

Skeletal development of the caudal complex in Caspian roach, *Rutilus caspicus* (Yakovlev, 1927) (Teleostei: Cyprinidae)

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Abstract. Study of skeletal development of economic fish species can help early diagnosis of the skeletal abnormalities. Caspian roach (*Rutilus caspicus*) is a native species of the Caspian Sea and commercially valuable species as a promising candidate for sea ranching programme. This study was conducted to describe the ontogeny of its caudal complex. A total of 300 larvae and fry specimens of the Caspian roach were randomly sampled from hatching up to 90-days post hatching (dph). The specimens were cleared and stained in order to examine their osteological features. The larvae were devoid any caudal elements at the hatching and the development of the caudal skeleton began with formation of the hypural 1, 2 and 3 at 5 dph. The flexion was occurred at 8 dph. Formation of the caudal skeleton was nearly completed at 90 dph. Compared with other teleost fishes, the Caspian roach was followed a pattern of relatively similar osteological development.

Key words: Tail, Ontogeny, Osteology, Cyprinidae, *Rutilus caspicus*.

Introduction

Phylogenetic homology is implied when the morphological structures of two different organisms are given the same name or identity (Doosey & Wiley 2015). In this regard, the ontogeny of osteological structures can reveal the systematic relationships of fishes by providing valuable keys to understand homology of various skeletal elements (Doosey & Wiley 2015). Ontogeny of osteological structures also helps our interpretation about osteological characters in adults (Fritzsche & Johnson 1980, Goslin 1961, Javidan & Schilling 2004, Koumoundorous et al. 1995, 1997, 1999, 2001, Nibelin 1973). Therefore, ontogeny of fins' skeleton are informative characters to identify fish larvae at different taxonomic levels (Valdes et al. 2001).

From aquaculture point of view, understanding of the skeletal development of economic fish species can help early diagnosis of the skeletal abnormalities, since most of the skeletal deformities are occurred during early developmental stages (Boglion et al. 2001, Koumoundorous et al. 1997). Skeletal deformities are crucial in aquaculture since they decrease the quality of produced fish by affecting their external morphology, growth and survivals. There are many reports about skeletal malformation in various fish species (Gavaya et al. 2002, Fraser et al. 2004, Koumoundorous et al. 1997, Sfakianakis et al. 2004). Therefore, production of healthy larvae and preventing the occurrence of skeletal deformities need a basic knowledge regarding the ontogenic process of the skeletal system (Koumoundorous et al. 1997, Lewis & Lall 2006).

Caspian roach (*Rutilus caspicus*) is a native species of the Caspian Sea and commercially valuable species as promising candidate for sea ranching programme. Since there is no information in available regarding the ontogeny of this fish species, therefore this study was conducted to describe the ontogeny of its caudal complex.

Material and methods

The Caspian roach larvae were obtained from a semi artificial breed-

ing of 20 brood stocks in an earth pond (Sijval, North of Iran). The larvae were fed by fertilizing the pond. Water quality of ponds during rearing period, including dissolved oxygen, temperature, and pH were ranged 6.5-8 ppm, 21.4-24.4°C and 7.6-8.4, respectively. The semi extensive condition, similar to the natural condition was used to ensure the normal development of the fish, since many deformities and body malformations are associated with intensive hatchery production (Lewis & Lall 2006).

A total of 300 larvae and fry of Caspian roach were randomly sampled from hatching up to 90 dph (days post hatch). The sampling was carried out daily till 15 dph and then every five days up to 90 dph by sampling ten specimens every morning of sampling day. Larvae were anaesthetized with 1% clove oil and fixed in phosphate buffered 5% formalin for 24 hours and then stored in 72% ethanol. The specimens of 0-40 dph were cleared and stained according to Darias et al. (2010), whereas those of 45-90 dph larvae were cleared and stained based on the Taylor & Vandyke (1985). To examination the osteological features, the stained specimens were preserved in glycerol by adding a small piece of Thymol crystal to prevent fungi contamination (Gavaya et al. 2002).

