Int. J. Aquat. Biol. (2016) 4(2): 117-124
ISSN: 2322-5270; P-ISSN: 2383-0956
Journal homepage: www.ij-aquaticbiology.com

Original Article

The complete description of the skeletal structure of Hafez loach, *Turcinoemacheilus hafezi* (Cypriniformes, Nemacheilidae)

Nasrin Nikmehr, Soheil Eagderi*, Hadi Poorbagher, Hamid Farahmand

Department of Fisheries, Faculty of Natural Resources, University of Tehran, Karaj, Iran.

Abstract: The osteological characteristics is an important tool for clarification of the phylogenetic statue of the family Nemechelidae. Since any information is available about osteological features of the genus *Turcinoemacheilus*, hence the present study provides a detailed osteological characteristics of the *Turcinoemacheilus hafezi* as representative of this genus. Ten specimens of *T. hafezi* were collected from the Shalamzar Stream, Tigris basin, Iran and cleared and stained for osteological examination. According to the results, *T. hafezi* is osteologicaly characterized by absence of the preethmoideum-I and postcleithrum, having four basibranchials, presence of the sesamoid ossification, free and short epural with the reduced neural process and pleurostyle, connection of the hypural-1 to the parahypural, no connection of the hypurals 3, 4, and 5 to the pleurostyle, and no bony bridge between the parietal.

Article history:
Received 21 January 2016
Accepted 17 April 2016
Available online 25 April 2016

Keywords:
Nemacheilidae
Osteology
Loach
Turcinoemacheilus
Iran

Introduction

Nemacheilids are small and benthic species that found in flowing waters of Europe, Asia, and northeast of Africa (Coad, 2016). Loaches of the genus *Turcinoemacheilus*, a member of the family Nemacheilidae, is easily distinguished from other members of this family by a more anterior position of the anus (Bânârescu and Nalbant, 1964; Freyhof et al., 2011). *Turcinoemacheilus* was a monotypic genus for 47 years (Conway et al., 2011; Golzarianpour et al., 2013). *Turcinoemacheilus kosswigi* Bănărescu and Nalbant, 1964 was recorded for the first time in Iran from the Karoun River drainage (Golzarianpour et al., 2009). *Turcinoemacheilus hafezi*, a new species from Karoun and Dez rivers drainages of Iran (Golzarianpour et al., 2013), was second species of this genus from Iran. Also, Esmaeili et al. (2014) described two new species of the genus *Turcinoemacheilus* from Iran, including *T. bahaii* from the Zayandeh River and *T. saadii* from the Karoun River drainage in Iran.

Turcinoemacheilus hafezi Golzarianpour et al. 2013 is distinguished from the other species of this genus by the more posterior position of the anus, which is closer to the anal-fin origin than to the pelvic-fin origin (distance from anus to anal-fin origin 0.2-0.4 times in distance from pelvic-fin origin to anal-fin origin), a mottled colour pattern or a prominent irregular dark brown or black mid-lateral stripe disconnect from blotches and saddles on back, a slightly emarginated caudal fin and a completely scaleless body (Golzarianpour et al., 2013).

Due to small size and low economic value, the members of the family Nemacheilidae are less known (Kottelat, 1990) and their classification is still complicated; therefore, ichthyologists are trying to reveal their phylogenic status (Prokofiev, 2010). Hence, the osteological characteristics can play an important role in this regard (Sawada, 1982; Mafakheri et al., 2015). The only comprehensive osteology and phylogenetic studies on nemacheilids performed by Prokofiev (2009, 2010), but in these studies, the *Turcinoemacheilus* did not include.

* Corresponding author: Soheil Eagderi
E-mail address: soheil.eagderi@ut.ac.ir

Figure 1. *Turcinoemacheilus hafezi* from the Shalamzari Stream River.

Since, there is no information is available about the osteological features of the genus *Turcinoemacheilus*, therefore, this study aimed to provide a detailed osteological description of Hafez loach, *T. hafezi* from the Karoun river drainage.

