

# Osteological Development of the Larvae and Juvenile of *Luciogobius grandis* (Pisces: Gobiidae)

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## Conflict of interests

The authors declare no potential conflict of interest.

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## Abstract

This study is intended to be used as the basic material for the taxonomic research by observing the stages of skeletal development of *Luciogobius grandis* larvae compared to the skeletal development patterns of the same fish family of Gobiidae. 3 days after hatching (DAH), the preflexion larvae was 4.01±0.11 mm (n=5) in average total length (TL) and the frontal began to ossify in the skull. 17 DAH, the advanced postflexion larvae was 5.37±0.05 mm (n=5) in average TL the supraoccipital and epiotic were ossified in the cranial bone. 36 DAH, the juvenile was 12.2±0.20 mm (n=5) in average TL and the urohyal was ossified in the hyoid arch. In addition to one hypural bone being ossified, the first, second, third and fourth were combined and were made three bone fragments and then, the bone ossification of all skeletons was completed.

**Keywords:** Gobiidae, Larvae, *Luciogobius grandis*, Osteological, Skeleton

## INTRODUCTION

The fish family Gobiidae are known as about 1,975 species in 275 genera around the world (Nelson et al., 2016), and about 76 species in 39 genera are reported in Korea (NIBR, 2017). *Luciogobius grandis* is a fish belonging to the family Perciformes Gobiidae, and is known to be distributed in the coastal and intertidal zone of the East and South Seas of Korea. (Kim et al., 2005).

The characteristics of osteological development of larvae is essential to detect and eliminate skeletal abnormalities occurring in early breeding in the process of seed production (Koumoundouros et al., 1997a, b), and, providing systematic basic traits of the young stage, the researches in this area are active (Mook, 1977; Potthoff et al., 1987, 1988; Potthoff & Tellock, 1993; Liu, 2001; Sfakianakis et al., 2004; Çoban et al., 2009).

The studies on *L. grandis* have done with the egg development and the larvae morphology development (Yun et al., 2008), and the studies on the skeleton of the fish family Gobiidae are *Chaenogobius laevis* (Kim & Han, 1989), *Periophthalmus cantonensis* (Lee, 1990), *L. guttatus* (Kim et al., 1992), *Tridentiger trigonocephalus* (Han et al., 2018), *T. obscurus* (Hwang et al., 2018). The fish family Gobiidae is difficult to classify its species because there are species that have the same habitats or are similar externally. *L. grandis* is very similar externally to *L. guttatus* and the taxonomic studies are

**Ethics approval**

This article does not require IRB/IACUC approval because there are no human and animal participants.

needed to identify differences between the two species. Therefore, this study is intended to be used as the basic material for the taxonomic research, as observing the stages of skeletal development of *L. grandis* larvae by the stages of growth, and comparing and examining the skeletal development patterns of the same fish family of Gobiidae.

## MATERIALS AND METHODS

### 1. Collection and species identification

The samples used in the study were collected from individuals showing allomothering and transported to the laboratory at the spawning site for spawned eggs and species identification under a rock in a small river connected to the sea located in Ocheon-dong, Yeosu-si, Jeollanam-do Korea in May 2006. It followed Kim et al. (2005) for the species identification.

### 2. Fertilized egg and rearing larvae

The fertilized eggs attached to the rock was housed in a square tank (45×30×20 cm), and was gently aerated by aeration after medicine bathing with 5% neutral formalin. It was maintained the breeding water temperature at 18 °C–21 °C (average 19.5±1.5 °C), and salinity at 30–33 psu (average 31.5±1.5 psu), from the salt concentration (YSI 556) was measured using a water quality meter. 21 days after hatching (DAH), it was fed *Brachionus rotundiformis* to the hatched larvae after completing yolk absorption, and fed 1–2 *Artemia* sp. nauplius per mL. From 42 DAH, feeding was carried out while mixing and feeding the microdiet particles (Dry feed, Jeilfeed, haman, Korea).

