

# Comparative osteology of three endemic cichlids (*Iranocichla* spp.) (Actinopterygii, Perciformes, Cichlidae) from southern Iran

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## Abstract

Iranian cichlids are isolated disjunct populations of the African cichlids group, restricted to the Hormuz Strait region in the Hormuz basin, and are a very important group from both zoogeographic and phylogenetic points of view. Thus, the osteological structures, as one of the most reliable structures, of the three nominal species of Iranian cichlid fishes, *Iranocichla hormuzensis* Coad, 1982, from the Mehran River, *Iranocichla persa* Esmaeili, Sayyadzadeh et Seehausen, 2016, from Khorgo hot spring, and *Iranocichla* sp., from the Kol River were described and compared. Ten specimens of each species were cleared and stained with Alcian blue and Alizarin red. A digital camera was used for taking pictures and CorelDraw X6 software for preparing drawings. The three species have some differences in the caudal fin skeleton, shape of the urostyle, neural spine of preural 2, hypural spines 2, and 3, anterior part of parhypural and hypurapophysis, the size of neural spines of preural 2 and 3, hypural 3 and 4, and epurals. Although there are some differences among the species, osteology of these species is very conservative and other traits like behavioral and molecular should be used.

## Keywords

Biodiversity, bone, Cichlidae, *Iranocichla*, osteology

## Introduction

The family Cichlidae contains about 202 genera and more than 2000 species. The geographical distribution of freshwater cichlids includes Jordan Valley, southern Iran, Sri Lanka and southern India, Cuba and Hispaniola, Madagascar, Africa, central, southern, and North America (Kullander 1998; Nelson et al. 2016). The Iranian cichlid, *Iranocichla* genus has three species, two described, *Iranocichla hormuzensis* Coad, 1982, *Iranocichla persa* Esmaeili, Sayyadzadeh et Seehausen, 2016, and one undescribed, *Iranocichla* sp. (see Schwarzer et al. 2016). *Iranocichla hormuzensis* distributed in the Mehran River, *I. persa* in Shur, Hasanlangi and Minab rivers and *Iranocichla* sp.,

in the Kol River drainages (Keivany et al. 2016; Esmaeili et al. 2017), flowing into the Persian Gulf at the Strait of Hormuz. Osteological studies help to understand processes such as feeding, respiration and swimming abilities through cognition jaw bones, branchial bones and fins, also osteology is necessary for understanding the phylogenetic relations among fishes and their classification (Helfman et al. 2009). Only a few osteological works on *I. hormuzensis* is available. Esmaeili and Teimory (2006) mentioned the morphology of the urohyal bone and its importance in the taxonomy of freshwater fishes of Iran, including *I. hormuzensis*. Stiassny et al. (2010) in studying a new species of *Danakilia* (Cichlidae) compared the lower pharyngeal, posterior neurocranium and anterior vertebral elements of

*D. dinicolai* with those of *I. hormuzensis*. Therefore, the aims of this study is to provide a detailed osteological description of *I. hormuzensis* and compare it to other Iranian cichlids, *I. persa* and *Iranocichla* sp.

