Thesis for the Degree of Master of Science

Morphological and osteological development of the larvae and juvenile of *Sebastes koreanus*

by

Hyo Jae Yu

Department of Marine Biology

The Graduate School

Pukyong National University

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[황해볼락（*Sebastes koreanus*) 자치어의 외부형태 및 골격 발달]

Advisor: Prof. Jin Koo Kim

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Hyo Jae Yu

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Approved by:

(Chairman) Dr. Ki Wan Nam

(Member) Dr. Chung Bae Kang

(Member) Dr. Jin Koo Kim

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황해볼락 (*Sebastes koreanus*) 자치어의 외부형태 및 골격 발달

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부경대학교 대학원 해양생물학과

요약

2011년부터 2013년 동안 황해에서 채집된 황해볼락(*Sebastes koreanus*) 자어[6.11–11.10 mm body length(BL)]와 치어(18.60 mm BL)를 대상으로 분자 동정을 실시하고, 성장에 따른 외부 형태 발달 및 내부 골격 발달을 상세히 기술하였다. 또한, 황해볼락 친어에서 얻은 부화 직전 알과 난황자어의 외부 형태를 자세하게 기재하였다. 황해볼락의 부화 직전 알은 구형을 띠고, 배체에는 어떠한 흑색소포도 없는 것이 특징이며, 난황자어 역시 복부 위쪽 일부를 제외하고 흑색소포가 나타나지 않았다. 척색장 6.74 mm 자어에서 처음으로 척색 말단의 굴곡이 시작되고 두부극이 나타나기 시작하였으며, 척색장 8.84 mm 자어에서 척색 말단의 굴곡이 완료되었다. 또한 척색장 7.66 mm 자어에서 등지느러미 및 뒷지느러미가 처음 분화되어 나타났다. 자어기부터 두정부와 복강에 점상 또는 선상의 흑색소포가 출현하였으며, 일부 개체는 후두부 뒤쪽으로 흑색소포가 나타나기도 하였으나, 이는 종내 개체변이로 사료된다. 치어기에는

체측에 3 개의 불분명한 흑색소포가 가로 띠를 형성하며, 등쪽과 배쪽에 뚜렷한 흑색소포가 분포하였다. 따라서 황해볼락 자치어는 흑색소포의 분포 및 형태에서 볼락속의 다른 종류와 뚜렷하게 구분되었다. 황해볼락의 최초 골화는 척색장 6.27 mm 자어에서 시작되었으며, 체장 18.60 mm 치어에서 모든 골격의 골화가 완성되었다. 최초 골격 발달은 악골, 새개부, 견대, 설궁에서 진행되었는데, 이는 섭식과 호흡, 유영에 필수적인 골격 요소에 우선적으로 골화되는 경향을 나타내었다. 또한, 두개골의 골화는 액골, 노정골, 익이골에서 최초로 시작되었으며, 이후 부설골과 기저후두골의 골화가 진행되었다. 치어에서 견대부의 견갑골과 가장 위쪽 사출골이 가깝게 연접해있지만, 아직 융합되지 않았다. 또한, 치어에서 미골의 하미축골이 서로 융합되어 3 개(1+2, 3+4, 5)로 골화되었다. 황해볼락의 초기 골격 발달에서 부설골과 상설골의 골화가 늦게 시작되는 점에서 다른 볼락속 및 쏨뱅이속 어류와 뚜렷한 차이를 보였다. 자치어의 형태 및 골격 발달 관점에서, 황해볼락은 다른 볼락속 어류와 달리 황해라는 독특한 환경에 고유하게 진화되어 온 분류군임을 확인할 수 있었다.

I. Introduction

The rockfish *Sebastes* belonging to the family Scorpaenidae (or Sebastidae sensu Nakabo 2013) comprises approximately 110 species worldwide (Nelson 2006). Of there, more than 30 species are found in the northwest Pacific, including in Korea (Kim 2011; Yu and Kim 2014) and Japan (Nakabo 2013). This genus is commercially important all over the world (Leet et al. 1992; Love et al. 2002; Kim et al. 2004; Nakabo 2013).

The rockfish is an ovoviviparous fish; as such, after mating, the mother releases free-swimming larvae which, after a pelagic stage, settle to the sea floor or on to drift algae (Helvey 1982; Boehlert and Yoklavich 1984; Shinomiya and Ezaki 1991; Love et al. 2002; Koya and Muñoz 2007). Generally, the larvae and juveniles of *Sebastes* have a relatively large head, strong spines on head or check, short preanal length, and tapering tail during larval stage, which are well distinguished from those of the other genera of the family Scorpaenidae (Matarese et al. 1989; Kendall 1991) or other familys (Carangidae, Serranidae, etc.) (Okiyama 2014). Larvae and juveniles of *Sebastes* species can be identified based on pigmentation pattern, head-spine development, and pectoral-fin shape (Moser et al. 1977; Kendall 1991). However, their identification on the basis of morphological features has difficult, as little or insufficient information at early life stages is available and significant intraspecific variations in phenotype exist (Sakuma et al. 2005). Therefore, the application of molecular techniques, such as DNA sequencing, can help resolve species identification problems (Rocha-Olivares et al. 2000; Taylor and Watson 2004; Kendall et al. 2007; Kim et al. 2010; Ko et al. 2013).

Osteological development in teleost fishes involves a sequence of remarkable morphological and functional changes, occurring in different developmental stages (Löffler et al. 2008; Kang et al. 2012; Ott et al. 2012). These ontogenetic changes strongly influence the feeding, breathing and swimming behaviors of both larvae and juveniles. These are therefore useful in functional and ecological analyses and inferences about phylogenetic relationships among teleost taxa (Omori et al. 1996; Faustino and Power 1999; Koumoundouros et al. 2000, 2001a, b; Liu 2001; Lima et al. 2013; Voskoboinikova and Kudryavtseva 2014). Practically speaking, an accurate knowledge of skeletal development is essential for the detection and elimination of skeletal deformities appearing during artificial seedling production, and to promote effective aquacultural and population assessment (Koumoundouros et al. 1997a, b).

Sebastes koreanus Kim and Lee 1994 was originally described as a new species by Kim and Lee (1994) based on specimens collected from the eastern margin of the Yellow Sea. However, in recent years, *S. koreanus* has been collected from the Shandong Peninsula, China (Choi and Yang 2008), and, accordingly, its distribution appears to be expanded to the western margin of the Yellow Sea. Although *S. koreanus* has been regarded as representative species in the Yellow Sea, few studies have been conducted, except for brief comments on its spawning season (Kim et al. 2004) and a report on the complete mtDNA sequence (Jang et al. 2014). Furthermore, the very restricted distributional range of the species may cause the collapse of the population, resulting in an environmental pollution and/or climate change in the Yellow Sea. In this respect, these need artificial seedling production for species conservation.

Therefore, the objective of the present study was to describe and compare the external morphological traits and osteological development of the wild-captured larvae and juvenile of *S. koreanus* revealed by molecular method with those of congeneric species. In addition, This study aims to provide morphological information on eggs, and yolk-sac larvae obtained from a gravid fish of *S. koreanus* and extrapolate the phylogenetic inferences based on their ontogenetic characteristics. In addition, we estimated the release season of *S. koreanus* larvae.

II. Materials and methods

1. Sample collection

All individuals were collected off the west coast of Korea, on the eastern margin of the Yellow Sea (Fig. 1). Larvae of *Sebastes koreanus* were collected off the Taean Peninsula in June 2011 [6.11–11.10 mm body length (BL), *n* = 32] and May 2013 (6.41–8.90 mm BL, *n* = 11), using a bongo net (0.6 m mouth opening, with 330 µm and 500 µm mesh size; bottom depth 15–24 m), and the juvenile of *Sebastes koreanus* (18.60 mm BL, *n* = 1) was collected off Gang-hwa-do in July 2012, using a stow net. The individuals were preserved in 99 % EtOH and 5% formalin immediately after collection. And the specimens fixed at formalin were washed with distilled water, and had preserved in 99 % EtOH. We also collected a gravid fish of *S. koreanus* [168.60 mm standard length(SL), *n* = 1] off Heuk-san-do in May 2013, using a long line. After transport to the laboratory under conditions of ice storage, the eggs and yolk-sac larvae were obtained by pressing on the abdomen of the gravid fish and upon release immediately preserved in 99 %

EtOH. The gravid fish was deposited at Pukyong National University (PKU), and the eggs, larvae, and juvenile were deposited at the Ichthyoplankton Laboratory of PKU (PKUI).

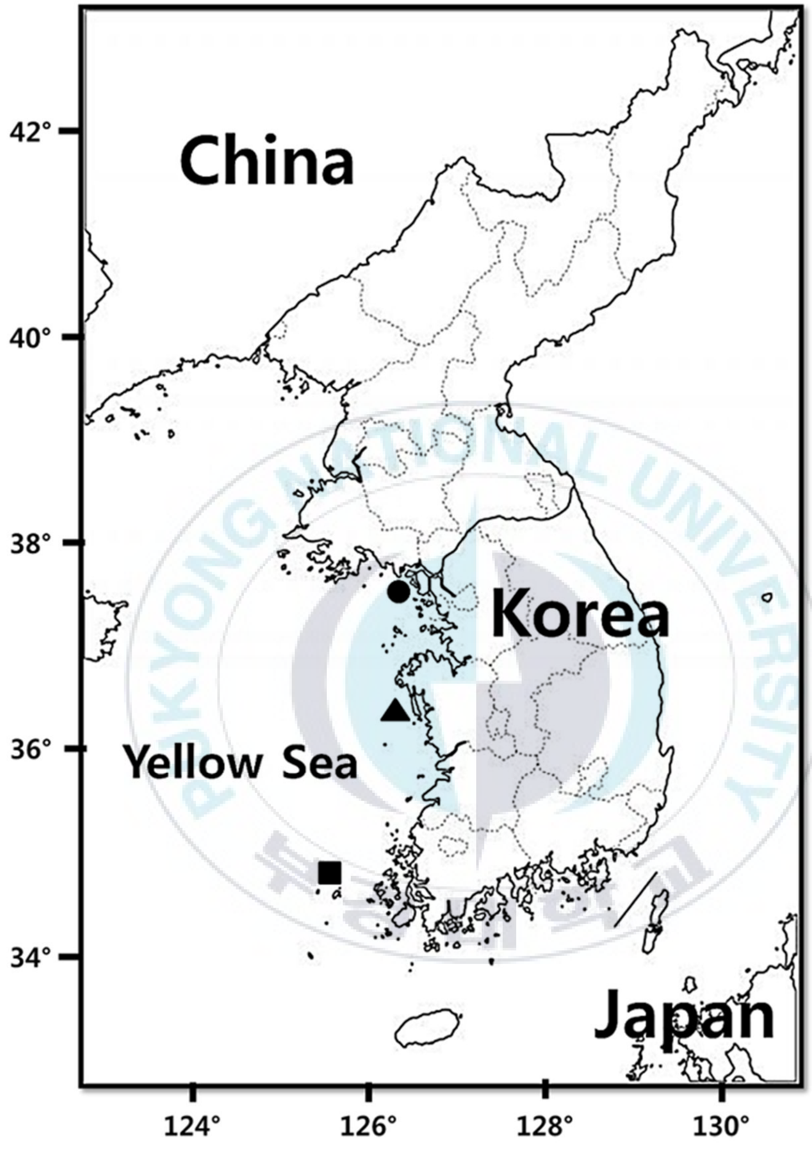

Fig. 1. Map showing the sampling sites. *Circle* indicates the sampling site of juvenile of *S. koreanus*. *Triangle* indicates the sampling site of larvae of *S. koreanus*. *Rectangle* indicates the sampling site of gravid fish of *S. koreanus*.