### 3. Skeleton staining

In order to observe the skeletal development of larvae, 5 specimens were collected at intervals of 3 to 10 days from just immediately after hatching to 51 days and measured their sizes to 0.01 mm with a profile projector (V-12B, Nikon, Tokyo, Japan), and, after fixing them in 5% neutral formalin, the tibia was stained according to the staining method of Kawamura & Hosoya (1991). The shape of the skeleton was sketched after observing with an anatomy microscope (NM-40, Nikon), and the name of each part of the skeleton was according to Kim et al. (1992).

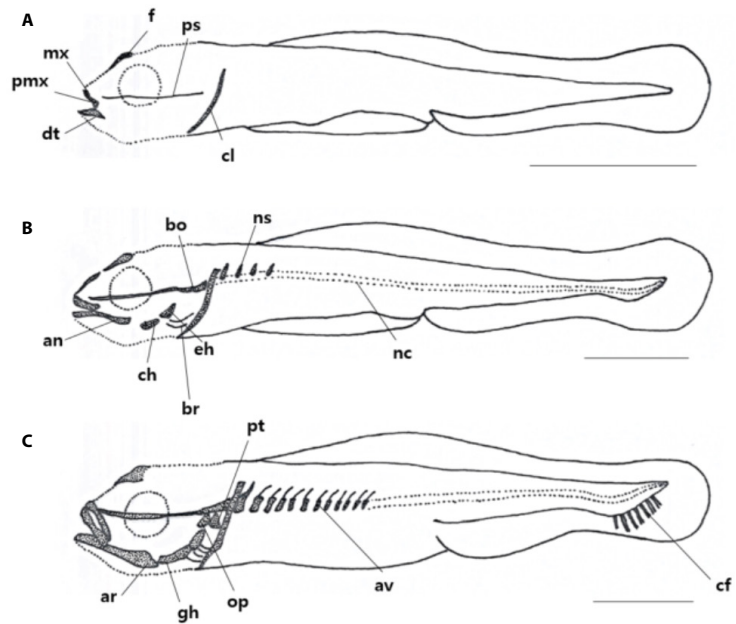
## RESULTS

### 1. Species identification

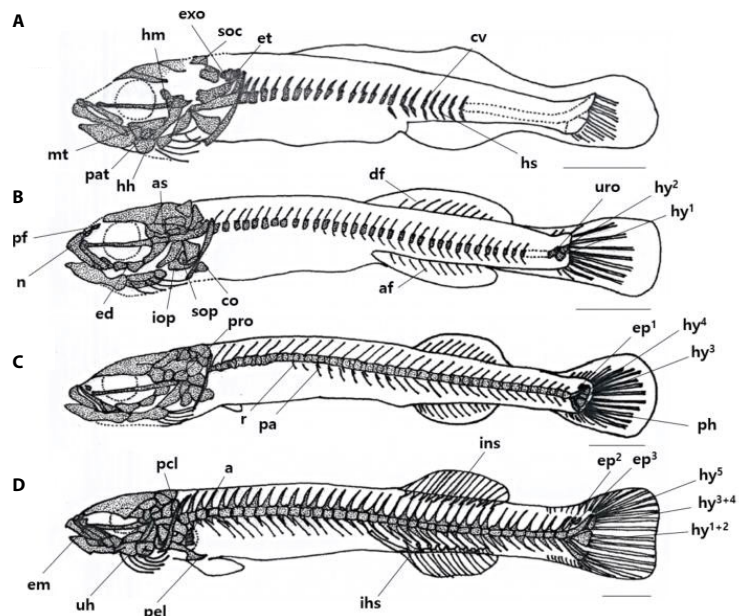
The head was a flat shape with an opercle, the dorsal fin was located at the back from the center, and the caudal fin edges were rounded. The meristic characters were 16 dorsal fins and 16 anal fins, and the lateral line scales were not observed. In the pectoral fin, two or more separated fin-rays appeared, showing a difference from the similar species, *Luciogobius guttatus* identified as *L. grandis* (Kim et al., 2005).

