

Caudal osteology and its application to reconstruct phylogenetic relationship in the genus *Garra*

Kaudal osteoloji ve *Garra* cinsi filogenisi uygulaması

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Abstract: Among cyprinid family, little information is available about the phylogenetic relationships of the members of genus *Garra* in Iran. The structure of caudal skeleton as a valuable source in taxonomic studies of fishes can help to determine their systematic position. Hence, this study was conducted to compare the osteological features of caudal fin skeleton and reconstructing phylogenetic tree of the Iranian members of genus *Garra*. For this purpose, five specimens of every selected (except Iran blind carp with two specimens from each morphotype) taxon were cleared and stained to examine the osteological characteristics of their caudal fin skeleton. Also, Kura barbel (*Barbus cyri*) and Barzam (*Capoeta trutta*) were designed as outgroup. The results showed that members of *Garra* constitute a monophyletic group. The results revealed that the caudal skeleton features cannot discriminate the members of genus *Garra* at the level of species because of small number of extractable characters and their states.

Keywords: Phylogeny, osteology, Iran blind carp, *Garra*

Öz: Cyprinidae familyası içerisinde, İran'da dağılım gösteren *Garra* cinsi üyelerinin filogenetik ilişkileri ile ilgili az sayıda araştırma bulunmaktadır. Kaudal yüzgeç iskeleti yapısı, balıkların sistematik pozisyonlarının belirlenmesine yardımcı olan, taksonomik çalışmalarda kullanılabilen bir karakterdir. Bu sebeple, bu çalışmada *Garra* cinsinin İran'daki türlerinin filogenetik ilişkilerini belirlemek amacıyla kaudal yüzgeç iskeletlerinin kemik yapısı karşılaştırılmıştır. Bu amaçla seçilen taksonlara ait beşer bireye kaudal yüzgeç iskeletlerinin osteolojik karakterlerinin belirlenmesi amacıyla şeffaflandırma yöntemi (cleared-stained) uygulanmıştır. Çalışmada *Barbus cyri* ve *Capoeta trutta* dış grup olarak kullanılmıştır. Elde edilen sonuçlara göre; *Garra* üyeleri monofiletik bir grup oluşturmıştır. Sonuçlar kaudal yüzgeç iskeletlerinin *Garra* cinsi için tür düzeyinde ayırımında kullanılamayacağını göstermiştir.

Anahtar kelimeler: Filogeni, osteoloji, İran kör balığı, *Garra*

INTRODUCTION

Caudal skeleton of fishes consists of the vertebral columns, vertebral processes and fin rays, and they are valuable structures in taxonomic studies of fish taxa (Fujita, 1990). In this regard, Monod (1968), and Schultze and Arratia (1989) used the caudal-fin skeleton as a taxonomic source for the classification of teleost. Development of techniques in the osteological studies such as clearing and staining, made it possible to use caudal skeletal structure for taxonomic studies in fishes.

About 300 fish species have been reported from Iranian inland waters, of which 111 belong to the family Cyprinidae (Esmaili et al., 2018). Members of this family are important in terms of aquaculture and scientific studies (Nelson, 2006) and their phylogeny, ecology, physiology and distribution are of great importance (Chen and Mayden, 2009). Among the cyprinids, members of the genus *Garra* are found from the

Southeast Asia to Africa (Coad, 2019). About 75 species of this genus have been reported, with 10 members in the inland waters of Iran (Esmaili et al., 2016; Mousavi-Sabet et al., 2016a, b; Hashemzadeh Segherloo et al., 2017). The members of this genus have small to medium-size body, almost fusiform, with inferior mouth in crescent shape (Coad, 2019) and horny-lip corners (Stiassny and Getahun, 2007), and 1-2 pairs of the short barbels. They are found in mountainous streams with high-velocity currents and can be fixed their position by sucker-mouth disc, although they have also been reported in low-flow rivers (Esmaili et al., 2016; Coad, 2019).

This study aimed to investigate the osteological structure of caudal fin in different populations of seven *Garra* species in Iran and the possibility of using the caudal skeleton characters to study their phylogeny based on cladistic method. Hence, this study investigate the efficacy of the caudal-fin skeleton

application to study phylogeny of Iranian cyprinids and the results may help to clarify the relationships of this genus in Iran as well.