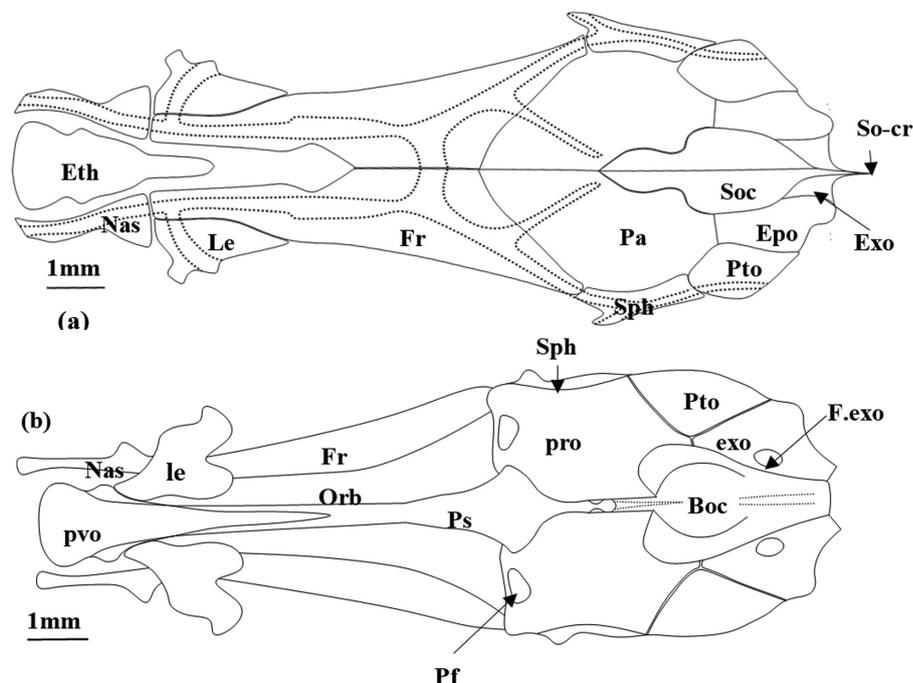
## Materials and methods

Thirty specimens of *Iranocichla* spp. with 3–5.5 cm total length collected from Mehran and Kol rivers and Khorgo hot spring in southern Iran, were examined. They were kept in ethanol (70%) and stored at Isfahan University of Technology Ichthyology Museum (IUT-IM). They were cleared and stained with Alizarin red and Alcian blue according to Taylor and Van Dyke (1985) protocol. A digital camera (Tucson) was used for taking pictures of them. Then, they were drawn using CorelDraw X6 software. The terminology of the bones is based on Rojo (1991).

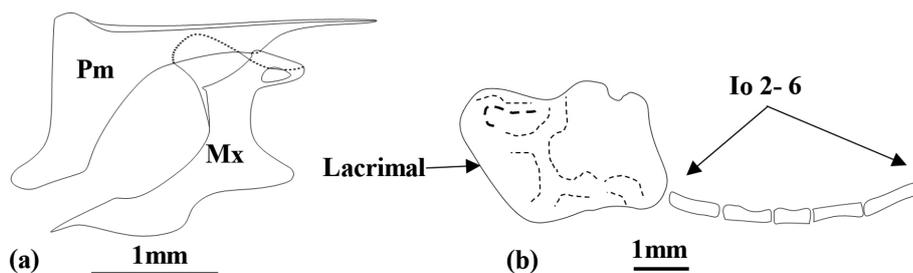
## Results

**Cranium** (Fig. 1). The posterior part of the skull is wider than its anterior part and its roof includes the ethmoid, nasal, frontal, parietal, sphenotic, epiotic, pterotic and supraoccipital and its crest (Fig. 1a). The ethmoid region consists of the paired nasals, lateral ethmoids and unpaired ethmoid, and prevomer. The nasal is short and attached to the frontal. The prevomer is horizontally triangular, elongated posteriorly and bears a strong connection to the parasphenoid. The lateral ethmoid is connected to the prevomer via an anterior process and, in dorsal view, is connected to the frontal (Fig. 1b).

The orbital region consists of the paired orbitosphenoids, circumorbital series, frontals and the unpaired parasphenoid. The frontal is a large element of skull roof with an elongated posterior edge which is linked to the sphenotic and parietal (Fig 1a). In the ventral view, the



**Figure 1.** Dorsal (a) and ventral (b) views of the cranium in *Iranocichla hormuzensis*. Boc: Basioccipital; Epo: Epiotic; Eth: Ethmoid, attached to the surroundings by cartilage not shown in the figure; Exo: Exoccipital; Fr: Frontal; F.exo: foramen exoccipital; Le: Lateral ethmoid; Nas: Nasal; Os: Orbitosphenoid; Pa: Parietal; Pf: Prootic foramen; Pro: Prootic; Ps: Parasphenoid; Ptr: Pterotic; Pvo: Prevomer; Sph: Sphenoid; Soc: Supraoccipital; So-cr: Supraoccipital crest.



**Figure 2.** Internal view of the upper jaw (a) and circumorbital series (b) of *Iranocichla hormuzensis*. Abbreviations: Aap: Anterior ascending process; Io 2–6: Infraorbitals 2–6; Mx: Maxilla; Pmx: Premaxilla; Rc: Rostal cartilage.

parasphenoid connects to the prootic by its posteriolateral edges and is elongated posteriorly and bears two foramina in this part (Fig. 1b). The two orbitosphenoids are attached to the frontal laterally and to the parasphenoid dorsally. The infraorbital series includes the lachrymal and five abutting elements (Fig. 2b). The lachrymal is almost oval-shaped and the biggest element which is the anterior most part of infraorbital series. A sensory canal traverse amid the lachrymal. Infraorbitals 2–6 are short and narrow (Fig. 2b).

The parietals are situated between the supraoccipital and frontal which are linked to each other in the midline. Epiotic is located between parietal, supraoccipital, exoccipital, and pterotic. This bone has an enlarged process in the posteriodorsal edge. The pterotic is nearly triangular which is ventrally connected to the parietal and epiotic, and laterally to the prootic. The prootic ventrally contacts the orbitosphenoid and frontal, dorsally the sphenotic and posteriorly the basioccipital and pterotic. There is a foramen in the prootic for the passage of auditory nerves and blood vessels. The sphenotic bears a small process anterodorsally. The supraoccipital is wide in the middle and linked to the parietal by its anterior process and posteriolaterally to the exoccipitals and epiotic and bears a blade-shaped crest. The exoccipital is linked to the pterotic laterally and bears a foramen on its ventral part. The basioccipital is almost jug-shaped that is cheeky in its middle portion. This bone is located between the prootic and exoccipitals in the lateral view of the skull (Fig. 1b). The posterior part of the basioccipital is connected to the first centrum.

