Embryonic, larval, and juvenile development of reared Cobitis striata fuchigamii (Cypriniformes: Cobitidae)

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Embryonic, larval, and juvenile development of reared *Cobitis striata fuchigamii* (Cypriniformes: Cobitidae)

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Abstract In this study, the early development of *Cobitis striata fuchigamii* was described using aquarium-spawned eggs and juveniles. Egg yolk diameter after water absorption was 1.0–1.1 mm, and the spherical, demersal eggs had a light-yellow yolk, no oil droplets, and low viscosity. Newly hatched larvae [4.0–4.5 mm in total length (TL)] had 44–45 (27–28 + 17–18) myomeres (26–27 between the pectoral fins and the middle of the anus), two pairs of short tubular outer gill filaments on the cheek, and only a few melanophores on parts of the eye. Notochord flexion began at 5.1–6.8 mm TL and ended at 10.0 mm TL. The number of fin rays on each fin was finalized at approximately 25.0 mm TL. Lateral line spots of the larvae were indistinct, and juveniles formed blotches at approximately 25.0 mm TL. The barbels were formed in the following order: third, second, and then first. The formation of membranous fins occurred in the following order: pectoral, caudal, dorsal, anal, and then pelvic; full fin ray complements occurred in the caudal, dorsal, anal, pectoral, and pelvic regions. Some individuals (5.1–7.5 mm TL at the flexion stage) had free lateral neuromasts on the caudal region, each with a short cupula.

Keywords: Cobitidae · Striped loach · Larvae · Juveniles · Endangered species

Introduction

The Japanese striped loach, *Cobitis striata fuchigamii* (Cypriniformes, Cobitidae), is an endemic subspecies inhabiting the middle and lower reaches of the Onga River system in northern Kyushu, Japan (Nakajima 2012; Nakajima and Uchiyama 2017). Its distribution is currently limited to narrow areas in the river system owing to the negative impact of past coal mine development (Nakajima 2017), recent river dredging, and farmland modernization (Fukuoka Prefecture 2014), causing it to be designated as 'Endangered' in the Red List of Japan (Ministry of the Environment of Japan 2020) and in the Red Data Book of Fukuoka Prefecture (Fukuoka Prefecture 2014).

Nakajima (2012) described this as a new subspecies due to certain features, such as an egg yolk diameter of approximately 1.0 mm; the lamina circularis at the base of the pectoral fin of the adult male simple roundish plate; a prepelvic myotome number (PMN) of 13; line system L5 organized in 11–14 roundish blotches in the non-spawning season; L5 forming obvious longitudinal stripes in adult males during spawning season; black spots in upper and lower regions of the caudal base, and the lower spot is either faint or missing.

Several studies, including those under the assorted names, have indicated the distribution and population status of this fish (Nakajima et al. 2006, 2008, 2011; Kitagawa et al. 2009; Nakajima 2017). In addition, Nakajima and Uchiyama (2017) presented photographs of the postflexion larvae and juvenile forms. However, no study has described the morphological characteristics of the early life stages. Our study investigated and described the morphology of eggs, larvae, and juveniles of *C. striata fuchigamii* based on specimens reared in the laboratory.

Materials and methods

Parental fish, larvae, and juvenile collection. Parental *Cobitis striata fuchigamii* fish were collected using a hand net (mesh size: 2 mm; net width: 350 mm) and a dip net (mesh size: 3 mm; net width: 900 mm) in a river of the Onga River system, Fukuoka Prefecture, northern Kyushu, Japan, on 22 June 2021. First, we confirmed that this fish inhabits a continuous, 2-km section of this river. Subsequently, more than 50 individuals were captured in a 45-minute collection window in a 50-m section of river, suggesting a population of more than 2,000 individuals in this river. Based on these results, we decided that the number of parent fish to be captured for the experiment should be less than 1% of the population.

The collected females had their body weight (BW) and standard length (SL) measured in the field, and after calculating the conditional factor ($CF = BW/SL^3$), six females (71.0–85.5 mm SL) with high CF values ($> 6.8 \times 10^{-3}$) were selected as parental fish. In males, the blotches along the middle of the body change to a broad stripe in the spawning season (Nakajima 2012). Six larger males (56.5–61.8 mm SL) with a linear pattern were selected as parental fish from the collected individuals. The parental individuals were maintained without water temperature and daylight controls in a glass aquarium (60 L) at the Fisheries Research Laboratory, Kyushu University.

Artificial breeding. On 25 June 2021 (15:00 h), human chorionic gonadotropin (HCG) (ASUKA Pharmaceuticals, Japan; 3,000 units for gonadotropin intramuscular injection) dissolved in 0.6% NaCl solution was injected into the abdominal cavity of the six *C. striata fuchigamii* females at the base of the ventral fin (Terumo Corporation, Japan; Terumo syringe for tuberculin 1 mL, injection needle 27G × 3/4). The administration method and dose (10 units per 1 g of body weight) followed those of Noguchi et al. (2009). Before dosing, females were anesthetized in a clove oil solution (Pedrazzani and Neto 2014) diluted in water to reduce the stress on the fish. A glass aquarium (60 L) was set up in the same laboratory, separated from the rearing aquarium, equipped with aeration and a netted fish tank made of a

horticultural pot net (3-mm mesh) to prevent adult fish from cannibalizing the eggs (Noguchi et al. 2009). Six males and six females were placed in the aquarium without water temperature control (23.9–25.9ºC) and in daylight.

