

The Description of Osteological Characters of *Turcinoemacheilus saadii* (Nemacheilidae) in Connection with Phylogenetic Relations of the Genus

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Abstract—This study provides a detailed description of osteological characters of *Turcinoemacheilus saadii* with an attempt to evaluate their significance for the grouping of different genera. In general, a comparative osteological analysis of *T. saadii* and other previously studied species of *Turcinoemacheilus* and the related genus *Paraschistura*, as well as the genera *Paracobitis* and *Oxynoemacheilus*, shows the absence of diagnostic features for the currently generally accepted genera or any other groups of species. However, some osteological characteristics are of diagnostic value at the species level, primarily the numbers of basibranchial and hypural bones, vertebrates, pterygiophores, unbranched and branched fin rays, as well as the shape and position of some bones and their connections.

Keywords: loaches, osteology, phylogenetic value, diagnostic value, *Turcinoemacheilus saadii*, Iran

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INTRODUCTION

Loaches of the suborder Cobitoidei are bottom living fishes, distributed mainly in Europe and Asia (with a few species in Africa). The monophyly of this group is consistently recovered in recent morphological and molecular phylogenetic studies (Tan and Armbruster, 2018). The last classification of Cobitoidei based on molecular data (Šlechtova et al., 2007; Bohlen and Šlechtova, 2009) was presented by Kottelat (2012), who distinguished the family Nemacheilidae, which was confirmed as monophyletic by phylogenetic studies (Tang et al., 2006; Šlechtova et al., 2007; Bohlen and Šlechtova, 2009; Chen et al., 2009; Mayden and Chen, 2010; Liu et al., 2012; Hirt et al., 2017), but not supported by morphological synapomorphies (Sawada, 1982; Conway, 2011). Kottelat (2012) adopted a total of 46 valid genera in the Nemacheilidae; six of them were included in the synonymy by Tan and Armbruster (2018), who in turn added five more genera (four of them were described after Kottelat, 2012). The phylogenetic relationships between these genera are still unclear; most of them have not been studied genetically. However, Prokofiev (2010) presented an attempt to reconstruct their phylogenetic relationship based on morphological characters. As a result, five

tribes were distinguished within Nemacheilidae (adopted by the author as Nemacheilinae). But phylogenetic support for these taxa leaves much to be desired, and molecular phylogenetic studies reject them as monophyletic (Tan and Armbruster, 2018).

The loaches of the genus *Turcinoemacheilus* Bănărescu et Nalbant, 1964 are very widespread in the Middle East, and distinguish from other nemacheilids by having the pelvic fin origin situated in front of the dorsal fin origin, an anterior position of the anus, a particular colour pattern, and a very slender body (Bănărescu and Nalbant, 1964; Breil and Bohlen, 2001; Freyhof et al., 2011; Golzarianpour et al., 2013; Esmaili et al., 2014; Çiçek et al., 2020). In total, six species of this genus have been described (Esmaili et al., 2014; Nikmehr et al., 2020); the pictures of all Iranian species are presented in Nikmehr et al. (2020) with the provision of additional morphological distinctive features of *T. kosswigi*, the type species of the genus.

In the phylogenetic tree based on the mitochondrial COI barcode region (Esmaili et al., 2014), four studied *Turcinoemacheilus* species represented two subclades, the first included *T. hafezi* Golzarianpour, Abdoli, Patimar et Freyhof, 2013, and *T. bahaii*

Esmacili, Sayyadzadeh, Özulug, Geiger et Freyhof, 2014, and the second consisted of *T. saadii* Esmacili, Sayyadzadeh, Özulug, Geiger et Freyhof, 2014, *T. koss-wigi* Bănărescu et Nalbant, 1964 and *T. minimus* Esmacili, Sayyadzadeh, Özulug, Geiger et Freyhof, 2014. In addition, Nikmehr et al. (2019, 2020) recently presented the phylogenetic studies of the genus *Turcinoemacheilus*, based on the osteological characteristics, according to which their reconstructed tree is consistent with that of Esmacili et al. (2014).

Poor modern awareness of the phylogenetic relationships of different genera of Nemacheilidae stimulates their further research by different methods. Therefore, this study aimed to provide the first detailed osteological description of the species *Turcinoemacheilus saadii*, with particular attention to the characters used in the phylogeny of this genus, as well as in other Cobitoidei taxa, and to try to assess their significance for the grouping of species and genera.

MATERIALS AND METHODS

Ten specimens of *T. saadii* (30.4–47.2 mm standard length, *SL*) were collected by electrofishing device from the Gamasiab River, Tigris River drainage (Kermanshah Province, western Iran). After capture, the specimens were anesthetized in 1% clove oil solution and fixed in 10% buffered formaldehyde. For osteological examination, the specimens were cleared and stained using alcian blue and alizarin red based on Taylor and Van Dyke (1985). Images of the stained skeletal structure were obtained by a scanner (Epson V600) equipped with a glycerin bath.

The skeletal structures were observed and examined under a stereomicroscope (Leica MS5). Drawings of the skeletal elements were made in CorelDraw X6 software. The nomenclature of the skeletal elements was taken from Sawada (1982) and Conway (2011).

RESULTS

Osteological Description of Turcinoemacheilus saadii

Neurocranium (Fig. 1). The neurocranium is narrow anteriorly, wide and flat posteriorly, widest at the otic region. The ethmoid region is composed of the paired lateral ethmoid and unpaired prevomer, and supraethmoid-ethmoid complex (Fig. 1a). The supraethmoid-ethmoid complex is ventrally fused with the prevomer and firmly connects to the frontals by a zig-zag suture posteriorly. The paired lateral ethmoids are L-shaped, their anterior part is not completely ossified. These bones are firmly attached to the anterolateral part of the orbitosphenoid, unmovable, and laterally extend forward to form a projection (Fig. 1c). The prevomer is a flat, paddle-shaped median bone that forms the anterior part of the mouth roof; posteriorly

it connects to the orbitosphenoid and parasphenoid. Preethmoid-I is absent.

