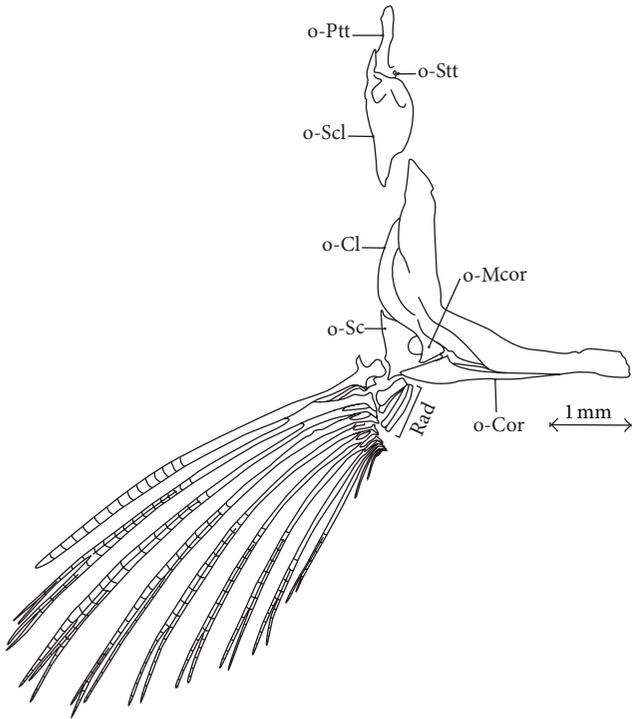


FIGURE 7: Medial views of the pectoral girdle of *Oxynoemacheilus chomanicus*. Cl: cleithrum; Cor: coracoid; Mcor: mesocoracoid; Ptt: posttemporal; Rad: ossified pectoral radial; Sc: scapula; Scl: supracleithrum; Stt: supratemporal.

six hypurals of which first two ones are large. The hypural-2 is fused to the centrum and others are inserted into the groove of the pleurostyle. The last hypural is small. The small epural is located between the pleurostyle and neural rudimentary. The pleurostyle is elongated and fused to the centrum. The principal caudal rays are supported by the hypurals.

**4. Discussion**

The members of the genus *Oxynoemacheilus*, with 11 reported species, are distributed in the most of the inland waters basins of Iran and their identification is difficult based on their external morphology. Hence, the osteological features are suitable characteristics for their distinction [12, 18]. In addition, the osteological data can be well interpreted in taxonomy and phylogenetic relationships of fish species even in higher level [23]. There are various works on the phylogenetic relationships of the superfamily Cobitoidea using osteological characteristics containing important information [12, 13, 18, 24–27]. Therefore, the present study provided detailed osteological characteristics of *O. chomanicus* for further taxonomical studies of these taxa.

Prokofiev [12] has pointed out that the coronomeckelian is located on the base and dorsal edge of the coronoid processes in the genus *Oxynoemacheilus*, whereas it is attached to the articular in *O. chomanicus* as mentioned by Sawada [13]. In addition, Prokofiev [12] mentioned that the lateral ethmoid is stationarily jointed with the supraethmoid-ethmoid, while

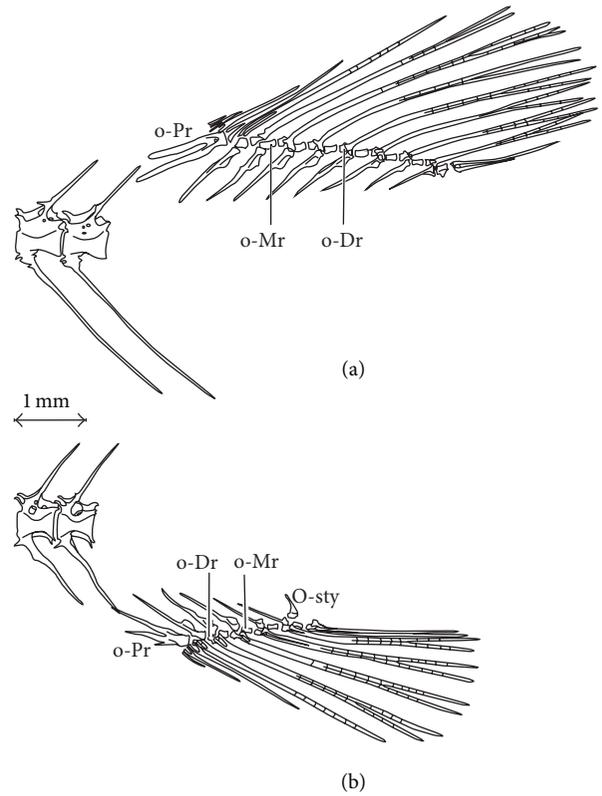


FIGURE 8: (a) Dorsal fin and (b) anal fin of *Oxynoemacheilus chomanicus*. dr: distal radial; mr: medial radial; pr: proximal radial; sty: stay.