Rearing of eggs and larvae. After spawning, approximately 900 eggs were transferred to a Petri dish (D100 × H20 mm), each containing approximately 100 eggs. The water temperature in the Petri dish ranged from 23.6–24.1ºC depending on the room temperature, which was maintained at 24ºC, and only water injection was performed until hatching (approximately 2 days). Hatched larvae were transferred to another Petri dish (D250 × H70 mm) with aeration. The larvae were fed rotifers (Clean wamushi, Kyorin Food Industries, Ltd., Japan) twice per day for two weeks from the third day after hatching. The remaining food was sucked out with a dropper and water was replenished only by the amount lost. After two weeks, the larvae were moved to an aquarium (60 L) with fine sand and decayed *Zizania latifolia* collected from the same location where the parental fish were collected, and they were kept until the end of the experiment (water temperature: 24.9–26.2ºC). We expected the promotion of growth of larvae and juveniles by creating a similar environment to their wild habitat, especially the corroded pieces of *Z. latifolia*, and the microorganisms generated by their decomposition would be used as feed for larvae and juveniles.

Observations and measurements. The *C. striata fuchigamii* eggs were photographed and observed (0.5–4 h cycle) under a stereomicroscope, and embryonic development was described. The larvae and juveniles were examined microscopically every day after sedation with clove oil and photographed when changed developmental stages. Nine individuals were sketched based on the images. Fresh specimens were preserved in 5% buffered formalin solution. Several specimens were stained with Alizarin Red for barbel observation. The developmental stages were classified according to Kendall et al. (1984), Fujimoto et al. (2006), and Saitoh and Hosoya (2014a, b, c). A line system (lines L1 to L5) and the prepelvic myotome number (PMN), which are used as taxonomic characters in *Cobitis*, were described

based on Nakajima (2012). Images of eggs, larvae, and juveniles, including a ruler photographed under a stereomicroscope, were enlarged on a PC, and egg and yolk diameters, and the total length (TL), head length (HL), pectoral fin length (P_1L), and predorsal length (PDL) of larvae and juveniles were measured. The fixed eggs, larvae, juveniles, and parental fish were registered at the Kyusyu University Museum (KYUM-PI-00007296 to 00007311).

Results

Eggs. *General development and morphology.* After spawning, eggs were scattered over at the bottom of the aquarium. Demersal, spherical, weakly viscous chorion, 2.1–2.3 (mean ± SD = 2.2 ± 0.1, n = 10) mm in diameter after water absorption of fertilized eggs; yolk diameter 1.0–1.1 (1.0 ± 0.0) mm, pale yellow, translucent, no chromatophores and oil globules. The time from fertilization to hatching and the main developmental changes elapsed as follows: 0.5 h after insemination, blastodisc formation (Fig. 1a); 1.5 h, 2-cell stage; 2 h, 4-cell stage; 2.5 h, 8-cell stage; 3 h, 16-cell stage (Fig. 1b); 9 h, germ ring stage; 11 h, somite stage, few somites and rudimentary optic vesicles formed (Fig. 1c); 15 h, increased somites; 17.5 h, a horizontal line entered the optic primordium, and a pair of otic vesicles formed in the head area, and Kupffer's vesicle formed in the tailbud region (Fig. 1d); and 20 h after insemination, Kupffer's vesicle almost disappeared, the yolk extension separated from the yolk ball, and the anus was depressed. Fluid circulation began to appear, and the body moved slightly (Fig. 1e). At 25 h, the optic vesicles developed into optic cups, lenses formed, and spinning occurred inside the egg (Fig. 1f). At 34 h after insemination, extremely short tubular outer gill filaments, pectoral fin buds, and a heartbeat were observed. Hatching occurred 37–40 h after fertilization at 23.6–24.1ºC. The hatching rate of the 900 eggs was approximately 20%.

Larvae and juveniles. *General development and morphology.* Total lengths (TL) of

larvae and juveniles at each developmental stage are presented in Table 1. Newly hatched larvae: 4.0–4.5 (4.3 ± 0.2, *n* = 4) mm TL; 27–28 + 17–18 = 44–45 myomeres (26–27 between the pectoral fins and the middle of the anus) close to constant in number. Yolk sac very large (68–70% TL), oval along the body axis (Fig. 2a), becoming smaller with growth; yolk absorbed in the flexion larvae above approximately 6.0 mm TL (2 days after hatching, Fig. 2d). Newly hatched larvae had both dorsal and ventral fin folds (Fig. 2a). Dorsal and anal fin folds separated from the caudal fin in juveniles above approximately 20.0 mm TL (35–77 days after hatching, Fig. 2g, h). Two pairs of short tubular outer gill filaments on the side near the operculum of newly hatched larvae, becoming elongated and increased with growth (0–2 days after hatching, Fig. 2a–c); tubular outer gill filaments completely retracted at the flexion larvae above 10.0 mm TL (5 days after hatching, Fig. 2d). The mouth opened in the preflexion larvae above 4.5 mm TL (0–1 days after hatching, Fig. 2b), and the alimentary tract and anus connected in flexion larvae above 6.0 mm TL (Fig. 2d); began to feed on rotifers. A gill opened and a pair of nostrils formed at the preflexion larvae above 5.1 mm TL (1–2 days after hatching, Fig. 2b, c); anterior and posterior nostrils separated at the postflexion larvae above approximately 17.7 mm TL (35 days after hatching, Fig. 2g). In several individual flexion larvae (5.1–7.5 mm TL; 2–3 days after hatching); a free neuromast with short cupulae along the body axis on the side posterior to the base of the pectoral fin. The PMN reached 13 during the juvenile stage.