Materials and Methods

Ten specimens of *T. hafezi* with average standard length of 38.22(±0.64) (Mean±SD) were collected by electrofishing device from the Shalamzar Stream (Charmahal-e Bakhtiari Province, Iran) (Fig. 1). Then, the specimens were anesthetized in 1% clove solution and fixed in 10% buffered formaldehyde. For osteological examination, the specimens were cleared and stained using alcian blue and alizarn red based on Taylor and Van Dyke (1985). Images of the stained skeletal structure obtained by a scanner (Epson V600) equipped to a glycerin bath. Skeletal structures were observed and studied by a stereomicroscope (Leica MS5). Drawings of the skeletal elements were performed using CorelDraw X6 software. Nomenclature and abbreviation of the skeletal elements were based on Prokofiev (2009).

Results

The neurocranium is wider posteriorly, forming the maximum width of the skull at the level of the pterotic. The ethmoid region is comprised of a pair of the lateral ethmoid, and unpaired prevomer and supraethmoid-ethmoid bones (Fig. 2A). The paired L-shaped lateral ethmoids posteriorly form the anterior part of the orbit. The prevomer is relatively flat having two processes with rounded edges antero-laterally. This bone is connected to the orbitosphenoid and parasphenoid posteriorly (Fig. 2B). The supraethmoid-ethmoid is narrower in the middle part. The kinethmoid is a small and free bone situated between two maxillae. This bone has two pointed lateral processes in the middle part, forming its maximum width.

The orbital region includes the frontal, orbitosphenoid, pterosphenoid, parasphenoid, lachrymal, and sclerotic bones. The paired large frontals are asymmetric and have two processes in latero-middle part; its anterior part is narrow. These bones include about half of the length of the neurocranium separating by the fontanel posteriorly. The frontal is connected to the orbitosphenoid antero-laterally. The posterior margins of the frontals contribute in formation of the fontanel. The pterosphenoid is connected to the frontal dorsally and to the sphenoid posteriorly, forming the posterior part of the orbit. The parasphenoid is bifurcated at two ends and its middle part is connected to the pterosphenoids. It has two small lateral and one large middle pores. The orbitosphenoid is connected to the lateral ethmoid ventrally, to the parasphenoid antero-laterally, and to the pterosphenoid posteriorly (Fig. 2).

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. In addition, no bony bridge present between the parietal and pterotic. The parietal is roughly rectangular. The pterotic is almost triangular in shape. There is no connection between the parietals due to a long fontanel. The sphenotic bears a lateral process and a

