

Developmental Characteristics of a Freshwater Goby, *Micropercops swinhonis*, from Korea

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ABSTRACT—The embryonic and larval development of a freshwater goby, *Micropercops swinhonis* (Odontobutidae; Gobioidae), from Korea are described. One female spawned several times from mid-April to early July. About 100 to 500 eggs per batch were laid on the under surface of the nest guarded by a male. Eggs hatched 12 days after spawning. Larvae, about 3.7 mm in total length (TL) just after hatching, passed the exogenous feeding larval stage, and reached the juvenile stage of about 16 mm TL in about one month. The developmental process of *M. swinhonis* is quite different from that of the fluvial *Odontobutis* of the same family, which skips the exogenous feeding larval stage, but is rather similar to that of amphidromous Gobiidae. On the other hand, the left and right pelvic fin buds of *M. swinhonis* appear distant from each other as in *Odontobutis*, rather than Gobiidae.

INTRODUCTION

The Gobioidae is a large suborder in the Perciformes comprising more than 2,000 species classified variously into three (Hoese and Gill, 1993), six (Pezold, 1993) or eight families (Nelson, 1994). They exhibit a wide range of life style from benthic to slow swimming life, and most species of specialized groups such as Gobiidae transform their two pelvic fins into a sucker as compared with less specialized groups such as Rhyacichthyidae and Odontobutidae which have separate pelvics (Hoese and Gill, 1993).

A small freshwater goby, *Micropercops swinhonis* (Günther), is distributed in China from the Sungari River to the Zhujiang River, and also in a narrow restricted area of western middle Korea (Zhu, 1995; Kim and Kim, 1997). The genus *Micropercops* is monospecific and is a member of Odontobutidae, which is the second most plesiomorphic family next to Rhyacichthyidae (Hoese and Gill, 1993). According to the diagnosis of the newly elected family Odontobutidae (Hoese and Gill, 1993), however, it does not have its own synapomorph but is characterized by some synplesiomorphs

with Rhyacichthyidae and some synapomorphs with Gobiidae. Moreover, genetic (mtDNA) data does not support the monophyly of this family (Akihito *et al.*, in press). Therefore, the phylogenetic position of *Micropercops* is rather ambiguous among Gobioidae.

M. swinhonis lives a moderately benthic life, and spawns small eggs (Kim and Kim, 1996) just like many other gobioid fishes (Okiyama, 1988). On the other hand, intensely benthic *Odontobutis*, the type genus of the family Odontobutidae, is known to spawn much larger eggs yielding large larvae that can grow to juveniles without exogenous feeding (Iwata *et al.*, 1987, 1988a, 1988b). Therefore, a comparison of their embryos and larvae would be very interesting for understanding the adaptational and/or phylogenetic implications of early development of this family.

Kim and Kim (1996) briefly reported the spawning habits and egg development of *M. swinhonis*. We describe the larval as well as embryonic development of this species more carefully, and discuss the differences and similarities of the developmental characteristics of *Micropercops* and *Odontobutis*.

MATERIALS AND METHODS

The parental fish were caught in the Mankyung River, Ha-ri, Samre-up, Wanju-gun, Chollabuk-do, Korea, in March, 1997. One

