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Growth of the Medaka (VI) – Development of Oral Skeletons and Teeth During Metamorphosis

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ABSTRACT

The present paper describes observational results on development of the oral skeletons including general features of dentition in the period of metamorphosis in the medaka, *Oryzias latipes*. The oral teeth develop progressively in parallel with the development of the dentigerous dentary in the lower jaw and the dentigerous premaxilla and edentulous maxilla in the upper jaw. Shortly after hatching, the first two teeth in lower jaw already bud on the ossified thin dentary. On the other hand, the first teeth in the upper jaw are observed in the larva about 6.5 mm total length (TL). Newly formed replacement teeth bud in the inside or outside of the exiting tooth row in the upper and lower jaws. The developmental process of the structural changes in oral teeth and the skeletal elements that support the dentary and premaxilla was morphometrically examined in metamorphic phases from the larval to the juvenile. The size of the skeletons and teeth increases with ossification in proportion to body growth. After hatching, the ossification process of the oral skeletons advances in the following order: the dentary, maxilla, retroarticular, palatine, quadrate, premaxilla, anguloarticular and ethmoidal bones.

Keywords: oral skeleton, tooth, dentition, metamorphosis, medaka

INTRODUCTION

Oral teeth have undergone extensive morphological variations in the process of evolution, and each animal has its unique and very informative characters, respectively. Therefore, they are recently regarded as an excellent model for paleontological and developmental studies. However, a very small freshwater medaka fish *Oryzias latipes* does not remain as a fossil, so that no paleontological information is available.

In nonmammalian gnathostomes, the teeth are found on all the dermal bones in the oral-pharyngeal cavity. In many groups of fishes, adaptations for diverse manners of feeding involve in the size and structure of the mouth and teeth. The tooth structure of the jaws in the medaka also has been examined morphologically in different species (Parenti, 1987). Atherinomorph medaka (beloniform, cyprinodontiform and atherinoid; Rosen and Parenti, 1981) do not have large and strong teeth in the front of the jaws for grapsing the prey.

Although there are some reports on the partial or whole morphological observations of the head skeletons in the adult medaka (Kulkarni, 1940; Rosen, 1964; Iwamatsu and Hirata, 1980; Rosen and Parenti, 1981; Yabumoto and Ueno, 1984), few studies have been devoted to post-embryonic development. Specifically, the developmental process of the oral skeletons has not been described precisely. So far, there are some morphological investigations on sexual characters (Egami, 1957; Takeuchi, 1966; Yabumoto and Ueno, 1984; Parenti, 1987), development of the head skeleton (Langille and Hall, 1987, 1988), formation (Takeuchi, 1967b) and development (Debiais-Thibaud *et al*., 2007) of the oral teeth in the medaka. Recently, the genetic pathways involved in development of the oral and pharyngeal dentition have been investigated in the medaka (Atukorala *et al*., 2010/2011; Mantoku *et al*., 2016). These studies support the hypothesis that the pharyngeal and oral teeth are serially homologous.

The development of the whole head skeletons including oral regions has been morphologically examined in the medaka, *Oryzias latipes,* by Langille and Hall (1987). However, little attention has been paid to the development of the oral skeletons

and teeth. To obtain a detailed knowledge of the dynamics of the tooth pattern formation in odontogenesis, the time and order of appearance of teeth in jaws must be examined. The anatomical data of developing oral skeletons in the growing period from larva to young fish will help to answer intriguing questions such as the relationship between morphology and function of adult oral skeletons. Here, I tried to execute a morphometric examination on the anterior portion of the cranium in the medaka. The present anatomical description will also contribute to provide indispensable data for the analysis of gene expression which cannot be interpreted without a detailed information of well-defined dentition in the process of development during morphogenesis.

MATERIALS and METHODS

The medaka fish, *Oryzias latipes* (d-rR strain), used in the present study were reared in a rectangular glass aquarium (60×35×30 cm, 3 females and 2 males per about 60 liters of water) under reproductive conditions (L14, D10; 26–28°C). During the rearing period, fish were fed a balanced diet (see Iwamatsu, 2014). Under these conditions, mature females mate with males and spawn 20–30 eggs every morning. After females were netted, fingers were used to pluck off a cluster of chorion-hardened fertilized eggs that were hanging by chorionic filaments from their urogenital pores. Fertilized eggs were placed in petri dishes with water (26–27°C) containing methylene blue (0.05 mg/liter) to prevent fungal growth (Langille and Hall, 1988). After hatching, larvae were reared by being fed the balanced diet in rainwater containing green algae in a polycarbonate rat cage (26–28°C).

For skeletal preparation, fish were sacrificed by overdose of the anesthetic (saturated solution of phenylurethane 7: ethanol 3). After total length (TL) of deeply anesthetized fish was measured, larvae and juveniles were treated with 0.5% NaOH containing 0.5% formalin for about one hour at room temperature, prior to whole skeletal staining. The NaOH-treated specimens were adequately rinsed in water, and then stained with a diluted alizarin red solution for a few hours, and an excessive staining was destained by rinsing repeatedly in water. Then, the stained specimens were finally cleared in 50% glycerol for observation and morphometry. Skeletons were observed and measured using a stereoscopic microscope (Olympus SZX12) equipped with a calibrated ocular micrometer. Growth stages of fish were assigned following the developmental criteria (Iwamatsu, 1994; Iwamatsu, 2004; Iwamatsu et al., 2003). The mean values of each size of the bones morphometrically examined was represented graphically. Names used for skeletal structures follow Owen (1984), Yabumoto and Ueno (1984) and Parenti (1987).