Pigmentation. Melanophores deposition on eyes found immediately after hatching (Fig. 2a), completed in the preflexion larvae (0–2 days after hatching, Fig. 2b, c). Head and dorsal melanophores existing in preflexion larvae (Fig. 2b), melanophores increasing more, spread head and laterally along the body axis in flexion and postflexion larvae (Fig. 2c–f). Melanophores of the lateral indistinct patchy pattern at approximately 18.5 mm TL (35 days after hatching, Fig. 2g); identifiable in lines L1 to L5, oval blotches in line L5 at 25.4 mm TL (77 days after hatching, Fig. 2h). The black oblique band from the upper part of the eye to the

snout tip appeared during the flexion larval stage (Fig. 2d), becoming darker and more distinct in the postflexion larvae and juveniles. A black oblique band of cheeks in postflexion larvae (10 days after hatching, Fig. 2e). Two small, indistinct black spots on the upper and lower caudal fin base in the flexion larvae (Fig. 2d), distinct on the upper and indistinct on the lower lobe (Fig. 2g); the lower spot almost disappeared above approximately 25.4 mm TL in juveniles (Fig. 2h). One or two distinct black arch-shaped bands on the dorsal and caudal fin membranes during the juvenile stage (Fig. 2g, h).

Fin formation. Dorsal and anal fin anlagen appeared above approximately 6.0 mm TL in flexion larvae (3–5 days after hatching, Fig. 2d), fin ray formation began above approximately 9.5 mm TL in postflexion larvae (10 days after hatching, Fig. 2e), attaining full complement (10 and 8 for the dorsal and anal fin, respectively) above approximately 20.0 mm TL in postflexion larvae (35 days after hatching, Fig. 2g). The dorsal fin anlage and fin rays appeared slightly earlier than those of the anal fin. Caudal fin ray formation started above approximately 7.0 mm TL in flexion larvae (4–5 days after hatching, Fig. 2d), attaining full complement (16) above approximately 10.0 mm TL in postflexion larvae (10 days after hatching, Fig. 2f). Newly hatched larvae had small pectoral fin buds (Fig. 2a). The early pectoral fin bud elongated, reached a maximum a few days after hatching, and then gradually decreased in size (Fig. 3). Pectoral fin ray formation began above 11.0 mm TL in postflexion larvae (10 days after hatching, Fig. 2f), attaining full complement (8) above approximately 20.0 mm TL in juveniles (Fig. 2g). The pelvic fin bud appeared above approximately 9.5 mm TL in postflexion larvae (10 days after hatching, Fig. 2f), and fin ray formation began above approximately 17.7 mm TL in postflexion larvae (35 days after hatching, Fig. 2g), attaining full complement (8) above approximately 24.5 mm TL in juveniles (35–77 days after hatching, Fig. 2h).

Barbel and suborbital spines. The mouth remained closed of the newly hatched larvae. The mouth, with the transparent tissue of the rostrum, opened like a sucker at 4.5–5.1 mm TL

in preflexion larvae 0–1 days after hatching (Fig. 4a). The rostral tissue disappeared as soon as the barbel formation began. The third barbels formed above approximately 5.1 mm TL in preflexion larvae 2 days after hatching (Fig. 4b), and many small, spinous papillae surrounded the mouth, including barbels (Fig. 4b–d), and appeared beneath the operculum (Fig. 2c, d). The second barbels formed above approximately 6.7 mm TL in flexion larvae 4 days after hatching (Fig. 4d). The first barbels formed above approximately 9.5 mm TL of postflexion larvae 10 days after hatching, and the small spinous papillae surrounding the mouth either decreased or disappeared (Fig. 4e, f).

Suborbital integument depression appeared above 11.0 mm TL in postflexion larvae 10 days after hatching. Suborbital spines formed in the depressed area above 17.5 mm TL (35 days after hatching, Fig. 2g).

Swimming behavior. Newly hatched larvae stayed at the bottom of the aquarium, sometimes suddenly surfaced after stimulation, such as vibration. Preflexion larvae stabilized at the bottom of the aquarium, and several individuals attached the aquarium wall perpendicular to the water surface by the transparent tissue of the rostrum. With the elongation of the pectoral fins, the body gradually raised, but the center of gravity on one side of the body. Postflexion larvae crawled along the bottom of the aquarium while fluttering their pectoral fins.

Discussion

In this study, we described for the first time the morphological changes at each developmental stage of *Cobitis striata fuchigamii*. According to Nakajima and Uchiyama (2017), 20 species and subspecies of the genus *Cobitis* inhabit Japan. Of these, egg and/or yolk sizes have been documented in 13 species/subspecies, whereas newly hatched larval sizes have been reported

in only six species/subspecies (Table 2). These numbers do not include *Cobitis biwae*, reported by Okada and Seiishi (1937), because we could not identify these species/subspecies in the latest classification (Nakajima and Uchiyama 2017). In addition, we noted that *C. striata* (Saitoh 1990; Saitoh and Hosoya 2014c), which will be described later in this discussion section, corresponds to *Cobitis striata striata* according to Nakajima and Uchiyama (2017).