Osteological developments of the specimens were examined using a stereoscopic dissecting microscope equipped with a 13 MP Nikon camera. The prepared specimens were scanned by a scanner (EpsonV700) equipped to glycerol bath. The skeletal elements were drawn from obtained images of the caudal complex using Core-DrawX5 software. Total lengths (TL) of specimens were measured to the nearest 1 mm and used as the reference point in the description of the ontogeny. Nomenclature and abbreviations of osteological features followed Lundberg & Baskin (1969) and Rojo (2009).

Results

Pre flexion stage

At hatching, mean TL of the sampled specimens were 6.096 ± 0.635 mm and devoid of any caudal elements (Fig. 1.A-B). The notochord was straight and primordial marginal fin fold was present (1-4 dph). The caudal fin development was started approximately at 8.624 ± 0.267 mm in TL (5 dph), with appearance of three cartilaginous plates beneath the notochord, including the hypurals 1, 2 and 3, respectively from left to right. The number of Principal Caudal Rays (PCR) was four (Fig. 1.C). The parhypural was appeared at 8.681 ± 0.356 mm in TL (6 dph) as a cartilaginous bud at the ventral

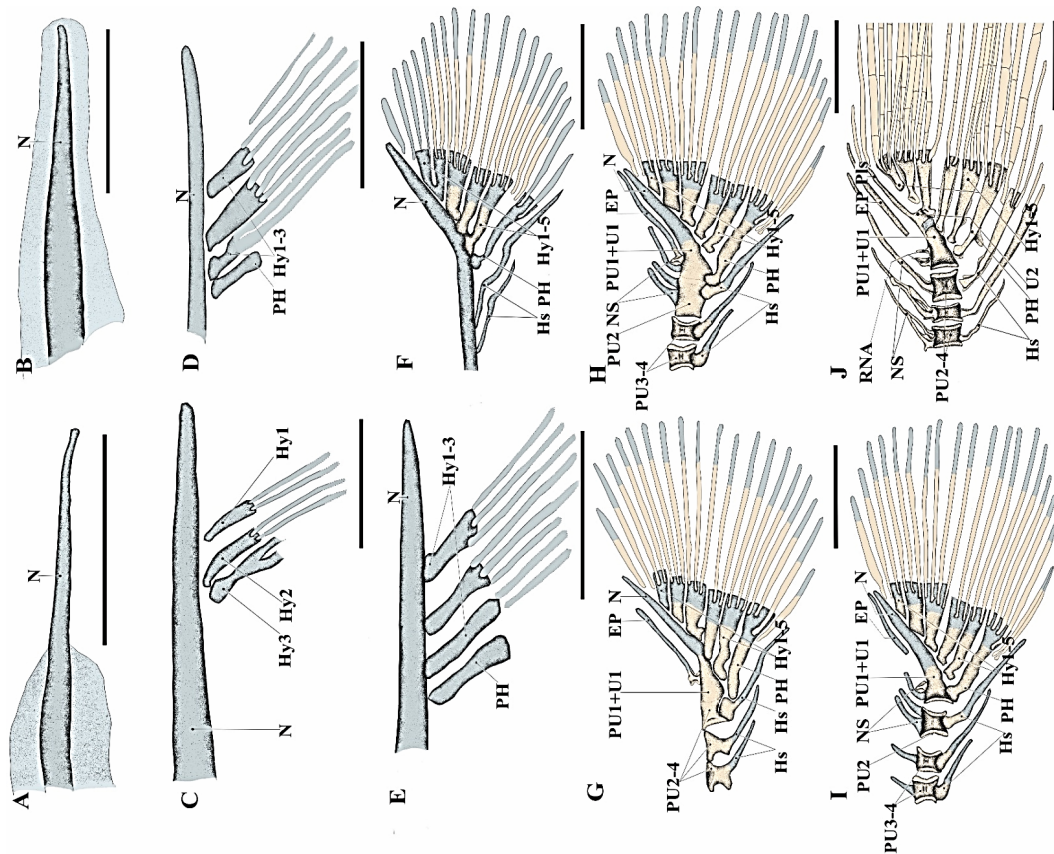


Figure 1. Development of the caudal skeleton in *Rutilus caspicus*. (A): 1 dph, (B): 2 dph, (C): 5 dph, (D): 6 dph, (E): 7 dph, (F): 8 dph, (G): 9 dph, (H): 10 dph, (I): 14 dph and (J): 25 dph. Blue area, cartilage; yellow area, ossification (bar = 0.50 mm).