### 2. The osteological development of larvae and juvenile

The skeleton of *L. grandis* larvae was divided into cranial, shoulder girdle, vertebrae, and coccyx, and the development process of the growth stage was shown in Figs. 1, 2; Tables 1–3. On the 3 DAH, the preflexion larvae was 3.86–4.13 mm in total length (TL) (average 4.01±0.11 mm, n=5), and the frontal began to ossify in the skull, and the parasphenoid ossified across the eyeball. The premaxillary and maxillary of the upper jaw were ossified in the jaw bones with intake function and



**Fig. 1.** Development stage of larvae skeleton in *Luciogobius grandis*. (A) 3 days after hatching (DAH) preflexion larvae, 4.01 mm in total length (TL), (B) 8 DAH preflexion larvae, 4.87 mm in TL, (C) 11 DAH preflexion larvae, 5.37 mm in TL. an, angular; ar, articular; av, abdominal vertebrae; br, branchiostegal; bo, basioccipital; cf, caudal fin; ch, ceratohyal; cl, clavicle; dt, dantary; eh, epihyal; f, frontal; gh, grosshyal; mx, maxillary; nc, notochord; ns, neural spine; op, opercle; pmx, premaxillary; ps, parasphenoid; pt, pterotic. Scale bars=1.0 mm.



**Fig. 2.** Development stage of larvae and juvenile skeleton in *Luciogobius grandis*. (A) 17 days after hatching (DAH) postflexion larvae, 5.73 mm in total length (TL), (B) 21 DAH postflexion larvae, 7.04 mm in TL, (C) 29 DAH preflexion larvae, 11.4 mm in TL, (D) 36 DAH juvenile, 12.2 mm in TL. a, actinost; af, anal fin; as, alisphenoid; co, coracoid; cv, caudal vertebrae; df, dorsal fin; ed, endopterygoid; em, ethmoid; ep, epural bone; et, epiotic; exo, exoccipital; hh, hypohyal; hm, hyomandibular; hs, hemal spine; hy, hypural bone; ihs, interhemal spine; ins, interneural spine; iop, interopercle; mt, metapterygoid; n, nasal; pa, parapophysis; pel, pelvic girdle; ph, parhypural bone; pat, parietal; pcl, postclavicle; pf, prefrontal; pro, prootic; r, rib; soc, supraoccipital; sop, subopercle; uh, urohyal; uro, urostyle. Scale bars=1.0 mm.

**Table 1.** The development process of cranium and shoulder girdle of *Luciogobius grandis*

Variable	Days after hatching (Average total length, mm)							
	3 (4.01)	8 (4.87)	11 (5.37)	17 (5.73)	21 (7.04)	29 (11.4)	36 (12.2)	
Cranium	Parasphenoid	■	■	■	■	■	■	
	Basioccipital		■	■	■	■	■	
	Exoccipital			■	■	■	■	
	Frontal		■	■	■	■	■	
	Parietal			■	■	■	■	
	Epiotic				■	■	■	
	Supraoccipital				■	■	■	
	Nasal					■	■	
	Alisphenoid					■	■	
	Prootic					■	■	
	Prefrontal					■	■	
	Lateral ethmoid						■	
	Shoulder girdle	Clavicle	■	■	■	■	■	■
		Coracoid					■	■
Pelvic girdle							■	
Actinost							■	
Post clavicle							■	

**Table 2.** The development process of vertebrae and caudal skeleton of *Luciogobius grandis*

Variable	Days after hatching (Average total length, mm)							
	3 (4.01)	8 (4.87)	11 (5.37)	17 (5.73)	21 (7.04)	29 (11.4)	36 (12.2)	
Vertebrae	Notochord		■	■	■	■	■	
	Abdominal vertebrae		■	■	■	■	■	
	Neural spine		■	■	■	■	■	
	Caudal vertebrae			■	■	■	■	
	Hemal spine				■	■	■	
	Parapophysis				■	■	■	
	Rib					■	■	
	Interneural spine					■	■	
	Interhemal spine						■	
	Caudal skeleton	Urostyle					■	■
		Hypural bone					■	■
5 <sup>th</sup>							■	
3 <sup>rd</sup> -4 <sup>th</sup>						■	■	
1 <sup>st</sup> -2 <sup>nd</sup>						■	■	
Parhypural bone							■	
Epural bone							■	
1 <sup>st</sup>						■		
2 <sup>nd</sup> -3 <sup>rd</sup>						■		