Eggs. The eggs of *C. striata fuchigamii* obtained in our study were spherical, demersal, and had a weakly viscous chorionic surface. These characteristics are similar to those of other species/subspecies of the all genus *Cobitis* in Japan (Nakajima and Uchiyama 2017). The yolk diameter of *C. striata fuchigamii* in our study was consistent with that reported by Nakajima (2012) and is considered to be mid-sized for all *Cobitis* species/subspecies (Table 2).
The hatching periods and water temperature showed differences among *Cobitis* species/subspecies: 1–2 days (25.0°C) for *Cobitis minamorii oumiensis* from Nakajima and Uchiyama (2017); 3–4 days (17.0–19.0°C) for *Cobitis* sp. BIWAE type D from Nagae et al. (2021); 4–5 days (18.0°C) for *Cobitis takatsuensis* from Shimizu et al. (1998); and approximately 2 days (23.6–24.8°C) in the *C. striata fuchigamii* of our study. Among these *Cobitis* species/subspecies, *C. striata fuchigamii* has a relatively short hatching period.

Larvae and juveniles. The following six species/subspecies of genus *Cobitis* in Japan have a description of newly hatched larvae: *C. striata fuchigamii* (4.3 ± 0.2 mm TL, the present study); *C. striata* (4.0 mm TL, Saitoh 1990), which corresponds to *C. striata striata* in Nakajima and Uchiyama (2017); *Cobitis magnostriata* (4.5 mm TL, Saitoh and Hosoya 2014a); *Cobitis minamorii minamorii* (3.5 mm TL, Saitoh and Hosiya 2014b); *Cobitis* sp. BIWAE type D (4.2 ± 0.6 mm TL, Nagae et al. 2021); and *C. takatsuensis* (5.7 mm TL, Shimizu et al. 1998), and the values from our study were greater than that of *C. minamorii minamorii*, less than that of *C. takatsuensis*, and similar to *C. striata*, *C. magnostriata*, and *Cobitis* sp. BIWAE type D (Saitoh 1990; Shimizu et al. 1998; Saitoh and Hosoya 2014a, b;

Nagae et al. 2021). A comparison of yolk diameter and hatched larval body size of reported species/subspecies suggests that the larger the yolk diameter, the larger the hatched larvae, indicating that hatched larval size is related to yolk diameter (Table 2).

The total number of myomeres in newly hatched larvae of *C. striata fuchigamii* was 44–45, and those of *C. magnostriata*, *C. minamorii minamorii*, and *C. striata* were 43, 40–41, and 43–44, respectively (Saitoh and Hosoya 2014a, b, c), suggesting a clear discrimination between this subspecies and *C. minamorii minamorii* in terms of total myomeres of newly hatched larvae. The number of myomeres between the pectoral fins and middle of the anus was 26–27 in this subspecies, 28–32 in *C. magnostriata*, 26–27 in *C. minamorii minamorii*, and 28–30 in *C. striata* (Saitoh and Hosoya 2014a, b, c). Although there was no overlap of *C. striata fuchigamii* with *C. magnostriata* and *C. striata* in these values, it may be difficult to distinguish these species based on the present observations because there is generally a range of myomere numbers within the same species/subspecies.

Newly hatched *C. striata fuchigamii* larvae had tubular outer gill filaments, similar to other *Cobitis* species/subspecies (Table 2), as well as other similar features, such as pectoral fin anlagen and eye melanophores. In addition, the barbels were formed in the order of third, second, and then first, and the number of fin rays developed in the order of caudal, dorsal, anal, pectoral, and then ventral, similar to other *Cobitis* species (Shimizu et al. 1998; Saitoh and Hosoya 2014a; Nagae et al. 2021). Several *Cobitis* species/subspecies in their early life stages inhabit areas with a high density of emerged plants (Bohlen 2003b; Nakajima and Uchiyama 2017) and hypoxic environmental conditions, as decayed plants are deposited at the bottom. The wild larvae and juveniles of *C. striata fuchigamii* inhabit similar environments in the Onga River system, and the outer gill filaments of *C. striata fuchigamii* are thought to develop as an adaptation to hypoxic environments, similar to *Cobitis taenia* (Bohlen 2003a).

Relationship between behavior and morphological traits. Figure 5 summarizes the

behavior and morphological characteristics of the early larval stages. Preflexion larvae of *C. striata fuchigamii* are shown 1–2 days after hatching, temporarily attached to the aquarium wall by the transparent rostrum tissue of the mouth. *Cobitis takatsuensis* exhibits the same behavior, suggesting that it adheres to objects to prevent flow (Shimizu 2005). After a few days, the transparent tissue disappeared, pectoral fin buds reached their maximum size, and flexion larvae began to feed in the aquarium. Elongation of pectoral fins may occur because of the migration from emerged plants to feeding areas, or to allow for a wider dispersion across various parts of the river (Fig. 3). The pectoral fin began to decrease immediately, and the period of migration and dispersal was expected to be short. From another point of view, the elongation of the pectoral fin overlaps with the start of feeding. Therefore, long pectoral fins may contribute to orientation and postural control during swimming.