2. Molecular analyses

Total DNA was extracted from the right eyeballs of larvae (*n* = 9) and the juvenile (*n* = 1) using a DNA Extraction Kit (Bioneer Trade Co. LTD, Korea). A polymerase chain reaction (PCR) was used to amplify the mitochondrial DNA cytochrome oxidase subunit I (mtDNA COI) gene using the VF2 (5′- TCG ACT AAT CAT AAA GAT ATC GGC AC -3′) and FishR1 (5′- TAG ACT TCT GGG TGG CCA AAG AAT CA -3′) PCR primers (Ward et al. 2005). The PCR was performed using a Thermal Cycler (Bio-rad MJ mini PTC-1148, USA) with a PCR solution containing 2 μL of total DNA, 3 μL of 10 × PCR buffer, 2.4 μL of 2.5 mM dNTPs, 1 μL of each primer, 0.1 μL of EX *Taq* polymerase (BioMedics, Seoul, Korea), and distilled water, to bring the final volume to 30 μL. The conditions of the PCR were as follows: initial denaturation at 95 °C for 5 min, 35 cycles of denaturation at 95 °C for 1 min, annealing at 50 °C for 1 min, extension at 72 °C for 1 min, and a final extension at 72 °C for 5 min. The PCR products were purified using a Davinch^{TM} PCR Purification Kit (Davinch-K Co., Korea). The DNA was sequenced on an ABI 3730XL Sequencer (Applied Biosystems Inc., USA) using the ABI BigDye Terminator Cycle Sequencing Ready Reaction Kit v3.1 (Applied Biosystems Inc., USA). The mtDNA COI sequences were aligned using Clustal W (Thompson et al. 1994) in BioEdit version 7 (Hall 1999). Genetic distances among specimens were calculated using the Kimura two-parameter model (Kimura 1980) as implemented in the software MEGA version 6 (Tamura et al. 2013). The neighbor-joining tree (Saitou and Nei 1987) was constructed with 1,000 bootstrap replications. For molecular comparisons, we obtained the mtDNA COI sequences of 20 species of the family Scorpaenidae from PKU and the National Center for Biotechnology Information (NCBI) (see Appendix).

3. Morphological analyses

Morphological characters were observed using a stereo microscope (Olympus SZX16, Japan). The following measurements and counts were obtained according to the methods of Leis and Carson-Ewart (2000). The following body parts were measured to the nearest 0.01 mm: body length (BL), standard length (SL), head length (HL), preanal length (PAL), snout length (SnL), eye diameter (ED), and body depth (BD). Analysis of melanophore shapes and distributions followed Russell (1976), the terminology of head spination followed Moser and Ahlstrom

(1978), and the terminology of developmental stages followed Kim et al. (2011). Sketches of the external shapes of specimens were made using a camera lucida (Olympus SZX-DA, Japan) attached to a microscope. For comparisons, measurements and counts were made using adult specimens of *S. koreanus* (89.12–168.55 mm SL, *n* = 10), deposited at PKU. In addition, we subdivided the pigmentation patterns of preflexion larvae into 22 positions, a modification of the 26 positions suggested by Kendall and Lenarz (1986) (Fig. 2).

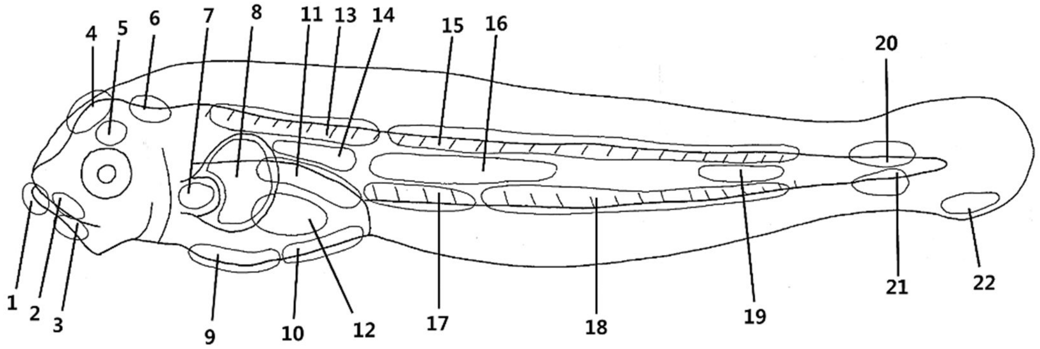

Fig. 2 Schematic drawing of a preflexion larva of *Sebastes* showing pigment positions. The numbering of positions is modified from Kendall and Lenarz (1986).

4. Skeletal staining

The skeletal staining technique was derived from the double staining protocol of Darias et al. (2010), and the anatomical terminology relating to skeletal structures follows Russell (1976). After staining, the specimens were examined on their right sides and dorsal sides with a stereomicroscope, and photographs taken with a camera lucida (Olympus SZX-DA, Japan) attached to the microscope. Drawings of the different skeletal parts were prepared from the photographs. We compared the skeletal structures of the larvae and the juvenile with those of adult *S. koreanus* specimens, to observe the precise locations and shapes of the skeletal elements. We also compared stained specimens with a stained *Sebastes inermis* complex juvenile (17.06 mm BL, n = 1) collected in the wild. The stained specimens were preserved in 100% glycerin in glass bottles, and were deposited at Pukyong National University (PKU).

5. Materials examined

i. Morphological development

Examined materials included eggs (1.42–1.77 mm egg diameter, *n* = 20; PKUI 188), yolk-sac larvae (3.59–4.36 mm BL, *n* = 20; PKUI 189), preflexion larvae (6.41–6.49 mm BL, *n* = 2; PKUI 190–191), flexion larvae (6.74–8.24 mm BL, *n* = 7; PKUI 192–198), postflexion larvae (8.84–8.90 mm BL, *n* = 2; PKUI 199–200), juvenile (18.60 mm BL, *n* = 1; PKUI 21), and adults of *S. koreanus* (89.12–168.55 mm SL, *n* = 10; PKU 2469, PKU 2471, PKU 2476, PKU 2479, PKU 4577, PKU 4583, PKU 4600, PKU 7138, PKU 7142, PKU 8815)

ii. Osteological development

Examined materials included preflexion larvae (6.11–6.27 mm BL, *n* = 2; PKUI 367–368), flexion larvae (6.43–8.40 mm BL, *n* = 19; PKUI 369–387), postflexion larvae (8.44–11.10 mm BL, *n* = 11; PKUI 388–398), juvenile (18.60 mm BL, *n* = 1; PKUI 21), and juvenile of *S. inermis* complex (17.06 mm BL, *n*=1; PKUI 399).

iii. Species examined in molecular comparisons

MtDNA COI sequences of 20 species of the family Scorpaenidae from PKU and the National Center for Biotechnology Information (NCBI)**.** *Sebastes koreanus* (PKU 2467), *Sebastes pachycephalus* (PKU 5733), *Sebastes nudus* (PKU 5728), *Sebastes hubbsi* (PKU 6), *Sebastes longispinis* (PKU 7), *Sebastes vulpes* (PKU 5727), *Sebastes zonatus* (PKU 4858), *Sebastes schlegelii* (PKU 1913), *Sebastes steindachneri* (PKU 1), *Sebastes inermis* complex (PKU 1204), *Sebastes thompsoni* (PKU 1065), *Sebastes owstoni* (PKU 10410), *Sebastes taczanowskii* (DQ678391), *Sebastes oblongus* (DQ678400), *Sebastes minor* (DQ638399), *Sebastes trivittatus* (DQ678389), *Sebastes matsubarae* (DQ678395), *Sebastiscus tertius* (PKU 2140, as an outgroup), *Sebastiscus marmoratus* (PKU 2166, as an outgroup), and *Scorpaena miostoma* (PKU 5718, as an outgroup).

III. Results

1. Molecular identification

A total of 578 base pairs of the mtDNA COI gene of nine larvae of *Sebastes* sp. and one juvenile of *Sebastes* sp. were compared with those of 20 other scorpaenid species. The resulting neighbor-joining tree showed that the 10 individuals clustered closely with adult of *S. koreanus* (pairwise genetic distance, *d* = 0.000–0.003) (Fig. 3). The 10 individuals differed from other species of *Sebastes*: *Sebastes pachycephalus* Temminck and Schlegel 1843 (*d* = 0.032–0.034), *Sebastes vulpes* Döderlein *in* Steindachner and Döderlein 1884 (*d* = 0.036–0.040), *Sebastes oblongus* Günther 1877 (*d* = 0.041–0.043), and *Sebastes taczanowskii* Steindachner 1880 (*d* = 0.045–0.047). The genetic distances between the 10 individuals and *Scorpaena miostoma* Günther 1877 were in the range of 0.194–0.196; these distances were greater than the distances between congeneric individuals. Based on the results of the DNA comparison, the 10 larval and juvenile individuals examined in this study were identified as *S. koreanus*.

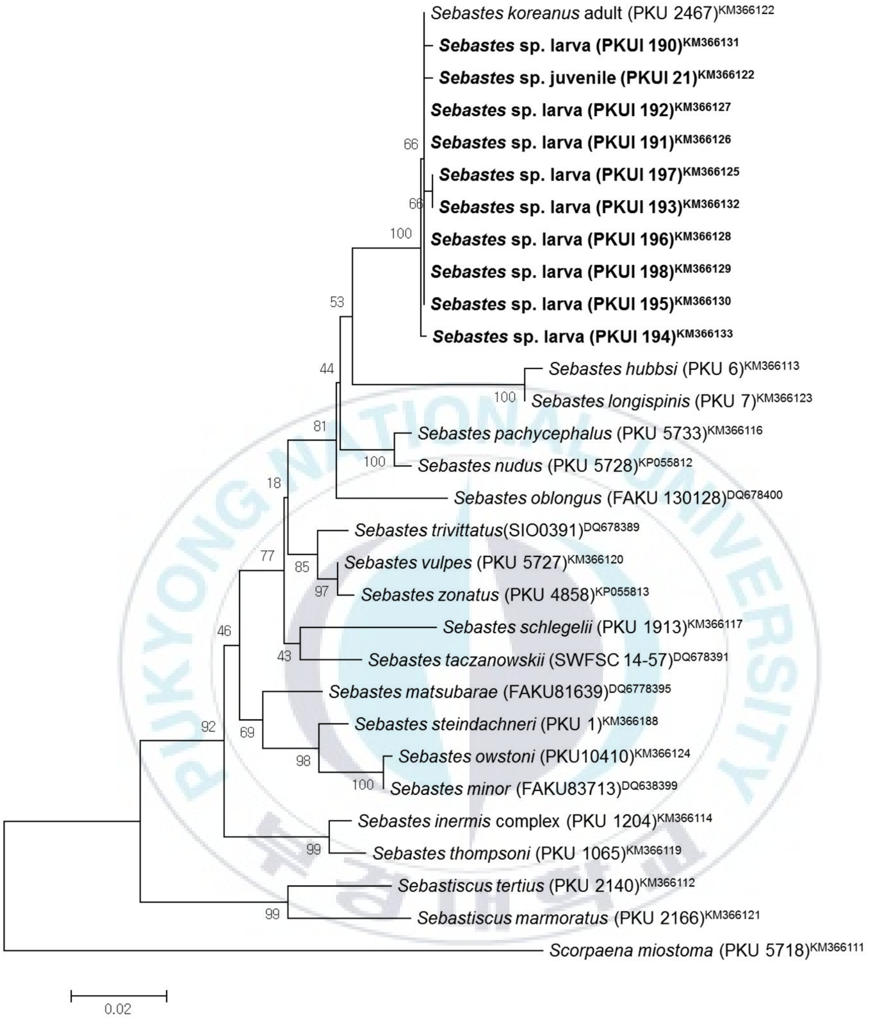

Fig. 3. Neighbor-joining tree based on partial mtDNA COI sequences, showing the relationships among larvae and a juvenile of *Sebastes* collected from the Yellow Sea and 20 scorpaenid species. The tree was constructed using the Kimura 2-parameter model and 1,000 bootstrap replications. The *bottom bar* indicates a genetic distance of 0.02. Parentheses and superscripts indicate the voucher number and NCBI registration number, respectively.

