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Early life history of the eastern Korean tetraploid spined loach, *Iksookimia yongdokensis* (Pisces: Cobitidae)

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Abstract. We investigated whether tetraploidy in *Iksookimia yongdokensis* adds more species specific variation in its early life history characteristics. To this end, morphological and temporal characteristics were analysed using artificial fertilisation and laboratory culture, and the results were compared to seventeen other species of the family Cobitidae. Extensive comparison of these characteristics among the cobitid species showed that tetraploid *I. yongdokensis* was apparently longer in egg diameter (1.54 ± 0.08 mm), hatching size (5.8 ± 0.15 mm in total length), and time to hatching (65 hours after fertilisation) than the other diploid cobitid species. With respect to these characteristics, *I. yongdokensis* was most similar to the congeneric species of *Iksookimia* (except *I. pacifica*). Next closest in similarity, were the other two species of different genera, *Kichulchoia brevifasciata* and *Cobitis takatsuensis*. Notably, all of the species with similar characteristics to *I. yongdokensis* were coincident in that they prefer a similar habitat, that being a bottom of pebbles and cobbles in middle to upper streams. In conclusion, the present study indicates that characteristics of the early life history of cobitid species are associated with both taxonomic closeness and habitat type.

Key words: artificial fertilisation, fecundity, mature egg size, hatching of the embryo, larva

Introduction

Loaches, which are fishes of the superfamily Cobitoidea (Teleostei: Cypriniformes), are small benthic freshwater fishes distributed widely across Eurasia to Morocco in northern Africa (Kottelat 2012, Nelson et al. 2016). A relatively large number of loach species (16 species in five genera of Cobitidae and three species in two genera of Balitoridae) inhabit Korea showing either an allopatric or sympatric distribution pattern, and among them, 13 species are endemic (Kim 1997, 2009, Kim & Park 2007, Vasil'eva et al. 2016). These cobitid species vary in their spawning periods and spawning grounds (Ko & Won 2015, Ko & Bang 2016), and occupy diverse habitats that have been associated with the distinctive structure of egg envelopes (Kim & Park 1995, 1996, 1997, Park 1996, Park & Kim 2001).

The eastern Korean spined loach, *Iksookimia yongdokensis*, belonging to the endemic genus *Iksookimia*, was established as a new species by Kim & Park in 1997, and it inhabits streams in the south-eastern region of Korea (Kim & Park 1997). *I.*

yongdokensis was reported to be the only tetraploid organism ($4n = 100$) among Korean cobitid fish (Lee et al. 1986, Kim et al. 1999). It inhabits the bottom of streams with cobbles, pebbles and sands, and it spawns between June and July (Ko et al. 2016). The structure of the egg envelope of this species has the shape of a granular form (Park & Kim 2001). From the perspective of conservation, *I. yongdokensis* has been evaluated as Least Concern (LC) in the Red Data Book of endangered fishes in Korea due to environmental pollution and development of rivers (Ko et al. 2011).

The early life history of fish are interesting biological themes and they are recognised as important factors for fish reproductive strategies. In particular, characteristics related to early egg development have been known for their potential association with various ecological environments. For instance, the number of eggs is known to be linked to fertility, frequency of spawning, parental care, egg size, population density, and other environmental factors (Bagenal 1978, Shimizu et al. 1998, Moyle & Cech 2000). In

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this context, some characteristics of early life history have been investigated among fish species inhabiting different aquatic environments (Balon 1981, Shimizu et al. 1998, Sado & Kimura 2002, Aoyama & Doi 2011, Ko & Won 2015).

Features of early life history are studied not only to characterise fish species but also to evaluate taxonomic relationships among closely related ones. Recently in Korea, these characteristics have been intensively investigated to obtain basic information for establishing culture technology aimed at the restoration and conservation of endangered and/or endemic fish species (Song et al. 2008, 2009, Ko et al. 2009, 2013, Ko & Park 2012, Ko & Bang 2014, Ko & Won 2015).