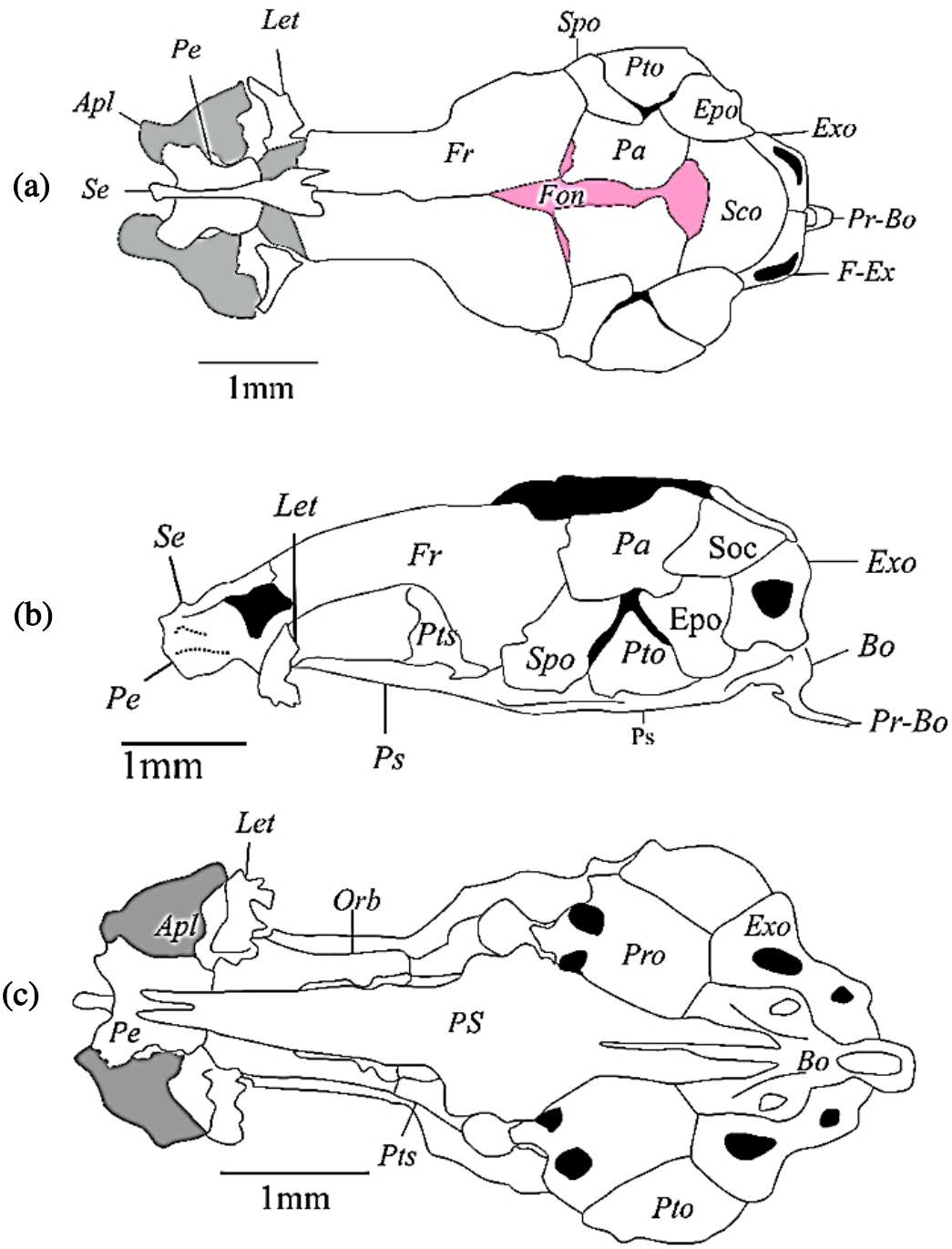

Figure 2. The neurocranium of *Turcinoemacheilus hafezi*. (a) Dorsal, (b) lateral, and (c) ventral views. Apl: autopalatine, fon: fontanel, fr-Exo: foramen exoccipital, pr-Bo: basioccipital process, Epo: epiotic, Exo: exoccipital, Fr: frontal, Let: lateral ethmoid, Orb: orbitosphenoid, Pa: parietal,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

latero-ventral facet.

The occipital region is composed of the supraoccipital, exoccipitals, and basioccipital. The supraoccipital has a pentagon shape; it is connected to the exoccipitals ventrally. The exoccipital encloses the foramen magnum and bears a small foramen laterally. The basioccipital bears a posterior process with a large cavity centrally. There is an occipital condyle at the posterior part of the basioccipital for connecting to the vertebrae.

In the branchiocranium, the upper jaw consists of the maxilla and premaxila (Fig. 3a). The maxilla is a

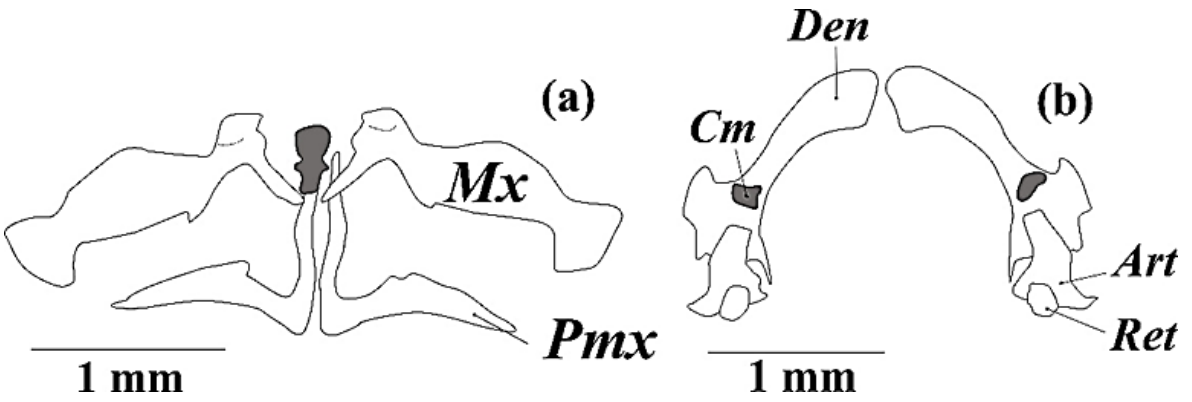

Figure 3. The upper (a) and lower (b) jaws of *Turcinoemacheilus hafezi*. Mx: maxilla, Pmx: premaxilla, Art: articular, Cm: coronomeckelian, Den: dental, and Ret: retroarticular.