The orbital region includes the paired frontal bones, orbitosphenoids, pterosphenoids, unpaired parasphenoid, and sclerotic bones. The frontals are the largest bones of the skull roof, flattened posteriorly and narrowed anteriorly (Fig. 1a). They connect to the orbitosphenoid, pterosphenoid, and sphenotic laterally, and to parietals posteriorly, where the frontals are separated by the anterior part of the fontanelle. The orbitosphenoid connects to the parasphenoid ventrally and pterosphenoid posterodorsally (Fig. 1b). The pterosphenoid connects to the frontal and sphenotic bones dorsally and posterolaterally, respectively; the posterior margin of the pterosphenoid is curved, forming an opening, bordered also by the prootic and parasphenoid bones (Fig. 1c). The parasphenoid is the longest bone at the base of the neurocranium, extending from the prevomer to the basioccipital bone; it is wider in the middle and bifurcated at both ends.

The otic region comprises of the parietal, sphenotic, pterotic, prootic, epiotic, posttemporal, and supratemporal bones (Figs. 1a, 1c). The parietal is square in shape and connects to the frontal anteriorly, to the supraoccipital and epiotic posteriorly, and to the pterotic and sphenotic laterally. The left and right parietals are separated by the fontanelle (Fig. 1a). The pterotic is the most lateral bone at the posterior part of the neurocranium. This bone is quarter-circle in shape and connects to the epiotic and sphenotic posteriorly, and to the prootic and exoccipital ventrally. The sphenotic is located at the rear of the orbit, forming the lateral wall of the skull (Fig. 1b); it connects to the pterotic ventrally and to the parietal posterodorsally. The prootics are the largest bones of the skull base and connect to each other lateroposteriorly; there is a foramen in the anterolateral part of the prootic (Fig. 1c). The epiotic is the most posterior element of the otic region (Fig. 1c). The posttemporal is a thin and long bone that connects ventrally to the upper part of the supraclithrum and dorsally articulates with the skull through the upper process. The supratemporal is a small bone located in the anterior part of the posttemporal.

The occipital region comprises of the exoccipital, supraoccipital, and basioccipital bones. The unpaired pentagonal supraoccipital bone connects to the exoccipital dorsally and forms the posterior margin of the fontanelle anteriorly. The exoccipital bones bear two foramens exoccipitalis in their middle and ventral parts. The unpaired basioccipital is located between exoccipitals and connects to the prootic anteriorly; posteriorly, it ends with a ring-shaped basioccipital process (Fig. 1b). The neurocranium has two facets for the articulation with the heads of the hyomandibular. The anterior facet is formed by the pterosphenoid,