Here, we investigate the early life history of *I. yongdokensis*, one of the endemic Korean cobitid species, in order to compare its features with an extensive taxon sample of cobitid species from Korea and Japan whose corresponding characteristics are available in the literature.

Material and Methods

We collected fish samples of the eastern Korean cobitid, *I. yongdokensis*, from the Chuksan stream, Sangwon-ri, Chuksan-myeon, Yeongdeok-gun, Gyeongsangbuk-do, Korea (36°30'34.5" N, 129°24'03.9" E) (Fig. 1) with hand nets (mesh size: 4 × 4 mm) and cast nets (mesh size: 6 × 6 mm) on June 15, 2015. In the laboratory, a dose of Ovaprim (Syndel, Canada; 0.5 mL/kg) was injected into individual mature females (n = 5) transferred from the field alive. After 12 hours, eggs were obtained by exerting gentle pressure on the abdomen of the female loaches, and they were separately fertilised with sperms diluted 100 times with Ringer solution, obtained from male fish (n = 5). We counted the total number of eggs present in each brood, and measured the egg sizes of 30 randomly selected eggs up to 0.01 mm.

Fertilised eggs were laid out in Petri dishes of 15 cm diameter and their development as well as the pre-larval stage were continuously monitored up to the hatching point. After hatching, the hatchlings were reared in an aquarium (sequentially transferred from a 20 litre tank to a 100 litre tank), and we observed the larval and juvenile periods. The hatchlings were fed with larvae of *Artemia* sp. from the point they absorbed most of the egg yolk and then up to 30 days after hatching (DAH). Following that point, they were fed with compound feed. While the water quality of the 20 litre tank was maintained through an exchange of half the volume of water daily, the water quality

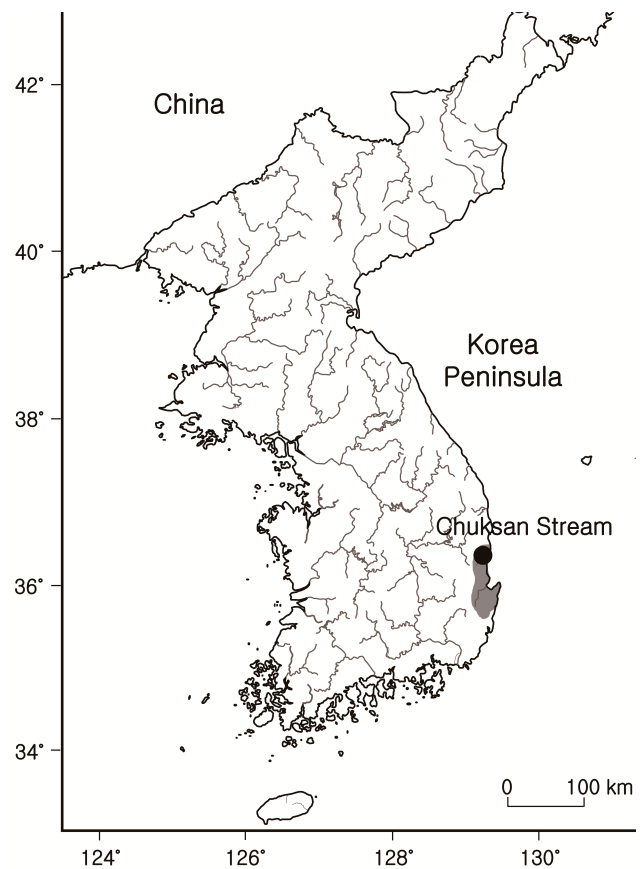


Fig. 1. Sampling site of *Iksookimia yongdokensis* in Chuksan stream, Sangwon-ri, Chuksan-myeon, Gyeongsangbuk-do, Korea. Grey area: distribution range of *Iksookimia yongdokensis* (Kim & Park 2007).

of the 100 litre tank, used for larger hatchlings of 30 DAH, was maintained by a circulation filtration system. The water temperature was maintained at 25 °C.