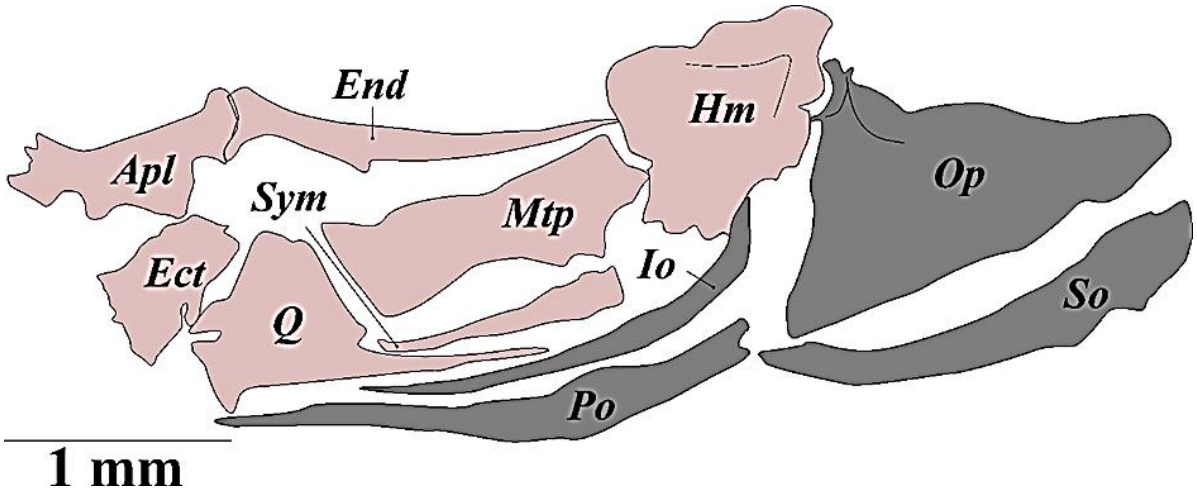

Figure 4. The suspensorium, palatine, and opercular series of *Turcinoemacheilus hafezi*. Apl: atutopalatine; Ect: ectopterygoid, End: endopterygoid, Hm: hyomandibular, Io: interopercle, Mtp: metapterygoid, Op: opercle, Q: quadrate, So: subopercle, and Sym: symplectic.

large laminar bone and its middle part is the wider; its anterior part is narrow with an antero-ventral process tilting ventrally. The premaxila is L-shaped comprising two horizontal and vertical parts. The horizontal part is arc-shaped and slightly wider in its middle part and the vertical part is narrower and longer. There is two small triangular process at the middle portion of the vertical part.

The lower jaw is composed of four bones, including the dental, angular, retroarticular and coronomeckelian (Fig. 3b). The dental is the largest element of the mandible, including a narrow antero-ventral branch and a deep section (process coronoideus) postero-dorsally; it is connected to the articular postero-dorsally and to the retroarticular dorsally. The coronomeckelian is triangular in shape and situated in the medial side of the dental beneath the process coronoideus.

The suspensorium is formed by the hyomandibular, ectopterygoid, endopterygoid, metapterygoid, symplectic, quadrate, and autopalatine (Fig. 4). The hyomandibular is a relatively large bone extending longitudinally that its dorsal part is wider. In addition, there are two anterior and posterior hyomandibular condyles on its dorsal margin. The quadrate bears a condyle antero-ventrally where it is articulated to the dental. The ectopterygoid, endopterygoid, metapterygoid, symplectic, and quadrate form a complex connecting to the anterior part of the neurocranium via autopalatine. The autopalatine is wider and posteriorly connected to the endopterygoid and anteriorly to the prevomer. The endopterygoid is pointed posteriorly connecting to the ectopterygoid