2. Morphological development

i. General morphology

Measurements and counts for all individuals are shown in Table 1. Also, drawings of egg, larvae, and juvenile are shown in Figs. 4–5. The embryos in the eggs possessed large eyeballs and appeared to be close to hatching. The eggs were spherical, with a mean diameter of 1.57 mm, and contained one oil globule (Fig. 4a). The yolk-sac larvae, with a mean length of 4.06 mm BL, each possessed a large yolk sac with a single oil globule (Fig. 4b), an unopened mouth, and opened anus. In the preflexion larval stage of 6.41 mm BL, the yolk and oil globule had been completely absorbed; the preflexion larvae possessed 25 or 26 myomeres and an opened mouth (Fig. 4c). The percentage of head length to BL and of preanal length to BL both increased with size (Fig. 13a and b, respectively). However, the percentage of eye diameter and body depth to BL showed less variance during growth (Fig. 13c and d, respectively). The flexion larva with a length of 7.72 mm BL possesse a single nostril that begins to split into two nostrils when the larva grows to 8.24 mm BL. The juvenile showed a body color similar to that of adult *S. koreanus* (Fig. 5d).

ii. Head spines

Development of the head spine is shown in Table 2. Although preflexion larvae as small as 6.49 mm BL lacked any head spines, flexion larvae more than 6.74 mm BL possessed a parietal spine prior to the head spine. A flexion larva with a length of 7.21 mm BL possessed three preopercular spines, and flexion larvae larger than 8.24 mm BL possessed four preopercular spines. A flexion larva with a length of 7.21 mm BL possessed postocular spines, and a flexion larva with a length of 7.66 mm BL possessed preocular spines and pterotic spines. A postflexion larva with a length of 8.84 mm BL possessed two opercular spines, three ocular spines, and one nuchal spine. The juvenile possessed prominent head and opercular spines: one parietal spine, one pterotic spine, one nasal spine, three ocular spines, five preopercular spines, and two opercular spines.

iii. Fin rays

Yolk-sac larvae possessed a primordial finfold, but lacked real fin rays. Pectoral-fin and caudal-fin rays first appeared in a preflexion larva with a length of 6.41 mm BL, and dorsal-fin and anal-fin rays were present in a flexion larva with a length of 7.66 mm BL. Dorsal-fin and anal-fin rays, reaching to XIV, 13 dorsal-fin rays and III, 6 anal-fin rays, respectively, in a postflexion larva with a length of 8.84 mm BL. A postflexion larva with a length of 8.84 mm BL possessed 5 pelvic-fin rays, but lacked a spine. In the juvenile with a length of 18.60 mm BL, the numbers of fin spines and rays were equal to those in adults: XIV, 12 dorsal-fin rays, 17 pectoral-fin rays, I, 5 pelvic-fin rays, and III, 6 anal-fin rays.

iv. Pigmentation

Melanophores on the head and body were completely absent in embryos just before hatching. After hatching, however, some yolk-sac larvae (7 of 20 individuals) possessed a row of 2–7 faint melanophores on the dorsal surface of the abdominal region. 9 to 20 punctate-shaped melanophores were present in the occipital region in flexion larvae as small as 6.83 mm BL, but these melanophores were transformed to donut-shaped melanophores in larvae more than 7.21 mm BL. All larvae possessed 4 to 10 branch-shaped melanophores in the abdominal region. Because only 2 or 3 larvae possessed melanophores behind the nape (positions 13 and 14, Fig. 2), this character may be difficult to use for species identifications. Similarly, 1 to 3 melanophores were present on the ventral contour of the tail, but were absent in most of the larvae examined here. On the other hand, the juvenile possessed a pigmentation pattern strikingly different from those in larvae smaller than 8.90 mm BL; some punctate-shaped melanophores were present around the eye, occipital, operculum, and jaw regions, and three distinct transverse stripes appeared in the trunk and caudal regions.

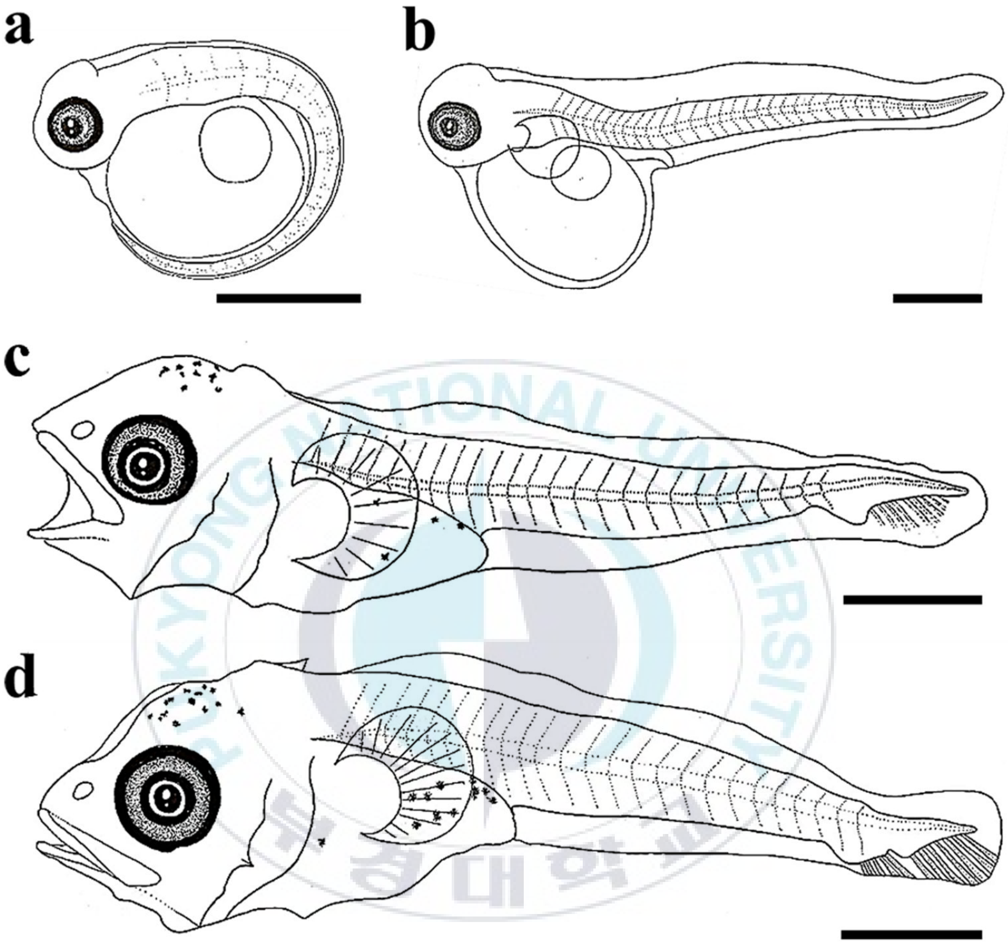

Fig. 4. Drawings of an egg, yolk-sac larva, and larvae of *Sebastes koreanus*. a Egg (diameter, 1.66 mm) (PKUI 188). b Yolk-sac larva (4.31 mm BL) (PKUI 189). c Preflexion larva (6.41 mm BL) (PKUI 190). d Flexion larva (6.75 mm BL) (PKUI 193). *Bars* 1 mm.

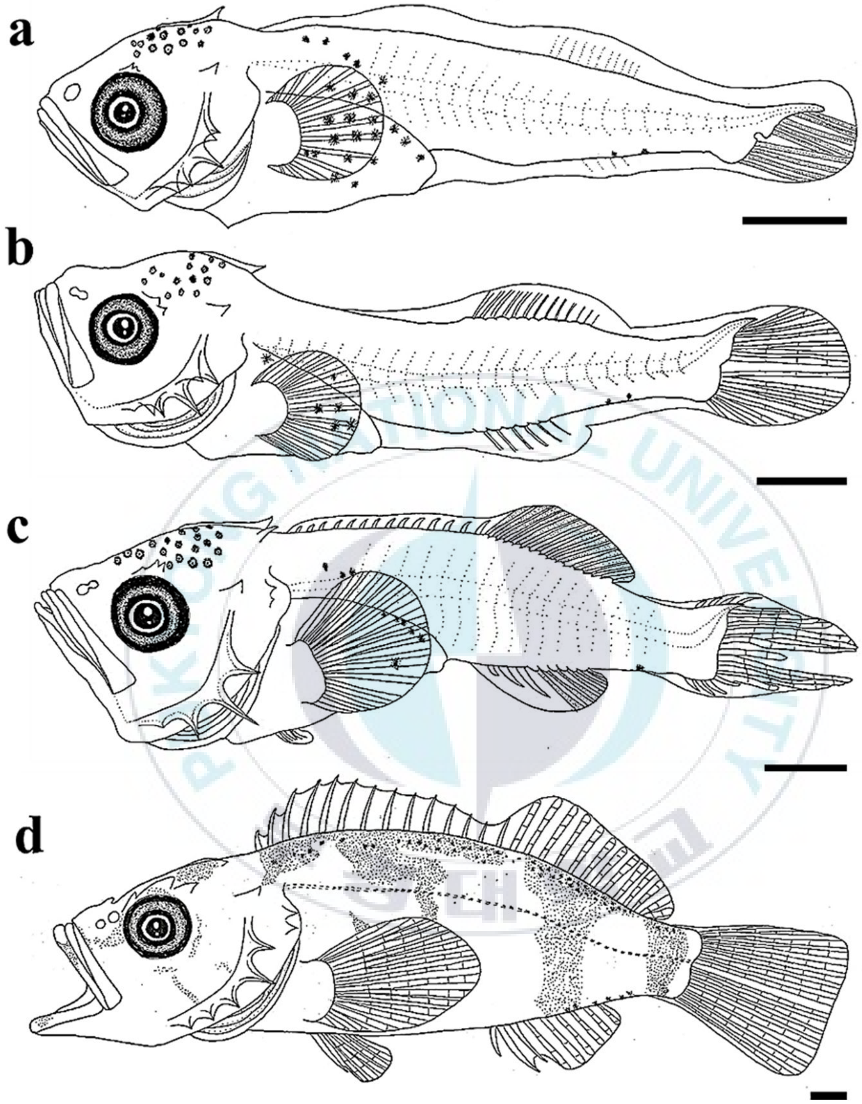

Fig. 5. Drawings of larvae and a juvenile of *Sebastes koreanus*. a Flexion larva (7.66 mm BL) (PKUI 196). b Flexion larva (8.24 mm BL) (PKUI 198). c Postflexion larva (8.84 mm BL) (PKUI 199). d Juvenile (18.60 mm BL) (PKUI 21). *Bars* 1 mm.

Table 1. Measurements and counts of larvae, juvenile, and adults of *Sebastes koreanus*.