Distinctions from closely related species in the Onga River system. *Cobitis striata fuchigamii* occurs only in the Onga River system, along with two native *Cobitis* species, *Cobitis matsubarae* and *C. takatsuensis* (Nakajima et al. 2011). Nakajima et al. (2011) reported different patterns of longitudinal distribution among these three native *Cobitis* species/subspecies, suggesting that they do not usually coexist. However, the occurrence of both *C. matsubarae* and *C. striata fuchigamii* was found at one site (Nakajima et al. 2011); therefore, it is necessary to compare the larvae and juveniles of both species and to indicate distinguishing characteristics between them. However, the early morphology of *C. matsubarae* has not yet been described. The images of adult fish skeletons published by Kano et al. (2018) were used to count the number of vertebrae in both species, but resulted in approximately equal numbers, and estimating the number of myomeres also produced similar results. One of the keys to distinguishing adults of *C. matsubarae* from *C. striata* is the black spot(s) on the caudal fin base, as the former has two and the latter only one (Hosoya 2013). The observation in this study shows that the black spot of this subspecies becomes clear at approximately 24.0 mm TL or greater. This result indicates that these *Cobitis*

species/subspecies may be distinguishable by the number of spots after reaching that size. *Misgurnus anguillicaudatus* also inhabits the Onga River system (Nakajima et al. 2011), and the exotic loach *Misgurnus dabryanus* has also been collected here (Sakai et al. 2008). However, the total number of myomeres of *M. anguillicaudatus* (44–47, Hosoya 2014) overlaps with *C. striata fuchigamii* (44–45). We summarized the position of the dorsal fin on the basis of sketches from previous studies (*C. biwae*, Okada and Seiishi 1937; *C. striata*, Saitoh and Hosoya 2014c; *C. magnostriata*, Saitoh and Hosoya 2014a; *C. minamorii minamorii*, Saitoh and Hosoya 2014b; *M. anguillicaudatus*, Hosoya 2014; *M. anguillicaudatus* and *M. dabryanus*, Takahashi and Shimizu 2016; *C. matsubarae*, Nagae et al. unpublished data); and the present study (*C. striata fuchigamii*), resulting in a clear difference between the genus *Misgurnus* (> 53% in predorsal length/total length) and genus *Cobitis* (< 52%; Fig. 6). After the juvenile stage, when the dorsal fin is completely developed, *M. dabryanus* and *Cobitis* species/subspecies are readily distinguishable.

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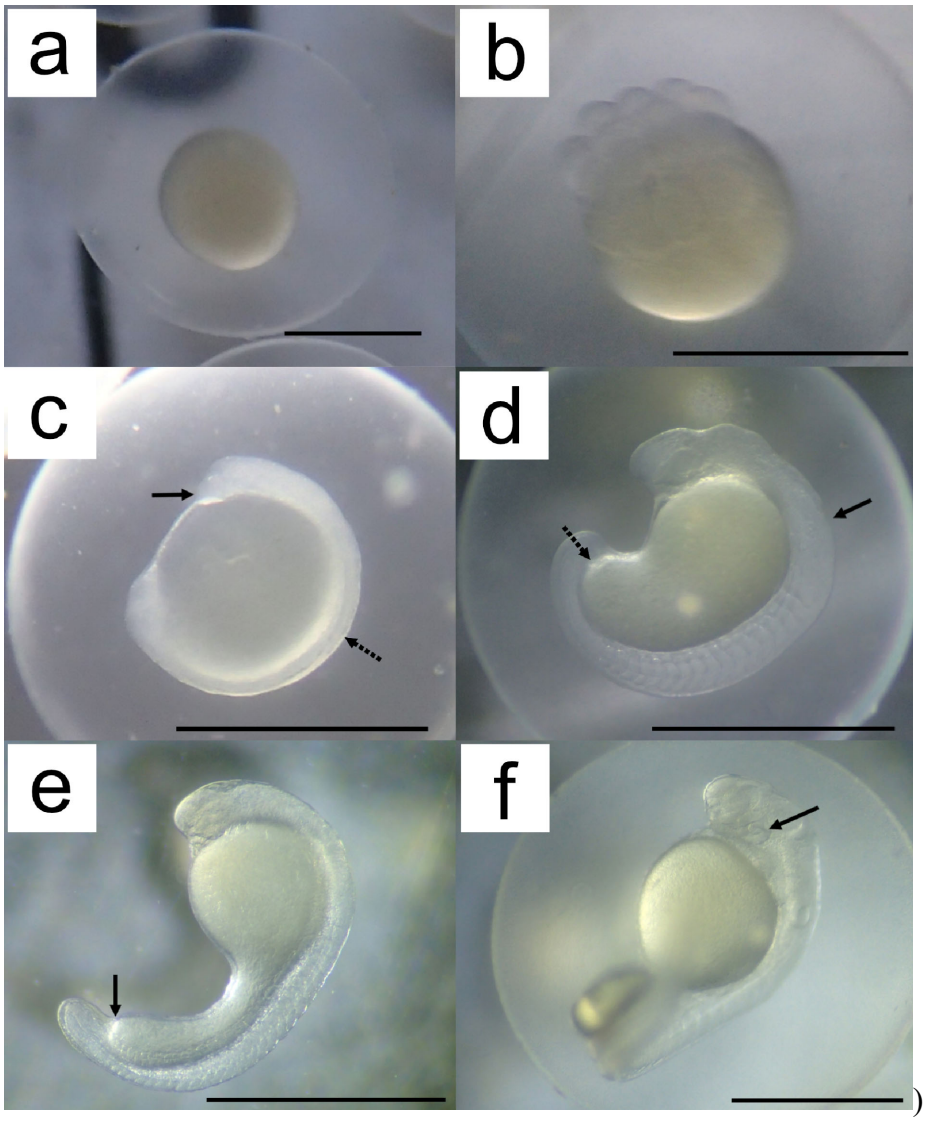