OBSERVATIONAL RESULTS and DISCUSSION

Shortly before hatching, the mouth of embryos opens. Larvae just after hatching have no tooth in upper and lower jaws but can be fed with green algae, *Paramecium* and so on. As seen in the small mouth of the just-hatched medaka (Fig. 1A; 4.5 mm TL), the lower jaw projects slightly beyond the upper jaw as a characteristic feature of *Oryzias* species belonging to Beloniform (Parenti and Soeroto, 2004; Herder and Chapuis, 2010). At the time of hatching , the lateral size of the mouth is about 200 μm. Shortly after hatching, the lateral size (breadth) of the mouth opening is about 470 μm and the lower jaw possesses two small tooth buds. The breadth of the upper and lower jaws increases linearly in proportion to body size during metamorphic period (Fig. 2). Breadths of the upper and lower jaws increase somewhat speedily when TL is smaller than 10 mm TL and then increase on a parallel with each other.

Development of oral skeletons

Skeletal sketches of the jaws and jaw supports of the mouth part are representative for each ontogenetic stage in Fig. 1A–C.

Skeletal development of the oral region occurs progressively after hatching, since the mouth and teeth have a special role in feeding. The opening of the mouth becomes wider as the body of the medaka grows. In the early larva, ossification in the oral skeleton begins first in the dentary of the lower jaw. Shortly after the initiation of this ossification, subsequent ossification of the maxillae commences in the lateral sides of the upper jaw, followed by the ossification of the premaxillae which form

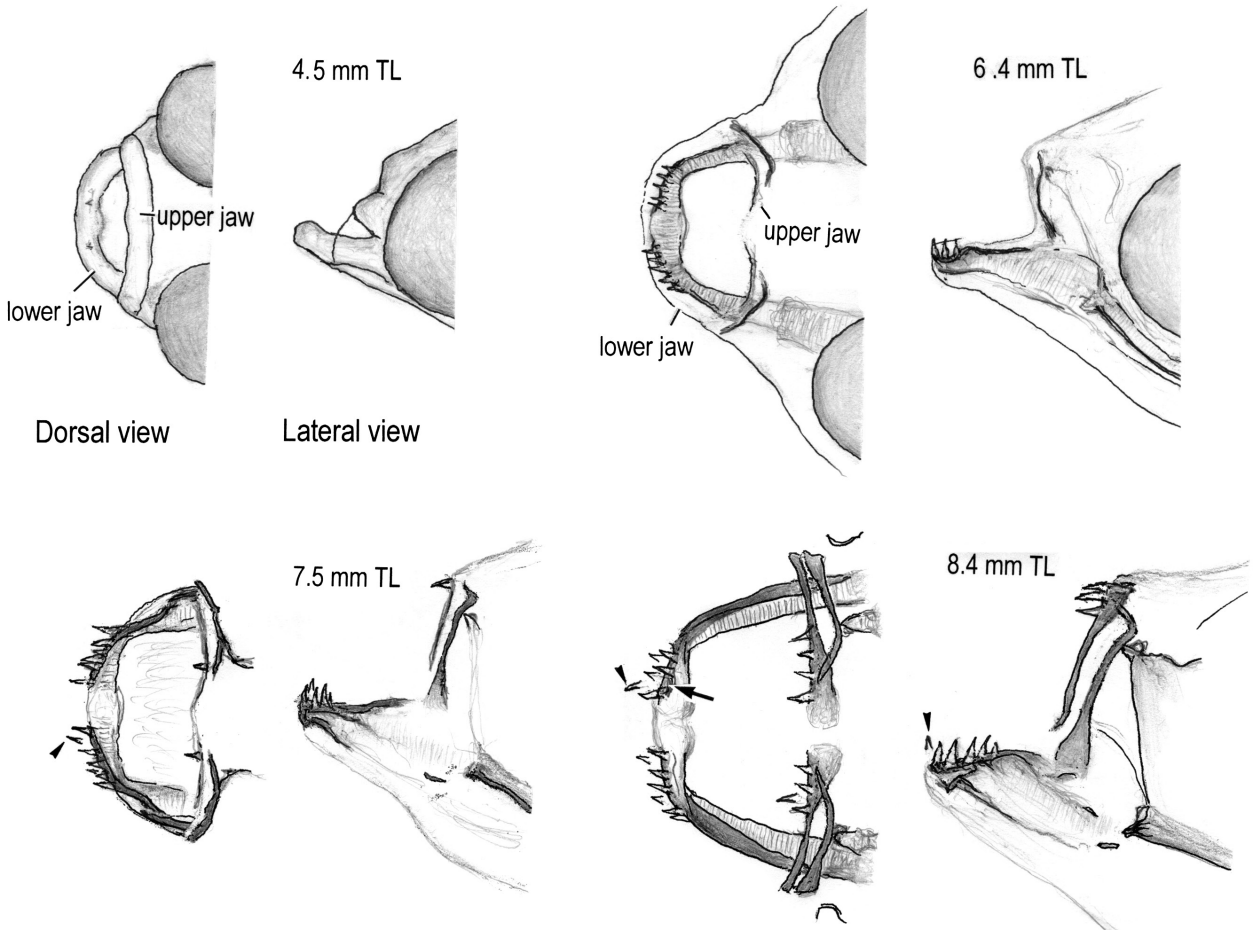

Fig. 1A Diagrammatic representation of skeletons of jaws and jaw suspensions in the growth period (4.5–8.4 mm TL). Note the position of a lost tooth (arrow) and newly erupted tooth (arrowhead).

the anterior region of the upper jaw. The early dentary is a thin bone that extends along the external edge of the lower jaw. Teeth (ca. 3.6 in number) in the lower jaw erupt in larvae of 4.3–4.6 mm in TL soon after hatching. When larvae grow to about 5.8 mm TL, three tooth buds form both in the ossified thin left and right dentaries.