**Table 3.** The development process of visceral skeleton of *Luciogobius grandis*

Variable		Days after hatching (Average total length, mm)							
		3 (4.01)	8 (4.87)	11 (5.37)	17 (5.73)	21 (7.04)	29 (11.4)	36 (12.2)	
Visceral skeleton	Upper jaw	Premaxillary							
		Maxillary							
	Lower jaw	Dentary							
		Articular							
		Angular							
	Hyoid arch	Epihyal							
		Branchiostegal rays							
		Ceratohyal							
		Glossohyal							
		Hypohyal							
		Urohyal							
	Palate	Hyomandibular							
		Metapterygoid							
		Palatine							
		Endopterygoid							
	Opercular	Opercle							
		Subopercle							
		Interopercle							

the dentary was ossified in the lower jaw. The clavicle was ossified in the shoulder girdle bone (Fig. 1A).

On the 8 DAH, the preflexion larvae was 4.77–4.95 mm in TL (average 4.01±0.11 mm, n=5), the basioccipital was ossified in the cranial bone, the angular was ossified in the palate and set in the dentary bone of the lower jaw. The epihyal and ceratohyal were ossified in the palatoglossal arch, and two branchiostegal rays began to ossify under the upper hyoid bone. The vertebrae that forming the central axis of the body was not ossified and consisted of notochord, and the neural spine began to ossify above the abdominal vertebrae (Fig. 1B).

On the 11 DAH, the advanced postflexion larvae was 5.30–5.44 mm in TL (average 5.37±0.05 mm, n=5), the palatine was ossified in the cranial bone, the articular was ossified in the palate and set in the angular bone. The glossohyal was ossified in the palatoglossal arch, the opercle began to ossify in the branchial arch. The vertebrae which consisted of the spine began to develop as the abdominal bone ossified. The caudal fin rays at the distal end of the body began to develop (Fig. 1C).

On the 17 DAH, the advanced postflexion larvae was 5.62–5.85 mm in TL (average 5.37±0.05 mm, n=5), the supraoccipital and epiotic were ossified in the cranial bone, the hyomandibular, metapterygoid, palatine were ossified in the palate, and the hypohyal was ossified in the palatoglossal arch. In the vertebrae, the caudal vertebrae began to ossify with the hemal spine as the abdominal bone was ossified with the nerve toward the tail. (Fig. 2A).

On the 21 DAH, the advanced postflexion larvae was 6.91–7.17 mm in TL (average 7.04±0.10 mm, n=5), the prefrontal, alisphenoid, and nasal were ossified in the cranial bone, the endopterygoid was ossified in the palate, and the subopercle, interopercle were ossified under the opercle in the opercular.

The coracoid was ossified in the shoulder girdle bone, and the dorsal fin rays and anal fin rays

developed as the primordial fin was divided into dorsal, anal, and tail fin. In the coccyx, the vertebral end was bent upward, and the urostyle began to ossify, and at the bottom, two hypural bones ossified (Fig. 2B).

On the 29 DAH, the advanced postflexion larvae was 11.2–11.8 mm in TL (average  $11.4 \pm 0.24$  mm,  $n=5$ ), the prootic was ossified in the cranial bone, and the rib and parapophysis were ossified under the abdominal vertebra. In the coccyx, 2 hypural bones were additionally ossified, the parhypural bone was ossified under the first hypural bone, and 1 epural bone was ossified between the neural spine and urostyle (Fig. 2C).

On the 36 DAH, the juvenile was 12.0–12.5 mm in TL (average  $12.2 \pm 0.20$  mm,  $n=5$ ), the lateral ethmoid was ossified in the cranial bone, and the urohyal was ossified in the hyoid arch, and the pelvic girdle was ossified in the ventral.

The actinost and the post cleithrum were ossified in the shoulder girdle bone, and the interneural spine and the interhemal spine were ossified in the pterygiophore supporting the dorsal and anal fins. Two pural bones were additionally developed in the coccyx, and one hypural bone was ossified in addition, and then, the first, second, third, and fourth were combining and made of three bone fragments, and the bone ossification of all skeletons was completed (Fig. 2D).