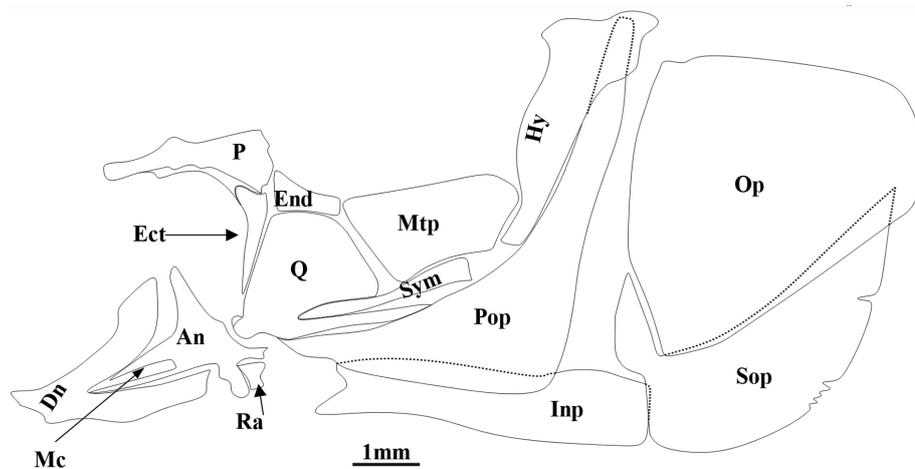
**Jaws** (Figs. 2, 3). Premaxilla bears teeth (not shown). The anterior part of premaxilla has a sharp ascending process which is longer than the premaxillary length. Having an ascending appendage with middle cartilage at its dorsal tip (rostral cartilage in Fig. 2a) helps the ethmoid to move forward and slide around as the mouth opens (Fujimura and Okada 2008). Both premaxillae are connected from the ascending to the middle part by a maxillary cartilage

linkage. The maxilla possesses a mid-lateral ascending process and a posterior descending process. This bone has a hole in the middle outer edge, connected to the middle long ascending process of the premaxilla. The lower jaw is triangle and connected to anteroventral part of the quadrate. The anguloarticular anterior shaft enters the dentary mid cavity. The retroarticular is a tiny bone linking to the posterior inner edge of the angular. The Meckel cartilage is elongated and located in the internal face of the angular (Fig. 3).

**Suspensorium** (Fig. 3). The palatine is small, with a round head, and is posteriorly flat. The ectopterygoid is thin and short, in part, anteriorly binds to the ventral surface of palatine and laterally to the quadrate. The endopterygoid is flat, small and located at the anterior part of the metapterygoid and above the posterior part of quadrate. The metapterygoid is broad and connected to the symplectic and hyomandibular. The quadrate is almost axe-shaped and the symplectic is bar-like. The upper part of hyomandibular is broad and inserted below the upper edge of the preopercle.

**Opercular Series** (Fig. 3). The opercle is nearly triangular and the largest component of the opercular series. The opercle slightly covers the upper rim of subopercle. The preopercle is L-shaped which is broader ventrally. The interopercle is broad and joined to the subopercle posteriorly. The posterior edge of hyomandibular covers the anterior border of preopercle and the ventral corner of the preopercle covers the ventral corner of the interopercle. The hyomandibular is connected to the pterotic through the hyomandibular fossa. The opercle is linked to the hyomandibular posteriorly. Subopercle is broad with a serrated edge and has a sharp and small ascending process that its inner face connects to posterior part of the opercle.

**Hyoid arch** (Fig. 4a). The basihyal is a short anteriorly flattened bar. The urohyal consists of a vertical and horizontal blade and has an anteriodorsal process. The hypohyals include the ventral and dorsal parts, the dorsal hypo-