The ossified maxillae first appear along the upper jaw, when TL of larvae becomes 5.0–5.4 mm. Subsequently, the slender remaxillae become observable in the anterior sides of the left and right maxillae. Thus, the maxillae and premaxillae with teeth form in the upper jaw of the larva of about 7 mm TL (Fig. 1A). The length of these bones increases in proportion to the body length (Fig. 3). The left and right maxillae that located at the posterolateral sides of the premaxilla remain to be laterally separated. At this stage, the distal tip of the cartilaginous palatine (autopalatine; Parenti, 1981) attaches to the mid region of the maxilla. The left and right maxillae curve at this mid region forming a joint with the anterior process of the palatine that forms a support for the maxilla. In the larvae at the end of this stage, the distal tip of the palatine capped with an articular cartilage can be barely detectable in contact with the bending point of the thinner maxilla (8.4 mm TL in Fig. 1A). As represented diagrammatically in the upper jaw in the subfamilies, Fundulinae and Rivulinae of the family Cyprinodontidae (Parenti, 1981), the ventral processes of the maxillaries lie beneath the flat and broad ascending processes of premaxillaries, and the both tips of the processes remain separated in midline (Fig. 1B–C). By the end of early juvenile phase, the palatine completes ossification and its articular tip fixed to the maxilla, which functions as a brace during the forward movement of the premaxillaries (Parenti, 1981).

The symphyses of the left and right dentaries and premaxillae are formed by a connecting cartilage on the adjacent side of these bones (Parenti, 1987). Until the ossified premaxillae appear in the larvae of about 6.5 mm TL, teeth in the upper jaw

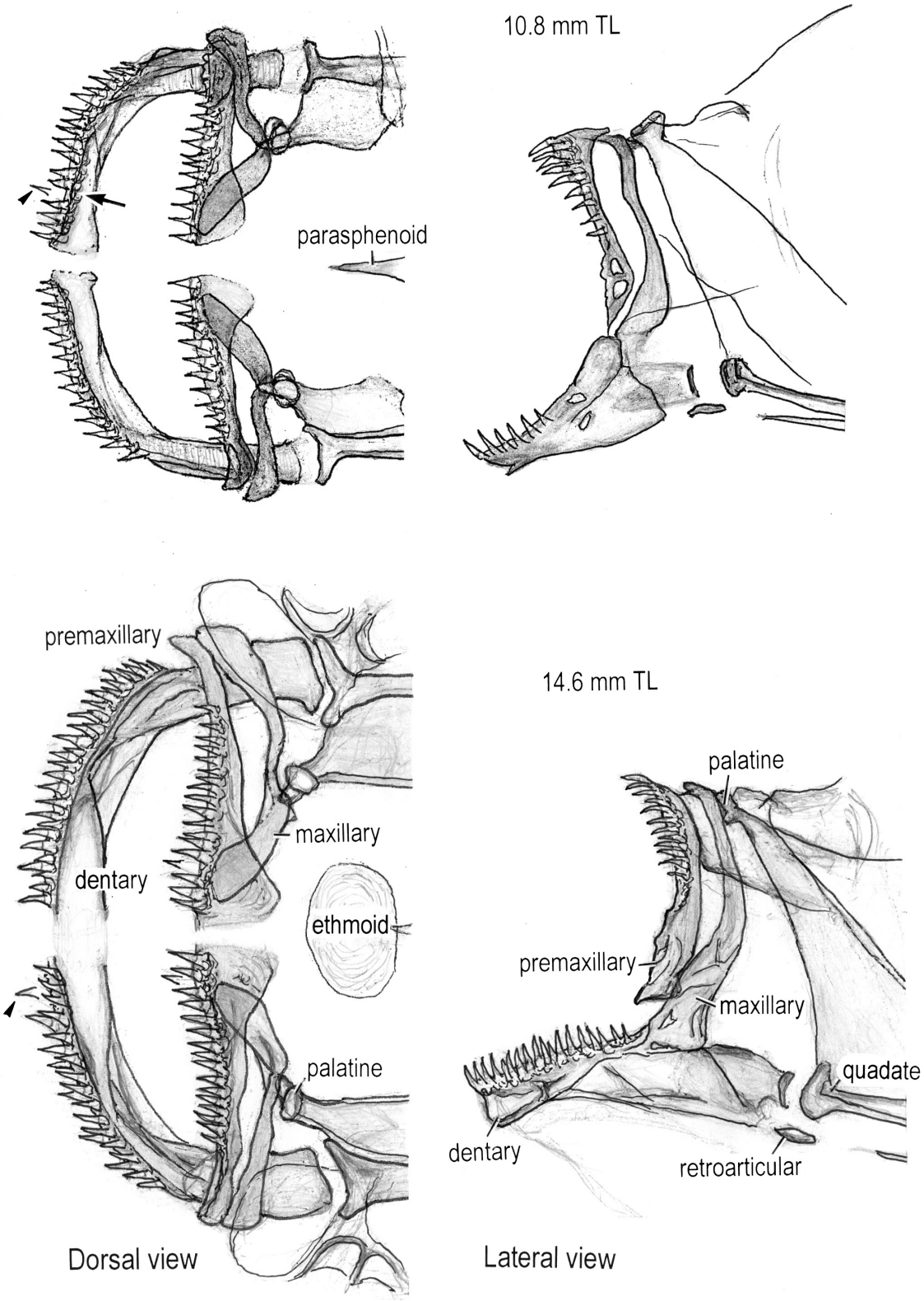

Fig. 1B Diagrammatic representation of skeletons of jaws and jaw suspensions in the growth period (10.8–14.5 mm TL).