Developmental distinctions were classified according to Balon (1975). The embryonic, larval and juvenile periods were observed and photographed using a digital camera (Olympus DP72, Japan) mounted on a dissecting microscope (Olympus SZX9, Japan). Subsequent temporal features of ontogeny were expressed as the time when 50% of individuals reached respective developmental steps. The total length (TL) of larvae and juveniles was measured in 10 randomly selected samples of each, after anaesthetisation with MS-222 (Syndel, Canada).

Results

The size of mature individuals and the characteristics of mature eggs

All captured males of *I. yongdokensis* (n = 5, 100-115 mm, total length (TL)) were mature, and all females (n = 5, 125-142 mm TL) were found not to have laid eggs in that season. The females ovulated 12 hours

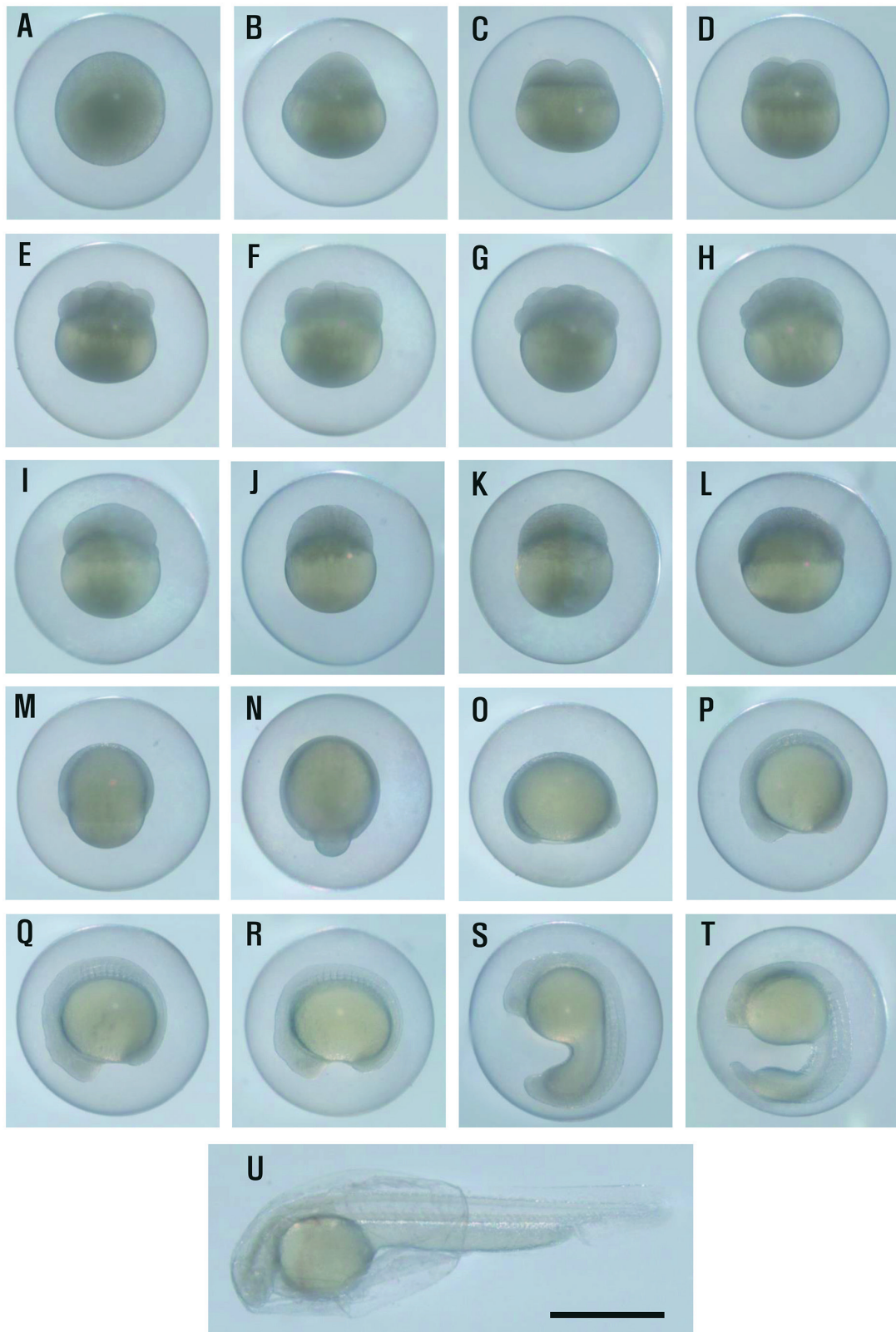


Fig. 2. Embryonic development and hatching of *Iksookimia yongdokensis*. The bar indicates 1 mm. A: 15 min after fertilization, swelling; B: 1 h, blastodisc; C: 1 h 20 min, 2 cells; D: 1 h 40 min, 4 cells; E: 2 h, 8 cells; F: 2 h 20 min, 16 cells; G: 2 h 40 min, 32 cells; H: 3 h, 64 cells; I: 3 h 20 min, 128 cells; J: 3 h 40 min, morula (256 cells); K: 4 h 40 min, blastula; L: 8 h, early gastrulation (50 % epiboly); M: 10 h 30 min, middle gastrulation (70-75 % epiboly); N: 12 h, late gastrulation (90-95 % epiboly); O: 14h 30 min, formation of the embryo; P: 17 h 20 min, 3-4 myotomes; Q: 20 h 10 min, 9-10 myotomes; R: 22 h 30 min, 17-18 myotomes; S: 25 h, 28-30 myotomes; T: 34 h, formation of the heart; U: 65 h, 50 % hatching.