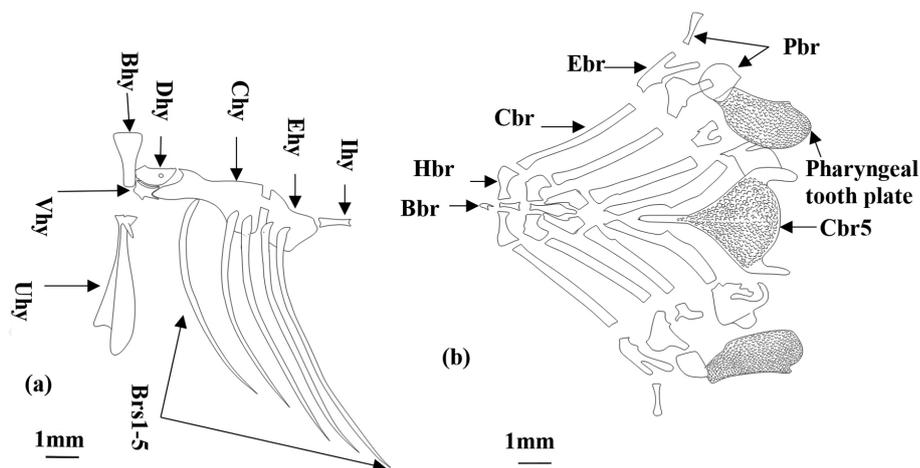
**Figure 3.** The suspensorium and opercular series and upper jaw in *Iranocichla hormuzensis*. Abbreviations: An: Angular; Dn: Dentary; Ect: Ectopterygoid; End: Endopterygoid; Hy: Hyomandibular; Inp: Interopercle; Mc: Meckel cartilage; Mtp: Metapterygoid; Op: Opercle; P: Palatine; Pop: Preopercle; Q: Quadrate; Ra: Retroarticular; Sop: Subopercle; Sym: Symplectic.

hyal bears a hole posteriorly. The (anterior) ceratohyal is posteriorly flatter than anteriorly and connected to the epihyal (posterior ceratohyal) with a small blade. The epihyal is almost triangular. The interhyal is cylindrical in shape. Five branchiostegal rays are present, two branchiostegals articulate with the epihyal and three with the ceratohyal.

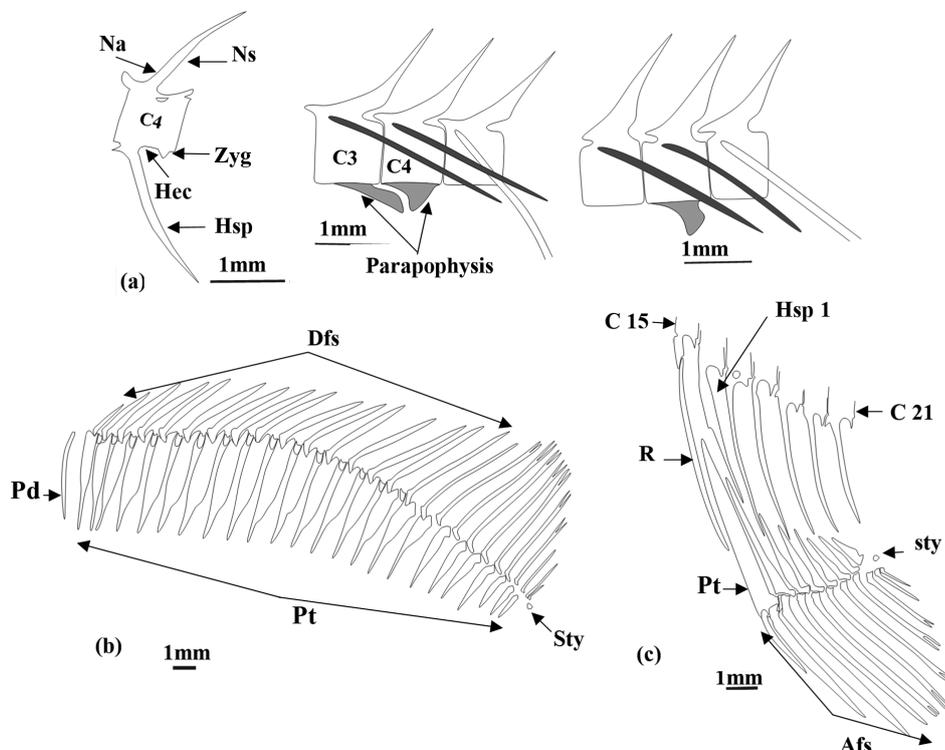
**Branchial arch** (Fig. 4b). There are three basibranchials that have different shapes. The first basibranchial is almost crescent-shaped and smaller than the others. The second and third basibranchials are rod-shaped; the second is flattened at the end and the third at the middle. The hypobran-

chials are three pairs, the third pair have been surrounded by the third basibranchial. The last ceratobranchials are covered by dermal toothplates. and the third and fourth pairs of pharyngobranchials are fused and covered by a single toothplate. The four epibranchials are tripartite.