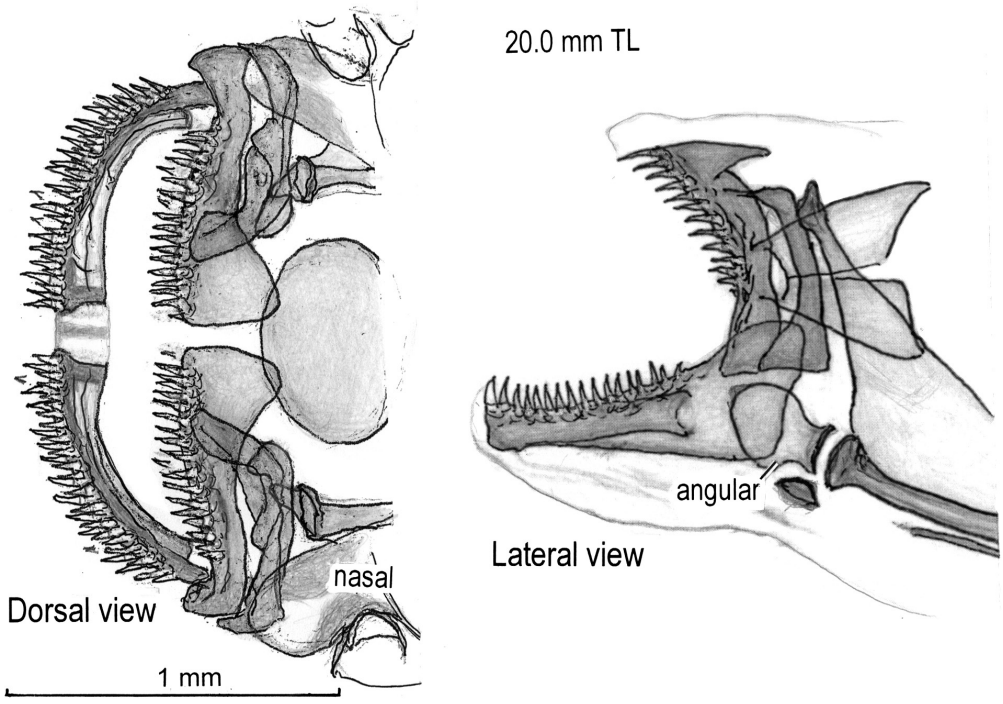

Fig. 1C Diagrammatic representation of skeletons of jaws and jaw suspensions in the growth period (20.0 mm TL).

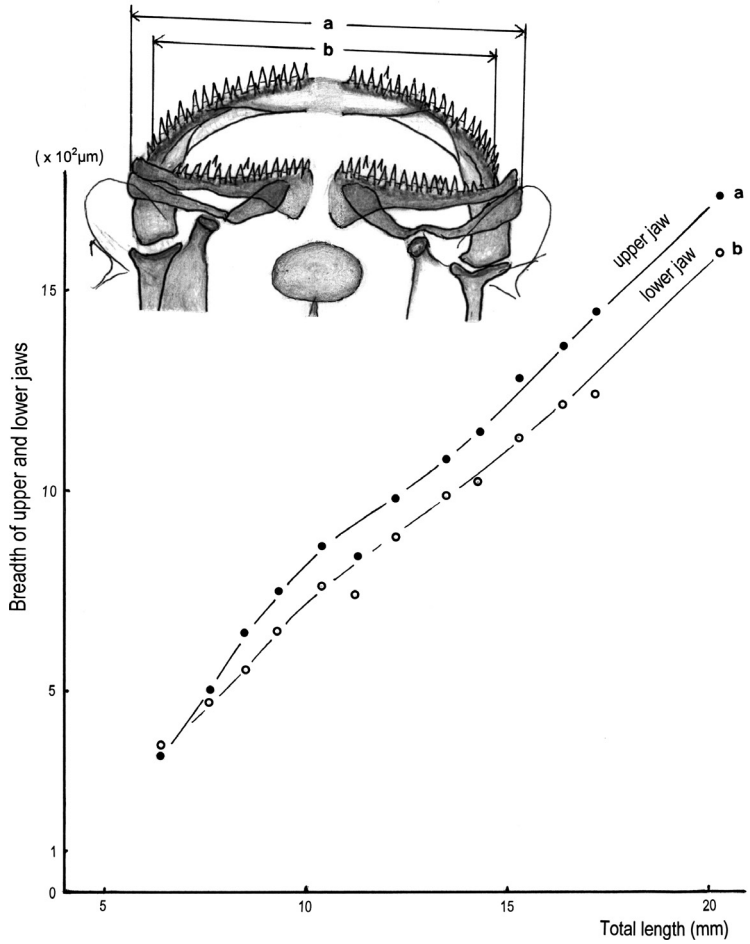

Fig. 2 Change in breadth of upper and lower jaws in the growth period.