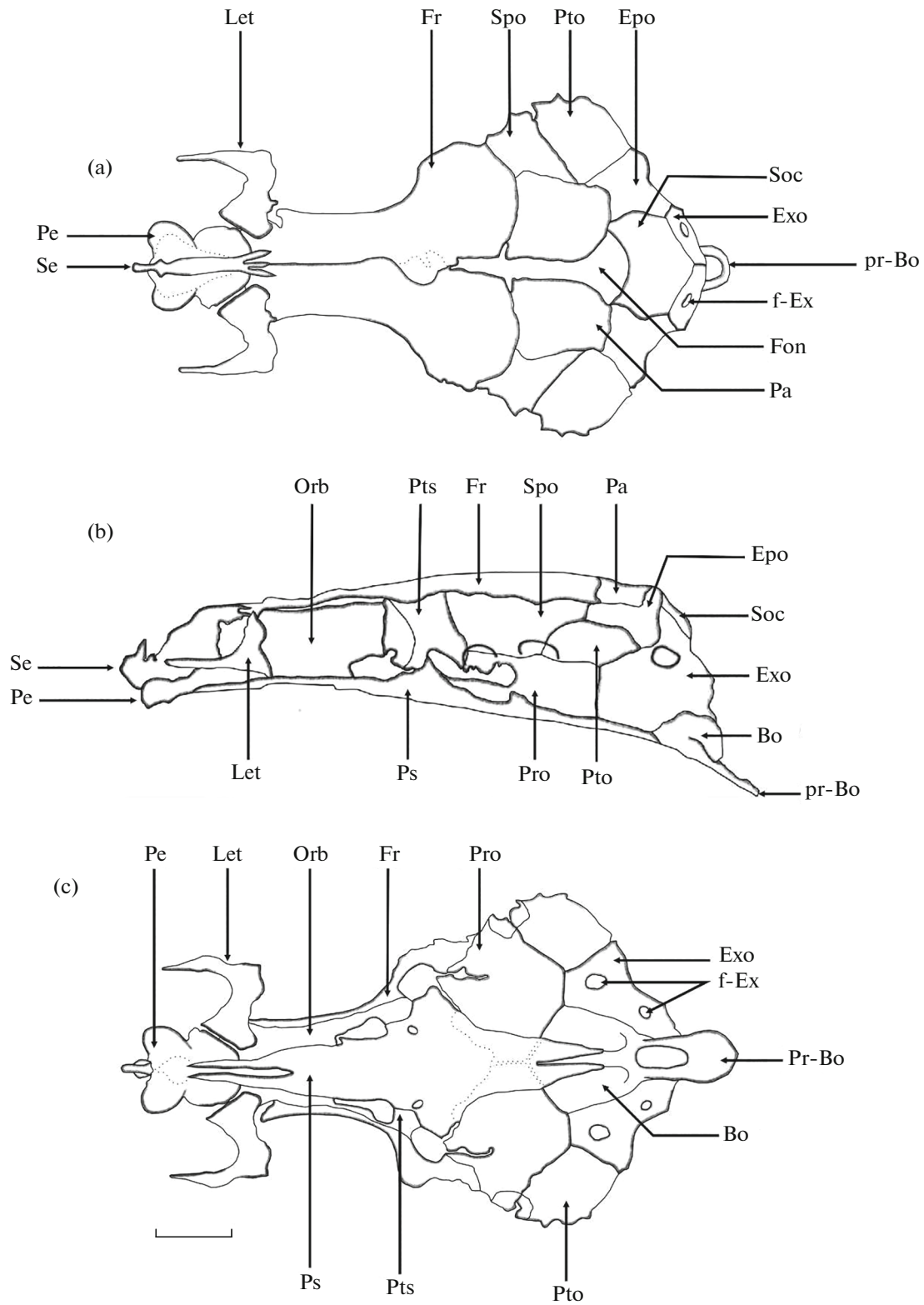


Fig. 1. Neurocranium of *Turcinoemacheilus saadii* in dorsal (a), lateral (left side) (b), and ventral (c) view. Bo—basioccipital, Epo—epiotic, Exo—exoccipital, f-Ex—foramen exoccipital, Fon—fontanelle, Fr—frontal, Let—lateral ethmoid, Orb—orbitosphenoid, Pa—parietal, Pe—prevomer, pr-Bo—basioccipital process, Pro—prootic, Ps—parasphenoid, Pto—pterotic, Pts—pterosphenoid, Se—supraethmoid-ethmoid, Soc—supraoccipital, Spo—sphenotic. Scale here and in Figs. 2, 4, 5: 1 mm.

sphenotic, and prootic, and the posterior one by the pterotic and sphenotic (Fig. 1b).

Jaws (Fig. 2). The mandibular arch consists of the premaxillary, maxillary, dentary, anguloarticular, coronomeckelian, retroarticular, kinethmoid, prepalatine, the second preethmoid and sesamoid bone. The upper jaw includes the maxillary and premaxillary (Fig. 2d). The premaxillary is a thin and narrow L-shaped bone; its large anterior ascending process distally connects to the dorsal end of the unpaired kinethmoid by a ligament. The horizontal part of the premaxillary is arcuate and slightly wider in the middle, while the ascending process is narrower and longer and has a small triangular process in the middle; anterior process (=processus dentiformis) is not developed (Fig. 2d). The maxillary is an irregularly shaped, thin bone articulated with the prepalatine and preethmoid-II (Figs. 2a, 2c, 2d). The preethmoid-II is short, rod-shaped, and connects to the prepalatine bone with its lateral outer part; posteriorly, this bone is connected to the anterior edge of the prevomer, and anteriorly to the maxillary; the paired small and round sesamoid bone is located between labial ligament, kinethmoid and maxillary. The lower jaw consists of the dentary, retroarticular, anguloarticular, and coronomeckelian bones (Fig. 2e). Dentary is the largest bone of the lower jaw, consisting of a narrow ramus dentalis and a wide coronoid process. This bone connects to the anguloarticular and retroarticular bones posterodorsally and dorsally, respectively. The anguloarticular connects to the dentary, retroarticular, and quadrate bones. A small subtriangular coronomeckelian is present medial to the lower jaw, locating in the dorsomedial part of the anguloarticular.

Suspensorium. The suspensorium includes the autopalatine, endopterygoid, ectopterygoid, metapterygoid, hyomandibular, quadrate, and symplectic bones (Fig. 2f). The autopalatine possesses a pointed ventral process and connects to the prevomer laterally, and prepalatine, preethmoid-II and endopterygoid anteriorly and posteriorly. Endopterygoid is an elongated rectangular bone that connects to the metapterygoid and ectopterygoid ventrally (Fig. 2f), and anteriorly to the autopalatine via a condyle. The metapterygoid is positioned between the hyomandibular and quadrate; its anterior part is wider; the mid-dorsal margin is slightly curved. The ectopterygoid is located between the endopterygoid and quadrate and has a pointed process in the anteroventral part. The hyomandibular is a wide rectangular bone connecting to the interhyal and symplectic ventrally, and metapterygoid anteriorly. Dorsally, the hyomandibular possesses two condyles for articulation with neurocranium. The quadrate is a flat triangular bone which posteroventrally forms a long taper process and anteriorly a condyle for the movable articulation with the

anguloarticular; dorsally and posteriorly, this bone connects to the endopterygoid and metapterygoid. The symplectic is a narrow and stretched bone located under the metapterygoid and the posterior margin of the quadrate bone (Fig. 2f).

Opercle series. The opercle is the largest bone in the opercle series; anteriorly it has a rod-shaped process articulating with the posterodorsal condyle of the hyomandibular. The subopercle is a thin blade-like bone lying ventromedial to the opercle. The interopercle is a thin blade-like bone lying ventromedial to the preopercle and the posterior process of the quadrate. The suprapreopercle bones are absent.

Branchial apparatus. The branchial apparatus consists of the basibranchial, hypobranchial, ceratobranchial, epibranchial, and infrapharyngobranchial bones (Fig. 3a). The number of unpaired basibranchials is four, the last of which is very small. Five pairs of the ceratobranchials are the largest bones of this series, the fifth of them is modified (= lower pharyngeal) and bears pharyngeal teeth. Three paired hypobranchial bones are located between the ceratobranchials and basibranchials. There are four pairs of the epibranchials and two pairs of the infrapharyngobranchials.