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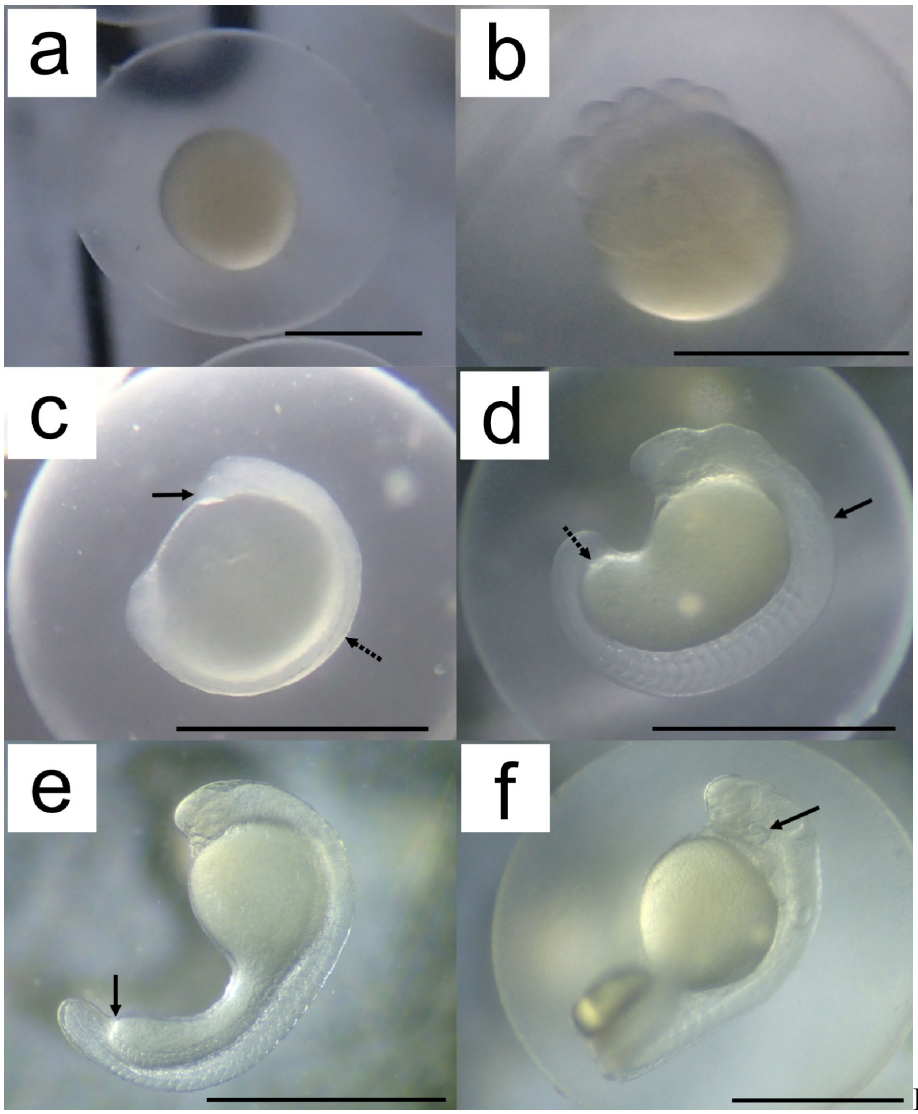

Fig. 1 Embryonic development of *Cobitis striata fuchigamii*. **a** 0.5 h after fertilization; **b** 3 h after fertilization; **c** 11 h after fertilization (*dotted arrow*, somites; *solid arrow*, rudimentary optic vesicles); **d** 17.5 h after fertilization (*dotted arrow*, Kupffer's vesicle; *solid arrow*, somites); **e** 20 h after fertilization (*solid arrow*, anus depressed); **f** 25 h after fertilization (*solid arrow*, rudimentary optic vesicles). Scale bar indicates 1.0 mm