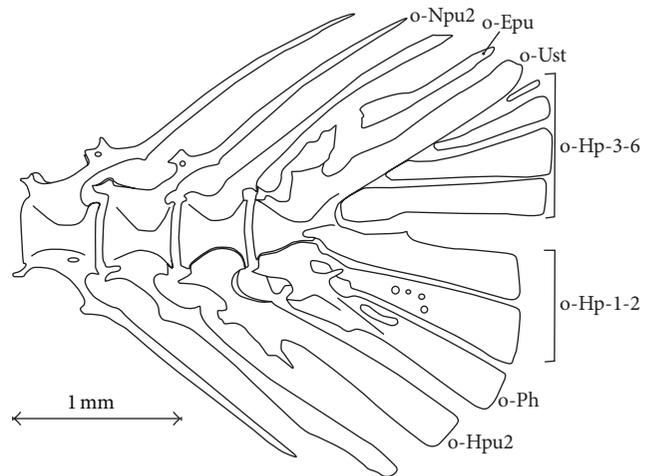


FIGURE 9: Caudal skeleton of *Oxynoemacheilus chomanicus*. Epu: epural; Hp: hypural; Hpu2: hemal process of the second preural centrum; Npu-2: neural process of the second preural centrum; Ph: parhypural; Ust: pleurostyle.

such a feature was not observed in *O. chomanicus*. In *O. chomanicus*, similar to other compared species of this genus, the prootic, pterosphenoic, and sphenotic form the anterior

articulatory facet, and the pterotic and sphenotic form the posterior articulatory facet, whereas Prokofiev [12] mentioned that, in the members of the genus *Oxynoemacheilus*, the anterior articulatory facet is restricted by the sphenotic and prootic, and the posterior articulatory facet is restricted by the sphenotic, prootic, and pterotic.

Based on the results, this species shows some osteological features that can be considered as its osteological identification key in comparison to *O. kiabii* [19], *O. persa* [21], *O. brandtii*, *O. kermanshahensis* [22], and *O. bergianus* [20]. There is an orbital shelf of the frontal in *O. chomanicus*, whereas such shelf is not present in *O. persa* [21]. The posterior process of the orbitosphenoid in *O. persa* is well developed [21] unlike that of *O. chomanicus*.

In *O. kiabii*, the supraethmoid-ethmoid is shorter and wider [19] than that of *O. chomanicus*. The connection pattern of the frontal and parietal in *O. kiabii* had a zigzag pattern [19] versus straight one of *O. chomanicus*. Furthermore, there is a small bone between the frontal and parietal in *O. persa* [21]. The shape of the metapterygoid in *O. chomanicus* is rectangular, while it is square-shaped in *O. kiabii* [19]. Furthermore, the hypohyals' processes of *O. kiabii* are well developed [19] unlike that of *O. chomanicus*. In *O. brandtii* and *O. bergianus*, the hemal and neural spines are wider and well developed [20, 22], while those of *O. chomanicus* are thin and narrow similar to *O. kiabii*, *O. persa*, and *O. bergianus*.

*Oxynoemacheilus chomanicus* has six hypurals, whereas *O. kiabii*, *O. brandtii*, *O. persa*, and *O. kermanshahensis* have five hypurals. The number of the vertebrae in *O. chomanicus* is 37, whereas *O. persa* and *O. kermanshahensis* have 34-35 and 39-40 vertebrae, respectively [21, 22]. The dorsal margin of the maxilla in *O. chomanicus* is smooth, whereas it is convex-shaped in *O. kermanshahensis* [22].

In *O. chomanicus*, the number of unbranched rays in dorsal and anal fins is five and four, respectively, which is different from the original description of this species (three and three) by Kamangar et al. [4]. These extra unbranched rays were visible only in cleared and stained specimens. The number of branched rays in dorsal and anal fins of *O. chomanicus* is  $8_{1/2}$  and  $5_{1/2}$ , respectively, corresponding to description of this species by Kamangar et al. [4]. The branched rays of the dorsal fin in *O. chomanicus* are more than those of *O. kermanshahensis* [19].

Some species of the genus *Oxynoemacheilus* were previously placed in other genera, including *Barbatula* and *Triplophysa*, but osteological investigations revealed their differences and ascribed them to related genera [12, 13]. Of the most important differences of the genus *Oxynoemacheilus* with the members of the genera *Barbatula* and *Triplophysa* are lack of the preethmoid-I and the number and shape of the radials of the pectoral fin. In the examined species, including *O. persa*, *O. bergianus*, *O. brandtii*, *O. kermanshahensis*, *O. kiabii*, and *O. chomanicus*, the preethmoid-I is absent. The numbers of the radials in the pectoral fins of *Triplophysa* is 3 and *Barbatula* is 4 with different shape compared to the members of the genus *Oxynoemacheilus*. The members of the genus *Oxynoemacheilus* bear two small sesamoid bones, whereas this bone is absent in *Triplophysa* and *Barbatula* [12, 13]. In *Oxynoemacheilus*, the number of the branched rays

of the anal fin is 5, whereas this number can be higher in *Barbatula* and *Triplophysa*.

Based on the results, the presence of the orbital shelf, number of the vertebrae, number of the hypurals, number of the unbranched rays in anal fin, features of the hemal and neural spines, and connection pattern of the parietal and frontal can be offered as identified keys for *O. chomanicus* from other examined species of the genus *Oxynoemacheilus* from inland water of Iran.

## Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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