Hyoid arch. The hyoid arch includes the unpaired urohyal and basihyal bones, paired dorsal and ventral hypohyal, ceratohyal, epihyal, and interhyal bones, as well as three pairs of the branchiostegals (Fig. 3b). The unpaired urohyal is lamellar laterally, but widened with a developed process from above view. There are two small parurohyals between the basihyal and urohyal bones. The basihyal is a T-shaped bone with a wider anterior part; the ceratohyal is the largest bone of the hyoid arch, which connects anteriorly to the ventral and dorsal hypohyals, and posteriorly to the epihyal. The epihyal is a flat triangular bone connected to the proximal end of the interhyal posterodorsally. The interhyal is a small cylindrical bone, dorsally connected to the hyomandibular and symplectic. There are three pairs of the branchiostegal bones; the first one connects to the ventral margin of the ceratohyal, the second one is connected to the ceratohyal and epihyal, and the third branchiostegal is articulated with the middle of the epihyal. The branchiostegal bones are extended to the dorsal margin of the subopercle (Fig. 3b).

Vertebral column. The total number of vertebrae is 36–37; the centra are cylindrical; the neural and haemal processes are not bifurcated. The four anterior centra form the Weberian apparatus and the bony swim-bladder capsule.

Weberian apparatus and swim-bladder capsule. The Weberian apparatus consists of four Weberian ossicles (claustrum, scaphium, intercalarium, and tripus) and the first to fourth vertebral elements (Fig. 4). Claus-

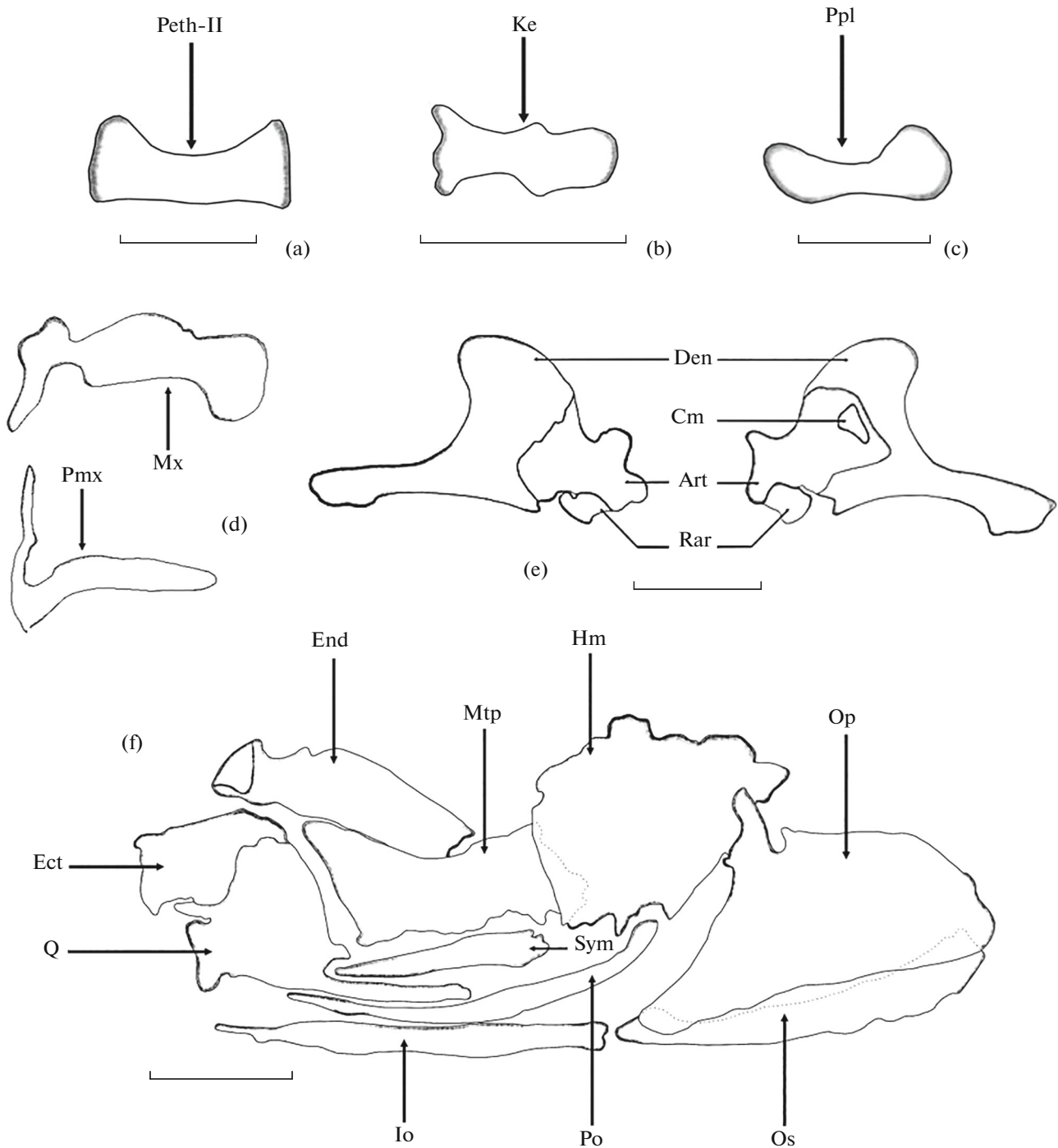


Fig. 2. Bones from mandibular arch—preethmoid (a), kinethmoid (b), prepalatine (c), upper jaw (d) and low jaw (e) and lateral view of suspensorium and opercle series (f) of *Turcinoemacheilus saadii*. Art—anguloarticular, Cm—coronomeckelian, Den—dentary, Ect—ectopterygoid, End—endopterygoid, Hm—hyomandibulare, Io—interopercle, Ke—kinethmoid, Mx—maxillary, Mtp—metapterygoid, Op—opercle, Peth-II—preethmoid-II, Pmx—premaxillary, Po—preopercle, Ppl—prepalatine, Q—quadrate, Rar—retroarticular, So—subopercle, Sym—symplectic. Scale: (a–c)—0.5, (d–f)—1 mm.