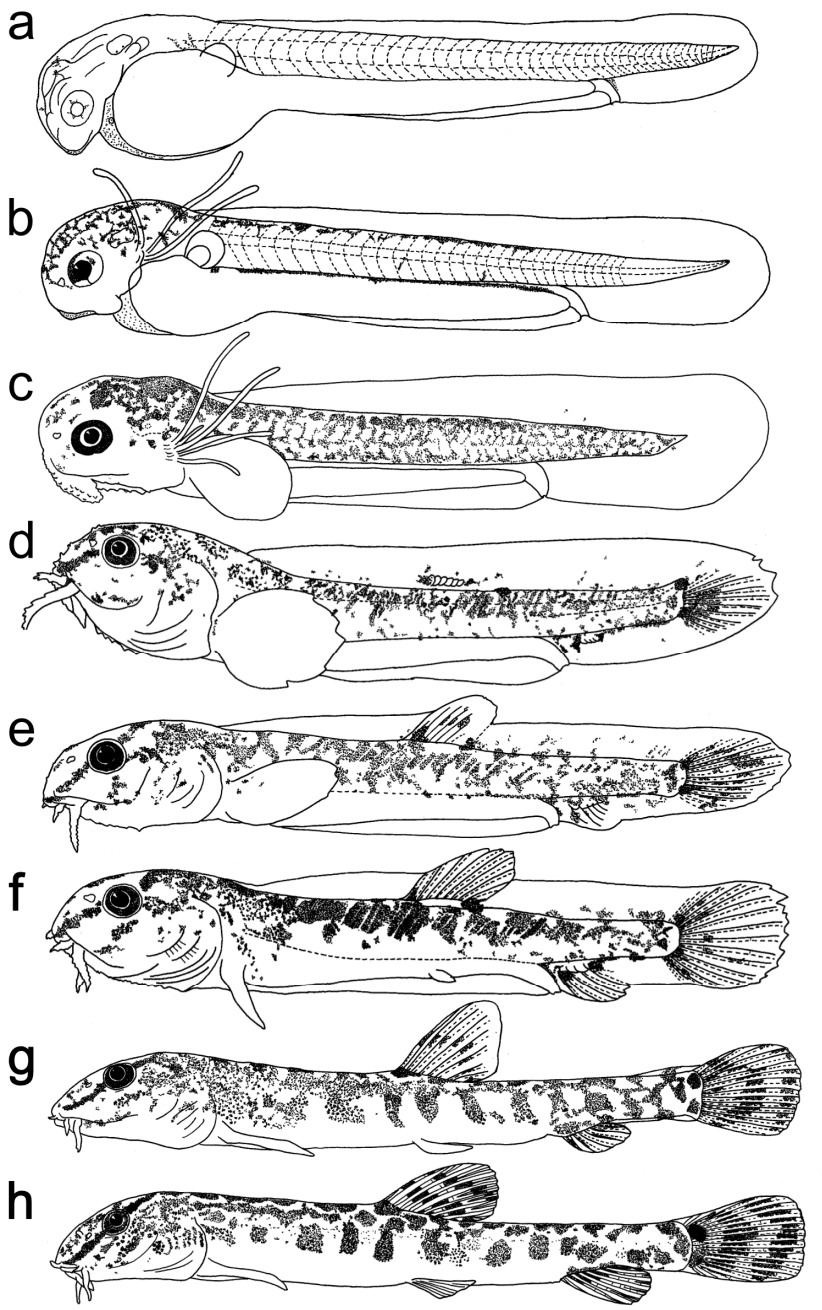

Fig. 2 Larvae and a juvenile *Cobitis striata fuchigamii* from artificial breeding. **a** Newly hatched larva, KYUM-PI 00007298, 4.4 mm in total length (TL); **b** preflexion larva, KYUM-PI 00007299, 4.5 mm TL (1 days); **c** preflexion larva, KYUM-PI 00007300, 5.1 mm TL (2 days); **d** flexion larva, KYUM-PI 00007304, 10.0 mm TL (5 days); **e** postflexion larva, KYUM-PI 00007306, 10.6 mm TL (10 days); **f** postflexion larva, KYUM-PI 00007307, 11.0 mm TL (10 days); **g** juvenile, 18.5 mm TL (35 days); **h** juvenile, KYUM-PI 00007308, 25.4 mm TL (77 days)

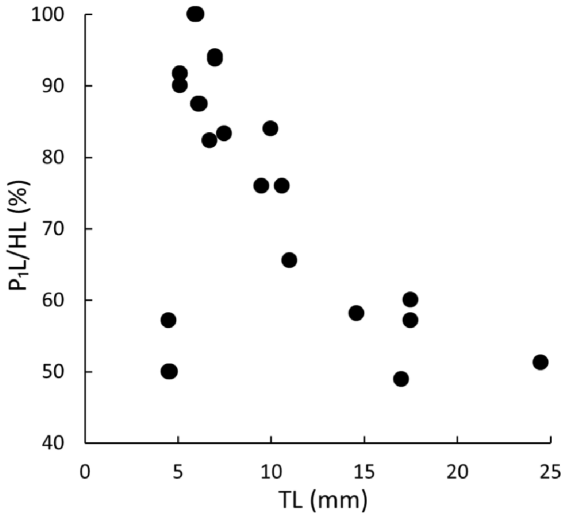

Fig. 3 Proportion of pectoral fin length (P_1L) to head length (HL) in reared *Cobitis striata fuchigamii*

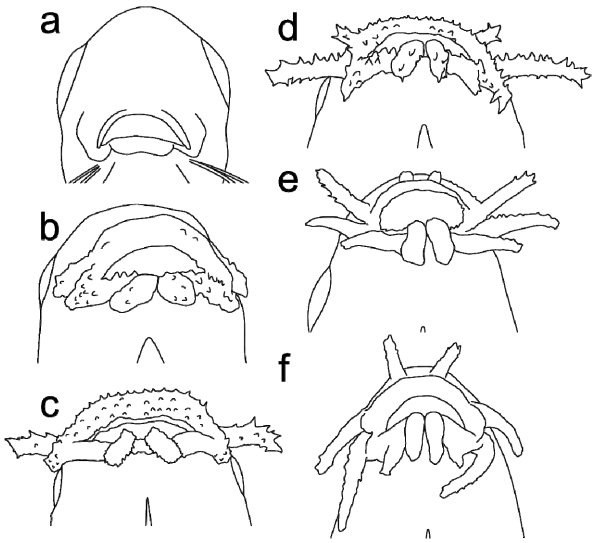

Fig. 4 Development of mouth and barbels of larval and juvenile *Cobitis striata fuchigamii*. **a** Preflexion larva (the specimen of Fig. 2b); **b** preflexion larva (the specimen of Fig. 2c); **c** flexion larva (7.5 mm TL, 4 days); **d** flexion larva (the specimen of Fig. 2d); **e** postflexion larva (the specimen of Fig. 2f); **f** juvenile (the specimen of Fig. 2h)

