Osteological characteristics of *Paraschistura nielseni* (Nalbant & Bianco, 1998) (Cypriniformes: Nemacheilidae)

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Abstract: *Paraschistura* Prokofiev, 2009 is a newly described genus based on osteological characters, but detailed information for all species of the genus is not available. To describe osteological characteristics of the endemic species of *Paraschistura nielseni*, twelve specimens were collected from the Shapur River of Persis basin which drains to the Persian Gulf and their osteological characteristics were examined. According to the results, *P. nielseni* is characterised by bearing a square-shaped prevomer, four basibranchials, a semi ossified sesamoid, a non-alveolar bony swim bladder capsule, and separated pelvic bone bases. Based on these features, *P. nielseni* could be distinguished from other loaches species.

Keywords: Osteology, Fish skeleton, Neurocranium, Loach, Persis basin.

Introduction

The members of the family Nemacheilidae, with about 72 genera and 651 species, are found across Eurasia with one species in northeast Africa (Nelson 2006; Eschmeyer & Fong 2015; Mafakheri et al. 2015). This family has a great diversity in Iranian interior waters (Coad 2015). Classification of these taxa are complex and researches are trying to determine their phylogenetic status (Prokofiev 2010; Mafakheri et al. 2015). Recent classifications of members of the family Nemacheilidae have been merely based on external morphology and to a small extent on anatomy, osteology and molecular data (Bănărescu & Nalbant 1995; Freyhof et al. 2015).

The first osteological work on this group of fishes was performed by Regan (1911), who separated the subfamily Nemacheilinae from Cobitidae. The only comprehensive phylogenetic study based on the osteology of nemacheilid fishes was carried out by Prokofiev (2010). Among the members of nemacheilids, *Paraschistura* Prokofiev, 2009 is a newly described genus, and therefore, not all of its species have been fully examined and ascribed to it or related genera (Coad 2015). Recently Vatandoust and Eagderi (2015) described *P. ilamensis* from Tigris River drainage as first species of the genus of *Paraschistura* from Iranian part of this basin. Freyhof et al. (2015) reviewed the genus *Paraschistura* from Iran and described six new species, including *P. abdolii* (from the Sirjan basin and the western tributaries of the Hamun-e Jaz Murian basin), *P. aredvi* (from the Zohreh drainage), *P. hormuzensis* (from the Minab drainage), *P. naumanni* (from the Kol and Mond drainages and Lake Maharlo basin), *P. pasatigris* (from the Karun and Karkheh drainages) and *P. susiani* (from the Jarahi drainage) based on the morphological and molecular (the mtDNA COI barcode region) data set.

Freyhof et al. (2015) provided the diagnostic characters for all eleven recognized species including *Paraschistura nielseni* and treated *Metaschistura* Prokofiev, 2009 as a synonym of *Paraschistura* Prokofiev, 2009.

Many members of this genus were previously described in the genus *Schistura* (McClelland 1838). Species of the genus *Paraschistura* are small and
Paraschistura nielseni (Nalbant & Bianco 1998) (Fig. 1) is endemic to Iranian inland waters commonly called sagmahi-ye Nielseni (Coad 2015). In 1966, this species was described by Bărănescu and Nalbant as Nemacheilus bampurensis (Coad 2015; Freyhof et al. 2015). Paraschistura nielseni is known from the Helleh and Mond River drainages (Persis basin) which drain to the northern Persian Gulf (Freyhof et al. 2015). Due to difficulties in using external morphology of the members of this genus for studying their taxonomy and limited information on these species, the present study was conducted to provide a detailed description of osteological characteristic of P. nielseni. Since, osteological features are important in the taxonomy of the family Nemacheilidae; the results of this study can be used as a reference to more accurately compare and distinguish different species of the genus.

Materials and Methods
Twelve specimens of P. nielseni (38-65 mm in standard length) were collected by electrofishing from the Shapur River (Helleh drainage, Persis basin, Fars Province, Iran) (51°33′06″E, 29°45′44″N). The collected specimens were anesthetized in 1% clove solution and then fixed in 5% buffered formalin. For osteological examination, specimens were cleared and stained using alcin blue and alizarin red based on Taylor & Van Dyke (1985). The skeletal structures were dissected and photographed using a scanner (Epson v600) equipped with a glycerol bath. The skeletal structure of cleared and stained specimens were observed and studied by an MS5Leica stereomicroscope. The skeletal elements were drawn based on digital pictures using CorelDrawX6 software. Nomenclature and abbreviation of the skeletal elements were based on Prokofiev (2009).