	Yolk-sac larvae	Preflexion larvae		Flexion larvae							Postflexion larvae		Juvenile	
	PKUI 189 *n* = 20	PKUI 190 *n* = 1	PKUI 191 *n* = 1	PKUI 192 *n* = 1	PKUI 193 *n* = 1	PKUI 194 *n* = 1	PKUI 195 *n* = 1	PKUI 196 *n* = 1	PKUI 197 *n* = 1	PKUI 198 *n* = 1	PKUI 199 *n* = 1	PKUI 200 *n* = 1	PKUI 21 *n* = 1	adults *n* = 10
Total length													23.31	106.31–202.30 (160.58)
Standard length														89.12–168.55 (134.78)
Body length	3.59–4.36 (4.06)	6.41	6.49	6.74	6.75	6.83	7.21	7.66	7.72	8.24	8.84	8.90	18.60	
Measurements (% of BL)														
Head length		26.8	26.5	26.7	27.4	25.6	29.5	27.4	28.1	29.7	35.2	34.6	37.4	35.7–39.0 (37.3)
Preanal length		50.4	52.9	51.2	49.3	48.2	51.6	50.0	54.0	51.8	56.0	55.3	72.1	61.6–72.2 (66.0)
Snout length		7.8	8.3	7.3	7.9	8.5	8.2	7.3	7.4	8.0	10.4	9.1	9.8	7.5–9.4 (8.4)
Eye diameter		9.2	10.2	11.0	10.1	10.5	9.7	9.7	9.1	9.7	10.6	10.8	11.4	9.1–11.2 (10.3)
Body Depth		22.5	25.4	24.3	23.1	23.3	23.7	23.4	24.6	23.4	28.4	28.0	33.5	35.4–38.9 (36.9)

Table 1. Continue.

	Yolk-sac larvae	Preflexion larvae		Flexion larvae							Postflexion larvae		Juvenile	adults *n* = 10
	PKUI 189 *n* = 20	PKUI 190 *n* = 1	PKUI 191 *n* = 1	PKUI 192 *n* = 1	PKUI 193 *n* = 1	PKUI 194 *n* = 1	PKUI 195 *n* = 1	PKUI 196 *n* = 1	PKUI 197 *n* = 1	PKUI 198 *n* = 1	PKUI 199 *n* = 1	PKUI 200 *n* = 1	PKUI 21 *n* = 1	
Total length													23.31	106.31–202.30 (160.58)
Standard length														89.12–168.55 (134.78)
Body length	3.59–4.36 (4.06)	6.41	6.49	6.74	6.75	6.83	7.21	7.66	7.72	8.24	8.84	8.90	18.60	
Counts														
Dorsal-fin rays	X	X	X	X	X	X	X	11	11	0,13	XIV,13	XIV,12	XIV,12	XIV,12–13
Anal-fin rays	X	X	X	X	X	X	X	4	4	6	III,6	III,6	III,6	III,6
Pectoral-fin rays	X	9?	9?	10?	13	14	15	14	15	16	17	16	17	16–17
Pelvic-fin rays	X	X	X	X	X	X	X	X	X	X	5	5	I,5	I,5

X absent; *?* present but indistinct or damaged

Parentheses indicate means

Table 2. Ontogenetic variations in head spination of *Sebastes koreanus*.

	Body length (mm)											
	Preflexion larvae		Flexion larvae							Postflexion larvae		Juvenile
	6.41	6.49	6.74	6.75	6.83	7.21	7.66	7.72	8.24	8.84	8.90	18.60
Parietal spine			+	+	+	+	+	+	+	+	+	+
Nuchal spine										+	+	+
Pterotic spine							+	+	+	+	+	+
Nasal spine												+
Preocular spine							+	+	+	+	+	+
Supraocular spine										+	+	+
Postocular spine						+	+	+	+	+	+	+
Upper opercular spine										+	+	+
Lower opercular Spine										+	+	+
Second anterior preopercular spine						+	+	+	+			
Fourth anterior preopercular spine						+	+	+	+			
First posterior preopercular spine						+	+	+	+	+	+	+
Second posterior preopercular spine			+	+	+	+	+	+	+	+	+	+
Third posterior preopercular spine						+	+	+	+	+	+	+
Fourth posterior preopercular spine									+	+	+	+
Fifth posterior preopercular spine												+

+ presence of spine

3. Osteological development

The osteological development of *S. koreanus* at various developmental stages was described in the following skeletal regions: neurocranium, jaw bones, palate series, opercular series, hyoid arch, pectoral girdle, infraorbital bone, caudal skeleton, and vertebrae. The development results are summarized in Table 3.

i. Neurocranium

The development and ossification of the neurocranium for individuals at different developmental stages are illustrated in Figs 6 and 7. In the smallest larvae (6.11 mm BL; preflexion stage), no skeletal structures of the neurocranium were visible. Ossification of the neurocranium started at 6.27 mm BL, with ossification of the parietal, frontal, and pterotic bones (Fig. 6A); ossification of these elements appeared to begin at the tips of the spines. In the 7.11 mm BL larva, the skeletal elements that had appeared in earlier stages continued to ossify, but no ossification of additional elements was observed (Fig. 6B). At 8.11 mm BL, the posterior of the parasphenoid and the anterior of the basioccipital had started to ossify, and the two elements were joined. At the same time, the exoccipital began to ossify along its posterior margin (Fig. 6C). At 9.06 mm BL, ossification of the frontal had extended to the dorsal area of the neurocranium, and then the frontal boundary line joined the parietal. In addition, the supraoccipital, sphenotic, and prootic elements had started to ossify along their margins at this stage (Fig. 6D). At 10.20 mm BL, the lateral ethmoid had started to ossify along its dorsal margin, and the parietal, pterotic, parasphenoid, and basioccipital were almost fully ossified (Fig. 7A). At 11.10 mm BL, ossification of the frontal had extended to most regions, and ossification of the sphenotic and supraoccipital was complete. The epiotic, which appeared relatively late compared with the other neurocranial elements, had started to ossify at this stage (Fig. 7B), and the vomer and medial ethmoid appeared simultaneously along their anterior margin. At this stage, although all the elements of the neurocranium had started to ossify, some elements continued to ossify in the cartilaginous regions. In the juvenile stage (18.60 mm BL), the ossification of the neurocranium was fully complete (Fig. 7C).

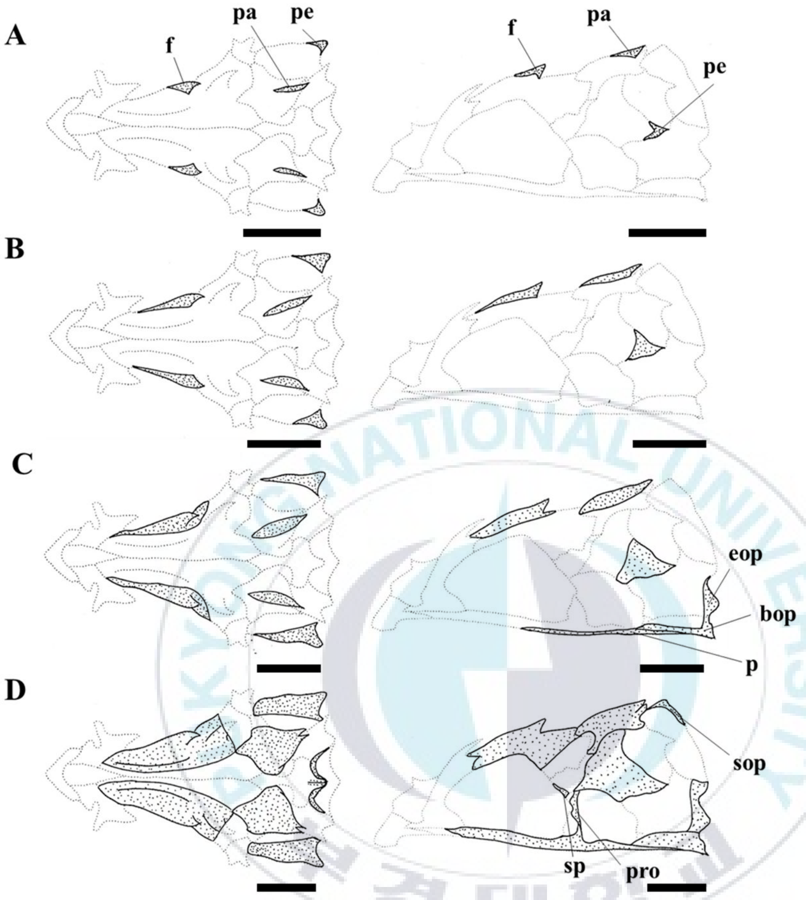

Fig. 6. Developmental sequence of the neurocranium of *Sebastes koreanus*, showing dorsal (left) and lateral (right) views of preflexion to postflexion larvae. (A) Preflexion larva; 6.27 mm BL. (B) Flexion larva; 7.17 mm BL. (C) Flexion larva; 8.17 mm BL. (D) Postflexion larva; 9.06 mm BL. Dotted lines show the outlines of skeletal structures in the adult. Open areas show nonskeletal structures. Solid lines show the boundaries of ossified areas. Dotted areas show ossified elements. bop, basioccipital; eop, exoccipital; f, frontal; p, parasphenoid; pa, parietal; pe, pterotic; pro, prootic; sop, supraoccipital; sp, sphenotic. *Bars* 0.5 mm.

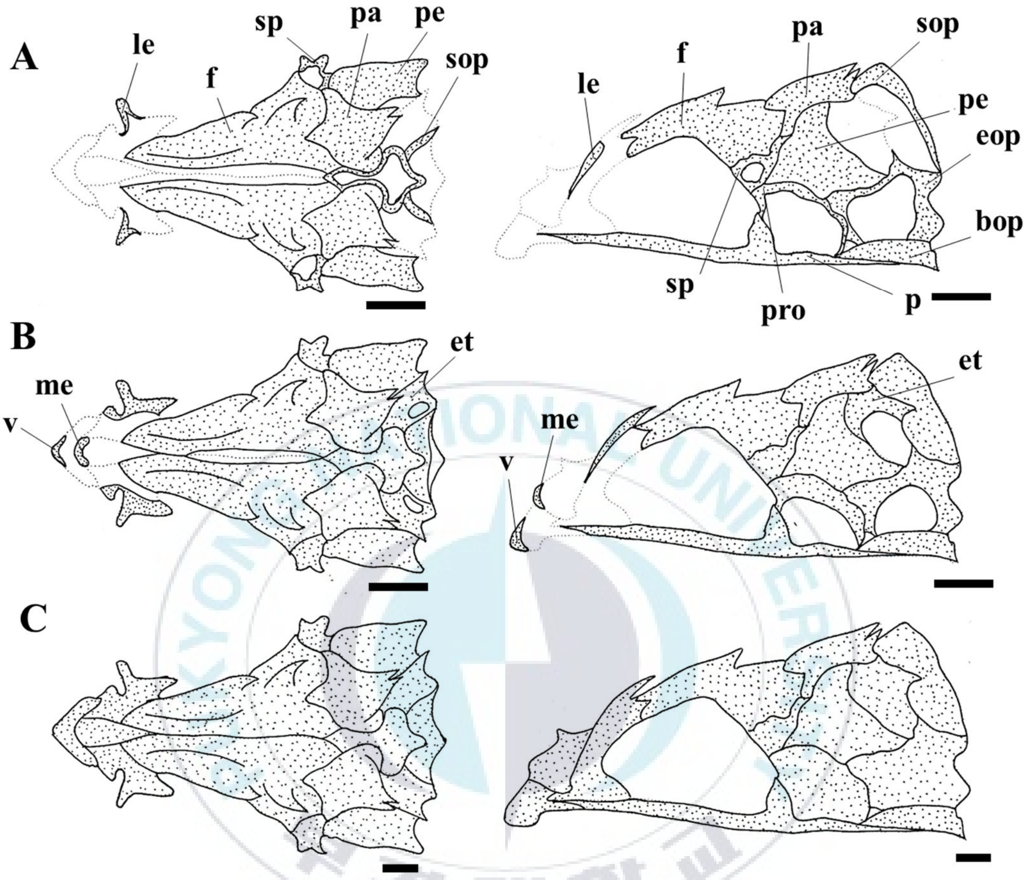

Fig. 7. Developmental sequence of the neurocranium of *Sebastes koreanus*, showing dorsal (left) and lateral (right) views of postflexion larval to juvenile stages. (A) Postflexion larva; 10.20 mm BL. (B) Postflexion larva; 11.10 mm BL. (C) Juvenile; 18.60 mm BL. Dotted lines show the outlines of skeletal structures in the adult. Open areas show nonskeletal structures. Solid lines show the boundaries of ossified areas. Dotted areas show ossified elements. bop, basioccipital; eop, exoccipital; et, epiotic; f, frontal; le, lateral ethmoid; me, medial ethmoid; p, parasphenoid; pa, parietal; pe, pterotic; pro, prootic; sop, supraoccipital; sp, sphenotic; v, vomer. *Bars* 0.5 mm.