after injection of Ovaprim. Mature eggs were slightly adhesive with a light yellowish colour. The number of eggs or fecundity ($n = 5$) was 2668 ± 399 ($\bar{x} \pm SD$) (min-max, 2220-3240), and the diameter of eggs ($n = 30$) was 1.54 ± 0.08 mm (min-max, 1.45-1.63 mm).

The embryonic period

The cleavage phase – fifteen minutes after fertilisation, the eggs absorbed water and swelled to 2.7 ± 0.06 mm ($n = 30$) in diameter (Fig. 2A). One hour after fertilisation, the cytoplasm was pulled toward the animal pole, and a blastodisc was formed (1-cell stage) (Fig. 2B). Cleavage of the blastodisc occurred 80 minutes after fertilisation (2-cell stage) (Fig. 2C). After the first latitudinal cleavage, cells divided every 20-minutes: the 4-cell stage by meridional cleavage at 100 minutes after fertilisation (Fig. 2D), the 8-cell stage by latitudinal cleavage at 120 minutes after fertilisation (Fig. 2E), the 16-cell stage by meridional cleavage at 140 minutes after fertilisation (Fig. 2F), the 32-cell stage at 160 minutes after fertilisation (Fig. 2G), the 64-cell stage at 180 minutes after fertilisation (Fig. 2H), the 128-cell stage at 200 minutes after fertilisation (Fig. 2I), and the 256-cell stage or morula at 220 minutes after fertilisation (Fig. 2J). Continuous cleavage led to a blastula at 280 minutes after fertilisation (Fig. 2K). Upon passage of eight hours, a gastrula was formed and its epiboly started to extend toward the vegetal pole (Fig. 2L). In the middle of the gastrula stage, at 10.5 hours, the growing epiboly covered 70-75 % of the vegetal pole (Fig. 2M), and then by 12.5 hours it covered 90-95 % of the vegetal pole, reaching the late gastrula stage (Fig. 2N).