Results
Neurocranium: The anterior part of the neurocranium is narrower and its posterior part is approximately oval-shaped. The maximum width of the skull is formed at the level of the pterotic. The ethmoid region comprises of the paired lateral ethmoid and, unpaired prevomer and supraethmoid-ethmoid (Fig. 2a). The supraethmoid-ethmoid is a stretched bone that is vertically fused to the prevomer and firmly connected to the frontal by a zigzag gap posteriorly. In the anterior part of the neurocranium, the paired L-shaped lateral ethmoids are present. The lateral ethmoid possesses a well-developed anterior process and also possesses a rod-like processes posteriorly. The prevomer is almost square-shaped and connected to the orbitosphenoid and parasphenoid posteriorly (Fig. 2b).

Several small and free bones are connected to the
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ethmoid part of the neurocranium, including the unpaired kinethmoid, paired preethmoids-II and prepalatines (Fig. 3). The preethmoid-II is rod-like connecting to the prepalatine laterally (Fig. 3a). Also, this bone is connected to the anterior edge of the prevomer posteriorly and to the maxilla anteriorly.
The kinethmoid is a small and free bone that it is positioned between the two maxilla bones (Fig. 3b). The prepalatine is a small bone connecting to the preethmoid-II dorsally and maxilla anteriorly (Fig. 3c). The paired sesamoids are semi-ossified in the ethmoid region.

The orbital region is composed of the frontal, orbitosphenoid, pterosphenoid, parasphenoid, and sclerotic bones. The paired frontals are the largest bones of the skull roof and connected to the orbitosphenoid, pterosphenoid and sphenotic laterally, and parietal posteriorly (Figs. 2a‒2b). These bones include about half of the length of the neurocranium which are separated by the fontanel posteriorly. The orbitosphenoid is connected to the parasphenoid ventrally and pterosphenoid posterodorsally (Fig. 2b). The pterosphenoid is connected to the frontal dorsally and sphenoid posterolaterally. The posterior margin of the pterosphenoid is curved, creating a cavity with the prootic and parasphenoid (Fig. 2c). The parasphenoid is the longest bone in the base of the neurocranium, and extended from the prevomer to the basioccipital. This bone is wider in the middle part and bifurcated at the two ends (Fig. 2c).

The otic region comprises the parietal, sphenotic, pterotic, prootic and epiotic (Fig. 2). The posterior margin of the parietal is connected to the supraoccipital and epiotic, and its lateral margin is connected to the pterotic and sphenotic. Also, these paired bones are anteriorly connected to the frontal and separated from each other by the fontanel (Fig. 2a). The pterotic is quarter-circle in shape and connected to the epiotic and sphenotic posteriorly and to the prootic and exoccipital ventrally. The sphenotic forms part of the lateral wall of the skull and connected to the pterotic ventrally and to the parietal postero-dorsally (Fig. 2b). The prootic is the largest bone of the skull base. Its anterior part contacts the parasphenoid, its upper edge contacts the sphenotic and its posterior part contacts the pterotic, exoccipital and basioccipital (Fig. 2c). There is a foramen in the anterolateral part of the prootic (Fig. 2c). The epiotic is the posterior most element of the otic region, and positioned between the occipital region and pterotic (Fig. 2c).

The occipital region comprises of the exoccipital, supraoccipital, and basioccipital. The supraoccipital is pentagon-shaped and its anterior margin is contributed in the formation of the fontanel. In addition, this bone is connected to the exoccipital dorsally and fontanel anteriorly. The exoccipital bones bear exoccipitalis foramen. The basioccipital is positioned between the two exoccipitals and connected to the prootic anteriorly (Fig. 2c). Also, this bone has a ring-like process posteriorly (process basioccipital) (Fig. 2b). The neurocranium has two facets for articulation with the heads of the hyomandibular. The anterior facet is formed by the...
pterosphenoid, sphenotic and prootic, and the posterior one by the sphenotic and pterotic. The parietal, frontal, and supraoccipital are contributed in the formation fontanel that is covered by a connective tissue and extended longitudinally (Fig. 2a).

**Jaws:** The upper jaw comprises the maxilla and praemaxilla (Fig. 4a). The premaxilla is a narrow L-shaped bone and composed of two parts, i.e. ascending and alveolar premaxilla processes. The horizontal part is arc-shaped and vertical part is narrower and longer (Fig. 4a). The maxilla is a large laminar bone and slightly twisted along its longitudinal axis. The lower jaw is composed of the dental, retroarticular, articular and coronomeckelian (Fig. 4b). The dental is the largest of this set and includes two parts i.e. the narrow ramus dentalis and wider coronoid process. This bone is connected to the articular postero-dorsally and to the retroarticular dorsally. The articular is connected to the dental anteriorly, retroarticular ventrally and quadrate posteriorly. The coronomeckelian is a small and triangular bone which is positioned in the dorso-medial part of the dental (Fig. 4b).