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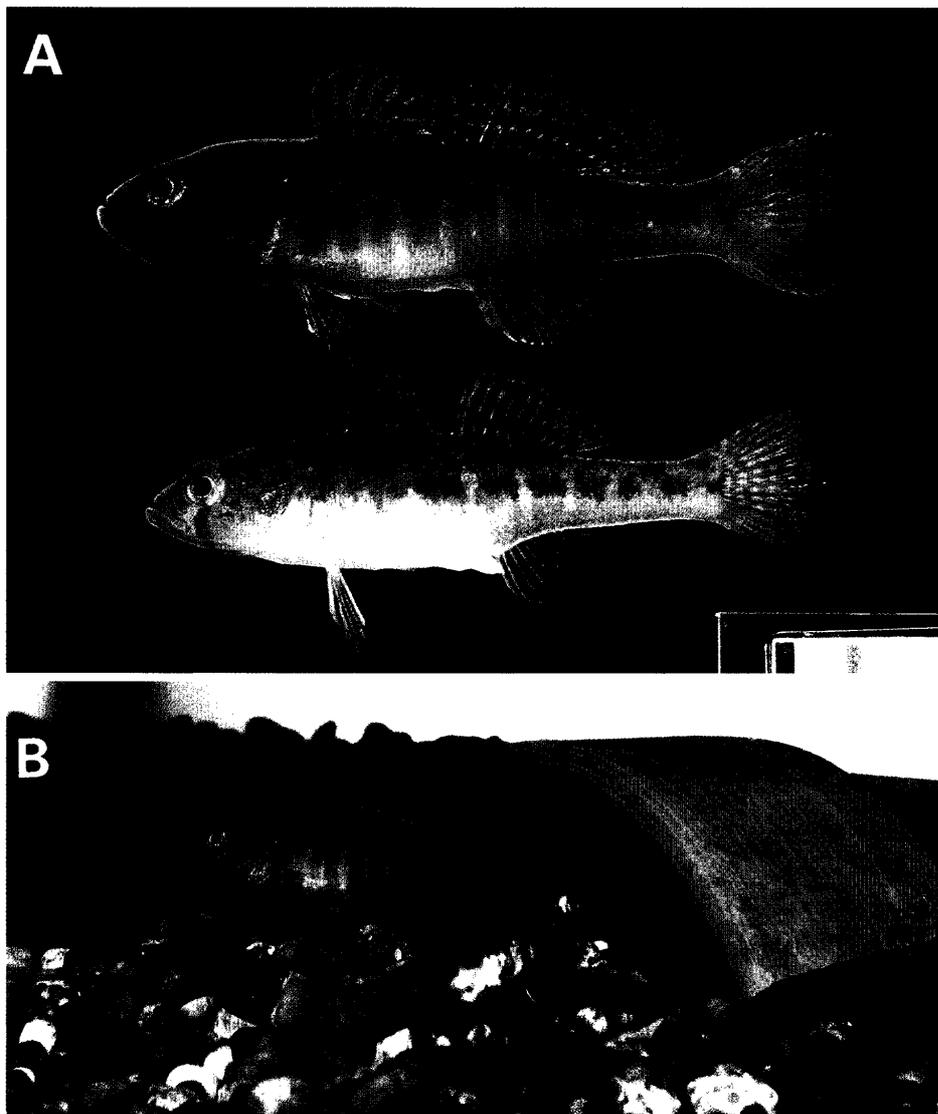


Fig. 1. A) *Micropercops swinhonis*, male (upper), 37.0 mm in standard length (SL), and female (lower), 34.0 mm SL, BLIH (Biological Laboratory, Imperial Household) 1997049; B) lateral display of a male (left) against a female (right) at the entrance of the nest.

male, about 40 mm in standard length (SL), and two females, about 35 mm SL were accommodated in each of six aquaria (60×30×30 cm) with a broken piece of a flowerpot as a spawning nest (Fig. 1). The fish were fed tubifex and water fleas.

One female spawned several times from mid-April to early July. Eggs were attached to the under surface of the nest in a one layer patch and guarded by the male. One batch amounted to about 100 to 500 eggs.

The egg patch was taken away from the guarding male just after spawning (April 17) and incubated in another aquarium (30×15×15 cm) after disinfection with a 10 ppm malachite green solution for 10 minutes. The incubating water was gently circulated in order to sway the eggs with a slow water flow. Some eggs were picked up and observed everyday. Some embryos were removed from the egg capsules and observed.

The egg patch spawned on April 18 by another pair in another aquarium was separated from the guarding male just before hatching (hatched on May 1). The hatched larvae were reared for 49 days in an aerated aquarium (30×15×15 cm) and fed with *Tropocyclops prasinus* and *Daphnia pulex* of various sizes depending on the growth of the larvae. Because of the small number of individuals in a single batch, another series of larvae from eggs spawned on April 23 was also reared and observed and the data supplemented the observation of the former series. Water temperature fluctuated between 18–23°C throughout the observations. The larvae from the latter batch

was reared under a little warmer conditions and therefore grew a little faster than the larvae from the former batch. An asterisk is added in front of the days after hatching for the larvae of the latter batch in the description.

The live eggs and larvae were placed in a shale, sketched utilizing a dissecting stereomicroscope with a drawing tube, and measured (total length, TL) with an ocular micrometer attached to the microscope. The larvae were anesthetized by dropping a 10% p-aminobenzoate solution of ethyl alcohol into the shale. The development of fin rays and scales was observed in fixed specimens (5% formalin) slightly dyed with a suminol cyanine solution (C₂₉H₃₅N₂).

RESULTS

Embryonic development

Immediately after spawning (Fig. 2A): The egg capsule was oval, 1.06–1.22 mm (20 eggs, mean 1.15 mm) in the long axis and 0.81–0.90 mm (mean 0.85 mm) in the short axis. The perivitelline space was comparatively narrow. Many small oil globules gathered in a mass.

One day after spawning (Fig. 2B): Late morula stage.