The embryonic phase – at 14.5 hours, the blastopore closed forming an embryo (Fig. 2O). At 17.3 hours, three to four myotomes and an optic vesicle were formed (Fig. 2P). At 20.2 hours, nine to ten myotomes developed (Fig. 2Q). At 22.5 hours, 17-18 myotomes, auditory vesicle, and Kupffer's vesicle developed (Fig. 2R). At 25 hours, 28-30 myotomes, lenses of the eyes, and the brain developed, Kupffer's vesicle disappeared, and movement started (Fig. 2S). At 34 hours, the heart developed and it began to beat (Fig. 2T). At 50 hours, a fish began to hatch out as its tail broke through the chorion. At 65 and 75 hours, 50 % and 100 % of the eggs hatched, respectively (Fig. 2U).

The eleutheroembryonic phase – the size of larvae that had just hatched ($n = 10$) were 5.8 ± 0.15 mm (min-max, 5.5-6.0 mm) in total length, with a colourless body, closed mouth and anus, a spherical yolk sac in the anterior part of the body, and an embryonic finfold that had started to form (Fig. 3A). One day after

hatching (DAH), it grew to 6.7 ± 0.12 mm ($n = 10$) in total length, pigmentation of the eye started, and black dots began to appear on the body. More than half of the yolk in the anterior part of the body was absorbed and five pairs of external gills appeared (Fig. 3B). Two DAH (7.3 ± 0.16 mm TL, $n = 10$), we observed a pair of barbels and the body shape flattened as the yolk was absorbed (Fig. 3C). Three DAH (7.9 ± 0.36 mm TL, $n = 10$), external gills began to regress, barbels grew up to the diameter of the eye, and pectoral fins became longer than the length of the head (Fig. 3D). Four DAH (8.3 ± 0.26 mm TL, $n = 10$), we observed that three pairs of barbels had appeared and most of the yolk was absorbed. The larvae's mouth and anus became open, so that they could feed on the larvae of *Artemia* sp. (Fig. 3E).

The larval period

Five DAH (8.5 ± 0.15 mm TL, $n = 10$), larvae entered into the larval period as the yolk was completely absorbed. External gills became so regressed that they were covered by gills. Three to four anal fin rays appeared, and anal and dorsal fin ray buds were observed (Fig. 3F). Seven DAH (8.9 ± 0.32 mm TL, $n = 10$), a caudal fin was observed and it consisted of 7-8 rays, and barbels had become longer (Fig. 3G). Thirteen DAH (9.8 ± 0.24 mm TL, $n = 10$), the number of rays of the caudal fin increased to 10-12 (Fig. 3H). Twenty DAH (10.3 ± 0.4 mm TL, $n = 10$), 4-5 dorsal fin rays and 14-15 caudal fin rays were observed (Fig. 3I).

The juvenile period

Twenty three DAH (10.9 ± 0.45 mm TL, $n = 10$), larvae entered the juvenile period, and the number of rays of the dorsal and anal fins increased to 7-8 and 5-7, respectively (Fig. 3J). Thirty DAH (12.6 ± 1.32 mm TL, $n = 10$), black dots appeared, and they generated lateral pigmented spots on the middle part of the body flank and 1-2 lines of spots on the caudal fin (Fig. 3K). Fifty DAH (17.3 ± 1.34 mm TL, $n = 10$), primordial finfolds were almost completely absorbed except for the posterior part of the dorsal fin, and the black dots became darker and more apparent yielding a number of conspicuous spots on the dorsal side (min-max, 10-12) and the body flank (min-max, 9-11) (Fig. 3L). Similarly, rows of spots appeared on the dorsal fin (1-2 rows), anal fin (1 row), and caudal fin (2-3 rows) (Fig. 3L). Seventy DAH (20.9 ± 1.94 mm TL, $n = 10$), the lateral pigmented spots on the middle part of the body flank became distinct (Fig. 3M). One hundred DAH (32.1 ± 4.11 mm TL, $n =$