**Vertebral columns** (Fig. 5a). *Iranocichla hormuzensis* has 25–27 vertebrae. Parapophyses are located in the posteriolateral part of the third and fourth vertebrates, this position was observed in three specimens from the Mehran River, in eight from Khorgo hot spring and in seven from the Kol River (in one specimen from the Kol River



**Figure 4.** (a) Hyoid arch of *Iranocichla hormuzensis*. Bhy: Basihyal, Brs 1–5: Branchiostegal 1–5; Chy: Ceratohyal, Dhy and Vhy: Dorsal and Ventral hypohyal; Ehy: Epihyal; Ihy: Interhyal; Uhy: Urohyal. (b) Branchial apparatus of *I. hormuzensis*. Bbr: Basibranchial; Cbr: Ceratobranchial (Cbr5: Pharyngeal bones); Ebr: Epibranchial; Hbr: Hypobranchial; Pbr: Pharyngobranchials.



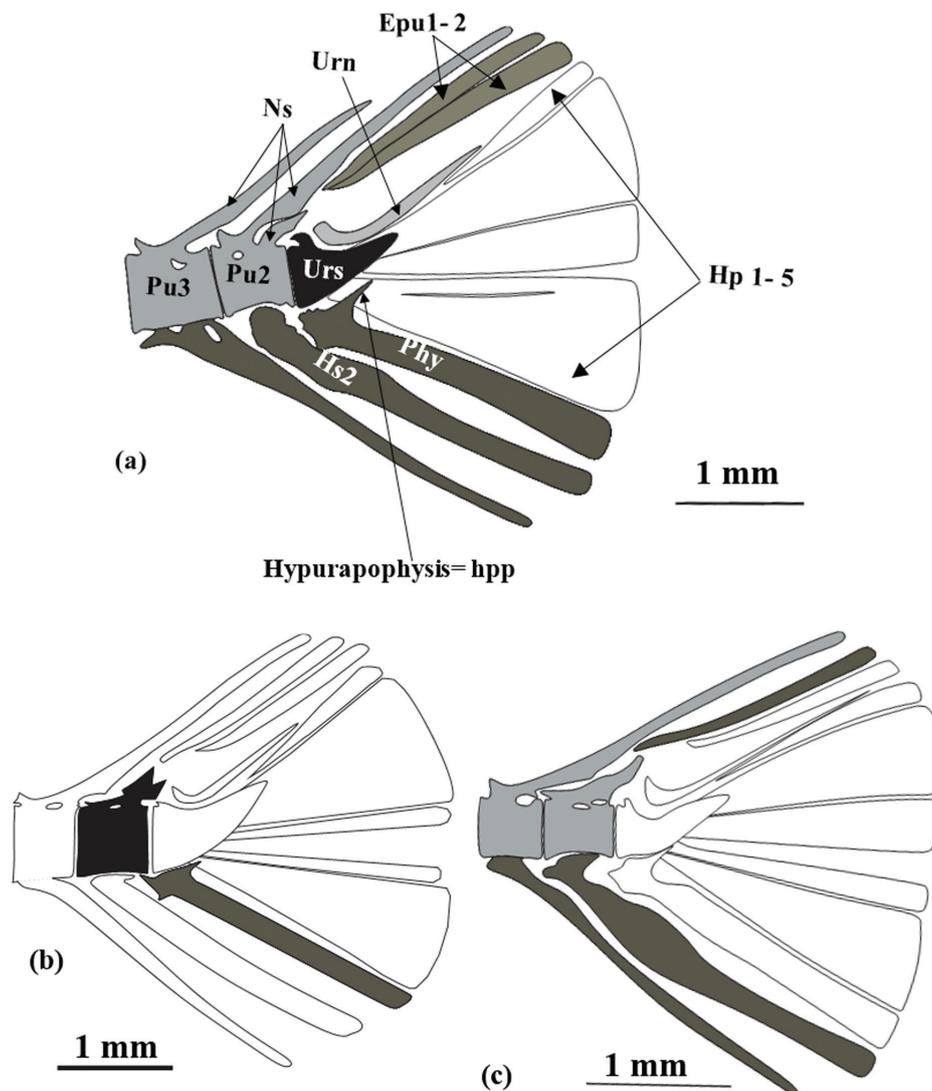
**Figure 5.** Lateral view of vertebrae (a), dorsal fin (b) and anal fin (c) of *Iranocichla hormuzensis*. Abbreviations: Afs: Anal fin spine; C3–C4, C15–C21: Centrum 3–4, Centrum 15–21; Dfs: Dorsal fin spine; Dpt: Distal part of the pterygiophore; Hec: Hemal canal; Hsp (1): Hemal spine (1); Na: Neural arch; Ns: Neural spine; Pd: Predorsal; Pt: Pterygiophore; R: Rib; Sty: Stay; Zyg: Zygapophysis.

and Khorgo hot spring, there was only the fourth centrum and in the Mehran River only the third or fourth or the centrum is absent).