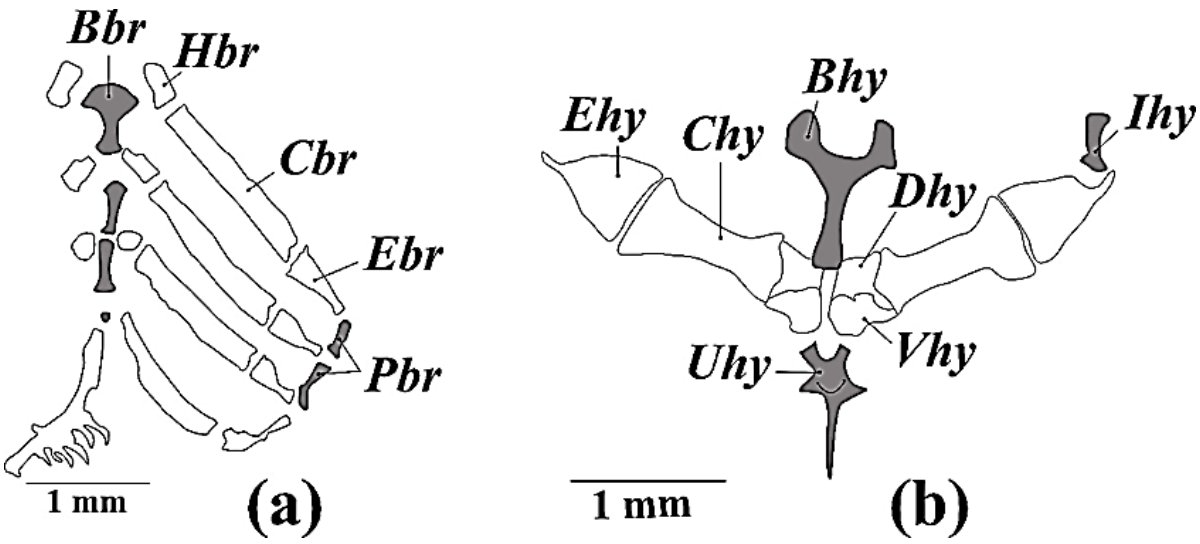

Figure 5. Dorsal view of the branchial apparatus (a) and hyoid arch (b) of *Turcinoemacheilus hafezi*. Bbr: basibranchial, Cbr: ceratobranchial, Ebr: epibranchial, Hbr: hypobranchial, Pbr: pharyngobranchial, Bhy: basihyal, Chy: ceratohyal, Dhy and Vhy: dorsal and ventral hypohyal, Epi: epihyal, Ihy: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

antero-ventrally and metapterygoid posteriorly. The metapterygoid bears several pores on its lateral face. The ectopterygoid is situated anterior to the quadrate.

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 is located ventral part to the opercle. The narrow preopercular is pointed anteriorly and bears a notch at its posterior rim. The interopercular is a curved bone with pointed anterior part.

The branchial apparatus includes five pairs of the ceratobranchial, four pairs of the epibranchiasls, three pairs of the hypobranchials and pharyngobranchials, and four unpaired basi-branchials (Fig. 5a). The anterior part of the first and second basibranchials are the wider. Two first hypobranchials are similar in shape and the last one is the smallest. Four pairs of the ceratobranchials are situated between the hypobranchials and epibranchials and the last ceratobranchials are modified as the tooth plats. The last epibranchial curves dorsally and its anterior part is narrower.

The hyoid arch consists of the paired interhyal, epihyals, ceratohyals, dorsal and ventral hypohyals, and the unpaired urohyal and basihyal, and three pairs of the branchiostegals (Fig. 5b). The urohyal is T-shaped and anteriorly bifurcated. The basihyal is also T-shaped with a deep notch on its anterior end. The dorsal and ventral hypohyals are connected firmly. The ceratohyals are the largest elements of the hyoid arch. There are three curved long branchiostegals; the first one is attached to the ceratohyal, the second one connected between the ceratohyal and epihyal, and the third one is attached to the epihyal. The interhyal is small with pointed posterior end (Fig. 5b).