One day after spawning (Fig. 2C): Gastrula stage, blastoderm covering about 50% of the yolk.

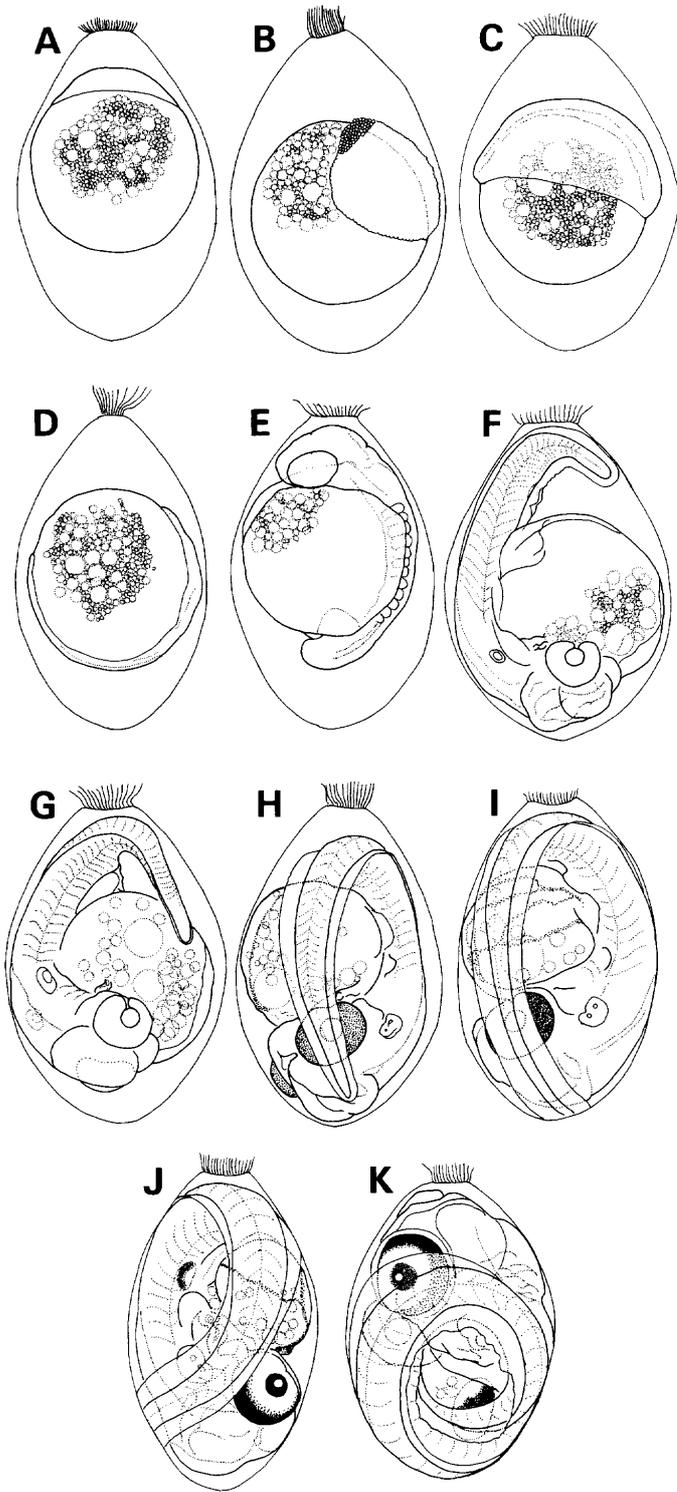


Fig. 2. Egg development of *Micropercops swinhonis*. A) immediately after spawning; B) 1 day (late moular); C) 1 day (gastrula); D) 2 days (neurula); E) 3 days; F) 4 days; G) 4 days; H) 5 days; I) 6 days; J) 8 days; K) 11 days.

Two days after spawning (Fig. 2D): Neurula stage, blastopore closed and embryonic body formed.

Three days after spawning (Fig. 2E): Head, a pair of optic vesicle and Kupffer's vesicle were differentiated. Myomeres counted 14.

Four days after spawning (Fig. 2F): A pair of otic vesicle and eye lenses were formed. Heart beat.

Four days after spawning (Figs. 2G, 3A): Tail was elon-

gated, and dorsal and anal finfolds started to develop. Embryo was 2.08 mm TL.

Five days after spawning (Figs. 2H, 3B): Eyes began to acquire black pigmentation. Pectoral fin buds and Cuvierian duct appeared. Dorsal, anal and preanus finfolds became higher. Myomeres completed 30. Embryo was 2.53 mm TL.