trum is a round bone that meets the mid-side of the scaphium and connects to the supraneural-2 dorsally. Intercalarium is a small bone lying between the scaphium and the tripus. The first vertebral centrum is

not involved in the formation of the bony capsule; its short lateral processes are connected to the upper end of the cleithrum. The second centrum is fused with the third centrum, both of them, as well as the fourth cen-

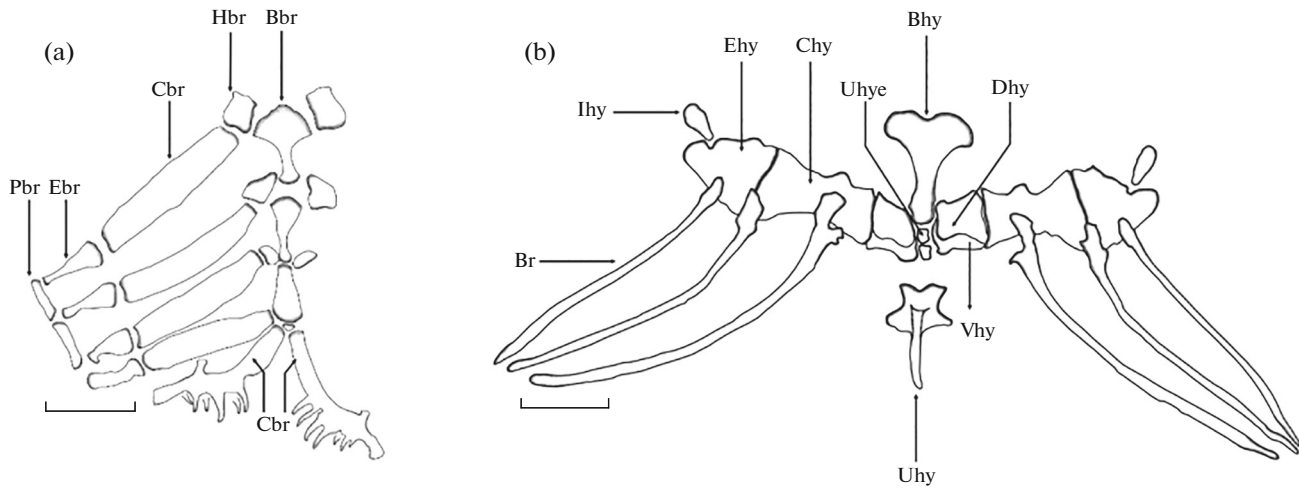


Fig. 3. Branchial apparatus (a) and hyoid arch (b) (dorsal view) of *Turcinoemacheilus saadii*. Bbr—basibranchials 2–5, Bhy—basihyal, Br—branchiostegals 1–3, Cbr—ceratobranchials 1–5, Chy—ceratohyal; Dhy, Vhy—dorsal and ventral hypohyals; Ebr—epibranchials 1–4, Ehy—epihyal, Hbr—hypobranchials 1–3, Ihy—interhyal, Pbr—infrapharyngobranchial, Uhy—urohyal, Uhye—parurohyal.