**Suspensorium:** The suspensorium comprises the autopalatine, endopterygoid, ectopterygoid, metapterygoid, hyomandibular, quadrate and symplectic bones (Fig. 5). The autopalatine possesses a blade-like process in the middle and it is connected to the prevomer laterally. Also, this bone is connected to the prepalatine, preethmoid-II and endopterygoid anteriorly and posteriorly, respectively. The endopterygoid is elongated and it is connected to the metapterygoid and ectopterygoid ventrally. This bone is connected to the autopalatine by a condyle anteriorly. The metapterygoid is almost rectangular in shape and positioned between the hyomandibular and quadrate. The hyomandibular is extended longitudinally and its dorsal part is wider. This bone has two developed processes in its anterior and posterior margins and connected to the interhyal and symplectic ventrally and to the metapterygoid anteriorly. There are two hyomandibular condyles in the dorsal margin of the hyomandibular for articulation to the neurocranium.

The quadrate has a pointed and stretched ventral process inclined posteriorly (Fig. 5). Also, this bone is connected to the endopterygoid dorsally and metapterygoid posteriorly. The symplectic is almost triangular in shape and situated under the endopterygoid and posterior to the quadrate. The ectopterygoid bears a pointed process anteriorly and a downward process ventrally.

**Opercular series:** The opercular series consist of the opercle, preopercle, subopercle, and interopercle (Fig. 5). The opercle is the largest element in this series and has a rod-shaped process antero-dorsally for connecting to the levator operculi muscle. The ventral margin of the opercle is connected to the subopercle. The subopercle is a stretched bone and connected to the interopercle anteriorly. The preopercle is narrow and arc-shaped, and situated on the interopercle. The interopercle is elongated and wider at its middle part.

**Branchial arches:** The branchial arch consists of the unpaired basibranchial and paired hypobranchial, ceratobranchial, epibranchial, and pharyngobranchials (Fig. 6). There are four basibranchials in this series that the fourth one is very small. Five ceratobranchials are the largest elements of the branchial arch that fifth one is modified into the pharyngeal teeth. The numbers of the epibranchial
are four; also there are two pharyngobranchials and three hypobranchials.

**Hyoid arch:** The hyoid arch is composed of the basihyal, hypohyals, ceratohyal, epihyal, interhyal, urohyal and branchiostegal rays (Fig. 7). The unpaired basihyal is T-shaped and its anterior part is wider. The hypohyals are consisted of the dorsal and ventral parts. The ceratohyal is the largest bone in the hyoid arch which is situated between the hypohyal and epihyal. The epihyal is almost triangular in shape with a pointed process posteriorly. The interhyal is a cylindrical bone connecting to the epihyal ventrally, and to the hyomandibular and symplectic dorsally. The unpaired urohyal has two ventral and dorsal parts. The dorsal part is blade-like and perpendicular to the ventral part. The branchiostegal rays are narrow and extended to the dorsal margin of the subopercle. The first and second branchiostegal rays are connected to the middle of the ceratohyal and at the junction of the ceratohyal and epihyal, respectively. Also, the third one is situated in the middle of the epihyal.

**Pectoral girdle:** The pectoral girdle includes the cleithrum, supraleithrum, coracoid, mesocoracoid, scapula, posttemporal, supratemporal and radials (Fig. 8). The small supratemporal is positioned antero-lateral to the elongated posttemporal. The supraleithrum is wide and it is connected to the cleithrum dorso-ventrally. The posttemporal is a long bone sitting between the supraleithrum and supratemporal. Also, this bone is connected to the epiotic posteriorly, through which the pectoral girdle, is connected to the neurocranium. The cleithrum is the largest element of the pectoral girdle and it is connected to the supraleithrum dorsally and to the coracoid through mesocoracoid latero-medially. The anterior part of the coracoid is narrow, while its posterior part is wide. The scapula has a large opening (scapula foramen) and it is positioned between the cleithrum and coracoid. The radials are four in number among which the third one is significantly wider than the others.

**Dorsal fin skeleton:** The dorsal fin skeleton bears 10 pterygiophores and one stay. The first pterygiophore is positioned in the front of the seventh centrum and
a stay is located at the end of the tenth pterygiophore (Fig. 9a). The numbers of unbranched and branched rays are four and 8½, respectively.