Six days after spawning (Figs. 2I, 3C): Eyes began to acquire silvery pigmentation. Blood vessels were branched around the yolk. The head was clearly detached from the chest. Embryo was 2.94 mm TL and turned freely in the egg capsule.

Seven days after spawning (Fig. 3D): Eyes were silver. Hatching glands appeared on the front head. Air bladder bud appeared with visible melanophores. Xanthophores appeared on the dorsal head. Embryo was 3.08 mm TL.

Eight days after spawning (Figs. 2J, 3E): Pectoral fin buds began to shift forward. Blood vessels reached to the last myomere. Embryo was 3.23 mm TL.

Nine days after spawning (Fig. 3F): The branchial arches and lower jaw were differentiated. Pectoral fin folds appeared. Melanophores appeared on the upper and lower part of the gut and on the lower part of the tail. Yellowish green liquid was visible in the end of gut. Embryo was 3.33 mm TL.

Ten days after spawning (Fig. 3G): Nasal pores were differentiated. Lower jaw reached to under the front end of the eye and was slightly movable. Melanophores appeared on the front end of the yolk. Embryo was 3.42 mm TL.

Eleven days after spawning (Figs. 2K, 3H): Pectoral fins were movable. Melanophores appeared on the dorsal fin fold base, the dorsal part behind the otic vesicles, and the lower and lateral parts of the yolk. Embryo was 3.48 mm TL, wound two times in the egg capsule. All embryos were normally breech birth.

Larval development

Immediately after hatching, twelve days after spawning (Fig. 4A): Front tip of the lower jaw extended to the same level as the front head. Yolk was slightly smaller than the head, and an oil globule as large as the otic vesicle and small ones the same size as the nasal pores were visible in it. Blood vessels reached to near the tail tip. The dorsal fin fold was lower than the anal fin fold. The gas bladder had no air. Xanthophores were scattered over the entire dorsal part. 3.75 mm TL.

One day after hatching (Fig. 4B): Gas bladder was inflated, oval and about the same size as the eye. Larvae were suspended in the water and sometimes dashed for a short distance. The gut wriggled and yellowish green liquid was excreted from the anus. Only a small number of oil globules as large as the pupil existed in the yolk. 3.80 mm TL.

Two days after hatching (Fig. 4C): The lower jaw was projected and exogenous feeding began. The yolk was reduced to the same size as the eye. The future caudal skeleton area swelled slightly. The middle part of the dorsal and anal fin folds began to drop lower. 4.05 mm TL.

Six days after hatching (Fig. 4D): The yolk was the same size as the pupil. Caudal fin support apparently began to form.