ii. Jaw bones, Palatine and Opercular series

The development and ossification of the jaw bones, palatine, and opercular series for individuals at different developmental stages are illustrated in Fig. 8. No skeletal structures were visible in the smallest preflexion larva (6.11 mm BL). At 6.27 mm BL, the maxillary and premaxillary had both begun to ossify at their anterior and ventral margins, respectively (Fig. 8A). The dentary also started to ossify along its V-shaped anterior margin. The hyomandibular started to ossify at opposite medial margins (Fig. 8A). At the same time, the strongest three preopercular spines on the preopercle began to ossify, and the opercle had simultaneously ossified at its anterior margin (Fig. 8A). At 7.17 mm BL, the quadrate and the symplectic started to ossify in the region in which the two elements join (Fig. 8B). At 8.17 mm BL, the interopercle and preopercle had begun to ossify at their margins (Fig. 8C). The premaxillary, maxillary, and dentary also continued to ossify, and then the premaxillary had formed the ascending process and articular process. At 9.06 mm BL, the angular had ossified (Fig. 8D), and the endopterygoid and ectopterygoid had started to ossify along their adjacent margins. In particular, the upper part of the hyomandibular had quickly and fully ossified, and the opercle had extended to the strongest first spine. At 10.20 mm BL, the articular had started to ossify, and the maxillary and premaxillary had fully ossified and assumed their adult forms (Fig. 8E). The palatine started to ossify along its anterior margin, but the degree of ossification was small. At 11.10 mm BL, the ossification of the jaw bones was complete, and the opercular series was almost fully ossified at this stage, except for small parts of the subopercle and interopercle (Fig. 8F). At the juvenile stage (18.60 mm BL), the ossification of the jaw bones, palatine, and opercular series was complete (Fig. 8G).

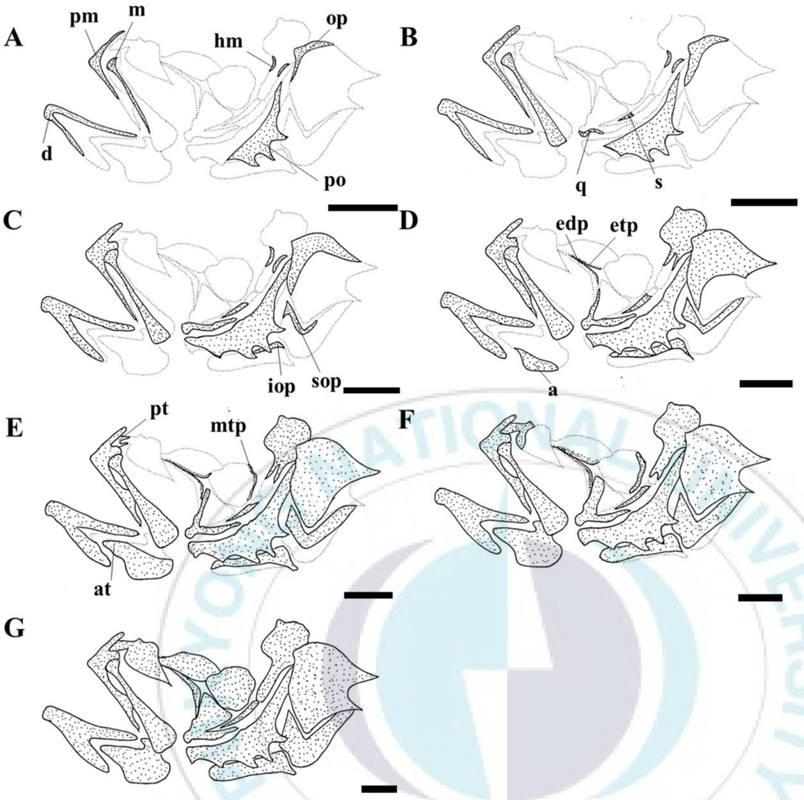

Fig. 8. Developmental sequences of the jaw bone, palate, and opercular series of *Sebastes koreanus* in preflexion larval to juvenile stages (lateral views). (A) Preflexion larva; 6.27 mm BL. (B) Flexion larva; 7.17 mm BL. (C) Flexion larva; 8.17 mm BL. (D) Postflexion larva; 9.06 mm BL. (E) Postflexion larva; 10.20 mm BL. (F) Postflexion larva; 11.10 mm BL. (G) Juvenile; 18.60 mm BL. Dotted lines show the outlines of skeletal structures in the adult. Open areas show nonskeletal structures. Solid lines show the boundaries of ossified areas. Dotted areas show ossified elements. a, angular; at, articular; d, dentary; edp, endopterygoid; etp, ectopterygoid; hm, hyomandibular; iop, interopercle; m, maxilliary; mtp, metapterygoid; op, opercle; po, preopercle; pm, premaxilliary; pt, palatine; q, quadrate; s, symplectic; sob, soborbital. *Bars* 0.5 mm.

iii. Hyoid arch and Pectoral girdle

The development and ossification of the hyoid arch and pectoral girdle in individuals at all stages of development are illustrated in Figs 9 and 10. At 6.27 mm BL, development of the hyoid arch and pectoral girdle had begun, with the ossification of the branchiostegal ray and clavicle, respectively (Figs 9A and 10A). The fifth branchiostegal ray, which was the first branchiostegal ray to begin ossification, started to ossify in its middle region, and the clavicle was fully ossified as a long needle-shaped. At 7.17 mm BL, the fourth branchiostegal ray and the upper clavicle had started to ossify (Figs 9B and 10B). At 8.17 mm BL, all of the branchiostegal rays, except the first ray, had started to ossify, and the ceratohyal had started to ossify along its dorsal and ventral margins (Fig. 9C). The posttemporal and supratemporal had also started to ossify, and were connected to the upper clavicle (Fig. 10C). At 9.06 mm BL, the first branchiostegal ray had started to ossify, and the other branchiostegal rays were fully ossified (Fig. 9D). The upper postclavicle and lower postclavicle of the pectoral girdle had also started to ossify and were connected to one other (Fig. 10D). At 10.20 mm BL, the ceratohyal had enlarged anteriorly and the posttemporal was fully ossified (Figs

9E and 10E). At 11.10 mm BL, the anterior parts of the ceratohyal and clavicle were fully ossified, and the seven pairs of branchiostegal rays had approached their adult number and shape (Figs 9F and 10F). In the juvenile stage (18.60 mm BL), the hyoid arch and pectoral girdle were fully ossified (Figs 9F and 10F). However, although the scapula and uppermost radial of the pectoral girdle were nearly joined, they had not fused (Fig. 10F).

iv. Infraorbital bone

The development and ossification of the infraorbital bone in individuals at all stages of development are illustrated in Fig, 11. At 9.06 mm BL, the infraorbital bone elements on the preorbital had started to ossify (Fig. 11A). At 10.20 mm BL, the area of ossification of the preorbital had increased, but no additional elements were visible (Fig. 11B). At 11.10 mm BL, the first and second suborbital bones had started to ossify along their dorsal margins (Fig. 11C). In the juvenile stage (18.60 mm BL), the infraorbital bone was fully ossified (Fig. 11D).

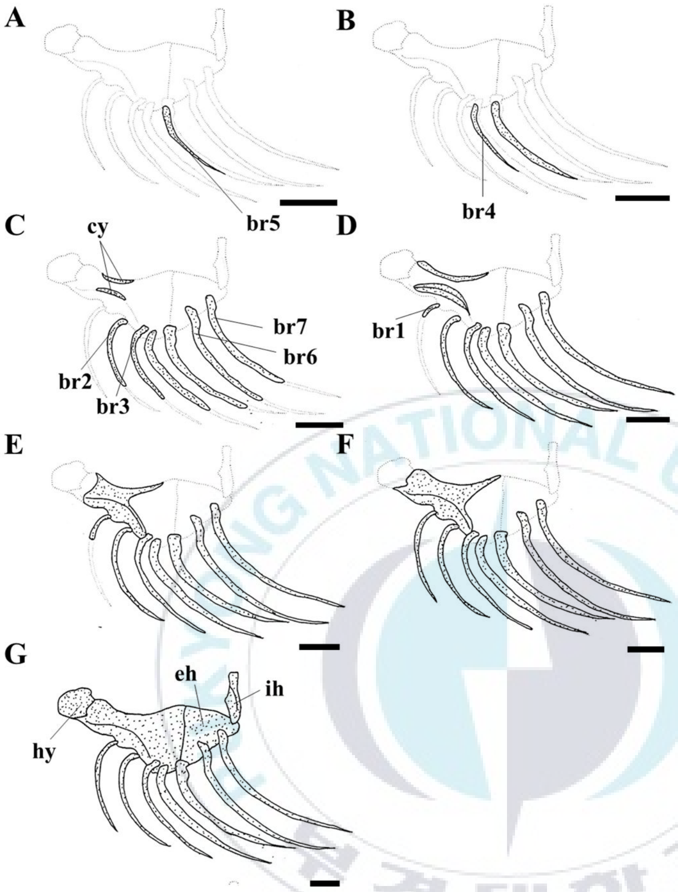

Fig. 9. Developmental sequence of the hyoid arch of *Sebastes koreanus* in preflexion larval to juvenile stages (lateral views). (A) Preflexion larva; 6.27 mm BL. (B) Flexion larva; 7.17 mm BL. (C) Flexion larva; 8.17 mm BL. (D) Postflexion larva; 9.06 mm BL. (E) Postflexion larva; 10.20 mm BL. (F) Postflexion larva; 11.10 mm BL. (G) Juvenile; 18.60 mm BL. Dotted lines show the outlines of skeletal structures in the adult. Open areas show nonskeletal structures. Solid lines show the boundaries of ossified areas. Dotted areas show ossified elements. br, branchiostegal ray; cy, ceratohyal; eh, epihyal; ih, interhyal; hy, hypohyal. *Bars* 0.5 mm.

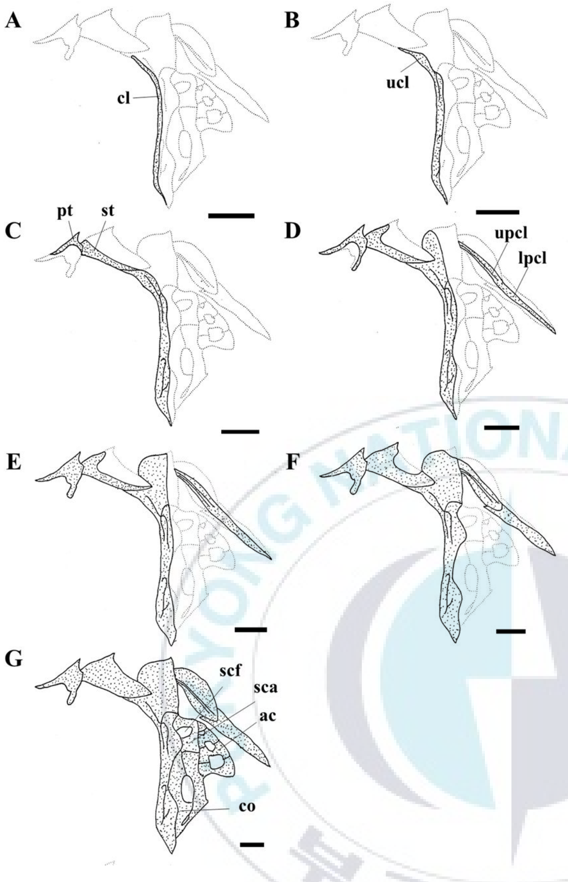

Fig. 10. Development of the pectoral girdle of *Sebastes koreanus* in preflexion larval to juvenile stages (lateral views) (A) Preflexion larva; 6.27 mm BL. (B) Flexion larva; 7.17 mm BL. (C) Flexion larva; 8.17 mm BL. (D) Postflexion larva; 9.06 mm BL. (E) Postflexion larva; 10.20 mm BL. (F) Postflexion larva; 11.10 mm BL. (G) Juvenile; 18.60 mm BL. Dotted lines show the outlines of skeletal structures in the adult. Open areas show nonskeletal structures. Solid lines show the boundaries of ossified areas. Dotted areas show ossified elements. ac, actinost; cl, clavicle; co, coracoids; lpcl, lower postclavicle; pt, posttemporal; sca, scapula; scf, scapula foramen; st, supratemporal; ucl, upper clavicle; upcl, upper post clavicle. *Bars* 0.5 mm.