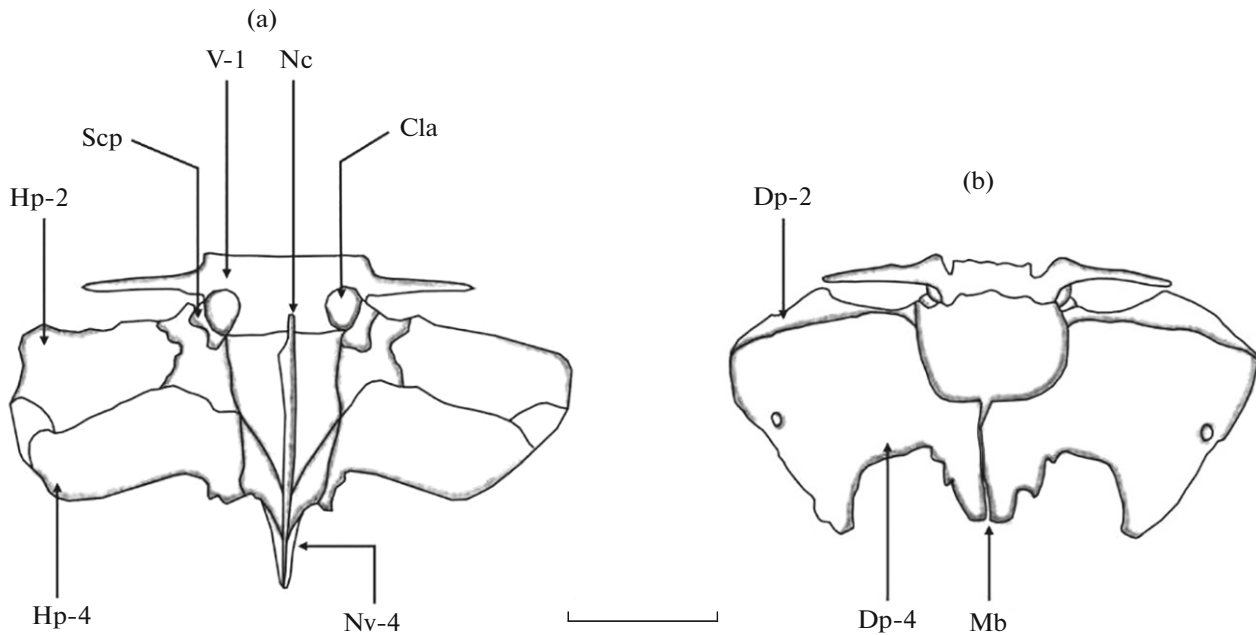


Fig. 4. Bony capsule of the swim-bladder in *Turcinoemacheilus saadii* in dorsal (a) and ventral (b) view. Cla—claustrum; Dp-2, Dp-4—descending processes of the second and fourth centra; Hp-2, Hp-4—horizontal processes of the second and fourth centra; Mb—manubrium, Nc—neural complex, Nv-4—neural process of the fourth centrum, Scp—scaphium, V-1—the first centrum.

trum, are involved in the formation of the bony capsule. The neural processes of the second and third centra merge into an expanded plate (complex neuralis). The parapophysis of the fourth centrum is modified and has similar processes as the second centrum, which is included in the structure of the posterior part of the bony capsule. The bony capsule is almost trapezoidal, with wing-like posterolateral processes; when

viewed from the lateral side, two openings are visible, the posterior one is larger than the inner one; the surface of the capsule is non-alveolar, and its right and left lobes are separated by the manubrium (Fig. 4).

Pectoral girdle. The pectoral girdle includes the cleithrum, supracleithrum, coracoid, mesocoracoid, scapula, and radials; the postcleithrum is absent (Fig. 5a). The cleithrum is the largest bone of the girdle; the

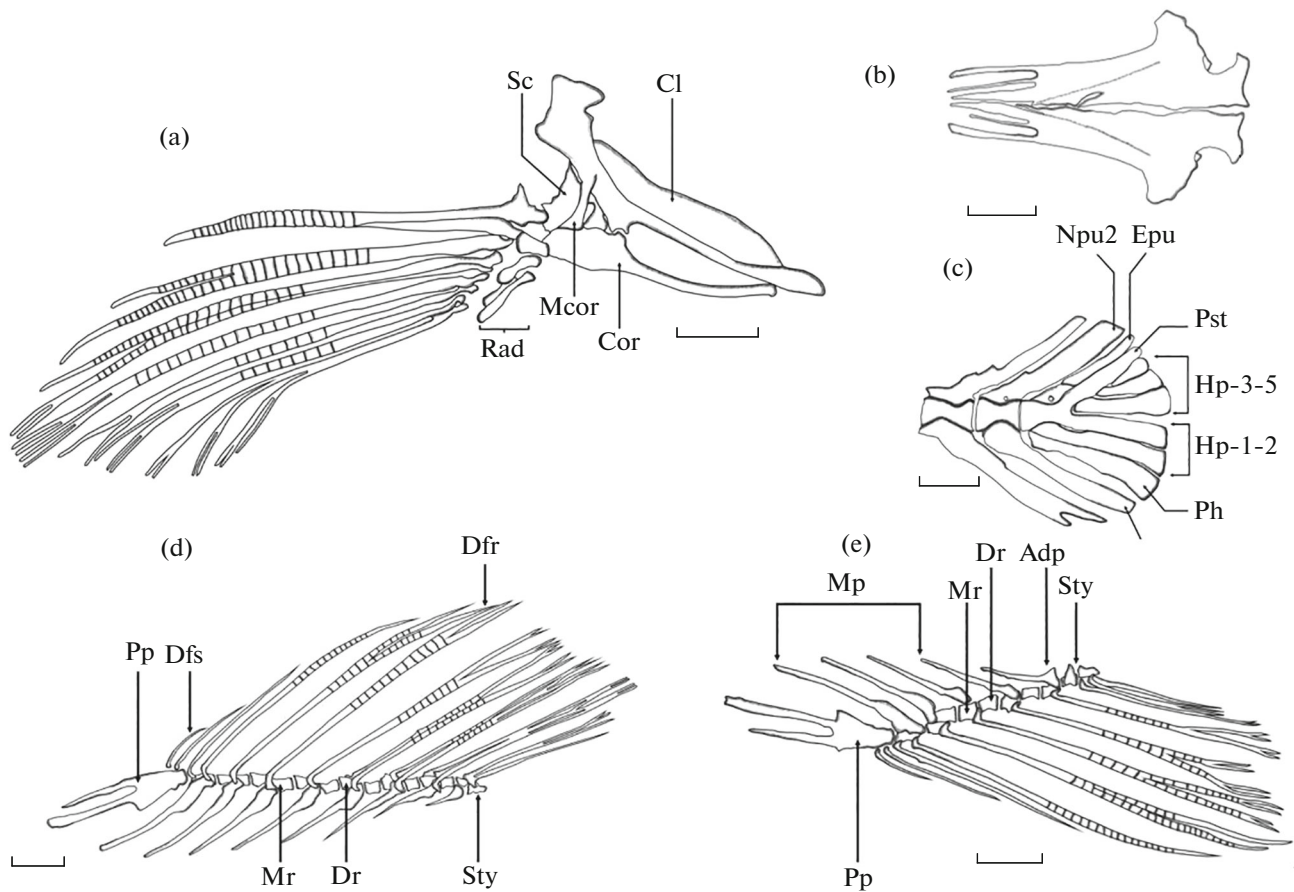


Fig. 5. Pectoral girdle (a), pelvic girdle (b), caudal skeleton (c), and skeletal elements of dorsal (d), and anal (e) fins of *Turcinoemacheilus saadii*. Adp—anal distal pterygiophore, Cl—cleithrum, Cor—coracoid, Dfr—dorsal fin rays, Dfs—dorsal fin spinous rays, Dr—distal radial, Epu—epural, Hp 1–2 and 3–5—hypurals, Hpu2—hemal process of the second preural centrum, Mcor—mesocoracoid, Mp—medial pterygiophore, Mr—medial radial, Npu2—neural process of the second preural centrum, Ph—parhypural, Pp—the first pterygiophore, Pst—pleurostylar, Rad—ossified pectoral radials, Sc—scapula, Sty—stay.

