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Development of the Caudal Skeleton in the Tetraodontid Fish, *Takifugu niphobles*

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The osteology of tetraodontid fishes has already been studied by many authors, e.g. Gregory (1933), Kuronuma (1943), Abe (1952a, b), and Tyler (1970, 1980), for the purposes of classification and understanding of phylogenetic relationships. However, developmental osteology as such, was not covered in these papers.

Components of the caudal skeleton of adult tetraodontid fishes show a high degree of fusion (Tyler, 1980; Fujita, 1990). Ontogenic studies of the caudal skeleton will facilitate identification of osteological structures of the fused bones. The purpose of this study is to describe the osteological development of the caudal skeleton of the tetraodontid, *Takifugu niphobles*, and to clarify the ontogeny of the caudal skeleton.

Materials and methods

The specimens of *Takifugu niphobles* examined in this study were obtained from eggs artificially inseminated at Kominato, Chiba Prefecture on 9 June 1990 and hatched at the Laboratory of Ichthyology, Tokyo University of Fisheries. Incubating and rearing temperatures ranged from 18.5 to 26.5℃ (mean, 21.0℃). Larvae and juveniles were fed with *Artemia* nauplii. About five specimens were sampled at one- or two-day intervals starting from hatching until 40 days after hatching. They were preserved in 5% buffered formalin. A total of 170 specimens ranging from 1.9 mm in notochord length (NL) to 12.6 mm in standard length (SL) was used. Clearing and staining followed Dingerkus and Uhler (1977). Observations and illustrations were made by means of a dissecting microscope equipped with a camera lucida.

The terminology of the caudal skeleton follows Fujita (1990).

Results

The caudal complex of *Takifugu niphobles* consists of an epural, an autogenous parhypural, the first and second hypurals, which are fused with the urostyle at the proximal base, an autogenous, compound, third hypural, expanded, large neural and hemal spines of the second preural centrum, and 11 caudal rays (5 in upper and 6 in lower lobe) (Fujita, 1990).

There were no caudal elements, except for the straight notochord, in larvae immediately after hatching (about 1.9 mm NL). Specimens between 1.9 and 3.8 mm NL had a straight notochord. Notochord flexion had occurred in some larvae from 4.0 to 4.7 mm NL. All specimens of 4.9 mm NL and larger had a flexing or flexed notochord.

Caudal rays with a count of 5 (2 upper, 3 lower) first appeared in some larvae of 3.6 to 4.3 mm NL, before notochord flexion (Fig. 1D, E). The rays were formed ventrally to the cartilaginous, compound, first and second hypurals (HY1+2) and the cartilaginous third hypural, which fused with the posterior hypurals (HY3+). Caudal rays increased in number during notochord flexion, and 10 rays (4 upper, 6 lower) were seen in specimens ranging from 4.6 to 5.3 mm NL (Fig. 1G, H). The full complement of caudal rays, 11 (5 upper, 6 lower), had been attained after full notochord flexion in some 5.0 mm SL larvae (Fig. 1I).

Two hypurals, the first and second (HY1 and HY2), developed in cartilage beneath the straight notochord at 2.9 mm NL (Fig. 1A). A cartilaginous parhypural (PH) was present just in front of the first hypural at 3.0 mm NL (Fig. 1B). The first and second hypurals were fused distally with each other, and proximally with the parhypural in a 3.4 mm NL larva. Posterior to the compound cartilage was the cartilaginous third hypural, which was fused with the posterior hypurals (HY3+) (Fig. 1C). The middle sections of the compound hypural (HY1+2) and HY3+ had begun to ossify in a 5.1 mm NL larva (Fig. 1G), and were nearly fully ossified in some 6.3 mm SL larvae (Fig. 1L). During such development among some specimens, the cartilaginous parhypural became fused to the posterior portion of the hemal spine of PU2 (Fig. 1H). This fusion is considered aberrant.

The epural first developed as a small cartilage above the point of flexion of the notochord at 5.3 mm NL (Fig. 1H). During and after notochord flexion, the cartilaginous epural became larger, and had started to ossify in some 6.3 mm SL specimens (Fig. 1L).

A cartilaginous hemal arch of the second preural centrum (HAPU2) had appeared just anterior to the