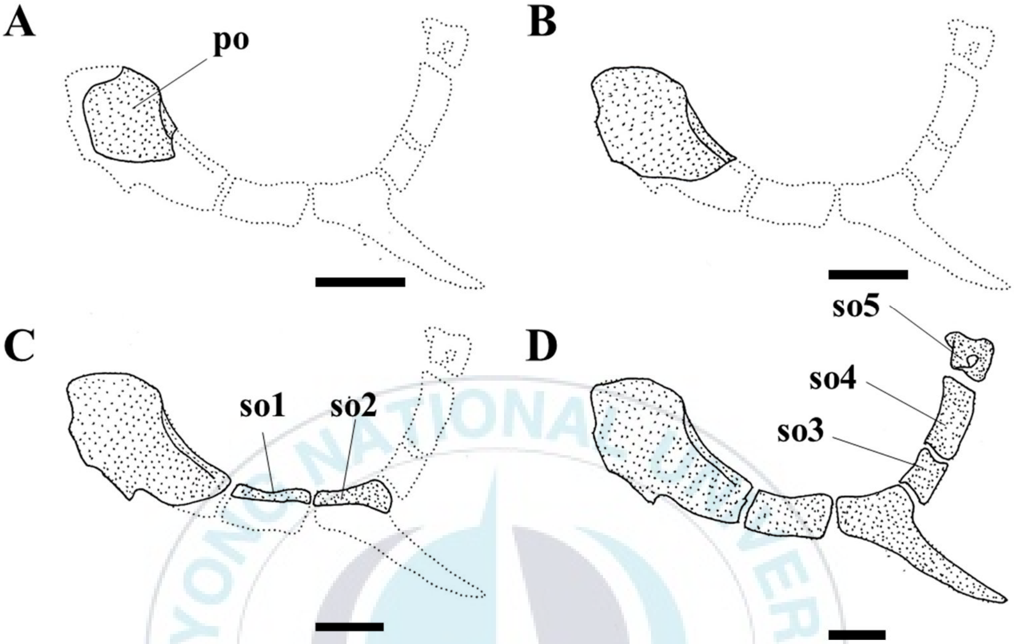

Fig. 11. Development of the infraorbital bone of *Sebastes koreanus* in postflexion larval to juvenile stages (lateral views). (A) Postflexion larva; 9.06 mm BL. (B) Postflexion larva; 10.20 mm BL. (C) Postflexion larva; 11.10 mm BL. (D) Juvenile; 18.60 mm BL. Dotted lines show the outlines of skeletal structures in the adult. Open areas show nonskeletal structures. Solid lines show the boundaries of ossified areas. Dotted areas show ossified elements. po, preorbital bone; so, suborbital. *Bars* 0.5 mm.

v. Vertebrae and Caudal skeleton

The development and ossification of the vertebrae and caudal skeleton in individuals at all stages of development are illustrated in Figs 12. The skeletal elements of the vertebrae were first apparent at 7.17 mm BL (Fig. 12A). The first visible ossified elements of the vertebrae were the neural spines; no ossification of the centra was observed at this stage. The centra first started to ossify in the dorsal regions at 8.17 mm BL (Fig. 12B). After a centrum had formed, the neural spines appeared to elongate dorsally. The first hemal spines were observed at 9.06 mm BL, at which time ossification was visible in 10 centra, 14 neural spines, and three hemal spines (Fig. 12C). The development of the neural spines in the vertebrae occurred more rapidly than did the vertebral centra. Two to three ossified parapophyses appeared on the trunk centra at this stage. At 10.20 mm BL, the anterior centra, neural spines, and hemal spines were almost fully ossified, completely surrounding the notochord, whereas the posterior vertebrae continued to ossify consecutively towards the caudal complex (Fig. 12D). The urostyle had also started to ossify for the first time at this stage, along its anterior margin (Figs 12D). At 11.10 mm BL, despite the progressive ossification of consecutive vertebrae, a few posterior vertebrae were still only present as cartilaginous structures (Fig. 12E). In the caudal skeleton, the urostyle had fully ossified at this stage, but no additional ossification was visible in the caudal skeleton (Fig. 12E). In the juvenile stage (18.60 mm BL), all the vertebral centra had completely surrounded the notochord, and the adjacent neural spines and hemal spines were also ossified (Fig. 12F). In addition, the hypurals, equals, parahypurals, parapophyses, and uroneural in the caudal skeleton were fully ossified in the juvenile (Fig. 12F). At the juvenile stage, the first and second hypurals and the third and fourth hypurals had also fused to form, together with the fifth hypural, three hypural segments (hy 1 + 2, hy 3 + 4, and hy 5).

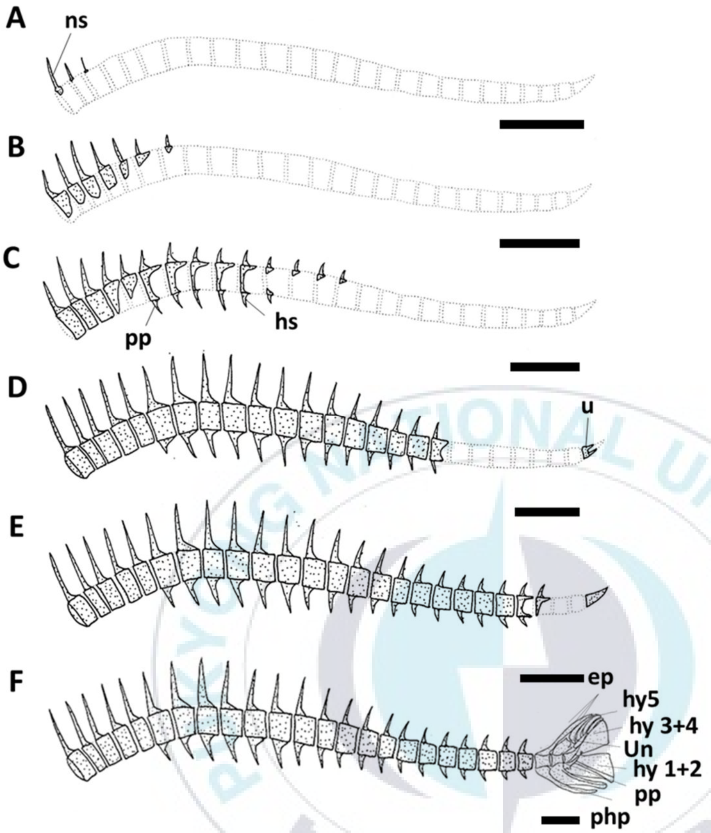

Fig. 12. Development of the vertebrae and caudal skeleton of *Sebastes koreanus* in flexion larval to juvenile stages (lateral views). (A) Flexion larva; 7.17 mm BL. (B) Flexion larva; 8.17 mm BL. (C) Postflexion larva; 9.06 mm BL. (D) Postflexion larva; 10.20 mm BL. (E) Postflexion larva; 11.10 mm BL. (F) Juvenile; 18.60 mm BL. Dotted lines show the outlines of skeletal structures in the adult. Open areas show nonskeletal structures. Solid lines show the boundaries of ossified areas. Dotted areas show ossified elements. ep, epural bone; hs, hemal spine; hy, hypural bone; pp, parapophysis; ns, neural spine; u, urostyle bone; un, uroneural. *Bars* 0.5 mm.

Table 3. Developmental sequence of ossification in *Sebastes koreanus*.

Elements	Body length (mm)								
	Preflexion larvae		Flexion larvae		Postflexion larvae				Juvenile
	6.11	6.27	7.17	8.17	9.06	10.20	11.10	· · · · · ·	18.60
Neurocranium									
parasphenoid				▶	—	—	—	—	—
exoccipital				▶	—	—	—	—	—
basioccipital`				▶	—	—	—	—	—
supraoccipita					▶	—	—	—	—
epiotic							▶	—	—
prootic					▶	—	—	—	—
frontal		▶	—	—	—	—	—	—	—
sphenotic					▶	—	—	—	—
pterotic		▶	—	—	—	—	—	—	—
vomer							▶	—	—
lateral ethmoid						▶	—	—	—
medial ethmoid							▶	—	—
parietal		▶	—	—	—	—	—	—	—
Jaw bone									
maxillary		▶	—	—	—	—	—	—	—
premaxillary		▶	—	—	—	—	—	—	—
dentary		▶	—	—	—	—	—	—	—
articular						▶	—	—	—
angular					▶	—	—	—	—
Hyoid arch									
ceratohyal				▶	—	—	—	—	—
epihyal								▶	—
hypohyal								▶	—
branchiostegal ray		▶	—	—	—	—	—	—	—
interhyal								▶	—

Table 3. Continue.

Elements	Body length (mm)								
	Preflexion larvae		Flexion larvae		Postflexion larvae				Juvenile
	6.11	6.27	7.17	8.17	9.06	10.20	11.10	· · · · ·	18.60
Palate									
palatine						▶	—	—	—
metapterygoid						▶	—	—	—
ectopterygoid					▶	—	—	—	—
endopterygoid					▶	—	—	—	—
hyomandibular		▶	—	—	—	—	—	—	—
synplectic			▶	—	—	—	—	—	—
quadrate			▶	—	—	—	—	—	—
Opercular									
opercle		▶	—	—	—	—	—	—	—
subopercle				▶	—	—	—	—	—
preopercle		▶	—	—	—	—	—	—	—
interopercle				▶	—	—	—	—	—
Pectoral girdle									
clavicle		▶	—	—	—	—	—	—	—
upper clavicle			▶	—	—	—	—	—	—
actimost								▶	—
coracoid								▶	—
scapula								▶	—
scapula foramen								▶	—
lower postclavicle					▶	—	—	—	—
upper postclavicle					▶	—	—	—	—
supratemporal				▶	—	—	—	—	—
posttemporal				▶	—	—	—	—	—

Table 3. Continue.

Elements	Body length (mm)								
	Preflexion larvae		Flexion larvae		Postflexion larvae				Juvenile
	6.11	6.27	7.17	8.17	9.06	10.20	11.10	· · · · ·	18.60
Caudal skeleton									
equral								▶	—
hypural								▶	—
parhypral								▶	—
urostyle						▶	—	—	—
parapophysis								▶	—
caudal bony plate								▶	—
Infraorbital bone									
preorbital					▶	—	—	—	—
suborbital							▶	—	—
Vertebrae									
neural spine			▶	—	—	—	—	—	—
hemal spine					▶	—	—	—	—
parapophysis					▶	—	—	—	—
centrum				▶	—	—	—	—	—

IV. Discussion

1. Morphological development

The present study is the first to provide detailed ontogenetic information on *Sebastes koreanus*, and these data are expected to provide insights into systematics of the species of *Sebastes*. In this study, larvae and a single juvenile of *Sebastes* sp., collected from the Yellow Sea, were identified as *Sebastes koreanus* based on mtDNA COI sequences and compared with larvae of 15 other species of *Sebastes*.