**Dorsal and anal fins** (Fig. 5b, c). D XV–XVI 10–12. There are 24–25 pterygiophores and one stays in the dorsal fin (Fig. 6b). The first pterygiophore is between 1<sup>st</sup> and 2<sup>nd</sup> vertebrae. The distal pterygiophores are linked to the branched rays which decline in size anterioposteriorly. One pretarsal is located before the first pterygiophore. The last two branched rays are not connected to the pterygiophore and a tiny stay support them. A III7–9. The first anal pterygiophore is located between the 15<sup>th</sup> and 16<sup>th</sup> centrum. There are 8–9 pterygiophores and one tiny stay. The first pterygiophore is largest and supports two spines (Fig. 5a).

**Caudal skeleton** (Fig. 6). There are five hypurals (in some specimens, hypural 1 and 2 or 3 and 4 are fused) (Fig. 6a). Hypurals 1–5 and parhypural directly support the caudal fin rays. The first and fourth hypurals are the largest. Two long epurals are bowed to the posterior part of hypural

plate to support the procurrent rays. Hypurapophysis is sharp and stretched toward the hypural 3. The uroneural is narrower than the hypural 5 and in the ventral part is bent to the urostyle. The Urostyle is thin and elongated at the end and part of the beginning is hooked and in general, this bone has insignificant differences in size and shape in different people of each population. The first neural spine of preural 2 is short and the second is elongated in *I. persa* and there is only a short neural spine in *Iranocichla* sp., but in *I. hormuzensis* in addition to this condition (short and elongated), there are also two other conditions; both short or both long. *Iranocichla* sp. and *I. persa* have a haemal spine in the preural 3 but in *I. hormuzensis* might be more than one. Epural 2 in *Iranocichla* sp. and *I. persa* is longer than epural 1 with a space between them, but in *I. hormuzensis* they are almost equal in size and attached all along. The differences in the caudal skeletons of all three species are shown in Fig. 6 and a comparison between them is summarized in Table 1.



**Figure 6.** Lateral view of the caudal skeletons of *Iranocichla hormuzensis* (a), *Iranocichla* sp. (b) and *I. persa* (c). Abbreviations: Epu 1–2: Epural 1–2; Hp 1–5: Hypurals 1–5; Hs2: Haemal spine 2; hpp: Hypurapophysis; Ns: Neural spine; Phy: Parhypural; Pu2 (3): Preurals 2 (4); Urn: Uroneural; Urs: Urostyle.

**Table1.** Comparison of the caudal skeletons of *Iranocichla hormuzensis*, *Iranocichla persa* and *Iranocichla* sp.

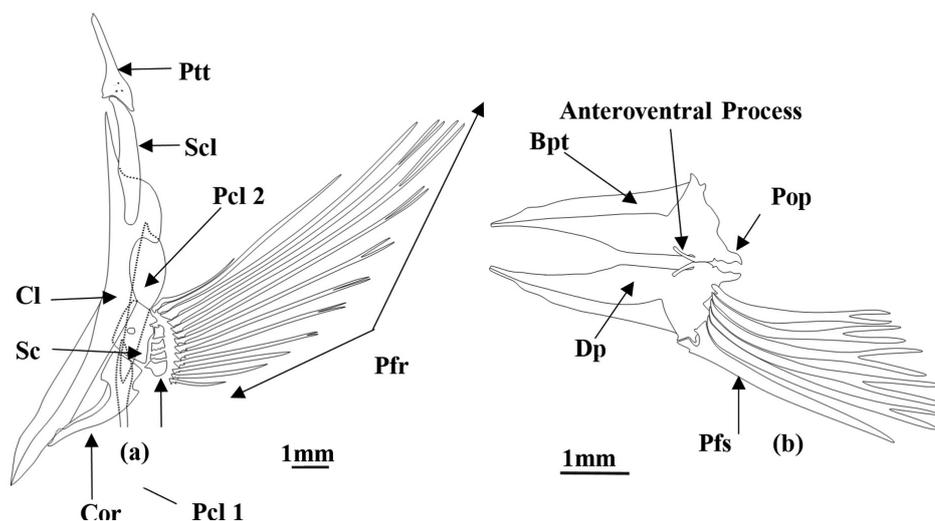
PHY, URN, URS, HS2	HS3	NS3		NS2		Epu1-2		Hyp5		Hyp1-4			Character /Species		
		No.	Length	No.	Length	No.	Status	No.	Status	Status					
										3,4	1,2				
Slight difference in shape	2 elements (first: elongated second: short)	6	Elongated	1	Both elongated	2 elements	2	Both free, second longer	2 elements	2	Wider posteriorly	All: free (wedge-shaped)	5	No fusion	No fusion
							1	First: free second: fused with pu2					1	Complete fusion	Complete fusion
							2	Attached to each other with curvature					1	Complete fusion	No fusion
							2	Attached to each other without curvature					1	No fusion	Incomplete fusion
							1	Fused to each other					1	Incomplete fusion	No fusion
							1	Without curvature					1	Incomplete fusion	Incomplete fusion
	1 element: elongated	4	Short	1	First: short, second: elongated (fused together)	1 element	2	Attached to each other without curvature	1 element	1	Anterior needle-shaped	All: free (wedge-shaped)	1	No fusion	Incomplete fusion
							1	Fused to each other					1	Incomplete fusion	No fusion
							1	With curvature					1	Incomplete fusion	Incomplete fusion
							1	Without curvature					1	No fusion	No fusion
							1	With curvature					1	No fusion	No fusion
							1	Without curvature					1	No fusion	No fusion
Slight difference in shape	1 element: elongated	6	Elongated	1	both Short	2 elements	6	Both free, second longer	2 elements	9	All free (wedge- shaped of different sizes)	5	No fusion	No fusion	
							3	First: short second: elongated				1	Complete fusion	No fusion	
							6	Short				3	No fusion	Incomplete fusion	
	1 element: short*	4	Short	6	Short	1 element	4	Attached to each other	1	Bar-shaped	All free (wedge- shaped of different sizes)	1	Incomplete fusion	Incomplete fusion	
							1	Attached to each other				1	Incomplete fusion	Incomplete fusion	
							1	Attached to each other				1	Incomplete fusion	Incomplete fusion	
Slight difference in shape	1 element: elongated	7	Elongated	3	First: short, second: elongated	2 elements	5	Both free, second longer with flat end	2 elements	10	All free (wedge-shaped of different sizes)	3	No fusion	No fusion	
							2	Complete fusion				2	Complete fusion	Complete fusion	
							1	Complete fusion				1	Complete fusion	No fusion	
	1 element: branched	3	Short	7	Short	1 element	5	Both free, second longer with hook-shaped end	2 elements	10	All free (wedge-shaped of different sizes)	1	No fusion	Complete fusion	
							3	No fusion				3	No fusion	Incomplete fusion	
							3	No fusion				3	No fusion	Incomplete fusion	