## DISCUSSION

The skeletal development of *L. grandis* was not observed just immediately after hatching. While growing, on the 3 DAH when the average TL was 4.01 mm, the frontal and the parasphenoid in the cranial bone began to ossify. In this period, as the yolk absorption was completed, the feeding function developed and the premaxillary, maxillary, and dentary were ossified. In addition, swimming ability developed as the clavicle of the shoulder girdle bone ossified. The skeletal development of *T. trigonocephalus* (Han et al., 2018), the same fish family Gobiidae, was began when the average TL was 4.44 mm on 7 DAH. The skeletal developments were began when *L. guttatus* (Kim et al., 1992) was 5.50 mm in TL on 11 DAH, when *C. laevis* (Kim & Han, 1989) was 6.00 mm in TL on 9 DAH, and when *T. obscurus* (Hwang et al., 2018) was 3.62 mm in TL on 8 DAH.

The fish family Gobiidae has an egg yolk right after hatching, so it survives for a period of time without feeding, during this period, the mouth opens for feeding and the swimming ability develops, and it is known that the development of feeding function and the role of clavicle are related (Wagemans & Vandewalle, 1999).

The shoulder girdle bone of *L. grandis* developed in the order of clavicle, coracoid, actinost, and post cleithrum when the average TL was 4.01 mm on 3 DAH, *T. trigonocephalus* (Han et al., 2018), a fish family Gobiidae, was ossified in the order of clavicle, coracoid, and actinost when the average TL of 4.44 mm on 7 DAH, and *L. guttatus* (Kim et al., 1992) ossified in the order of clavicle, coracoid, scapula, and actinost when the average TL was 5.50 mm on the 11 DAH. *C. laevis* (Kim & Han, 1989) ossified in the order of clavicle, scapula, and actinost when the average TL was 6.30 mm on 13 DAH, *T. obscurus* (Hwang et al., 2018) ossified in the order of clavicle, actinost, coracoid, and scapula on 13 DAH, as it showed a slight difference according to the order of ossification, but it was a relatively similar developmental pattern.

*L. grandis* was observed to have one pore formed in the scapula when the average TL was 12.2 mm on 36 DAH, mostly observed in the same fish family Gobiidae, and the forming pores in the scapula is known as a taxonomic trait that appears only in *L. japonicus*, but is considered a general trait observed in various fish (Han et al., 2018).

Specifically, the number of vertebrae has been used as a taxonomical character (Yamada et al., 2009). Table 4 compares the number of vertebrae in fish of the genus *Luciogobius*. The vertebrae development of *L. grandis* began to abdominal vertebrae ossification from head to tail when it was 4.87 mm on the 8 DAH, after the neural spine ossified, and when the average TL is 5.37 mm on 11 DAH. *T. trigonocephalus* (Han et al., 2018), the same fish family Gobiidae, began to ossify abdominal vertebrae at an average TL of 5.02 mm on 10 DAH, and when *L. guttatus* had an average TL of 6.10 mm on 16 DAH, the neural spine was ossified simultaneously with the ossification of the abdominal vertebrae. In *C. laevis* (Kim & Han, 1989), the abdominal vertebrae and caudal vertebrae ossified simultaneously at an average TL of 6.00 mm on 9 DAH. In *T. obscurus* (Hwang et al., 2018), the neural spine ossified after abdominal vertebrae ossification at 3.96 mm on the 10 DAH. *L. grandis* developed abdominal vertebrae after neural spine development, *L. guttatus*, *T. trigonocephalus*, and *T. obscurus* developed abdominal vertebrae and ossified neural spine, and hemal spine, and in *C. laevis*, abdominal vertebrae and caudal vertebrae were ossified at the same time, showing a difference from the order of development of *L. grandis*.