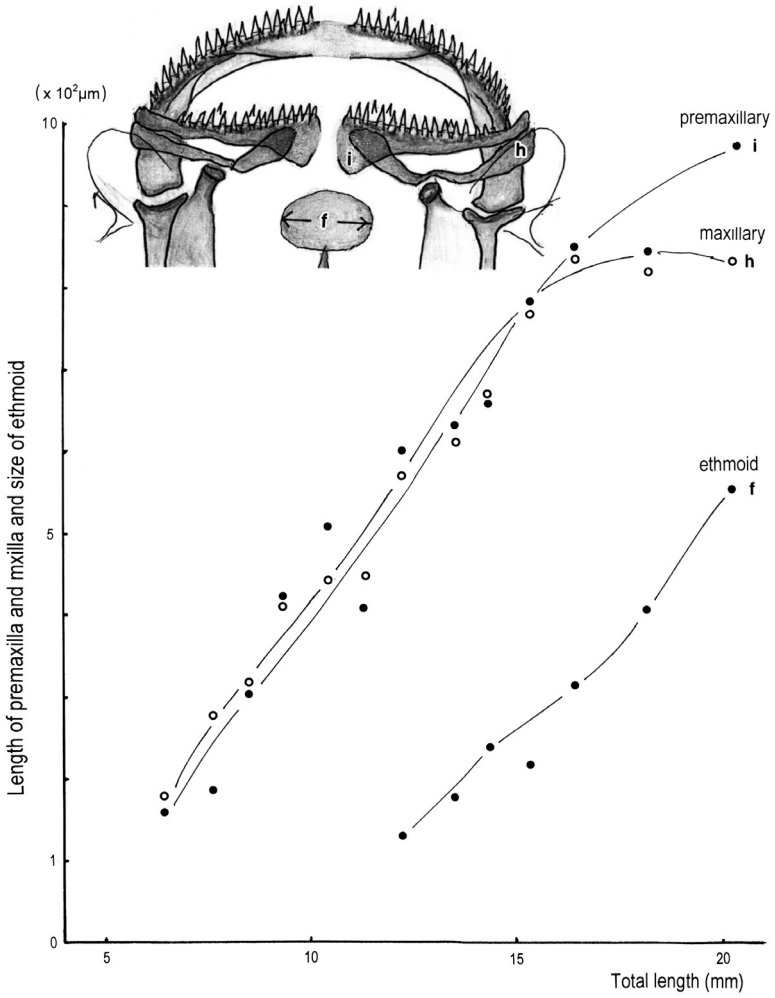

Fig. 3 Change in length of premaxilla and maxilla and size of ethmoid in the growth period.

do not erupt. In the larvae of more than 7 mm TL, the caniform teeth are observable in the mid region of the lower (ca. 10 in number) and upper (ca. 4 in number) jaws. In these larvae, premaxillae and dentaries with two irregular rows of the caniform teeth join at their lateral ends. When larvae grow up about 7 mm TL, the faint retro-articular is already detectable in front of the quadrate (Fig. 1A). In the larva of 8.4 mm TL, the maxilla bends slightly at the articular point where the distal tip of the palatine form a joint (Fig. 1A). The ossification of the angular bones also commences in the larvae about 10 mm TL. The ossified angular bone that articulates with the anterior region of the quadrate supports the posterior region of the dentary and can be recognized in larvae about 11 mm TL (Fig. 1B). Ossification of the flat maxilla progresses toward lateral sides and spreads to the central region of the upper jaw (Fig. 1A). In the advanced growth stages (10–15 mm TL), the anterior edges of the left and right premaxillae meet each other by further expansion and a plate of cartilage is produced in the interspace between them (Fig. 1B). In these stages, the anterior part of maxilla attaches beneath the central region of the expanded flat premaxilla. The ethmoid cartilage commences ossification and expands in the larvae of more than about 13 mm TL (Figs. 1B–C and 3). In the upper jaw of the juveniles 14.6 mm TL, the faintly ossified ethmoid plate appears as a flattened disc in the midline of the body and the ossified parasphenoid attaches to it (Fig. 1B). The expanded median ends of the paired membranous premaxillae nearly contact with each other. As described above, the ossification of cartilage bones in oral skeletons (the dentary, maxillary, retroarticular, palatine, quadrate, premaxillary, anguloarticular and ethmoidal bones) proceeds in sequence from the end of growth stage 40. Morphometric measurements of the growth of these skeletons are graphically represented in Figs. 2–5.

Increases in size of the premaxillae and maxillae are very similar during the growth (Fig. 3). The cartilaginous gap-space between the left and right premaxillae becomes somewhat narrower until the larvae grow to 9 mm in TL, whereas the gap between the left and right dentaries remains almost unchanged during metamorphosis (Fig. 4). The gap between the left and right anterior tips of palatines remarkably becomes wider, whereas the gap between the left and right maxillae increases just slightly. At the end of metamorphosis, the lower jaw consists of a dentigerous dentary, and the upper oral jaw is composed of edentulous maxillae and dentigerous premaxilla, as shown in Fig. 1C. The dentigerous dentary and premaxilla become supportive by their skeletal elements in young juveniles of about 10–15 mm TL (Fig. 1B). Parenti (1987) observed that Meckel's cartilage extends nearly the entire length of the dentary in 4–7 mm SL.