In the postcranial skeleton, the pectoral girdle consists of the cleithrum, supracleithrum, coracoid, mesocoracoid, scapula, posttemporal, supratemporal and radials (Fig. 6a). The largest element of this complex is the cleithrum that bears a longer vertical and a horizontal sections. The dorsal part of the supracleithrum is wider. The coracoid is connected to the cleihtrum posteriorly and middle-ventrally. The mesocoracoid is narrow and its ventral part ia wider and connected to the coracoid. The mesocoracoid is firmly connected to the cleithrum. The scapula is trapezoid in shape and connected to the coracoid ventrally. The pectoral girdle bears four radials that overlap together and first one is the widest. The pectoral fin has one unbranched and 9 branched rays.

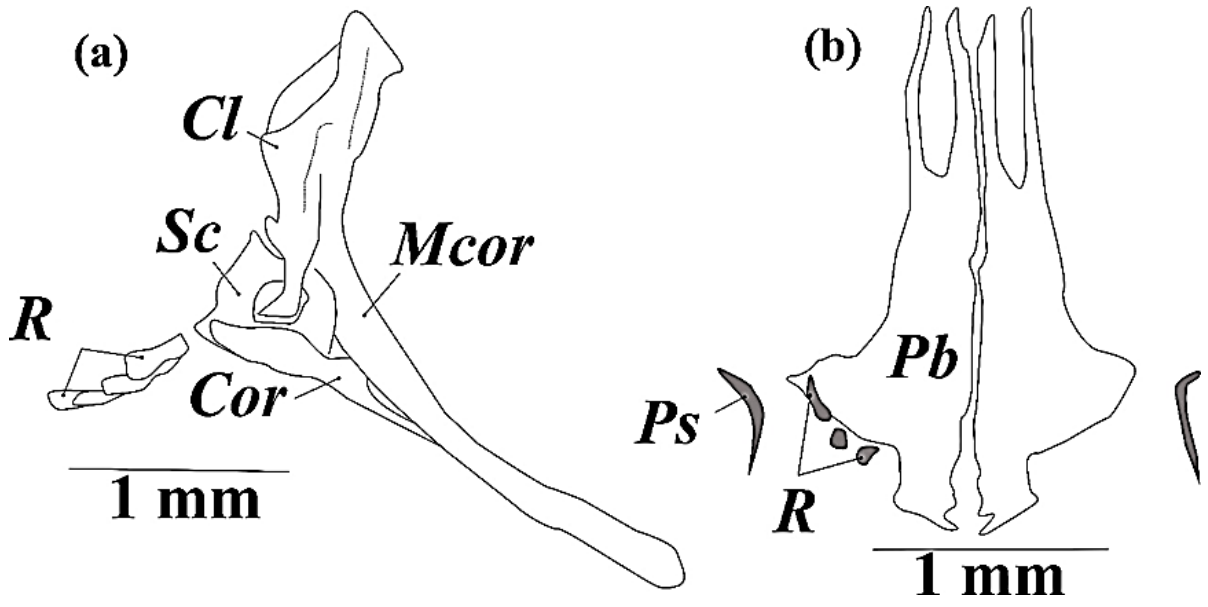

Figure 6. Medial view of pectoral girdle (a) and pelvic girdle (b) of *Turcinoemacheilus hafezi*. Cl: cleitherum, Cor: coracoid, Mcor: mesocoracoid, Pb: pelvic bone, Ps: pelvic splint, R: radials, and Sc: scapula.