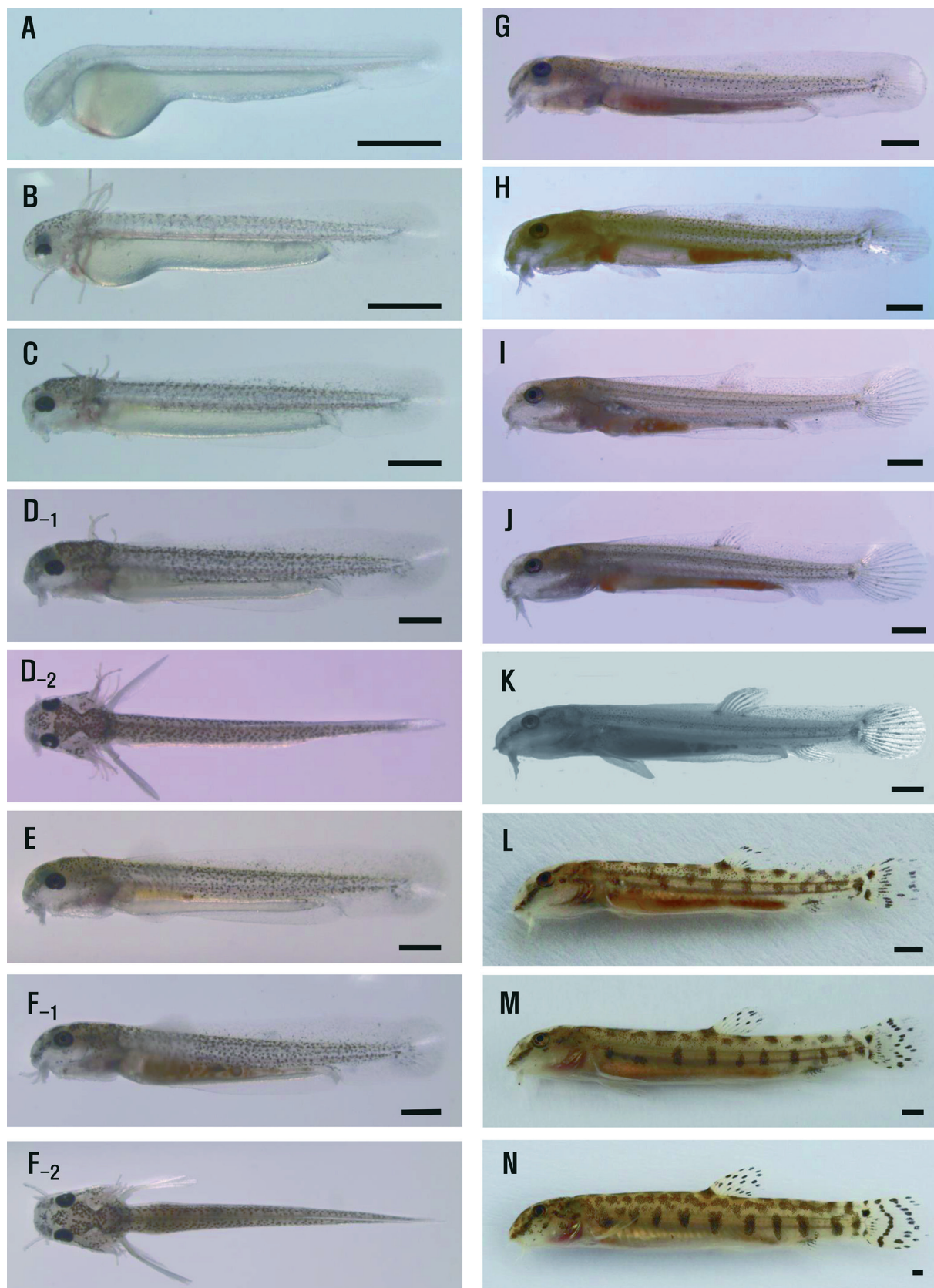


Fig. 3. Larval and juvenile development of *Iksookimia yongdokensis*. The bar indicates 1 mm. A: 0 day, the eleutheroembryonic phase, 5.8 ± 0.15 mm ($\bar{x} \pm \text{SD}$) total length; B: 1 day, 6.7 ± 0.12 mm; C: 2 days, 7.3 ± 0.16 mm; D: 3 days, 7.9 ± 0.36 mm; E: 4 days, 8.3 ± 0.26 mm; F: 5 days, the larval period, 8.5 ± 0.15 mm; G: 7 days, 8.9 ± 0.32 mm; H: 13 days, 9.8 ± 0.24 mm; I: 21 days, juvenile, 10.3 ± 0.43 mm; J: 23 days, the juvenile period, 10.9 ± 0.45 mm; K: 30 days, 12.6 ± 1.32 mm; L: 50 days, 17.3 ± 1.34 mm; M: 70 days, 20.9 ± 1.94 mm; N: 100 days, 32.1 ± 4.11 mm.

Table 1. Comparisons of early development among cobitid species found in Korea and Japan. * When all fin rays attained their full counts.

Species	Spawning (or maturity) period	Mature egg size (mm)	Swelling egg size (mm)*	Fecundity	Time to hatching (water temperature)	Hatching size (mm)	Outer gill filament	Days until full yolk absorption	Days until juvenile*	Main bottom structure	References
<i>Isookimia yongdokensis</i>	June-July	1.54 ± 0.08	2.67 ± 0.06	2668 ± 399 (2220-3240)	65 (50-75) h (25 °C)	5.8 ± 0.15	Present	5	23	Coble, pebble, sand	Present study
<i>I. koreensis</i>	June-July	1.40 ± 0.07	2.40 ± 0.03	2020 ± 518 (1339-2635)	50 (45-60) h (23 °C)	4.7 ± 0.21	Present	5	17	Coble, pebble	Kim 1978, Ko et al. 2012
<i>I. pumila</i>	June-July	1.27 ± 0.02	2.11 ± 0.07	1017 ± 334 (352-1440)	52 (47-55) h (23 °C)	4.7 ± 0.14	Present	5	17	Coble, pebble	Ko et al. 2013
<i>I. longicarpa</i>	June	1.45 ± 0.07	2.2 ± 0.13	1992 ± 925 (717-3862)	50-72 h (23-25 °C)	5.1 ± 0.23	Present	5	25	Coble, pebble	Ko et al. 2009
<i>I. hugowoffeldi</i>	June-July	1.35 ± 0.03	1.93 ± 0.03	1933 ± 530 (1350-2680)	56 (48-60) h (25 °C)	5.6 ± 0.18	Present	5	15	Coble, pebble	Ko & Bang 2016, Park 2016