Development of the dentition

According to Parenti (1987), in adult medaka all oral teeth have a fibrous connection to the pedicels of the attachment bone. Premaxillary teeth have a ring of unmineralized collagen between the dentine cone and bone of attachment. As

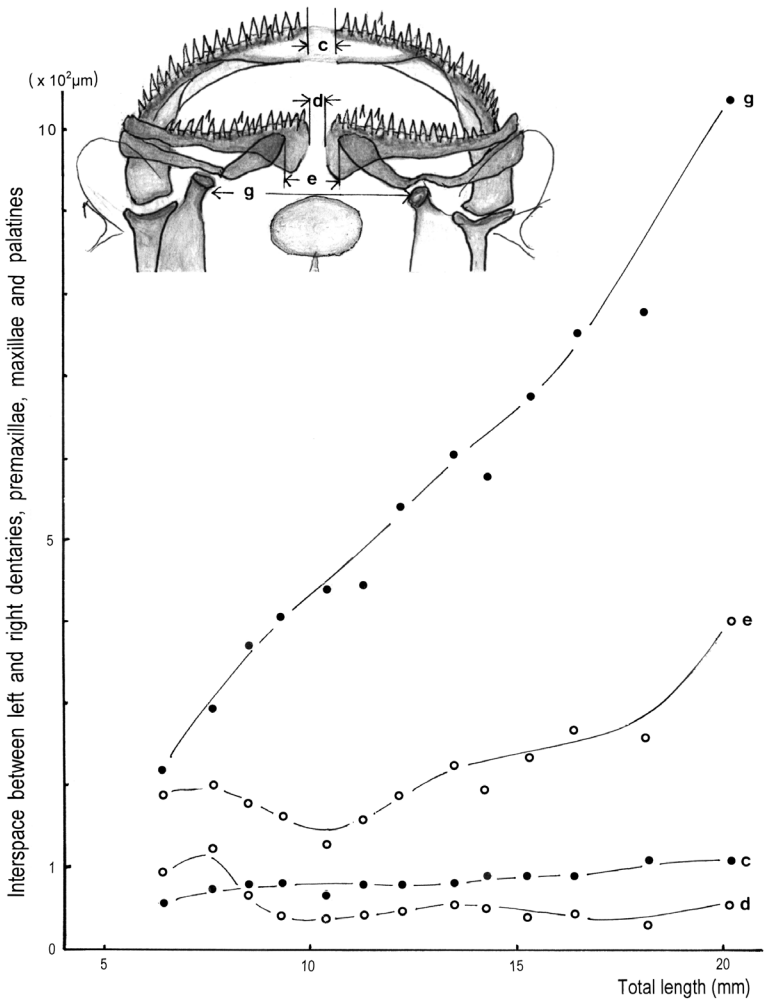

Fig. 4 Change in the interspace between left and right dentaries, premaxillae, maxillae and palatines in the growth period.

described previously, the lower jaw of a few larvae (4.5 mm TL just after hatching) already possesses two small tooth buds prior to appearance of the alizarin red-stainable dentary. A tooth bud soon displays a long cone-shaped cap and barrel-shaped pedicel of the attachment bone with unmineralized collagen when a dentary is (about 40–50 μmin length) faintly stained with alizarin red. The first two teeth that are positioned in the middle part of the dentary are about 20 μm in whole length (about 12 μm in diameter), in the larvae larger than 4.5 mm TL. The first or replacement tooth of the dentary and premaxilla is observed as an enamel-capped bud on a faint-stainable premaxilla in the larvae of about 7.5 mm TL (Figs. 1A and 6). According to Debiais-Tibaud *et al*. (2007), the first teeth of the upper jaw are observed as a dentine-capped buds between 16 and 17 days post-fertilization. In the premaxilla of larvae of about 6 mm TL in which uniserial teeth erupt, the root of early oral teeth (stage 6 tooth development by Takeuchi) attaches to the dentary by dentine that is not stained with alizarin red (Fig. 6). The whole length of teeth enlarges gradually up to about 100 μm (ca. 50–70 μm in long cap, dentine) in the juvenile of 15 mm TL and 200 μm (ca. 100 μm in long cap) in the adult (30 mm TL): the diameter of the pedicel also increases up to about 50 μm. This indicates that the teeth become larger with age. The mechanism of tooth growth (increase in tooth size) remains to be elucidated through understanding tooth development in molecular level.

The cone-shaped cap with enamel layer of tooth buds (Takeuchi, 1967) is stained with alizarin red. Normal developmental process of the tooth in the medaka can be divided into six stages by histological observations: (stage 1) the edge of the epithelium extends into the connective tissue and bud-like thickening appears, (stage 2) the papilla elongates and invaginates the tip (some odontoblasts appear in the hole made by the invagination, (stage 3) the enamel is formed, (stage 4) the formation of dentin starts, (stage 5) the crown is formed, and (stage 6) the root is formed and the crown erupts (finally, the base of the tooth attaches to the underlying bone) (Takauchi, 1967b). According to Debiais-Tibaud et al. (2007), oral teeth first appears anteriorly to the medial part of the lower jaw at aroud stage 36 (Matsui's stage; Yamamoto, 1975) and remained unattached until about 13 days post-fertilization. In this study, three steps of tooth developmental stages can be recognized under Nomarski light microscope after alizarin red staining: (i) the tooth bud is morphologically distinguishable but lacks mineralized tissue, (ii) a mineralized cap on the bud forms, (iii) the alizarin red-stained tooth attaches to the underlying bone. These teeth on the premaxilla and dentary are arranged in two zigzag tooth rows (distal and proximal rows). The unicuspid, conical teeth adjoin closely each other and form a queue on the dentaries and the premaxillae.

The sexual difference of teeth that are larger in the male than the female at the corner of the mouth is recognized in the individuals larger than 22 mm in TL (Takeuchi, 1966; Parenti, 1987). The large teeth on the posterior extent of the premaxilla and dentary of males (Egami, 1956; Takeuchi, 1966, 1967a; Yabumoto and Ueno, 1984; Parenti, 1987) appear after metamorphosis is completed.