As in other similar species of *Sebastes* [*Sebastes schlegelii* Hilgendorf 1880, *Sebastes thompsoni* (Jordan and Hubbs 1925), and *S. taczanowskii*], larvae and the juvenile of *S. koreanus* showed that the percentage of HL relative to BL increased with growth (Fig. 13a) and that these values approached those of adults (35.7–39.0 %) at a length of 89.12–168.55 mm BL (Nagasawa and Kobayashi 1995; Nagasawa and Domon 1997; Nagasawa et al. 2008).

Yolk-sac larvae of *S. koreanus* have single oil globules (Fig. 4b), which is also observed in the yolk-sac larvae of *S. schlegelii*, *S. pachycephalus* and *S. oblongus* (see Kim and Han 1991; Byun at al. 1995; Han at al. 1996). But, yolk-sac larvae of *S. thompsoni* and *Sebastes inermis* Cuvier 1829 have multiple oil globule (2–3 and 3–4, respectively) (Han 1991; Kim and Han 1993).

Development of head spination in larvae of *S. koreanus* was similar to that of *S. thompsoni*, *S. vulpes*, *S taczanowskii*, *S. schlegelii* and *S. inermis*, but some differences were found (Nagasawa and Kobayashi 1995; Nagasawa and Domon 1997; Nagasawa 2000; Nagasawa et al. 2000, 2008). In the larvae of *S. koreanus,* parietal spine and the second posterior preopercular spine appeared first on the head part (Table 2), whereas in five other species of *Sebastes* mentioned above, the third posterior preopercular spine appeared first. In the case of *S. thompsoni*, *S. vulpes*, *S taczanowskii*, and *S. schlegelii,* pterotic spine appears first. In *Sebastes inermis* pterotic and parietal spines appeared at the same time. The second and fourth anterior preopercular spines appeared in flexion larva of *S. koreanus* (7.21 mm BL) and disappeared in the postflexion larva of *S. koreanus* (8.84 mm BL) (Table 2). This seems to be similar to those of *S. thompsoni*, *S. vulpes*, *S taczanowskii*, *S. schlegelii,* and *S. inermis*, but the anterior preopercular spines of larvae of *S. koreanus* disappeared earlier than those of *S. thompsoni*, *S. vulpes*, *S* *taczanowskii*, *S. schlegelii,* and *S. inermis*. In the five species of *Sebastes* mentioned above, the anterior preopercular spines disappeared in the late postflexion and transforming larvae (Nagasawa and Kobayashi 1995; Nagasawa and Domon 1997; Nagasawa 2000; Nagasawa et al. 2000, 2008).

The most distinct feature of the embryos in the egg and yolk-sac larvae of *S. koreanus* is the absence of melanophores, except on the upper margin of the abdominal region in a few yolk-sac larvae, where the melanophores persist until the preflexion larval stage; subsequently, melanophores appear at the top of the head and in the abdominal region, but are absent on the remaining body parts. Three transverse stripes, which are present in the juvenile of *S. koreanus*, correspond to the coloration observed in adult. Such a pigmentation pattern is also observed in congeneric species (*S. vulpes*, *S. inermis*, and *S. thompsoni*) at similar sizes (16.5–20.2 mm BL) (Kim et al. 1993; Nagasawa and Kobayashi 1995; Nagasawa 2000). The examined larvae and juvenile of *S. koreanus* are clearly distinguished from the other 15 species of *Sebastes* by the distribution and shapes of the melanophores. A comparison of the pigmentation pattern among 15 species of *Sebastes* showed that *S. koreanus* was the only species with melanophores present in positions 4, 11, and 12; however, other species have melanophores in various positions (positions 5–16) (Table 4).

The sympatric species of *S. koreanus*, *S. pachycephalus*, *S. schlegelii*, and *S. thompsoni* show similar release seasons of larvae (Kokita and Omori 1998; Okiyama 1998), which may cause confusion in species identifications during early life stages; however, the species can be identified by the following morphological traits: larvae of *S. pachycephalus* possess many melanophores on the pectoral fins; larvae of *S. schlegelii* possess many melanophores on the dorsal and ventral edges of the tail; and larvae of *S. thompsoni* have melanophores along the mid-lateral region of the tail (Nagasawa and Kobayashi 1995; Han et al. 1996; Nagasawa and Domom 1997). *Sebastes koreanus* and *S. owstoni* are the most similar in the number of fin rays, but the two species are distinguished by the three distinct transverse stripes during a juvenile stage (present in *S. koreanus* vs. absent in *S. owstoni*) (Okiyama 1988; Kim et al. 2005).

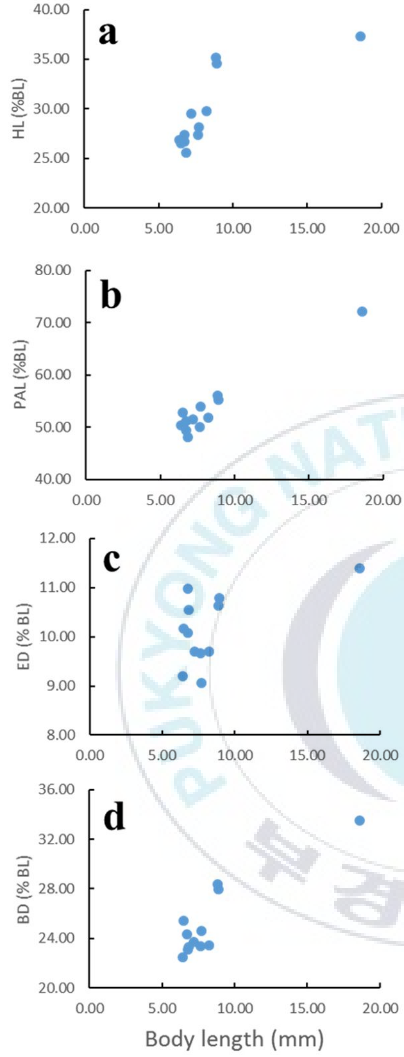

Fig. 13. Relative growth of various body parts in larvae and a juvenile of *Sebastes koreanus*. a Head length (HL) as a percentage of body length (BL) versus BL. b Preanal length (PAL) as a percentage of BL versus BL. c Eye diameter (ED) as a percentage of BL vs. BL. d Body depth (BD) as a percentage of BL vs. BL.

Table 4. Comparison of pigmentation pattern in preflexion larvae of 16 species of *Sebastes* from the northwest Pacific.

Species	Melanophore positon																						References
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
S. koreanus	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	Present study
S. pachycephalus	0	0	0	1	0	0	0	1	0	1	1	1	1	1	0	0	0	0	0	0	0	0	Han et al. 1996
S. thompsoni	0	0	0	1	0	1	0	0	0	0	1	1	0	0	1	0	0	1	1	0	0	0	Nagasawa and Kobayashi 1995
S. schlegelii	0	0	0	1	0	1	0	0	0	0	1	1	0	0	1	0	0	1	0	0	0	0	Nagasawa and Domon 1997
S. vulpes	1	0	0	1	1	1	0	1	1	1	1	1	1	1	0	0	1	1	0	0	0	1	Nagasawa 2000
S. longispinis	1	1	1	1	1	1	1	0	0	0	1	1	1	1	1	1	1	1	1	0	0	0	Okiyama 1988
S. hubbsi	1	0	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	0	0	Okiyama 1988
S. inermis complex	0	0	0	1	0	1	0	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0	Nagasawa et al. 2000
S. oblongus	0	0	0	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	0	0	0	Okiyama 1988
S. owstoni	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0	Okiyama 1988
S. taczanowskii	0	0	0	1	0	0	0	0	0	0	1	0	0	0	1	0	1	1	0	0	0	0	Nagasawa et al. 2008
S. steindachneri	0	0	0	1	0	0	0	0	0	0	1	1	0	0	1	0	0	1	0	0	0	0	Sasaki 1974
S. wakiyai	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	1	0	Sasaki 1976
S. minor	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	Nagasawa 1993
S. matsubarae	1	0	0	1	0	1	1	1	1	1	1	1	1	1	0	0	1	1	0	0	0	0	Okiyama 1988
S. trivittatus	1	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	1	1	0	Nagasawa 2014

0 absent; *1* present

2. Osteological development

This study is the first to examine and describe in detail the sequence of osteological development in *S. koreanus* collected in the wild, and to provide data with which to infer the phylogenetic relationships of species within the suborder Scorpaenoidei. In *S. koreanus*, ossification of the skeletal elements is first observed in the neurocranium, jaw bones, palatine, opercular, hyoid arch, and pectoral girdle of the preflexion larva with a length of 6.27 mm BL [6.45 mm Total length(TL)], and then the only one juvenile (18.60 mm BL) was fully completed the skeletal development of all elements (Table 3). In a previous study of early skeletal development in the genus *Sebastes*, ossification was first observed in *S. inermis* complex at 7 days (7.0 mm mean TL) (Kim et al. 1993), in *S. schlegelii* Hilgendorf 1880 at 6–8 days (6.85 mm TL) (Kim and Han 1991), and in *S. oblongus* Günther 1877 at 3 days after release (8.0 mm TL) (Byun et al. 2012). In a study of early skeletal development in *S. macdonaldi* (Eigenmann and Beeson 1893) from southern California, ossification was first observed in the smallest larva (6.11 mm BL) (Moser 1972). Like this, the ossification of wild-captured *S. koreanus* larvae was first observed even smaller larva than reared larvae of *S. inermis*, *S. schlegelii* and *S. oblongus* (but not in *S. macdonaldi*). These differences in the sizes of the larvae at the onset of ossification are probably related to the size at which the larvae are released from the adult, which is smaller than 6.11 mm BL in *S. koreanus* (this study), 6.12 mm TL in *S. inermis* (Kim et al. 1993), 5.52 mm TL in *S. schlegelii* (Kim and Han 1991), 7.2 mm TL in *S. oblongus* (Byun et al. 2012), and 4.5 mm BL in *S. macdonaldi* (Moser 1972). These differences may also be affected by external environmental factors, such as temperature and salinity (Fuiman 2002; Ložys 2004; Löffler et al. 2008; Ott et al. 2012), which may cause corresponding osteological differences (Matsuoka 1987; Wimberger 1993; Koumoundouros et al. 1997a) and meristic variations (Fowler 1970; Lau and Shafland 1982) between reared larvae and wild-captured larvae (Boglione 2001). Therefore, despite the similar size of the released larval of *S. koreanus*, *S. inermis* and *S. schlegelii*, the first ossification size is considered to differ from each other because the larvae of *S. koreanus* was collected in the wild. In addition, ossification was first observed in the reared larva of *Sebastiscus marmoratus* (Cuvier 1829) and *Sebastiscus tertius* (Barsukov and Chen 1978) at 3.35 mm TL and 4.4 mm TL, respectively, and these larvae were ossified even smaller than the larvae of species of *Sebastes* (Kim et al. 1997; Han et al. 2001).

These result was also probably related to the size at larval release, because the larval release size is smaller than larvae of *Sebastes* (Kim et al. 1997; Han et al. 2001).