supracleithrum is triangular and flattened in shape, connected to the dorsoventral part of the cleithrum. The anterior part of the coracoid is narrow sloping downward, while the posterior part is wide; lateromedially, it connects to the cleithrum through the mesocoracoid. The scapula is located at the rear of the cleithrum and dorsal to the mesocoracoid, and has a large opening in the middle and a depression in the posterior part for the connection with the first unbranched pectoral fin ray. There are four cylindrical radials supporting pectoral fin rays; two first radials are wider than the rest (Fig. 5a).

Pelvic girdle. The pelvic girdle consists of the paired pelvic bones and radials (Fig. 5b). The anterior part of the pelvic bones is narrower than the posterior part. These bones have two anterior processes (external and internal), and posterior ischiac process (Fig. 5b). Posterior to each pelvic bone, there are three small and rounded radials. The pelvic fin consists of the pelvic splint, and one unbranched and seven branched rays.

The pelvic splint is a curved unsegmented spine-like bone running along the outer surface of the outermost ray.

Dorsal fin skeleton. The dorsal fin includes 8 pterygiophores, 4 unbranched and 7½ branched rays (Fig. 5d). The first proximal elongated and bipartite pterygiophore is located in front of the nineteenth centrum.

Anal fin skeleton. The anal fin includes 6 pterygiophores, 4 unbranched and 5½ branched anal rays (Fig. 5e). The first pterygiophore is inserted in front of the twenty-third centrum.

Caudal skeleton. Caudal complex includes the following elements: the first preural centrum, second preural centrum, pleurostylar, neural spine and arch, last haemal spine and arch, hypurals, parhypural, uro-neural, epural, principal caudal rays and procurrent rays. The first preural centrum is the last vertebra bearing the pleurostylar posterodorsally (Fig. 5c). There are five hypurals and the proximal end of the hypural-1 connects to the urostyle; ventrally hypural-1 is connected to the parhypural. The parhypural is

relatively wide; its anterior end connects to the urostyle. The epural connects to the rudimentary neural arch of the first preural centrum. The caudal fin has 18 principal rays, the number of the dorsal procurrent rays is five, and the number of ventral rays is six.

DISCUSSION

The present study offers a detailed description of the skeleton of *T. saadii* from the Gamasiab River, with particular attention to the characters used in phylogenetic analysis of fishes of the suborder Cobitoidei (Ramaswami, 1953; Sawada, 1982; Prokofiev, 2004, 2009, 2010; Conway, 2011; Mabee et al., 2011). The obtained results confirm that the osteological features of this species do not contradict the conditions described for the family Nemacheilidae by Sawada (1982, accepted as a subfamily) as a monophyletic group, namely, the presence of the prepalatine bone and the absence of the deep subtemporal fossa formed by the prootic.

As mentioned above, the phylogenetic classification of the genera of nemacheilids, first developed by Prokofiev (2009, 2010) on the basis of the selected sets of morphological characters, contradicts the relationships of the species revealed by molecular analyses (Tang et al., 2006; Šlechtová et al., 2007; Liu et al., 2012; Tan and Armbruster, 2018). The genus *Turcinoemacheilus* was confirmed as monophyletic in a phylogenetic clade that included the genus *Paracobitis* (Esmaeili et al., 2014; Freyhof et al., 2016). Nikmehr et al. (2016, 2019, 2020) provided a detailed osteological description for *Turcinoemacheilus hafezi* and the relevant characters of *T. kosswigi*, *T. saadii*, and *T. bahaii* for their phylogenetic studies. Based on these data and our observations, *T. saadii* is distinguished by autopalatine bones symmetrical in the posterior part (vs. asymmetrical ones in *T. kosswigi*) and the first pterygiophore of the dorsal fin located in front of the 19th centrum (vs. 17th in *T. bahaii*, and 18th in *T. kosswigi* and *T. hafezi*). In addition, the epural bone in *T. saadii* is articulated with the rudimentary neural arch of the first preural centrum, in contrast to *T. hafezi* with a long epural bone not articulated with the neural arch; while in *T. kosswigi* the epural fuses with the urostyle. *T. saadii* also has the longest lateral projection of the lateral ethmoid bone, while *T. hafezi* has the lowest dentary at the level of coronoid process; the basihyal bone in *T. saadii* has a small notch at the anterior end, in contrast to the deep notch in *T. hafezi*. These differences must be confirmed by special studies of intraspecific variation, observed in representatives of the genus for some osteological features (Esmaeili et al., 2014; Azimi, 2014; Nikmehr et al., 2016, 2019, 2020). In any case, the comparative interspecific analysis

shows the absence of deep osteological divergence between the species of the genus *Turcinoemacheilus*.