Table 1. Anatomical abbreviation of the caudal complex.

| Abbreviation | Structure |
|--------------|--|
| EP | Epural |
| HS | Haemal spine |
| N | Notochord |
| PU1-2 | Preural centra 1, 2 |
| U1-2 | Ural centra 1, 2 |
| RNA | Rudimentary neural arch |
| PU1 + U1 | Compound centrum formed the first preural and the first Ural centrum |
| Hy | Hypural 1-6 |
| PH | Parhypural |
| NS | Neural spine |
| U1-2 | Ural centrum 1, 2 |
| Pls | pleurostyle |

distal portion of the notochord. Then, the number of principal caudal rays were increased to eight (Fig. 1.D-E).

Early flexion stage

By the flexion stage at 9.548 ± 0.482 mm in TL (8 dph), the notochord presented an upward curvature posteriorly. The hypurals 4 and 5, and two haemal spines were formed; therefore, eight cartilaginous plates beneath the notochord were corresponded to the two haemal spines, parhypural and five hypurals, respectively, from left to right concomitant with increasing PCR. Then, the ossifications of the hypural plates 1-3 and PCR were started (Fig. 1.F).

Late flexion stage

The notochord flexion was more developed at 10.961 ± 0.731 mm in TL (9 dph). A composite main caudal centrum comprising, the first preural centrum fused to the first ural centrum (PU1 and U1) was observed (Fig. 1.G). Following formation of the hypurals 1-3 at 9.548 ± 0.482 mm in TL (8dph), the parhypural and hypural 4 and 5 and haemal spines started ossifying partially. Ossification of the PU1+U1 and PU 2-3 began as well. Dorsal side of the notochord, the neural spines of the preural centrum 2 and 3 were appeared at 11.305 ± 0.630 mm in TL (10 dph). The preural centrum 2 and 3 and their haemal and neural spines were included as a part of the caudal complex (Fig. 1.H).

Post flexion stage

During this stage, development of the caudal complex was occurred as following:

a) 12.019 ± 0.571 mm in TL (14 dph): One narrow epural cartilage was appeared as neural spine of the PU1 while the trace of its neural arch remained as RNA. The development of the cartilaginous neural spines continued (Fig. 1.I).

b) 14.795 ± 0.685 mm in TL (25 dph): The large Pls covered the anterior margin of the hypurals. A second ural centrum was appeared similar to young specimens of the most ostariophysi as a chorda centrum (Lundberg & Baskin 1969) (Fig. 1.J).

c) 16.066 ± 0.513 and 20.762 ± 2.224 mm in TL (30 and 40 dph, respectively): The anterior half of the neural spine of the PU2 was appeared at 30 dph. This structure further