MATERIAL AND METHODS

For this study, *Garra* species, including *G. rufa* from the Tazeh-Ab River (Tigris river drainage) and Ilood Spring (Hormuzgan basin), *G. gymnothorax* from the Karkheh River (Tigris river drainage), *G. persica* from the Kash, Sanderk and

Sarbaz rivers (Makran basin), *G. rossica* from the Nahang River (Makran basin) and *G. lorestanensis* and *G. typhlops* from the Bagh-e Loveh, Lorestan Province (Tigris river drainage) as in-group and *Capoeta trutta* and *Barbus cyri* as out-groups were collected, each 10 with specimens except *G. lorestanensis* and *G. typhlops* with two specimens due to their conservation status (Table 1).

Table 1. Sampling stations of this study

Species	River	Basin	Latitude	longitude	Above Sea Level
<i>Garra rufa</i>	Ilood	Hormuzgan	27°12'28"N	54°40'9"E	438
<i>Garra gymnothorax</i>	Karkheh	Tigris	48°20'45"N	31°52'5"E	332
<i>Garra rufa</i>	Tazeh-Ab	Tigris	34°44'1"N	46°11'54"E	1099
<i>Garra persica</i>	Kash	Makran	25°5'57"N	57°44'57"E	155
<i>Garra persica</i>	Sanderk	Makran	26°50'24"N	57°15'54.7"E	54
<i>Garra persica</i>	Sarbaz	Makran	26°37'53"N	61°15'35"E	894
<i>Garra rossica</i>	Nahang	Makran	26°50'36"N	61°35'27"E	1345
<i>Garra typhlops</i>	Bagh-e Loveh	Tigris	33°4'38"N	48°35'33"E	812
<i>Garra lorestanensis</i>	Bagh-e Loveh	Tigris	33°4'38"N	48°35'33"E	812
<i>Capoeta trutta</i>	Semirom	Tigris	31°11'15"N	51°15'25"E	1548
<i>Barbus cyri</i>	Sefid	Caspian Sea	37°1'29"N	37°1'29"E	81

For osteological examination, the specimens were cleared and stained based on Taylor and Van Dyke (1985) using alizarin red and alcian blue for bone and cartilage staining, respectively. The caudal skeleton of all species were separated and examined under a stereomicroscope (Leica MS5). Their structures were then scanned by a scanner (Epson v600) equipped with a glycerin bath, and drawn and labeled using CorelDraw X6 software. Nomenclature of the skeletal elements is followed Rojo (2009), and Jalili and Eagderi (2014).

The hypothesis of phylogenetic relationships was performed based on cladistic method (Sworfford et al., 1996). By examination and comparison of the samples, the morphological characteristics of different taxa were selected and described as character states. Polarity of the characters were determined according to Watrous and Wheeler (1981). Seven character states were used in phylogenetic tree reconstruction to investigate the evolutionary model and unspecified data were labeled as? To understand the effect of unspecified data, parsimony analysis was performed separately with and without these data.

Phylogenetic reconstruction was performed based on maximum parsimony criterion using PAUP4 software (Sworfford, 1999). Data matrix were executed using Heuristic search algorithm by selecting 100 Tree-bisection-and-reconnection iterations and deleting in-informative characters. Character states distribution was evaluated using ACCTRAN (Accelerated Transformation) and bootstrap analysis with 100 iterations was performed using Heuristic search algorithm.

RESULTS

Comparison of the caudal-fin skeleton: The caudal fins of the studied taxa, similar to other cyprinids, possesses the hypural, epural, uroneural, parhypural and pleurostile bones (Figure 1h). The hemal spines of the first vertebrate i.e. ual centra are transformed as the hypural and parhypural bones to support the caudal-fin rays. In members of the genus *Garra*, number of the hypurals varies 5-7. *Garra rufa* has six hypurals, *G. gymnothorax* five (Figure 1a, b, c) and *G. persica* and *G. rossica* six, whereas those of *G. persica* from the Sanderk River have five. *Garra typhlops* and *G. lorestanensis* also have six or seven hypurals, respectively.

The first hypural is fused to the parhypural, and this complex is connected to the first vertebrate. The parhypural is long and almost wide bearing a hypurapophysis in its base. The hypural-II and pleurostile are also fused to the last vertebrate. The anterior part of the third hypural is attached to the urostyle, and the hypural IV-VII are freely located in the posterior part of the pleurostile. The epural is neural spine of the first vertebrate to be separated. This long bone is unpaired having different sizes and shapes. In all studied species, the ventral part of this bony element is wider than its dorsal part. In *G. persica* populations of the Kash River, the dorsal part is wider, but it has same width throughout its length as *G. rossica*.