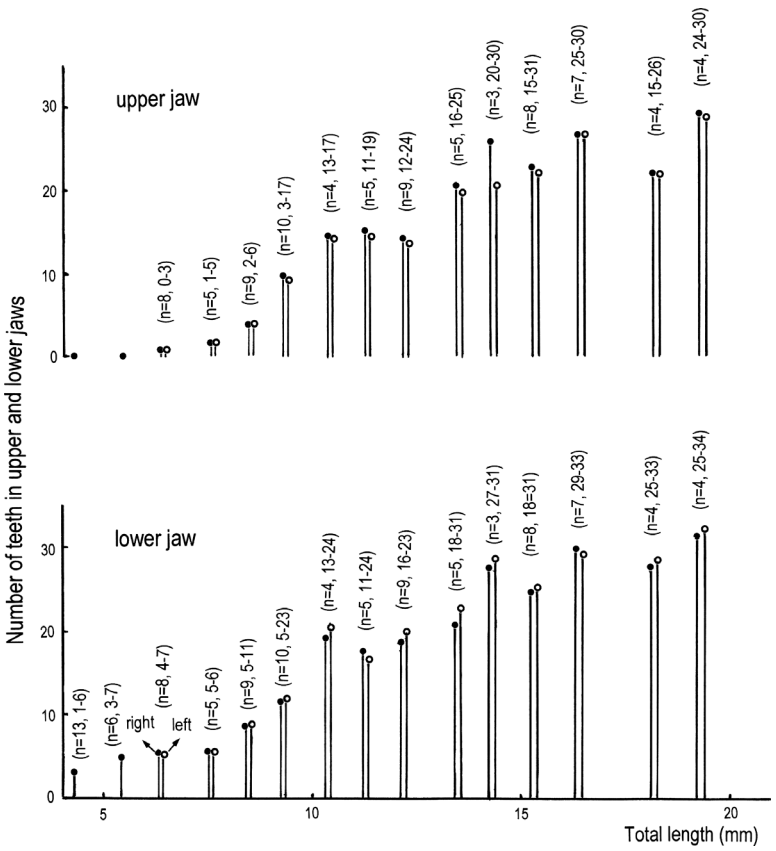

Fig. 5 Change in number of teeth in upper and lower jaws
Mean numbers of teeth in the left (open circle) and right (closed circle) dentary and premaxilla: the number in parentheses indicates the range of variation in tooth number.

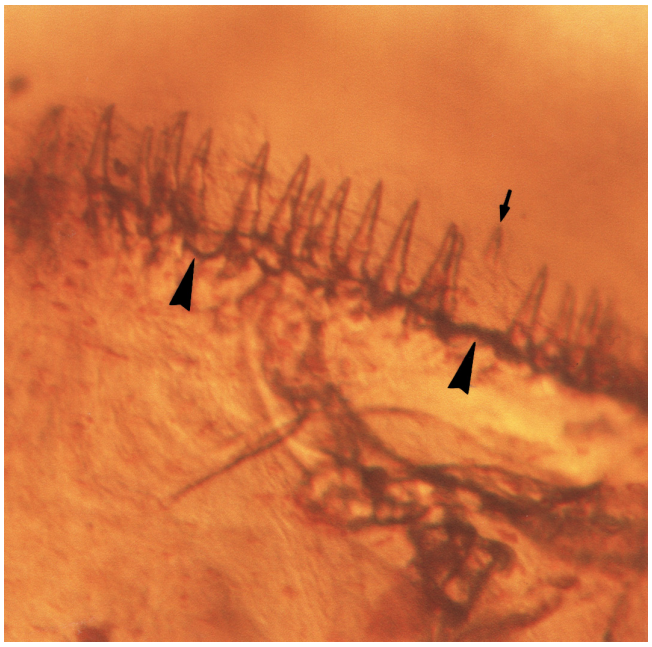

Fig. 6 Microphotograph of teeth in the premaxilla in young fish 15 mm TL. Arrowhead indicate the position of each lost tooth, and a newly budding tooth by a small arrow. X 120

Change in the number of teeth upon growth

Shortly after hatching, all larvae possessed two teeth on the lower jaw, as reported in Takeuchi (1966). Small larvae (4–5 mm TL) had 3–6 teeth on the lower jaw. In larvae about 6 mm TL, 4 teeth are seen in the left and right dentaries, respectively. New teeth are progressively added on the median and lateral margins of the dentary at the stages subsequent to hatching. The first teeth in the upper jaw appear in the larvae of more than 6.5 mm TL. In larvae about 7 mm TL, each dentary bears two staggered (zigzag) rows of unicuspid teeth (5–6 in number). Tooth replacement is observed before the tooth area in the dentary and premaxilla is fully occupied by teeth. The new teeth by replacement do not directly attach to the dentary but remain connected via the cartilage that is not stained with alizarin red at the early budding step. Additional replacement teeth separately protrude in the inner or outer side of the primary tooth row that bears larger teeth than the inner ones. In most cases, the replacement tooth is located at the same position as the lost tooth in rostrocaudal direction (Fig. 6). Growing fish retain a staggered pattern of the tooth row by the tooth replacement. In most fish, the mean number of teeth on the low and upper jaws increases linearly in proportion to the increase of TL, but varies remarkably among individuals (Fig. 5). This implies that tooth replacement rate may not increase with age. According to Takeuchi (1966), the number of small teeth of adult medaka is slightly greater in the female than in the male, but no sexual difference in number is recognizable in young medaka. This is consistent with the present observational results. No recognizable difference in the number between teeth of left and right dentaries and premaxillae is also observed (Fig. 5). The newly added teeth at the medial cartilage side at which the left and right premaxillae adjoin, first form around the period of sexual maturation. In young fish of 20–20.7 mm in TL, the averages of tooth number in the upper and lower jaws are 56 and 58, respectively.