A close phylogenetic relationship between the genera *Turcinoemacheilus* and *Paraschistura* has been demonstrated in several molecular analyzes (Freyhof et al., 2015; Ghanbarifardi and Yazdani-Moghaddam, 2017). The comparative analysis of osteological characters described in *T. saadii* and other members of *Turcinoemacheilus* (Nikmehr et al., 2016, 2019, 2020), with the characteristics presented by Prokofiev (2009) as phylogenetically important for the genus *Paraschistura* Prokofiev, 2009, but later studied in species *P. cristata* (Berg, 1898), *P. alta* (Nalbant et Bianco, 1998) and *P. nielsenii* (Nalbant et Bianco, 1998) (Azimi et al., 2015a, 2015b; Noroozei et al., 2019), testifies about the absence of osteological features separating these two genera. For example, the sesamoid bone lying above the preethmoid II in *Turcinoemacheilus* species have not previously been found in *Paraschistura*. (In this study, we accept *Metaschistura* Prokofiev, 2009 as a synonym of *Paraschistura* according to Freyhof et al., 2015). Sawada (1982) noted sesamoid ossifications only for studied fishes of Botiinae, however, Prokofiev observed them in *Dzihunia* Prokofiev, 2001, most of the species of *Paracobitis* Bleeker, 1863, and some specimens of loaches of the genus *Oxyneomacheilus* Bănărescu et Nalbant, 1966, and thus concluded that “phylogenetic significance of the character, most likely, is not large” (Prokofiev, 2010. P. 885). Later, these ossifications were also observed in *O. kiabii* Goltzarianpour, Abdoli et Freyhof, 2011 (Mafakheri et al., 2014), were not found in *Paracobitis hircanica* Mousavi-Sabet, Sayyadzadeh, Esmaeili, Eagderi, Patimar et Freyhof, 2015 (Azimi et al., 2015c), but were described in the three mentioned *Paraschistura* species found in Iran (Azimi et al., 2015a, 2015b; Noroozei et al., 2019).

Like most genera of the family Nemacheilidae, united by Prokofiev (2010) into the tribe Nemacheilini, *T. saadii* lacks preethmoid-I, as well as other studied *Turcinoemacheilus* (Nikmehr et al., 2016, 2019, 2020). It should be noted that the parurohyal that observed in *T. saadii* were not recorded in Nemacheilinae by Prokofiev (2009, 2010), but these bones as extra urohials were observed in *O. kiabii* and *O. bergianus* (Derjavin, 1934), *O. kermanshahensis* (Bănărescu et Nalbant, 1966), *O. chomanicus* Kamangar, Prokofiev, Ghaderi et Nalbant, 2014, *O. persa* (Heckel, 1847), *Paracobitis hircanica*, *Paraschistura cristata*, *P. alta* and *Turcinoemacheilus* species (Mafakheri et al., 2014, 2015a, 2015b, 2016; Azimi et al., 2015a, 2015b, 2015c; Jalili and Eagderi, 2015; Nikmehr et al., 2016, 2019, 2020). In addition, according to Prokofiev (2010. P. 866), the anterior articulating facet of the neurocranium in Nemacheilin fish “is restricted by

the sphenotic and prootic, and the posterior facet is restricted by the sphenotic, prootic, and pterotic". Whereas in *T. saadii*, *P. hircanica*, *P. cristata*, *P. nielsenii*, *O. kiabii*, *O. bergianus*, *O. kermanshahensis*, *O. chomanicus*, and *O. persa* the anterior facet is formed by the pterosphenoid, sphenotic and prootic, and the posterior one is formed by the sphenotic and pterotic (Mafakheri et al., 2014, 2015a, 2015b, 2016; Azimi et al., 2015a, 2015b, 2015c; Jalili and Eagderi, 2015).

In the scheme of "relationships within the subfamily Nemacheilinae" (adopted as a family in this study) proposed by Prokofiev (2010, Fig. 25), the genus *Oxy-noemacheilus* belongs to the group of genera that demonstrate the least relationship with the "clade" uniting *Parashistura*, *Metaschistura*, *Paracobitis*, and some other genera. (It should be mentioned again, that the phylogenetic position of all these genera within the family Nemacheilidae has not yet been studied.) Nevertheless, the investigated *Oxy-noemacheilus* species (Mafakheri et al., 2015a, 2015b, 2016; Jalili and Eagderi, 2015) do not show any diagnostic osteological features that distinguish them as a separate group from *Parashistura* and *Paracobitis* (Prokofiev, 2009; Azimi, 2014; Azimi et al., 2015a, 2015b, 2015c). Our study also demonstrates a high osteological similarity between *T. saadii* and the previously described *Oxy-noemacheilus* species. In addition to the features mentioned by Prokofiev (2009, 2010), this similarity is observed in the presence of the fused supraethmoid-ethmoid and prevomer bones, a small coronomeckelian located in the dorsomedial part of the anguloarticular (according to Prokofiev, 2010, in Nemacheilidae it is attached to the base and dorsal edge of the coronoid process of the dentary), and the presence of four unpaired basibranchials (Jalili and Eagderi, 2015; Mafakheri et al., 2015a, 2015b, 2016). However, in *Turcinoemacheilus hafezi*, the coronomeckelian is located on the medial side of the dentary under the process of coronoides (Nikmehr et al., 2016).

In general, a comparative analysis of reported osteological characters in studied Middle East nemacheilid species shows the absence of explicit diagnostic skeletal features for previously proposed (Prokofiev, 2010) tribes or genera. However, some osteological characteristics are of diagnostic value at the species level, for example, the numbers of basibranchial and hypural bones, vertebrates, pterygiophores, unbranched and branched fin rays, the shape and position of some bones and their connections. In some cases, the differences between congeneric species look so deep that the authors propose to separate the studied species into an independent genus, for example, in the situation with *Paraschistura cristata* (Azimi et al., 2015b).

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflict of interest.

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