The uroneural is small and narrow, except in *G. persica* of the Sarbaz River, which it is absent. In others, this bone is paired. The uroneural is situated on the anterior margin of the pleurostile having diverse sizes. In the antero-dorsal part of the

first vertebral center, a neural process is usually seen, which it is narrow and pointed at its end. In *G. rufa* of the Iloud Spring, the first vertebrate of the caudal skeleton bears two processes. In the members of the genus *Garra*, there are several differences in the structure and shape of the second neural spine (PU2).

Such a differences were also observed in members of the same species.

The second neural spine is usually broad at its base and narrow at the end. In *G. typhlops*, this spine is modified as a process, whereas the two neural spine are observed in *G. lorestanensis*, which the posterior one is narrower seems to be developed from the Zygapophysis. One specimen of *G. persica* of the Sanderk River had an inter-neural spin bone in the anterior part of PU2. A total of seven characters were defend as [Table 2](#).

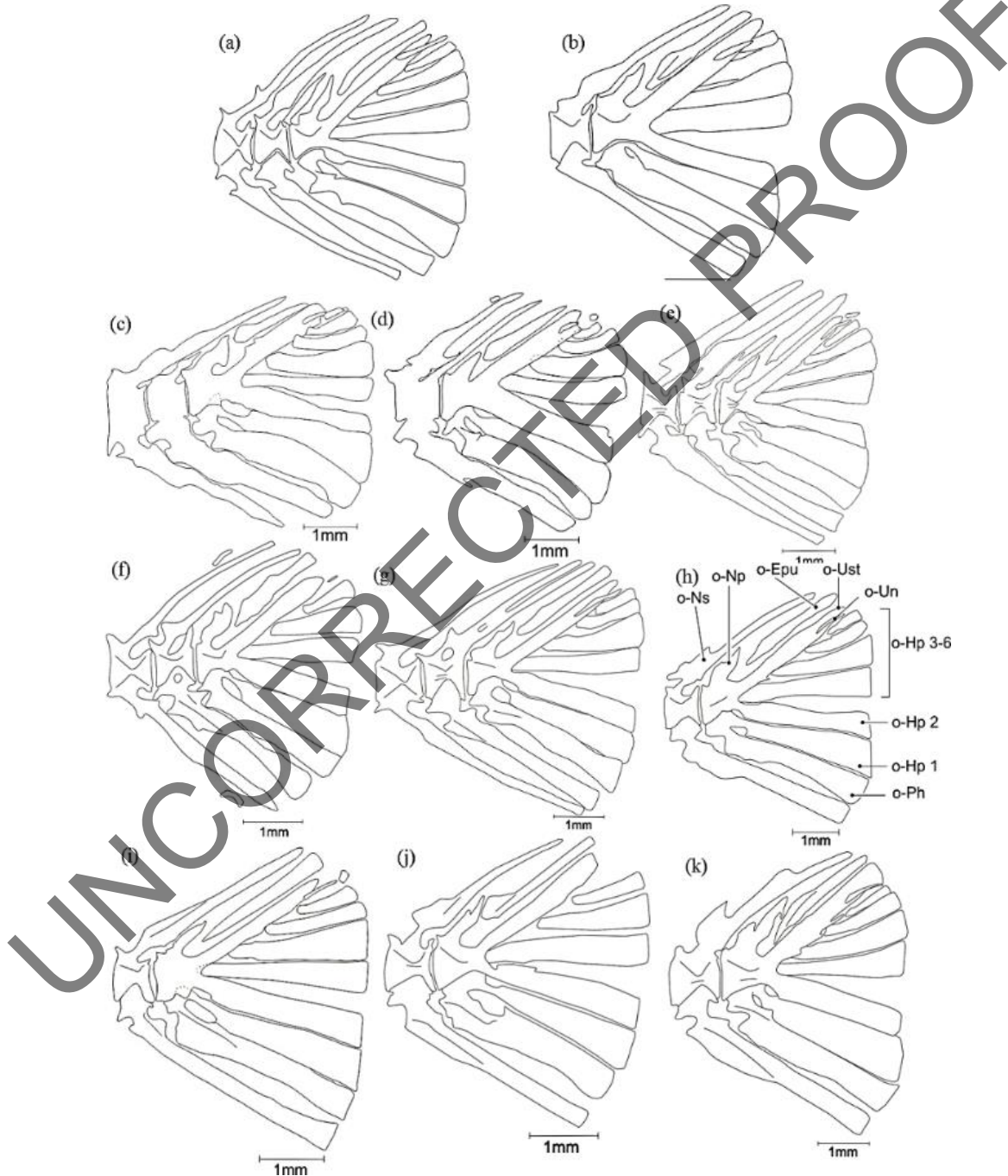


Figure 1. Caudal-fin skeleton of (a) *Barbus cyri*, (b) *Capoeta trutta*, (c) *Garra typhlops*, (d) *G. lorestanensis*, (e) *G. rossica*, (f) *G. gymnothorax*, (g) *G. rufa* (Iloud), (h) *G. rufa* (Tazeh-Ab), (i) *G. persica* (Kash), (j) *G. persica* (Sarbaz) and (k) *G. persica* (Sanderk) (Epu: epural; Hp: hypurals; Np: neural process; Ns: neural spine; Ph: parhypurale; Un: uroneural; Ust: pleurostile)

Table 2. Character states were defend

Character states	
Character 1	sixth hypural is large and complete (0), reduced (1), absent (2).
Character 2	caudal skeleton bears six (0), five (1), seven (2) hypurals.
Character 3	PU1 possesses one (0), two (1) neural spine.
Character 4	neural spine of PU2 is long (0), short (1).
Character 5	parhypural has ventral position bearing greater angle with vertebral column (0), has posterior position less angle with vertebral column (1).
Character 6	Zygapophysis of PU2 is small (0), well-developed (1).