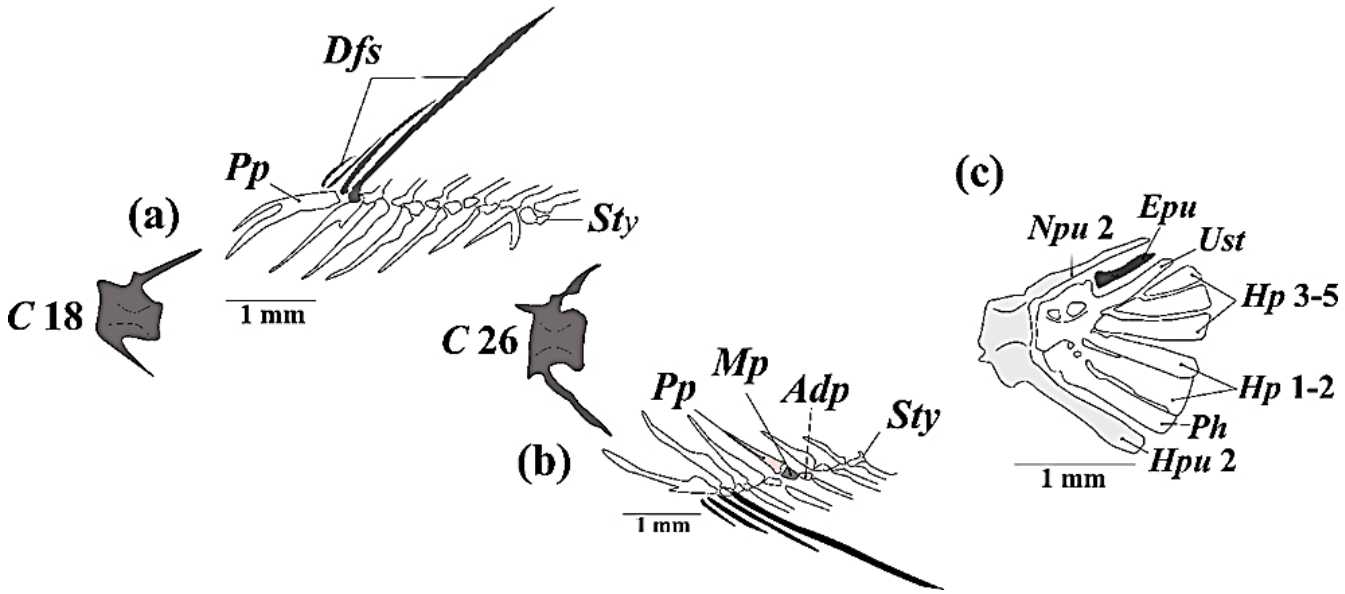

Figure 7. Dorsal (a), anal (b) and caudal (d) fin skeleton of *Turcinoemacheilus hafezi*. Adp: anal distal pterygiophore; C 18-26: 18th and 26th centrum; Dfs: dorsal fin spine; Mp: mesial pterygiophore; Ph; parhyporal; Pp: pterygiophore; Sty: stay; Epu: epural; Hp1–5: hypural plates; Npu 2: neural processes of the second preural centrum; Hpu 2: hemal processes of the second preural centrum; Ust: pleurostyle.

The pelvic girdle includes the paired pelvic bones, pelvic splint and radials (Fig. 6b). The pelvic bone is horizontally positioned in the middle part of the belly. It has two long processes anteriorly and its posterior part is wider. The pelvic bone has a round process latero-posteriorly. The pelvic girdle possesses three radials that the medial one is the smallest one. The styloid is situated lateral to the radials. The pelvic fin bears one unbranched and 7 branched rays.

The dorsal fin bears 4 unbranched and 6 branched rays, 8 pterygiophores and one stay bone. The first pterygiophor is next to the 17th or 18th vertebra. The largest bone of the dorsal fin is the first pterygiophores that supports 4 unbranched rays. The first and last petrygiophores are bifurcated ventrally. A triangular stay supports the last branched ray (Fig. 7a). The anal fine originates at 26th centrum; it has 4

unbranched and 5 branched rays and is supported by 6 pterygiophores and one small stays bone (Fig. 7b).

The caudal skeleton is made up of four centra along with the epural, parhypural, pleurostyle, uroneural and 5 hypurals (Fig. 7c). The anterior part of the hypural-1 is wider and its anterior end reaches to the last centrum. The hypural-1 is also connected to the parahypural ventrally. The parahypural is relatively flat, and its posterior end is connected to the last centrum. The hypurals 3, 4, and 5 are positioned between the pleurostyle and hypural-2. The neural process of the second pleural is long and narrow. The caudal fin bears 18 branched rays. The number of the dorsal procurrent is 5 and thar of the ventral procurrent is 6.