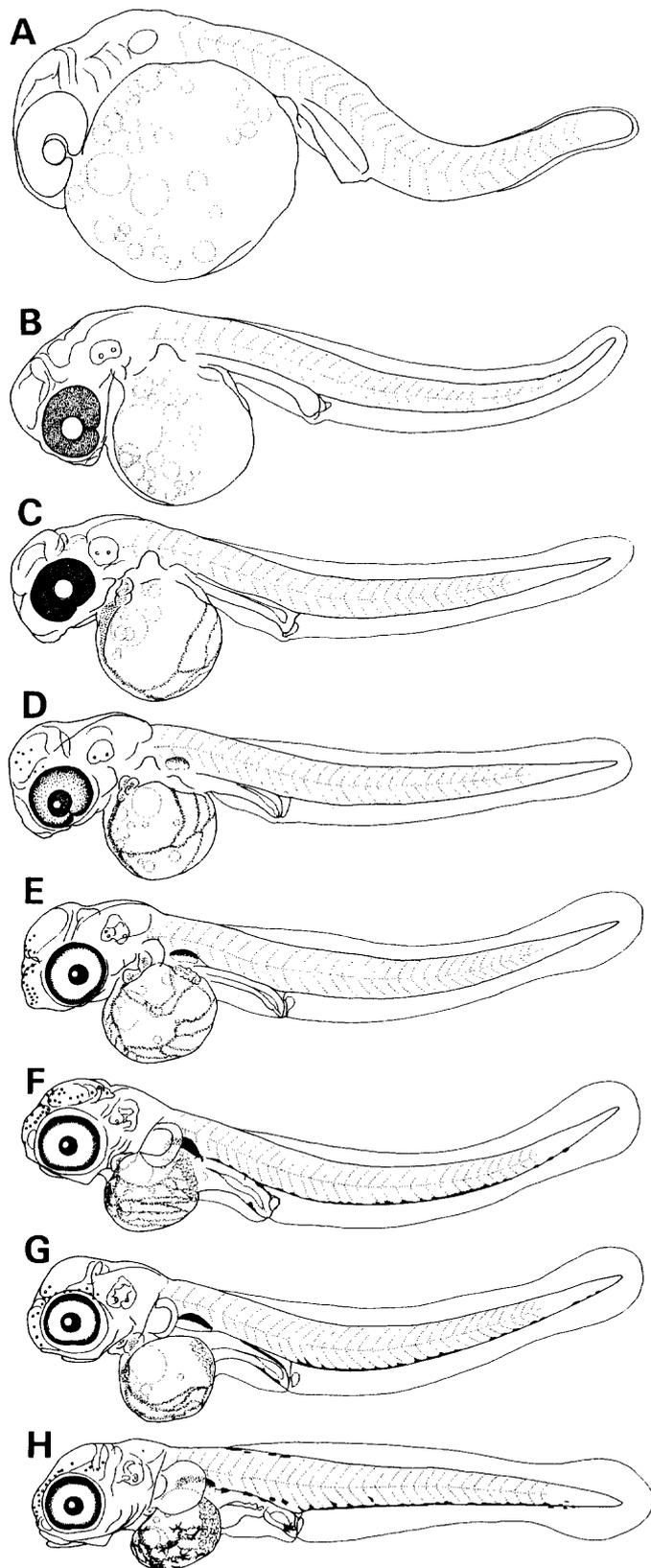


Fig. 3. Embryos in the eggs of *Micropercops swinhonis*. A) 4 days after spawning (2.08 mm TL); B) 5 days (2.53 mm TL); C) 6 days (2.94 mm TL); D) 7 days (3.08 mm TL); E) 8 days (3.23 mm TL); F) 9 days (3.33 mm TL); G) 10 days (3.42 mm TL); H) 11 days (3.48 mm TL).

Rudimentary anal fin support appeared. Gill lamellae were clearly observed. The lower part of the gas bladder inflated to swell downward. The middle part of the dorsal and anal fin folds were degenerating. Melanophores on the back increased

in number, and were also visible in the otic vesicles. 4.97 mm TL.

Nine days after hatching (Fig. 4E): Five caudal rays were differentiated. Rudimentary second dorsal fin support appeared. Oil globules in the yolk became smaller than nasal pore size. 5.33 mm TL.

Twelve days after hatching (Fig. 4F): Branchiostegals were formed. The spleen was differentiated. The first to eighth neural spines were recognized. Eight caudal rays and 5 anal rays were counted. Oil globules had disappeared. 5.62 mm TL.

Sixteen days after hatching (Fig. 4G): The notochord end started to flex upward. Rudimentary first dorsal fin support and pelvic fin buds appeared. All neural and haemal spines were observed. All fin folds were greatly reduced. Ten second dorsal rays, 8 anal rays, and 10 caudal rays were counted. Erythrophores appeared near the caudal rays in addition to melanophores. Xanthophores appeared on the dorsal part of the notochord end. 6.90 mm TL.

Twenty days after hatching (Fig. 4H): Seven first dorsal spines were counted. First spines of the second dorsal and anal fins were formed, and soft rays of both fins reached to the ends of the fin folds. Pelvic rays began to develop. All fin folds disappeared except those between the first and second dorsal fins. Intestine folding began. Melanophores appeared on both jaws. 7.83 mm TL.

*Twenty-one days after hatching (Fig. 4I): Notochord flexion was complete. Caudal fin rays began to segment. Myomeres were W-shaped. Melanophores appeared on the second dorsal rays, anal rays, and the upper and lower parts of the vertebrae. Xanthophores also appeared on the upper part of the vertebrae. 9.30 mm TL.

*Twenty-four days after hatching (Fig. 4J): Five pectoral rays were counted. The second dorsal and anal rays began to segment. The second dorsal, anal, caudal and pelvic fins became longer. The gas bladder again was oval. Erythrophores gathered on the caudal fin base. 10.64 mm TL.

*Twenty-four days after hatching (Fig. 4K): Nasal pores became long ellipses. The first dorsal spines began to elongate. Fifteen pectoral rays were counted. The peritoneum began to be tinted silvery. Swimming behavior was similar to that of the adult. 11.47 mm TL.

Twenty-eight days after hatching (Fig. 4L): Cycloid scales appeared on the caudal peduncle. Melanophores appeared on the first dorsal and pectoral fins. Melanophores were obvious on the dorsal part of the head, upper part of the opercle, snout, below the eye, and above the pectoral fin base. 11.83 mm TL.