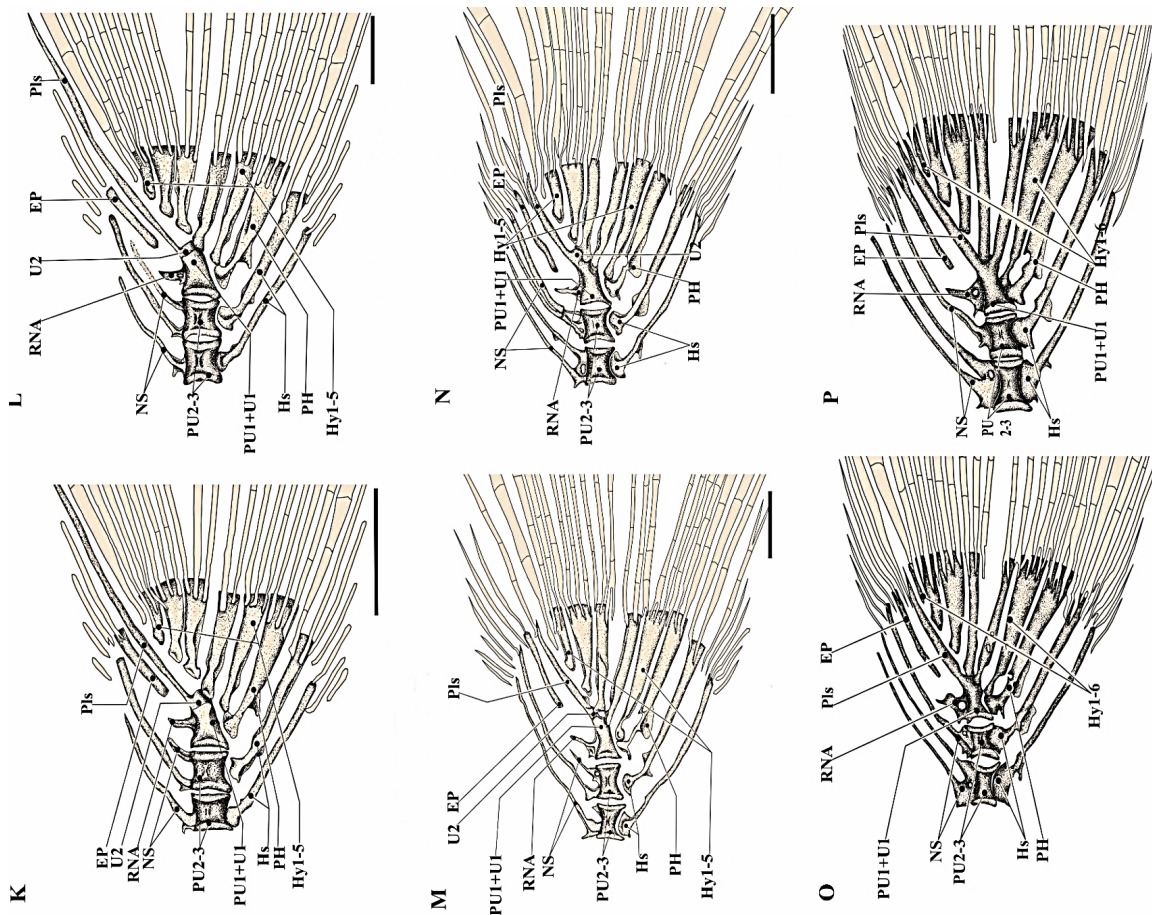


Figure 2. Development of the caudal skeleton in *Rutilus caspicus*. (K): 30 dph, (L): 40 dph, (M): 50 dph, (N): 60 dph, (O): 70 dph and (P): 90 dph. Blue area, cartilage; yellow area, ossification (bar = 0.50 mm).

developed at 40 dph. The position of the U2 was shifted anteriorly i.e. toward the PU1+U1 (Fig. 2.K-L).

d) 29.639 ± 2.003 and 32.695 ± 2.393 mm in TL (50 and 60 dph, respectively): The anterior half of the neural arch of the PU3 was appeared corresponding to development of the neural spines of the PU2. Two halves of the neural spine of the PU2 and neural arches of the PU3 attached to each other as unanimous neural spine and neural arch at 60 and 70 dph, respectively. The neural spines of the PU3 attached to each other after 90 dph based on examination of the adult specimens (Fig. 2.M-N).

e) 39.539 ± 4.030 mm in TL (70 dph): The U2 chorda centrum fused to the base of the hypural 3, hence a well-developed U2 auto centrum was absent in the adult specimens. This is represented in adults only by knob-like mass at the base the hypural 3 and positioned in a cavity on the posterior face of the compound centrum (Fig. 2.O).

f) 41.637 ± 3.343 and 40.021 ± 3.412 mm in TL (80 and 90 dph, respectively): The rims of the hypurals were not ossified, and the sixth hypural appeared in the dorsal side of the hypural 5 and beneath Pls at 80 dph. Fusion of the hypural 2 and compound centrum was begun at 90 dph (Fig. 2.P).