<i>I. pacifica</i>	July	1.09 ± 0.04	-	2968 ± 502	48 h (21-24 °C)	2.9 ± 0.05	Present	7	26	Sand	Lee et al. 2011
<i>Cobitis hankugensis</i>	July	1.29 ± 0.07	1.98 ± 0.06	2783 ± 1543 (812-6474)	45-52 h (23-25 °C)	4.5 ± 0.24	Present	5	25	Sand	Ko 2009, Ko & Park 2012
<i>C. tetralineata</i>	July	1.04 ± 0.03	1.88 ± 0.04	2646 ± 916 (1494-4096)	56 (45-65) h (25 °C)	4.6 ± 0.11	Present	5	25	Sand	Kim et al. 2006, Ko & Won 2015
<i>C. nalbanti</i>	July	0.99 ± 0.03	1.65 ± 0.04	1527 ± 410 (1121-2055)	52 (41-57) h (25 °C)	4.2 ± 0.22	Present	5	15	Sand	Ko & Park 2011, Ko & Bang 2013
<i>C. choii</i>	June-July	1.0 ± 0.08	1.2	2444 ± 838 (923-4202)	24 h (23-25 °C)	3.6	Present	4	35	Sand	Song et al. 2008, MEK 2011
<i>C. takatsuensis</i>	June-August	1.5	2.7 ± 0.2	150-300	4-6 d (18 °C)	5.7	Present	< 16	38	Pebble	Sakai et al. 1989, Shimizu et al. 1998
<i>C. biwae</i>	May-June	1.1-1.2	2.11	668 ± 176 (423-980)	2-3 d (23-26 °C)	4.6	Present	5-6	-	Sand	Okada & Seishi 1937, Okada 1959
<i>Misgurnus anguillicaudatus</i>	May-June	1.1	1.2-1.4	16300-40000	48-72 h (20 °C)	4.0	Present	4	-	Mud	Uchida 1939, Suzuki 1976
<i>M. mizolepis</i>	April-June	1.12	-	8500-13500	24 h (25 °C)	2.7	Present	4	24	Mud	Kim et al. 1987, 1992
<i>Koreocobitis naktongensis</i>	May-June	1.0 ± 0.05	1.1	22643 ± 4629 (15909-30323)	38 h (20 °C)	2.7	Present	7	50	Coble, pebble	Song et al. 2009, Hong et al. 2011
<i>Kichulchoia brevifasciata</i>	July	1.46 ± 0.07	2.25 ± 0.10	60 ± 35 (18-160)	66 (60-72) h (25 °C)	5.5 ± 0.07	Present	6	17	Pebble	Ko & Bang 2014
<i>K. multifasciata</i>	May	1.8	2.5	861 (798-901)	6 d (10-14 °C)	5.4	Present	16	85	Coble, pebble	Chong 1986, Kim & Lee 1995
<i>Niwaella delicata</i>	April-May	2.7	3.2	60-120	17 d (9 °C)	7.5	Absent	< 90	-	Coble, pebble	Suzuki 1966, Honjo & Taguchi 1974, Kano 2000