*Twenty-nine days after hatching (Fig. 4M): The nasal pores became dumbbell-shaped. Branching of the caudal rays began. Pelvic fins were greatly elongated. Scales extended to below the first dorsal fin. Six spots appeared on the back, and xanthophores were obvious on the spots. 14.41 mm TL.

Thirty-six days after hatching (Fig. 4N): The nasal pores were separated into anterior and posterior nostrils. The urogenital papilla was developed. Eight caudal rays were

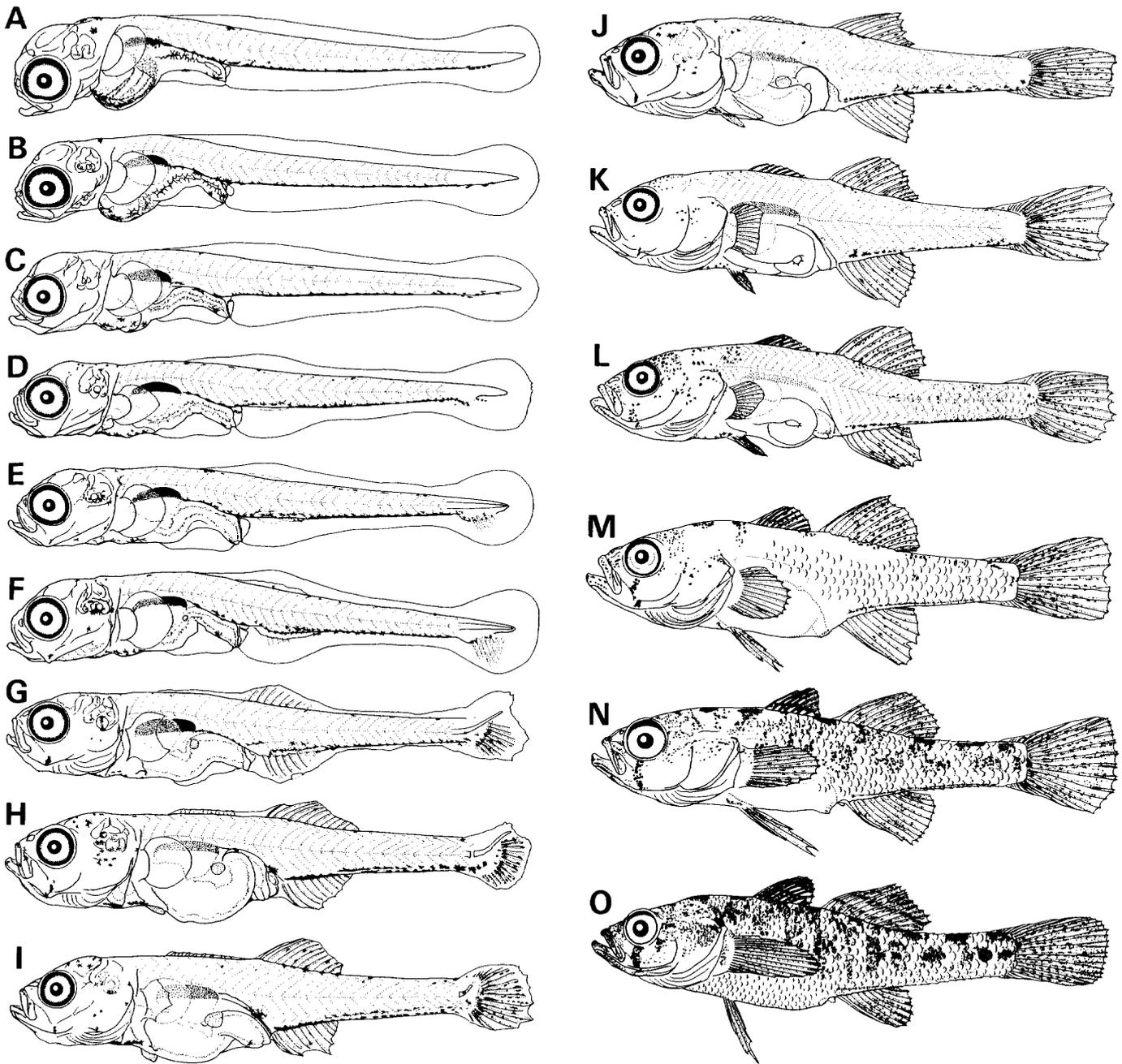


Fig. 4. Larval development of *Micropercops swinhonis*. A) just after hatching (12 days after spawning) (3.75 mm TL); B) 1 day (3.80 mm TL); C) 2 days (4.05 mm TL); D) 6 days (4.97 mm TL); E) 9 days (5.33 mm TL); F) 12 days (5.62 mm TL); G) 16 days (6.90 mm TL); H) 20 days (7.83 mm TL); I) *21 days (9.30 mm TL); J) *24 days (10.64 mm TL); K) *24 days (11.47 mm TL); L) 28 days (11.83 mm TL); M) *29 days (14.41 mm TL); N) 36 days (16.28 mm TL); O) 49 days (22.00 mm TL).

branched. Pelvic rays began to segment. The pectoral fin became larger. Most scales became ctenoid. Melanophores appeared on the upper part of the pectoral fin base. Eight spots appeared on the lateral body. 16.28 mm TL.