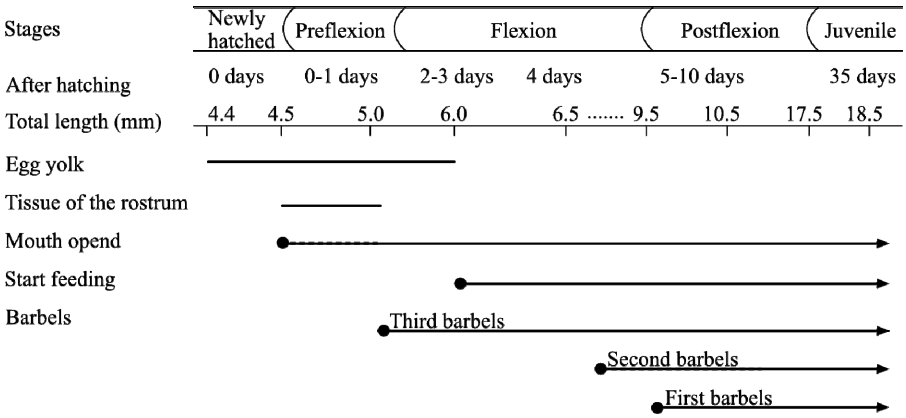

Fig. 5 Schematic diagram of the relationship between mouth tissue, barbels, and egg yolk in *Cobitis striata fuchigamii*

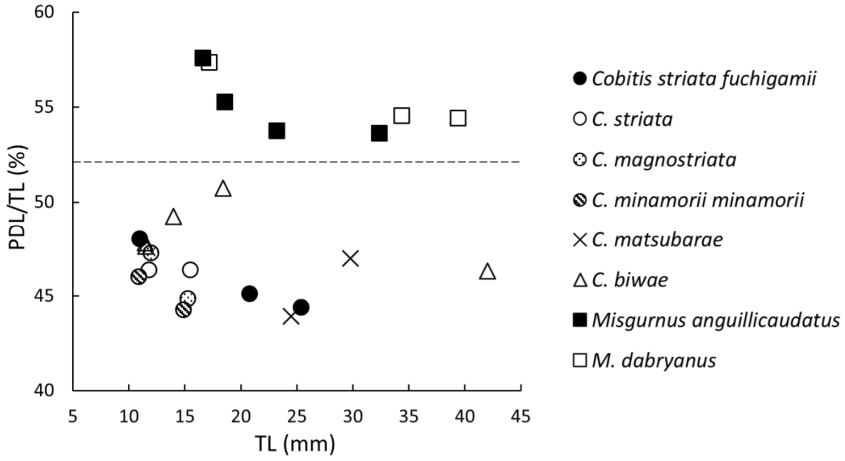

Fig. 6 Proportion of predorsal length (PDL) to total length (TL) of eight species and subspecies of Cobitidae in Japan – comparison with previous studies

Table 1 Total length of each developmental stage of *Cobitis striata fuchigamii*

Stage	Range (mm)	Mean ± *SD*	*n*
Newly hatched larva	4.0–4.5	4.3 ± 0.2	4
Preflexion larva	4.5–5.9	4.9 ± 0.5	7
Flexion larva	5.1–10.0	6.8 ± 1.3	10
Postflexion larva	9.5–17.5	14.0 ± 3.3	7
Juvenile	≥ 17.7	-	5

Table 2 Morphological features of newly hatched larvae of 14 species and subspecies of Cobitidae in Japan

Name	Egg yolk diameter (mm)	Total length (mm)	Pigmentation on eyes	Outer gill filaments	Pectoral fin	Total number of myomeres (between the pectoral fins and the middle of the anus)	References
Cobitis striata	1.0 ± 0.0	4.3 ±	existent	existent	bud	44–45 (27–	Present study,
fuchigamii	1.0 ± 0.1	0.2	-	-	-	28)	Nakajima
		-				-	(2012)
C. striata striata	-	4.0	existent ?	existent	bud	43–44 (28–	Saitoh (1990),
	-					30)	Saitoh and
	1.0 ± 0.1	-	-	-	-		Hosoya (2014c),
						-	Nakajima
							(2012)
zCobitis striata	1.0 ± 0.1	-	-	-	-	-	Nakajima
hakataensis							(2012)
Cobitis kaibarai	0.8 ± 0.0	-	-	-	-	-	Nakajima
							(2012)
C. magnostriata	-	4.5	existent	existent	bud	43 (28–32)	Saitoh and
	1.2 ± 0.1	-	-	-	-	-	Hosoya (2014a),
							Nakajima
							(2012)

C. minamorii	-	3.5	existent	existent	bud	40–41 (26–	Saitoh and
minamorii	0.9 ± 0.1	-	-	-	-	27)	Hosoya
						-	(2014b),
							Nakajima
							(2012)
C. minamorii	0.8 ± 0.0	-	-	-	-	-	Nakajima
oumiensis							(2012)
Cobitis minamorii	0.8 ± 0.0	-	-	-	-	-	Nakajima
tokaiensis							(2012)
Cobitis minamorii	0.9 ± 0.1	-	-	-	-	-	Nakajima
saninensis							(2012)
C. matsubarae	1.1	-	-	-	-	-	Nakajima and
							Uchiyama
							(2017)
C. biwae *	1.1	4.6	existent	existent	bud	44 (29)	Okada and
							Seiishi (1937)
Cobitis sp. BIWAE	1.1	4.2 ±	existent	existent	bud	46 (32)	Nagae et al.
type D *		0.6					(2021)
C. takatsuensis	2.7	5.7	existent	existent	bud	37 (26)	Shimizu et al.
							(1998)
Misgurnus	0.8	3.4	existent	existent	bud	-	Okada and
anguillicaudatus	-	3.4–4.0	-	-	-	44–47 (27–	Seiishi (1938),
						30)	Hosoya (2014)

- Not mentioned in previous literature

* The *Cobitis biwae* species complex contains four species (Nakajima et al. 2012); it is unknown which species of *C. biwae* corresponds to the report by Okada and Seiishi (1937)