Character 7	epural has same wideness along its length (0), it is broader ventrally (1)

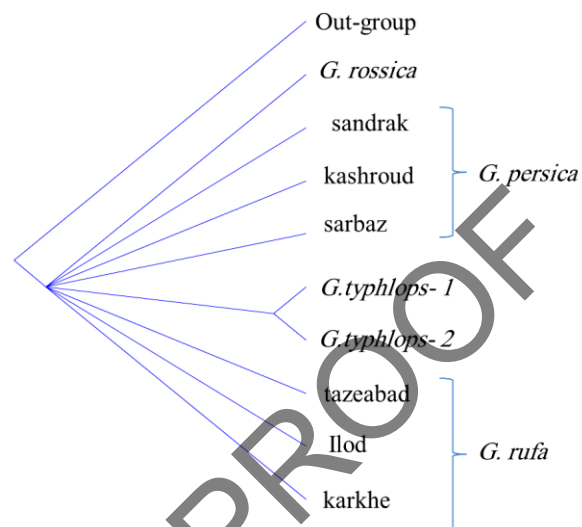
Phylogenetic analysis: The data matrix for 9 in-groups and 2 out-groups is presented in Table 3. The results of the parsimony analysis based on data matrix had 10,000 most-parsimony trees with a length of eight, consistency index (CI) of 0.875 and retention index (RI) of 0.8333. Due to the high number of parsimony trees, the consensus majority tree was selected based on values obtained from 100 replicates (Figure 2).

Table 3. Data matrix of 11 studied taxa, including 9 in-groups and 2 out-groups (0= plesiomorphics, 1 and 2 = synapomorphics)

Taxa	characters	1	2	3	4	5	6	7
<i>G. ruffa</i> (Iloud)		0	0	1	0	0	?	0
<i>G. gymnothorax</i> (Karkheh)		1	1	0	0	0	0	1
<i>G. ruffa</i> (Tazeh-Ab)		0	0	0	0	0	0	0
<i>G. typhlops</i>		0	2	0	0	1	1	1
<i>G. lorestanensis</i>		0	2	0	1	1	0	1
<i>G. persica</i> (Sarbaz)		2	0	0	0	0	0	1
<i>G. persica</i> (Kash)		0	0	0	0	0	0	1
<i>G. persica</i> (Sanderk)		0	1	0	0	0	0	1
<i>G. rossica</i> (Nahang)		0	0	0	0	0	1	0
<i>C. trutta</i>		0	0	0	0	0	0	1
<i>B. cyri</i>		0	0	0	0	0	1	0

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**Figure 2.** Cladogram of studied taxa of the genus *Garra* in Iran

DISCUSSION

The results showed that all members of the genus *Garra* are monophyletic (with 100% support) in which two blind cave species (with 81% support) based on trait (4: 1), i.e. the posterior hypural status, are considered as sister groups. Characters of 3 and 6 were different in two examined specimens of *G. typhlops*. Three characters were non-informative and four others were informative. Despite importance of the caudal-fin skeleton as a taxonomic source for classification of teleost (Schultze and Arratia, 1988), these characteristics could not separate the studied taxa of the genus *Garra* in Iran due to low number of available character states. Therefore, it needs to use more characters at least twice the number of the examined taxa, particularly those of cephalic region.

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