Forty-nine days after hatching (Fig. 4O): Four second dorsal rays, 4 anal rays, 9 caudal rays, and 3 pectoral rays were branched. The last ray of the second dorsal fin separated into two, but that of the anal fin did not. Pectoral rays reached below the first soft ray of the second dorsal fin. The upper part of the opercle, throat and under part of the belly were covered with cycloid scales; all other scaled regions were covered with ctenoid scales. The spotted pattern was same as the adult pattern. 22.00 mm TL.

DISCUSSION

The developmental process of *Micropercops swinhonis* is quite different from that of *Odontobutis*, a member of the same family, that lays very large eggs, yielding very large larvae (Iwata *et al.*, 1987, 1988a, 1988b). In *O. interrupta* (Iwata *et al.*, 1987), for example, notochord flexion occurs and pelvic fin buds appear before eye formation and jaw completion (notochord flexion began after eye formation and jaw completion, and pelvic fin buds appeared even later in *M. swinhonis*), intestine folding occurs before dorsal and anal fin formation (intestine was folded after completion of the dorsal and anal fin ray counts in *M. swinhonis*), first dorsal spines appear at

the same time as the second dorsal and anal rays (the first dorsal spines developed after the completion of the dorsal and anal fin ray counts in *M. swinhonis*), and larvae grow to juveniles without exogenous feeding (larvae began feeding before fin ray formation in *M. swinhonis*).

M. swinhonis neither lays large eggs nor lacks the exogenous feeding larval stage, and therefore its developmental process is similar to those of many amphidromous gobies of another family, Gobiidae, having planktonic sea going larval stages (see Moser *et al.*, 1984; Okiyama, 1988). Although *M. swinhonis* has a planktonic exogenous feeding larval stage, it never goes to sea. It usually lives in inlets or slow flowing ponds in rivers (Kim and Kim, 1996, 1997), and its larvae must also dwell there. Therefore, it can reside in rivers without going to the sea.

On the other hand, the developmental characteristics unique to *Odontobutis* must be caused by the heterochronic developmental shifts of organs involved in the enlargement of egg size that enable *Odontobutis* juveniles to skip the exogenous feeding larval stage and live a fluvial life (Iwata *et al.*, 1987, 1988a, 1988b).

However, two developmental characteristics, one minor and the other major, make *M. swinhonis* distinct from gobiid fishes. The minor one is that the middle part of the dorsal and anal fin folds drops lower before caudal fin ray differentiation (later than that in gobiid fishes) (see Okiyama, 1988).

The major difference is that the left and right pelvic fin buds appear distantly from each other (Fig. 5C). Notably, this characteristic is shared by *Odontobutis* (shown in Fig. 5B cited from Iwata *et al.*, 1987) in which the two fin buds appear much more distantly (Iwata *et al.*, 1987, 1988a, 1988b). On the other hand, the two fin buds are close in gobiid fishes (see Okiyama, 1988; an example is shown in Fig. 5A cited from Sakai and Yasuda, 1978). Gobiids with separated pelvics such as *Eleotris oxycephala* (Eleotrinae of Gobiidae by Hoese and Gill, 1993) (Dotu and Fujita, 1959) and *Oxyeleotris marmoratus* (Butinae of Gobiidae by Hoese and Gill, 1993) (Senoo *et al.*, 1994) also seem to develop their pelvic buds close, although only lateral views of larvae are presented in the reports.

It is possible that the distantly separate appearance of pelvic buds accompanies the developmental adaptation to fluvial life. That is, the larvae of fluvial gobies have much larger yolks than those of their amphidromous relatives, and this enables them to grow without going to sea; the two pelvic buds may appear distantly interrupted by the large yolk sack. However, *M. swinhonis* does not have such a large yolk, but the pelvic buds still appear distantly. Furthermore, even though their larvae have large yolk sacks, the two pelvic buds of fluvial *Rhinogobius* spp. from Okinawa Island develop close together (Hirashima and Tachihara, 2000).