In most cases, early skeletal development occurs in elements that are necessary for feeding and respiration, and therefore affect the survival of young larvae (Vandewalle et al. 1997; Wagemans and Vandewalle 1999). For example, the total resorption of the vitellus is essential for the transition from endogenous to exogenous feeding, because the efficiency of suction feeding increases with increasing prey size and the ossification of the related skeletal elements (Gluckmann et al. 1999). In *S. koreanus*, the skeletal elements that first start to ossify (at 6.27 mm BL) are the premaxillary, maxillary, dentary, preopercle, opercle, hyomandibular, and the fifth branchiostegal ray (Figs 8 and 9), and the order of ossification is initially defined by the importance of the skeletal elements to feeding, swimming and respiration. The cleithrum in the pectoral girdle ossifies in the same developmental stage (Fig. 10), and the early ossification of the clavicle produces an attachment site for the sternohyoideus muscle, which is important for feeding in subsequent growth stages (Wagemans and Vandewalle 1999; Koumoundouros et al. 2001a; Cloutier et al. 2011). Similar patterns of early skeletal development have been observed in other species of *Sebastes* (e.g., *S. inermis*, *S. schlegelii*, *S. oblongus*, *Sebastiscus marmoratus*, and *Sebastiscus tertius*). However, the timing of the ossification of the hyomandibular is highly variable (Kim and Han 1991; Kim et al. 1993; Kim et al. 1997; Byun et al. 2012). In most teleostei, the parasphenoid is the first element to ossify, except in some species in which the parasphenoid ossifies simultaneously with the frontals (*Pagrus major*; Matsuoka 1987) or the basioccipital (*Heterobranchus longifilis*; Vandewalle et al. 1997), or is ossified after the ossification of the frontals (*Scophthalmus maximus*; Wagemans et al. 1998). In *S. koreanus*, the first-ossified elements in the neurocranium (at 6.27 mm BL) are the spines of the parietal, frontal, and pterotic (Fig. 6). Subsequently, the parasphenoid and basioccipital begin to ossify at 8.17 mm BL (Fig. 6); these elements may help to reinforce the cranial floor to prevent damage to the neurocranium during feeding (Vandewalle et al. 1992) and to promote the balance needed for swimming (Weisel 1967). Therefore, the ossification of the parasphenoid and basioccipital during early skeletal development is important because they significantly affect feeding and swimming behavior, as do the jaw bones and clavicle.

The order of ossification of the neurocranial elements appears similar in different *Sebastes* species, but variations exist, particularly in the timing of the ossification of the parasphenoid. In many species of *Sebastes* and *Sebastiscus,* such as *S. macdonaldi, S. inermis, S. schlegelii, Sebastiscus marmoratus,* and *Sebastiscus tertius*, the parasphenoid is the first element to ossify (Moser 1972; Kim and Han 1991; Kim et al. 1993; Kim et al. 1997; Han et al. 2001), whereas in *S. koreanus,* the parasphenoid begins to ossify simultaneously with the basioccipital and exoccipital, just after the ossification of the parietal, frontal, and pterotic (present study), or in *S. oblongus*, the parasphenoid beings to ossify simultaneously with the supraoccipital, just after the ossification of the parietal and frontal (Byun et al. 2012). The pterotic and parietal also begin to ossify relatively early in some species, including *S. koreanus*, *S. macdonaldi, Sebastiscus marmoratus,* and *Sebastiscus tertius*, but no significant or clear differences between the species of *Sebastes* and *Sebastiscus* are apparent (Moser 1972; Kim et al. 1997; Han et al. 2001). In *S. koreanus*, the early ossification of the hyoid arch appears on the ceratohyal and branchiostegal rays, but there is no additional ossification of elements between 8.17 and 11.10 mm BL (Fig. 9). In contrast, the ossification of the hyoid arch is clearly different in many species of *Sebastes* and *Sebastiscus* from that observed in *S. koreanus*, and begins to occur at the same time as the ossification of the ceratohyal and epihyal (in *S. inermis*, *S. schlegelii*, *Sebastiscus marmoratus,* and *Sebastiscus tertius*), or the epihyal begins to ossify just after the ossification of the ceratohyal (as in *S. oblongus*) (Kim and Han 1991; Kim et al. 1993; Kim et al. 1997; Han et al. 2001; Byun et al. 2012).

Ossification of the pectoral girdle also shows a high degree of variability between different species of *Sebastes* and *Sebastiscus*. In *S. koreanus*, the ossification of the pectoral girdle begins with the clavicle, followed by the upper clavicle and soon thereafter by the supratemporal and posttemporal (Fig. 10). In contrast, in *S. oblongus*, the ossification of the clavicle first begins 3 days after release, and the ossification of the upper clavicle and posttemporal begin at 20 days, soon after the initial ossification of the supratemporal (Byun et al. 2012). In *S. inermis*, the first ossification on the clavicle begins 7 days after release, followed by the ossification of the postclavicle at 45 days and the upper clavicle at 50 days. The supratemporal begins to ossify at 65–69 days (Kim et al. 1993). In *Sebastiscus marmoratus*, the ossification of the clavicle first appears 5 days after release, and the ossification of the upper clavicle, posttemporal, scapula, and coracoid begin at 28 days (Kim et al. 1997). Therefore, it is difficult to determine a common ossification pattern for the pectoral girdle because of the observed variability between species. According to Ishida (1994), adults of *Sebastes*, *Sebastiscus,* and *Hozukius* (suborder Scorpaenoidei) share the derived characteristic of a fusion of the scapula and uppermost radial in the pectoral girdle. However, although the fusion of the scapula and uppermost radial was observed in adults of *S. koreanus* in this study, fusion was not observed in the *S. koreanus* juvenile (18.60 mm BL) (Fig. 10G). In some species of *Sebastes* (e.g., *S. oblongus*, Byun et al. 2012; *S. schlegelii*, Kim and Han 1991, Omori et al. 1996; *S. macdonaldi*, Moser 1972), fusion between the scapula and uppermost radial is not observed during skeletal development. The separation of the scapula and uppermost radial was also clearly observed in an *S. inermis* juvenile in this study (17.06 mm BL). Thus, it appears that the scapula and uppermost radial fuse slowly after (or starting in) the juvenile stage. Actually, the scapula and uppermost radial were closely adjoined along a thin boundary line in the *S. koreanus* juvenile observed in this study, presumably just prior to fusion. Similarly, in the *S. inermis* juvenile observed in this study (17.60 mm BL), the scapula was closely adjoined to the uppermost radial, although the two elements were not fused with one another. However, in *S. schlegelii*, the illustration of the pectoral girdle provided by Omori et al. (1996) showed no fusion of the scapula with the uppermost radial until a relatively later stage of juvenile development (30.10 mm TL). In this respect, any uncertainty in the timing of ontogenetic transformations, such as the timing of the fusion of the scapula and uppermost radial, can cause confusion about phylogenetic relationships. Thus, more extensive research into early skeletal development is needed to confirm the fusion time of these elements and the extent of the ontogenetic variations.

With respect to locomotion, the swimming of larvae immediately after their release from the adult is possible only through the antagonistic interactions of the notochord and trunk muscles (Ott et al. 2012). With growth, the notochord is gradually replaced by vertebrae, and the ossified vertebrae contribute stronger attachment sites for the powerful dorsalis trunci muscles, which are primarily responsible for swimming (Rojo 1991). In *S. koreanus*, after the ossification of the neural spine at 7.17 mm BL, the ossification of the vertebral centra mainly proceeds from the abdominal to the caudal vertebrae, and the urostyle is fully ossified before the ossification of the caudal vertebrae is complete (Fig. 12). This pattern is similar to that observed in other species of *Sebastes* and *Sebastiscus*, except in *S. schlegelii* (Kim and Han 1991; Omori et al. 1996), e.g., in *S. oblongus* (Byun et al. 2012), *S. inermis* (Kim et al. 1993), *Sebastiscus marmoratus* (Kim et al. 1997), and *Sebastiscus tertius* (Han et al. 2001). Furthermore, in adults, the caudal skeleton in *Sebastes* and *Sebastiscus* species is formed by three hypurals (hy 1 + 2, hy 3 + 4, and hy 5), because the first and second hypurals and the third and fourth hypurals are fused (Ishida 1994). A similar trend is observed in the skeletal development of *Sebastes* and *Sebastiscus* species, including *S. koreanus, S. inermis, S. schlegelii, S. koreanus, S. macdonaldi, Sebastiscus marmoratus,* and *Sebastiscus tertius* (Fig. 12F) (Kim et al. 1993; Omori et al. 1996; Kim et al. 1997; Han et al. 2001; Byun et al. 2012). Therefore, the ontogenetic characteristics reflect the taxonomic characteristics of the adults well. In particular, the hypural cartilages fuse before ossification, unlike the fusion of the scapula and the uppermost radial (Omori et al. 1996).

Park et al. (2015) provided a brief overview of the external and osteological development of *S. koreanus* based on the artificial breeding of hatched larvae, using a gravid adult collected from the southern Korean Strait. However, compared with present study, there are several differences, such as the pigmentation patterns and sequence of osteological development. Thus, we can be able to explain their differences in the three hypotheses. The first hypothesis is that it could be caused by the difference of population, because present study and

Park et al. (2015) are different in sampling site (Yellow Sea vs. Korean Strait). In this respect, there is a possibility of existence of the different population, that isn't realized to exist the southern population of *S. koreanus*. Similarly, Kim et al. (2010) confirmed that the two populations of *Ammodytes personatus* larvae showed the morphological differences in morphometric characters and pigmentation. The second hypothesis is that it could be caused by the difference in water temperature. The larvae and juvenile of *S. koreanus* (present study) was collected at the average water temperature 12.0–15.1°C from Taean Peninsula (May and June), whereas reared larvae and juveniles were reared at the water temperature 13.5–15.5°C. These water temperature difference (ca. 1–3.5°C) can especially become the cause of the ontogenetic differences (Löffler et al. 2008; Ott et al. 2012). Finally, the third hypothesis is that it could be caused by difference of food source between reared larvae and wild-captured larvae. Particularly, calcium deficiency at the food source induces a delay in the ontogeny of skeletal development without affecting final bone mineralization (Fontagne et al. 2009). In addition, we should be considered for the case that the specimens fixed in formalin or alcohol can be reduced or disappeared the pigmentation. Therefore, further research with microsatellite DNA and comparison of rearing is required to confirm the difference between reared larvae and wild-captured larvae.

In summary, although the sequences and periods of osteological development in *Sebastes* and *Sebastiscus* species show some variation, the early ossification of the skeleton proceeds in a sequence that prioritizes the elements required for feeding, swimming, and respiration. In this way, larvae are equipped with functional capacities that enhance the probability of their survival at this stage of their life cycle. Also, the ossification of parasphenoid and epihyal in the neurocranium appeared relatively later than congeneric species, this features indicated that *S. koreanus* is a unique species, which has been evolved in distinctive marine environment of the Yellow Sea.

3. Release season estimates

The release season of *S. koreanus* larvae has been reported to be during the winter (Kim et al. 2004), but the following data obtained in the present study indicate that the release season is during the spring (ca. April–June): (1) a fully gravid female of *S. koreanus* was collected in May; (2) larvae of *S. koreanus* were collected in May and June; and (3) a juvenile of *S. koreanus* was collected in July. The release time of *S. koreanus* larvae can be estimated from the size of larvae collected in the wild, based on data from previous studies on their early life history obtained under artificial conditions. Larvae of the *S. inermis* complex were grown to 16.1–19.2 mm TL, 55–69 days after their release (Kim et al. 1993); larvae of *S. thompsoni* were grown to 23 mm TL, 60 days after their release (Kokita and Omori 1998); and larvae of *S. oblongus* were grown to 20.0–24.5 mm TL, 50 days after their release (Kwak et al. 2006). According to Park et al. (2015) larvae of *S. koreanus* were grown to 15.5–16.2 mm TL, 40–45 days after their release. On this basis, the release time of the 18.60 mm BL (23.31 mm TL) juvenile of *S. koreanus* collected in the Gang-hwa-do area in July can be estimated to be May or June. Therefore, the release season of larvae of *S. koreanus* examined in this study is estimated to be during the spring.

V. References

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제가 이렇게 석사과정을 무사히 마칠수 있었던건, 2년 넘게 생활한 어

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