Discussion

The present study provided a detailed skeletal description of the *T. hafezi*. Based on the results, *T. hafezi* showed various differences with other members of the family Nemacheilidae. In the neurocranium, the lateral ethmoid, supraethmoid-ethmoid and prevomer are fused, as other loaches, with the exception of *Oreonectes platycephalus*, *Lefua* spp., *Yunnanilus pleurotaenia*, *Triplophysa microphthalma*, *T. tenuis* and *Schistura fasciolata* (Sawada, 1982). In contrast to *T. kosswigi*, the L-shaped lateral ethmoid of *T. hafezi* has a process posteriorly. *Turcinoemacheilus hafezi* bears a sesamoid ossification similar to *T. kosswigi* (Azimi, 2014), *Paracobitis malapterurus* (Azimi et al., 2015a), *P. longicauda*, *Dzihunia amudarjensis* and *Oxynoemacheilus* angorae (Prokofiev, 2004, 2009). However, the presence of the sesamoid ossifications in loaches had been rejected previously (Sawada, 1982). In *T. hafezi* similar to *T. kosswigi*, (Azimi, 2014), the supraethmoid-ethmoid is tightly attached to the frontals, as other loaches with the exception of *Indoreonectes evezardi* (Sawada, 1982). There are two opening on the ventral part of the exoccipital in *T. hafezi* similar to *T. kosswigi* (Azimi, 2014), *O. platycephalus*, *L. costata*, *Orthrias barbatulus*, *T. stoliczkae*, *T. alticeps*, *T. strauchii*, *D. amudarjensis*, *O. angorae*, *Micronoemacheilus pulcher*, *Iskandaria kuschakewitschi* and *Afronemacheilus abyssinicus*, with the exception of *P. cristata* (Prokofiev, 2010; Azimi et al., 2015b).

In branchial apparatus of *T. hafezi*, there are four basibranchials similar to *T. kosswigi* (Azimi, 2014), *O. angorae* and *O. brandti* (Mafakheri et al., 2015) that fourth one is very small; Prokofiev (2010) are reported three basibranchials in *Lefua* spp., *O. platycephalus*, *Y. pleurotaenia*, *Hedinicthys*, in the majority of species of the genus *Orthrias* (with the exception of *O. dgebuadzei*), in *I. kuschakewitschi*, *Paracobitis longicauda*, *P. malapterurus*, *Schistura* spp. *Paraschistura*, *Nemacheilus masyae*, *Nun*, *Seminemacheilus*, *Acanthocobitis botia*, and *I. evezardi* (Prokofiev, 2010; Azimi et al., 2015a, b). In addition, the basihyal of *T. hafezi* has a deep notch compared to that of *T. kosswigi* (Azimi, 2014; Azimi et al., 2015a, b).

In the caudal skeleton, the hypoural-1 of *T. kosswigi* attaches to the terminal centrum similar to *O. brandti* (Prokofiev, 2010; Mafakheri et al., 2015). The hypural-1 is connected to the parahypural ventrally in *T. hafezi* similar to *T. kosswigi* (Azimi, 2014), and unlike majority of loaches with the exception of *O. angorae*, *Triplophysa orientalis*, and *T. scleroptera* (Prokofiev, 2010). The epural of *T. hafezi* is long and not connected to the reduced neural process of the terminal centrum similar to *P. cristata* (Prokofiev, 2010; Azimi et al., 2015b), whereas in *T. kosswigi*, it is fused to the urostyle (Azimi, 2014).

According to Prokofiev (2010), the most stable and important diagnostic and phylogenic feature of the pectoral girdle in loaches is the number and shape of bony radials in the pectoral fin; unlike *O. platycephalus* and *Lefua* spp., which have two radials (Sawada, 1982), there are three pelvic radials in *T. hafezi* and *T. kosswigi* (Azimi, 2014) like other loaches. In loaches, the caudal skeleton has 5-6 hypurals (Prokofiev, 2010), which five hypurals were observed in *T. hafezi* similar to *T. kosswigi* (Azimi, 2014). Finally, since the identification of the species of the genus *Turcinoemacheilus* is difficult based on morphometric characteristics and color

pattern, the present study provides a useful distinguishing osteological features of this genus from the other members of the nemacheilid family.

Acknowledgments

We would like to express our sincere thanks to A. Jouladeh-Roudbar for his help during sampling, H. Mousavi-Sabet for providing some materials and P. Jalili for her help during clearing and staining of specimens. This study was financially supported by the University of Tehran.

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