**Anal fin skeleton:** The anal fin skeleton includes seven pterygiophores and one stay. The first pterygiophore is positioned in the front of the nineteenth centrum. In addition, this fin bears three unbranched and 5½ branched rays (Fig. 9b).

**Pelvic girdle:** The pelvic girdle consists of the paired pelvic bones and radials (Fig. 9c). These bones are
enclosed by muscles. The pelvic bones have three processes consisting the anterior (pubic) process, postero-lateral (iliacus) process that rays connected on it and posterior (ischiaicicus) process. The anterior part of the pelvic bones (pubic process) is narrower (Fig. 9c). On each side of the paired pelvic bones, there are three small and rounded radials positioning between the pelvic bones and rays. Furthermore, a developed styloid bone is found lateral to the unbranched rays.

**Caudal skeleton:** The hypural-1 of the caudal skeleton is wide and connected to the centrum by a rod-shaped process and parhypural ventrally (Fig. 10). The parhypural is relatively flat and it is connected to the centrum posteriorly. The hypurals-3, 4, and 5 are positioned between the pleurostyle and hypural-2. The epural is connected to the pleurostyle, and it is situated between the pleurostyle and rudimentary neural arch.

**Weberian apparatus and swim bladder capsule:** The Weberian apparatus comprises of the claustrum, scaphium, intercalarium, and tripus (Figs. 11a-b).
shape and its ventral surface is not alveolar. Also, the right and left lobes of the bony capsule are symmetrical and divided by the manubrium (Fig. 11b).

Discussion
In the present study, the skeletal system of *P. nielsenii* was described in detail. Based on the results, the length of the occipital region in *P. nielsenii* is less than one-third of the neurocranium length similar to the majority of the loach species (Prokofiev 2010). In *P. nielsenii*, the supraethmoid-ethmoid and prevomer are fused similar to other loaches, with the exception of *Leufa* spp., *Oreonecetes platycepalus*, *Yunnanilus pleurotaenia*, *Triplophysa microphthalmal*, *T. tenuis* (Prokofiev 2010) and *Schistura fasciolata* (Sawada 1982). The connection of the lateral ethmoid to the neurocranium in *P. nielsenii* is at the level of the anterior margin of the orbitosphenoid, similar to *Oxyoemacheilus kiabii* (Mafakheri et al. 2014) whereas, *O. bergianus* (Jalili & Eagderi 2015), the anterior facet is formed by the prootic, pterotic and sphenotic. Whereas, Prokofiev (2010) noted that the anterior facet is formed by the sphenotic and pterotic, and the posterior one by the sphenotic, prootic and pterotic.

The coronomeckelian of *P. nielsenii* is situated on the dorso-medial part of the articular similar to that of *O. kiabii* (Mafakheri et al. 2014) and *O. bergianus* (Jalili & Eagderi 2015), but Prokofiev (2010) noted that the coronomeckelian is connected to the base and dorsal edge of the coronoid process in loaches.

There are four basibranchials in *P. nielsenii* similar to *O. kiabii* (Mafakheri et al. 2014) and *O. bergianus* (Jalili & Eagderi 2015), but Prokofiev (2010) reported three basibranchials in the genus *Paraschistura*. The two extra urohyals were not find in *P. nielsenii*, however, in *O. kiabii* (Mafakheri et al. 2014) and *O. bergianus* (Jalili & Eagderi 2015), they exist. Also, these bones are not reported by Prokofiev (2010) for thee members of this family. *Paraschistura nielsenii* has five hypurals in its caudal skeleton, while *O. bergianus* (Jalili & Eagderi 2015) has six hypurals. In *P. nielsenii*, the base of the right pelvic bones is separated from the main body which is not observed in *O. kiabii* (Mafakheri et al. 2014) and *O. bergianus* (Jalili & Eagderi 2015). In *P. nielsenii*, the bony swim bladder capsule is rectangular-shaped and its ventral face is not alveolar similar to *O. bergianus* (Jalili & Eagderi 2015), while the ventral face in *O. kiabii* (Mafakheri et al. 2014) is alveolar.

In conclusion, the findings of this research showed that *P. nielsenii* has a number of distinct and important osteological characteristics including a square-shaped prevomer, four basibranchials, a semi-ossified sesamoid, a non-alveolar bony swim bladder capsule and separated pelvic bone bases that can be used to distinguish this species from other loach
species. It may also be possible to use these osteological characteristics as a reference in the identification, taxonomy and phylogenetic relationship of other loach species in this genus in the future.

References