\*The only exception in *Iranocichla* sp. population; haemal spine in preural 4 is short.

**Pectoral girdle** (Fig. 7a). The largest bony element of the pectoral girdle is the cleithrum. This bone is posteriorly attached to the coracoid, to the scapula anteriorly and to the supracleithrum ventrally. The coracoid is curved anteriorly making a large hole between itself and the cleithrum. The postcleithra are elongated and thin. The second postcleithrum ends into a sharp point. Two postcleithra are linked to each other behind the scapula. The first ray is slim and directly attached to the posteriodorsal part of the scapula. The scapula is almost square with a foramen in the middle. The elongated supracleithrum is located anteriodorsally on the cleithrum. The base of posttemporal is broadened to attach to the supracleithrum and has a pore on its wide part, but the anterior part of

the posttemporal is elongated and the sensory canal of the head passes through it. The first actinost is attached directly to the scapula, but other actinost are connected to the scapula by cartilage and the fourth actinost is the largest.

**Pelvic girdle** (Fig. 7b). The pelvic includes the paired distal processes, basiptyrgia, posterior processes, anteroventral processes, and fin rays. This fin is horizontally situated in the thoracic area and directly linked to pectoral fin and fixed to it by muscles and ligaments. Pelvic fins are attached together via the combined posterior processes. The anteroventral processes are rod like and thin that fused together between the posterior distal processes. The anterior part of the distal process is sharp but wide posteriorly. There is one spine that is forked at the junction with the distal process.



**Figure 7.** Lateral view of the pectoral girdle (a) and internal view of the pelvic (b) of *Iranocichla hormuzensis*. Abbreviations: Act: Actinost; Anp: Anteroventral Process; Bpt: Basipterygium; Cl: Cleithrum; Cor: Coracoid; Dp: Distal process; Pcl 1, 2: Postcleithrum 1, 2; Pfr: Pectoral fin ray; Pfs: Pelvic fin spine; Pop: Posterior process; Ptt: Posttemporal; Sc: Scapula; Scl: Supracleithrum.

## Discussion

The osteology of three endemic species of Iranian cichlid, *Iranocichla hormuzensis*, *I. persa*, and *Iranocichla* sp. is described and compared for the first time. Coad (1982) introduced the Iranian cichlid as a new genus and species from the Mehran River. Schwarzer et al. (2016), studied phenotypic and genetic diversity of populations of *Iranocichla* from the Mehran, Kol, Shur, and Minab rivers and found genetic differences between populations of the western and the eastern branches of the Kol River system. They suggested that the genetically differentiated populations with different nuptial coloration represent distinct biological species. Esmacili et al. (2016) introduced a new cichlid species as *I. persa* based on mitochondrial DNA sequence and male nuptial coloration from the Shur, Hasanlangi, and Minab river drainages flowing into the Persian Gulf and pointed out that *Iranocichla* sp. was closer to *I. hormuzensis* in terms of mitochondrial sequence and to *I. persa* in male nuptial coloration.