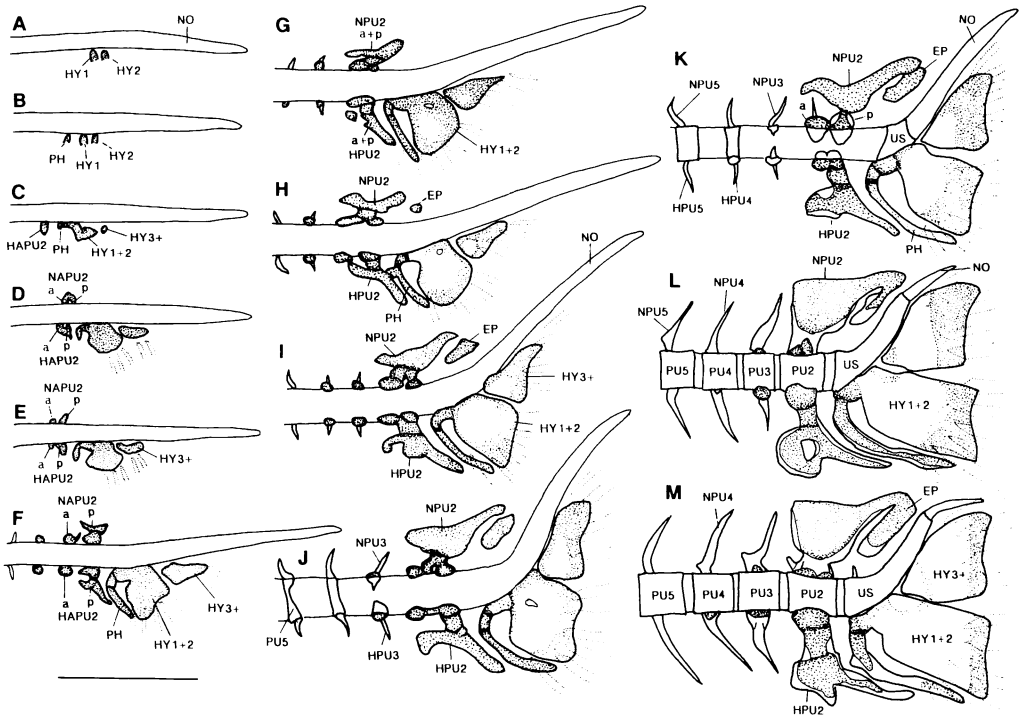

Fig. 1. Development of the caudal skeleton of *Takifugu niphobles*. A: 2.9 mm NL. B: 3.0 mm NL. C: 3.4 mm NL. D: 3.6 mm NL. E: 4.1 mm NL. F: 4.7 mm NL. G: 5.1 mm NL. H: 5.3 mm NL. I: 5.0 mm SL. J: 5.8 mm SL. K: 6.1 mm SL. L: 6.3 mm SL. M: 7.1 mm SL. EP, epural; HY1+2, hypural 1 plus hypural 2; HY3+, hypural 3 plus hypurals of upper lobe; HAPU2 a, anterior hemal arch of PU2; HAPU2 p, posterior hemal arch of PU2; HPU, hemal spine of preural centrum; NAPU2 a, anterior neural arch of PU2; NAPU2 p, posterior neural arch of PU2; NPU, neural spine of preural centrum; NO, notochord; PH, parhypural; PU, preural centrum; US, urostyle. Dense dots indicate cartilage. Sparse dots indicate intermediate condition between cartilage and bone. Undotted areas, except for notochord, indicate bone. Scale bar indicates 0.5 mm.

compound cartilage (PH+HY1+2) in a 3.4 mm NL larva (Fig. 1C). The cartilaginous neural and hemal arches developed on and beneath the straight notochord just anterior to the parhypural at 3.6 mm NL (Fig. 1D). These arches corresponded to the neural and hemal arches of the second preural centrum (NAPU2 and HAPU2). They had two proximal bases, anteriorly and posteriorly (Fig. 1D, a and p). In a 4.1 mm NL larva (Fig. 1E), another small cartilage was recognizable close to each of NAPU2 and HAPU2. The posterior neural and hemal arches (p) of PU2 subsequently grew longer, and cartilages corresponding to the neural and hemal arches of the third preural centrum (NAPU3 and HAPU3) developed on and beneath the flexing notochord (Fig. 1F). During notochord flexion, the anterior and posterior bases, and the neural and hemal arches of PU2 became fused, forming large cartilaginous neural and hemal spines (Fig. 1G, H, I and J). At this stage, the neural and hemal arches proceeding PU2 started to ossify at their tips. Preural centra, except for the second preural centrum and urostyle, had started to develop in a 5.8 mm SL specimen (Fig. 1J). In a 6.1 mm SL specimen (Fig. 1K), formation of PU2 had begun at the anterior and posterior bases of the neural and hemal arches. The urostyle had been formed, being fused to the proximal base of HY1+2. The length of the second preural centrum, with expanded neural and hemal spines, was twice as long as that of the third preural centrum at 6.3 mm SL (Fig. 1L). Also, it was formed from both anterior and posterior centers of ossification, which indicated that the second preural centrum is formed from two incipient centra. The third preural centrum was

equal in length to the second preural centrum in a larva of 7.1 mm SL (Fig. 1M).

With further development, the formation of the urostyle proceeded posteriorly to the entire proximal base of HY3+, and the notochord tip became shorter. The epural, parhypural, and expanded neural and hemal spines of PU2 ossified proximally from their peripheral areas and the two hypural plates became nearly fully ossified (Fig. 1L and M). In the largest specimen (12.6 mm SL) studied, the proximal base of the parhypural was separate to the lower hypural plate.

Remarks

According to Matsuura and Katsuragawa (1985), the larvae of the balistid, *Balistes capriscus*, have a urostyle consisting of two segments, indicating the likelihood of three original centra. However, during the ontogeny of the caudal skeleton of the tetraodontid, *Takifugu niphobles*, such segments could not be seen in the larval urostyle. A compound, third hypural (HY3+), probably consisting of three hypurals, occurs as one small cartilage, and the traces of fusion could not be confirmed. As in *Balistes capriscus*, there were no traces of uronurals during the ontogeny of *Takifugu niphobles*.

During the ontogeny of the caudal skeleton of *Takifugu niphobles*, the most interesting phenomenon is the development of the second preural centrum and its associated elements, the second preural vertebra apparently consisting of two fused vertebrae. Such a phenomenon has not been observed in *Balistes capriscus*. This evidence suggests that such fusion might be one of the means of reduction in vertebral number in advanced teleostean fishes.

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クサフグの尾部骨格の発達

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1990年6月9日に千葉県安房小湊でクサフグの卵を人工受精させ，その孵化仔魚を40日飼育し，定期的に仔稚魚をホルマリン固定し，軟骨・硬骨二重染色法で尾部骨格の発達を調べた．

尾部棒状骨は尾鰭椎前第1椎体と尾鰭椎とが癒合して形成されるとされているが，発達過程では尾部棒状骨が直接現れ，両者の癒合による形成の証拠は得られなかった．クサフグの成魚では尾鰭椎前第2椎体に著しく大きい神経棘および血管棘をそなえるが，それは個体発生の途上で2個の脊椎骨が癒合して形成されたためであることが明かとなった．

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