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REFERENCES

Atukorala, A.D.S., K. Inohaya, O. Baba, M.J. Tabata, R.A.R.K. Ratnayake, D. Abduweli, S. Kasuga, H. Mitani and Y. Takano (2010/2011) Scale and tooth phenotypes in medaka with a mutated ectodysplasin-A receptor: implications for the evolutionary origin of oral and pharyngeal teeth. *Arch. Histol. Cytol*., 73 (3): 139–148.

Debiais-Thibaud, M., V. Borday-Birraux, I. Germon and F. Bourrat (2007) Development of oral and pharyngeal teeth in the medaka (*Oryzias latipes*): Comparison of morphology and expression of *eve 1* gene. *J. Exp. Zool.* (Mol. Dev. Evol.), 308B: 693–708.

Egami, N. (1956) Notes on sexual difference of teeth of the fish, *Oryzias latipes*. *Jap. J. Zool.*, 12: 65–69.

Herder, F. and S. Chapuis (2010) *Oryzias hadiatyae*, a new species of ricefish (Atherinomorpha: Belonforms: Adrianichthyidae) endemic to Lake Masapi, Central Sulawesi, Indonesia. *Raf. Bull. Zool*., 58 (2): 269–280.

Iwamatsu, T. (1994) Stages of normal development in the medaka *Oryzias latipes*. *Zool. Sci*., 11: 825–839.

Iwamatsu, T. (2004) Stages of normal development in the medaka *Oryzias latipes*. *Mech. Develop*., **121**: 605–618.

Iwamatsu, T., H. Nakamura, K. Ozato and Y. Wakamatsu (2003) Normal growth of the "see-through" medaka. *Zool. Sci*., **20**: 607–615.

Iwamatsu, T. (2014) Growth of the medaka (III) — Formation of scales. *Bull. Aichi Univ. Educ.*, 63 (Nat. Sci.): 59–66.

Iwamatsu, T. and K. Hirata (1980) Comparative morphological study of three species of *Oryzias*. *Bull. Aichi Gakugei Univ. Educ.*, 29: 103–120. (In Japanese)

Kulkarni, C.V. (1940) On the systematic position, structural modifications, bionomics and development of remarkable new family of cyprinodont fishes from the province of Bombay. *Rec. Indian Mus*., 42: 379–423.

Langille, R.M. and B.K. Hall (1987) Development of the head skeleton of the Japanese medaka, *Oryzias latipes* (Teleostei). *J. Morph*., 193: 135–158.

Langille, R.M. and B.K. Hall (1988) Role of the neural crest in development of the cartilaginous cranial and visceral skeleton of the mdaka, *Oryzias latipes* (Teleostei). *Anat. Embryol.*, 1177: 297–305.

Mantoku, A., M. Chatani, K. Aono, K. Inohaya and A. Kudo (2016) Osteoblast and osteoclast behaviors in the turnover of attachment bones during medaka tooth replacement. *Dev. Biol.*, 409: 370–381.

Owen, R. (1984) On the anatomy of vertebrates: Fishes and reptiles. *Intern. Books & Period. Suppl. Serv.,* New York.

Parenti, L.R. (1981) A phylogenetic and biogeographic analysis of cyprinodoniform fishes (Teleostei, Atherinomorpha). *Bull. Amer. Mus. Nat. Hist*., 168: 341–557.

Parenti, L.R. (1987) Phylogenetic aspects of tooth and jaw structure of the medaka, *Oryzias latipes*, and other eloniform fishes. *J. Zool. Lond*., 211: 561–572.

Parenti, L.R. and B. Soeroto (2004) *Adrianichthys roseni* and *Oryzias nebulosus*, two new ricefishes (Atherinomorpha: Beloniformes: Adrinichthyidae) from Lake Poso, Sulawesi, Indonesia. *Ichthyol. Res*., 51: 10–19.

Rosen DE. (1964) The relationships and taxonomc position of the halfbeaks, killifishes, silversides, and their relatives. *Bull Amer Mus Nat Hist.,* 127: 219–268.

Rosen, D.E. and L.R. Parenti (1981) Relationships of *Oryzias*, and the groups of atherinomorph fish. *Am. Mus. Novit.*, No. 2719: 1–25.

Takeuchi, K. (1966) Sexual difference in medaka, *Oryzias latipes*, teeth. Zool. Mag., 75: 236–238.

Takeuchi, K. (1967a) Large tooth formation in female medaka, *Oryzias latipes*, given methyl testosterone. *J. dent. Res*., 46: 750.

Takeuchi, K. (1967b) Normal development of teeth of the medaka, *Oryzias latipes*. *Aichi-Gakuin, Shigaku-kaishi*, 5 (1): 81–83. (In Japanese)

Yabumoto, Y. and T. Ueno (1984) Ostelogy of the ricefish, *Oryzias latipes. Bull Kitakyushu Mus. Nat. Hist*., No. 5: 143–161.

Yamamoto, T. (1975) Medaka (Killifish): Biology and Strains. *Keigaku Publ. Co.*

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