The majority of the bony elements including the neurocranium and the branchiocranium were basically similar in all the three species and did not show any significant differences among the species. The mesethmoid is found in many species of tilapia but the genera *Sarotherodon*, *Oreochromis*, and *Iranocichla* are reported to lack this bone (Trewavas 1973, 1983), however, our findings indicate the presence of this bone in *Iranocichla* species. Like the genus *Gymnogeophagus*, it is assumed that there is no predorsal in the *Iranocichla* (see Reis and Malabarba 1988), but again, our observation indicates the presence of this bone in *Iranocichla* species, although it is limited to one. In the genus *Gymnogeophagus*, the outer part of the epiotic is connected to the posttemporal bone, but in *Iranocichla*, the outer part of the pterotic is connected to the posttemporal bone (Reis and Malabarba 1988). Some

little differences were found in the vertebral parapophyses of centrum 3 or 4. Stiassny et al. (2010) showed that there were two inferior vertebral apophysis on 3<sup>rd</sup> and 4<sup>th</sup> centroms of *Iranocichla hormuzensis*, as seen in our study. As Keivany (2014a) pointed out, the premaxillary ascending process although reduced in many eurypterygian taxa, is well developed in most of the higher percomorphs including Perciformes. The fourth pharyngobranchial is absent or reduced in most eurypterygian fishes (Keivany 2014c) but present in *Iranocichla* spp., however, in the majority of taxa, it bears a separate toothplate, but in *Iranocichla*, the third and fourth pharyngobranchials share a relatively large toothplate. The postcleithra could be present or absent in percomorphs (Keivany 2014d) including cichlids. Also, as in these species, the preopercle is L-shaped in most eurypterygians (Keivany 2014b). As Esmacili and Teimory (2006) denoted that the ventral surface of urohyal of *I. hormuzensis* is triangular, grooved and only a thorn in the dorsal surface can be seen. In the genus *Gymnogeophagus*, the interhyal is articulated with a separate cartilage to the symplectic and the hyomandibular, but in the genus *Iranocichla*, this bone connects to the interopercle (Reis and Malabarba 1988). Caudal skeleton probably is the most variable structure among the species. Generally, the elements of caudal fin of Eurypterygii (see Keivany 2017a) and the hypurals of some cichlids tend to fuse to each other (Vandewalle 1973; Keivany 2017a). In some cichlids of African lakes, this tendency to fuse with each other is seen first on a double plate and then on a single plate (Vandewalle 1973); this trend is apperceived in some individuals of the Mehran and Kol populations. The differences between caudal skeleton of populations were in the shape of the urostyle, neural spine of preural 2, hypural spine 2 and 3, anterior part of parhypural and hyporapophysis, the size and number of neural spines of preural 2 and 3, hypural 3 and 4 and epurals. Sebilia and

Andreata (1990) suggested that the shape of the parhypural, urostyle and haemal spine 2; the degree of ossification of the hyporapophysis and the number of caudal fin rays are suitable for taxonomy of cichlid fishes. As in the majority of eurypterygians (Keivany 2017b), the pelvic girdle is a simple structure in cichlids and featured by the presence of anteroventral process.

Sebilia and Andreata (1990) found that the characteristics of the total number of radius of the caudal fin and how they are located on the supporting bony elements, urostyle shape, parhypural, haemal spine 2, degree of ossification of the hypurapophysis, are valuable characteristics in the classification of cichlids. However, these characteristics do not seem to be suitable for the Iranian cichlid species due to the great variety in the structure of the caudal skeleton. Ottoni (2015) showed that the morphology of the anterior structure of the ceratohyal was not useful for the detection of *Laetacara* species as well as a key factor for the classification of Cichlasomatini members. Therefore, it is possible that in different species, only some structures be suitable for the separation of the species. In general, the osteology of these cichlids, like other cichlids, is quite stable and conservative and could not resolve the relations among the species. However, despite the findings of

Esmaceli et al. (2016) (Based on mitochondrial DNA sequencing and male staining during the breeding season), it seems that *Iranocichla* sp. and *I. persa* are more similar to each other than to *Iranocichla hormuzensis*.

The presence of two types of oral and pharyngeal teeth and the parental care system in Cichlids has increased various feeding strategies and increased the survival of larvae and population (Yoder et al. 2010). In addition, the difference in the structure of the tail stem causes more efficiency of swimming, reduces the cost of energy and mechanism and increases the power of moving forward in the river. It seems that the Iranian cichlid, using the three factors of having two types of teeth, the type of parental care system and the diversity in the structure of the caudal fin, has created motor strategies for living in harsh environmental conditions, thereby improving its survival and reproduction.

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