This developmental characteristic, distant pelvic fin buds, seems very rare even among perciform fishes, although pelvic fin development has been scarcely observed from under side. For example, the larvae of blennioids, another suborder of Perciformes which lives benthic life and has separate pelvics like odontobutids, seem to develop their two pelvic buds close

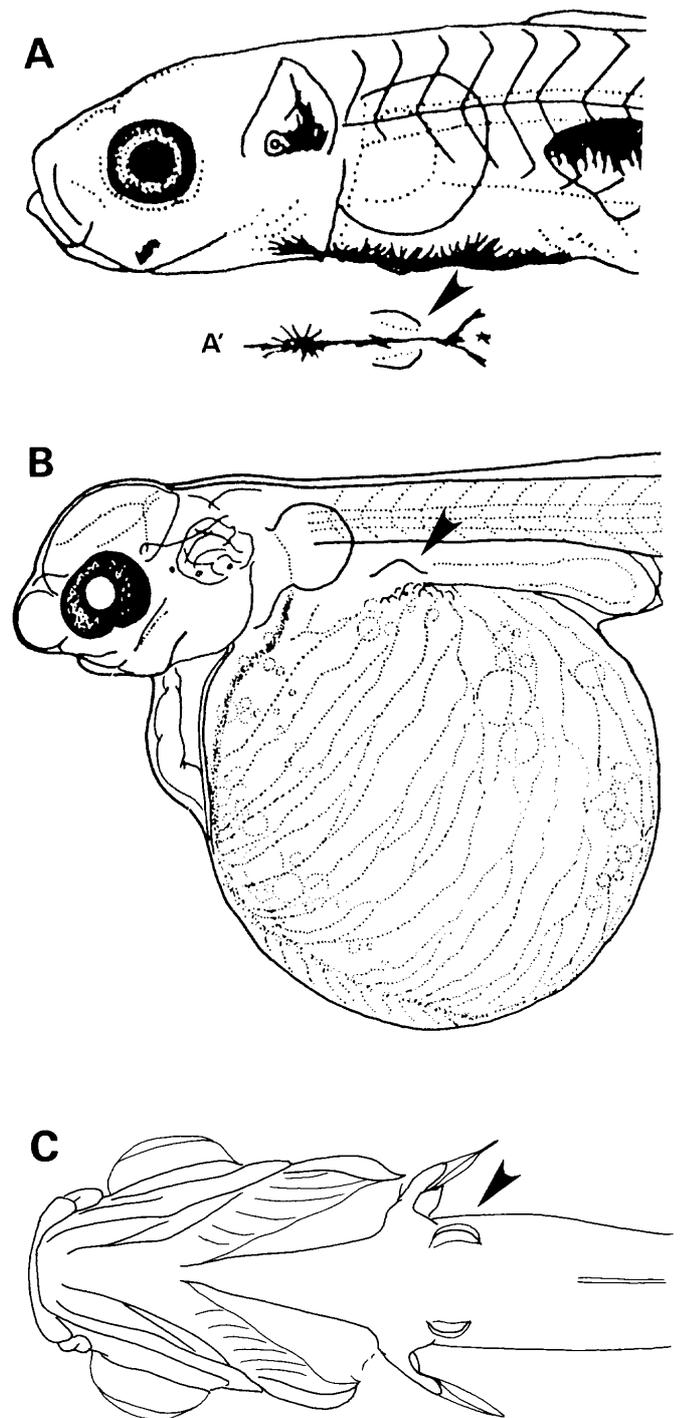


Fig. 5. First appearance of pelvic fin buds (arrows) in three gobies. A) *Rhinogobius* sp. CB (cited from Sakai and Yasuda, 1978); B) *Odontobutis interrupta* (cited from Iwata *et al.*, 1987); C) *Micropercops swinhonis*, the present study, 16 days after hatching.

to each other, as far as observed from lateral view (see Moser *et al.*, 1984; Okiyama, 1988). So, it may be uniquely synapomorphic to Odontobutidae.

However, neither the genetic (Akihito *et al.*, in press) nor the morphological data (Hoese and Gill, 1993) support the monophyly of Odontobutidae. Therefore, there also remain large possibilities that the characteristic is a synplesiomorph or a result of convergence. The question, synapomorphy, synplesiomorphy or convergence, will be concluded after investigating the larval development and the genetic relation-

ship of the last monospecific genus of Odontobutidae, *Perccottus glehni* from China and Russia. Further developmental and genetic comparisons with other gobioids especially Rhyacichthyidae will also help in understanding the phylogenetic implications of developmental characteristics.

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