The stems of dorsal fin and anal fin of *L. grandis* developed simultaneously when the average TL was 7.04 mm on 21 DAH, *T. trigonocephalus* (Han et al., 2018), the same fish family, began to develop the dorsal fin stem after the posterior dorsal fin development when the average TL is 6.40 mm on 16 DAH, *Mugilogobius abei* (Kim & Han, 1991) was when an average TL was 3.20 mm on 12 DAH, and *T. obscurus* (Hwang et al., 2018) began to develop stems on the dorsal and posterior fins on the back when the average TL was 7.99 mm on 28 DAH. *C. laevis* (Kim & Han, 1989) began to develop the stem of dorsal fin and anal fin when the average TL was 7.05 mm on 17 DAH, and *L. guttatus* (Kim et al., 1992) began to develop the stem of dorsal fin and anal fin with an average TL of 6.10 mm on 16 DAH. *L. grandis* had the same structure as *L. guttatus* with one dorsal and anal fin respectively, and the development patterns of them were similar.

*L. grandis* developed the fin stem at the latest among the same fish family, and the same fish families, *T. trigonocephalus*, *M. abei*, *C. laevis*, and *T. obscurus*, showed differences from the fin development process of *L. grandis* because the dorsal fin was divided into the front and back. In addition, the dorsal fin developed from the rear to the front and was similar to the general development pattern of *L. japonicus* (Johnson, 1984; Faustino & Power, 1999).

The pterygiophore development of *L. grandis* occurred after centrum development began, and

**Table 4.** Comparison of the number of vertebrae in fish of the genus *Luciogobius*

Species	Vertebrae number	Authors
<i>Luciogobius</i>		
<i>L. grandis</i> (Yeosu, Korea)	36–37	Present study
<i>L. grandis</i> (Shirahama, Japan)	40–42	Yamada et al. (2009)
<i>L. elongatus</i>	38–44	
<i>L. adapel</i>	50	
<i>L. platycephalus</i>	41–42	
<i>L. parvulus</i>	41–43	
<i>L. guttatus</i>	35–39	
<i>L. pallidus</i>	37	
<i>L. albus</i>	30–31	
<i>L. dormitoris</i>	36	
<i>L. brevipterus</i>	35	
<i>L. martellii</i>	33	

this developmental pattern appeared in the same fish family Gobiidae, and the pterygiophore was fully formed after completed the development of fin stem. The developing pterygiophore when the development of vertebrae and fins has been completed appears to be related to increased propulsion in fish swimming (Lee et al., 2001).

The coccyx of *L. grandis* consisted of the urostyle, the pural bone, the hypural bone, and the parhypural bone, the urostyle began to ossify when the average TL was 7.04 mm on the 21 DAH, it began to ossify that *T. trigonocephalus* (Han et al., 2018) was when the average TL was 9.32 mm on the 28 DAH, *L. guttatus* (Kim et al., 1992) was when the average TL was 7.60 mm on the 25 DAH, *C. laevis* (Kim & Han, 1989) was when the average TL was 7.25 mm on the 20 DAH, and *T. obscurus* (Hwang et al., 2018) was when the average TL was 7.99 mm on the 28 DAH, and *L. grandis* and *T. trigonocephalus* were similar in the period of urostyle and their TL.

The fusion of the hypural bone formed 3 hypural bones (1+2, 3+4, 5) when the average TL was 12.2 mm on the 36 DAH. It showed the differences as *T. trigonocephalus* (Han et al., 2018) formed three hypural bones (1+2, 3+4, 5), and *C. laevis* (Kim & Han, 1989) and *L. guttatus* (Kim et al., 1992) formed two hypural bones (1+2, 3+4+5).

Thus, as the shape of hypural bone appeared diverse in the same fish family Gobiidae, it is considered to be significant as the basic data for systematic research. The differences in the skeletal development of the larvae of *L. grandis* and *L. guttatus* are that *L. grandis* showed the faster ossification of the first skeleton, and the abdominal vertebra of vertebra *L. grandis* ossified after ossification of the neural spine, but, in *L. guttatus*, vertebrae and neural spine were ossified simultaneously and showed the difference. It could be verified the two similar species have exomorphic difference through the results of this study as *L. guttatus* was fused into two bone fragments, while the hypural bone of *L. grandis* was fused into three. In the future, the study of the skeletal development of larvae is thought to be used as a very significant basic data for understanding species identification and skeletal characteristics of an adult fish, and it seems that more researches have to be carried out to characterize the classification and skeletal characteristics of the fish family Gobiidae.

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