Discussion

The development of osteological structures in teleostei is of demonstrable value in phylogenetic studies (Nibelin 1973,

Gosline 1967, Koumoundorous et al. 1995, 1997, 1999, 2001). Osteological structures of the adult fish sometimes show a high degree of fusion, especially in advanced teleostei, making it difficult to correctly identify bony elements (Matsuura & Katsuragava 1985). Hence, this study was provided a detailed ontogeny of the caudal complex that can be used in future taxonomic studies of this taxon.

The flexion of the notochord accompanies the development of the caudal complex and subsequently alteration in the locomotors ability, swimming mode and velocity, body shape and feeding behavior (Koumoundorous et al. 1999). This was occurred at 8 dph for *R. caspicus*. At this point, eight cartilaginous plates beneath the notochord were corresponded to the two haemal spines, parhypural and five hypurals, respectively. The flexion stage is sometimes concomitant with ossification of the hypural plates as observed in Caspian roach and *Grunion leuresthes tenuis* (Valdes et al. 2001). Rudder-like structure of the hypurals and parhypurals can provide enough space for the attachment of the muscle mass resulting in sufficient force to move the body during flexion (Dasilao & Yamaoka 1988, Lundberg & Baskin 1969).

The second ural centrum is represented in the young of the most ostariophysian as a chorda centrum (Lundberg & Baskin 1969). It is generally believed that both the U1 and U2 are co-ossified with the first preural, as a typical condition of many advanced fishes (Lundberg & Baskin 1969). Whereas, the U2 is fused with the base of the hypural 3 and 4 or only hypural 3 in Cyprinidae (Lundberg & Baskin 1969) as seen in

Caspian roach. Although, galaxioid fishes possess U2, and very occasionally, even smaller ural centrum U3 (Mcdowall 2001). Based on our findings, the PU1+U1 (urostyle), the second and third preural centra contribute supporting of the caudal fin rays, similar to that of white perch (*Morone americana*) and striped bass (*Morone saxatilis*) (Fritzsche & Johnson 1980).

Phylogenetic reduction of the caudal complex commonly involved hypural fusions (1, 2 - 3, 4) as seen in Florida blenny (*Chasmodes saburrae*) (Peters 1981), losing posterior pairs of the uroneural and less frequently losing the epurals 1-3, various combinations or fusions of the hypurals with the parhypural or PU1+U1 (urostyle) as seen in Caspian roach, loss or fusion of the anterior uroneural pair and fusion of the haemal spines. These reductions are associated with increasing swimming ability (Koumoundourous et al. 1995, 1997, 1999, 2001).

The members of Cypriniformes have six independent hypural plates (Buhan 1972). Fusion of the hypurals into a broad plate begins with hypurals 3, 4, parhypurals and urostyle in *Prionotus evolans* (Yuschak 1985), hypural 3 to 5 and hypural 1 and 2 in flying fishes (*Cypselurus heterurus doederleini*), whereas there is no any fusion between hypurals and parhypurals in *R. caspicus* (only conjunction between hypural 2 and compound centrum was observed). Hyprals fusion is one of the main characters which is found in advanced marine teleostei (Doosey & Wiley 2015). Caspian roach, and Grey trigger fish (*Balistes capricus*) (Matsuura & Katsuragava 1985) have one epural, while many other species such as *Dentex dentex* (Koumoundourous et al. 1999) and flying fishes, *Cypselurus heterurus doederleini* (Dasilao & Yamaoka 1988) have three.

Simply naming the single epural of *R. caspicus* does not provide sufficient information about other phylogenetically homologous structures with it. Thus "epural 1" of Caspian roach is named epural of the preural centrum 1 (E-Pu1) to correctly indicate that it is an epural whose ontogenetic origin lies with the epaxial segment associated with the first preural centrum (Doosey & Wiley 2015). Fundamentally, more complex caudal skeleton in marine fishes indicate that Cypriniformes as fresh water fishes are ancestors of the more advanced marine fishes.

It is concluded that cartilaginous and bony elements in Caspian roach could be related to their ability to swim and evade predators which enable them to survive effectively during early development. This study provided a base for further phylogenetic and biological study regarding Caspian roach.

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