10), 8-12 spots on the middle part of the flank were vertically extended (Fig. 3N). Several rows of spots were observed on the dorsal fin (3 rows), anal fin (1 row), and caudal fin (3-4 rows). The ventral side had a silver-white colour, so that the overall appearance was similar to that of an adult fish (Fig. 3N).

Discussion

The present study on the early life history of the eastern Korean tetraploid cobitid, *I. yongdokensis*, is important as it completes and complements a body of research among all the described species of the genus *Iksookimia*. In addition to these six *Iksookimia* species, we compiled corresponding data from twelve other species for a comprehensive comparison of Cobitidae fish using all of the available information on this taxonomic group. As shown in Table 1, the data were entirely collected from cobitid species found in Korea and Japan, and early life history of cobitids have been characterised for several parameters including spawning period, mature egg size, fecundity, time to hatching, hatching size, and time to larval and juvenile periods. Closer examination of the spawning period and the type of habitat in their natural environment helped us to understand the developmental characteristics of early life of these fish in early life.

The spawning times of cobitids can be broadly categorised into three periods: spring period spawning from April to May (*Kichulchoia multifasciata*, *Niwaella delicata*), spring-summer period spawning from May to June (*Misgurnus anguillicaudatus*, *M. mizolepis*, *Koreocobitis naktongensis*), and summer period spawning from June to July (*Cobitis*, *Iksookimia*, *K. brevifasciata*) (Table 1). Our study species, *I. yongdokensis*, belongs to the summer type as it spawns from June to July. Most species of the genera *Iksookimia* and *Cobitis* with summer spawning (June to July), including *I. yongdokensis*, similarly take 2-3 days to hatch at 23-25 °C. However, there is a difference between the two spring period cobitids, *K. multifasciata* (water temperature 10-14 °C, six days) and *N. delicata* (water temperature 9 °C, 17 days) (Suzuki 1966, Kim & Lee 1995). Three spring-summer type period cobitids, *M. mizolepis* (water temperature 23-25 °C, 24 hours) (Kim et al. 1987), *K. naktongensis* (water temperature 20 °C, 38 hours) (Song et al. 2009), and *M. anguillicaudatus* (water temperature 20 °C, 48-72 hours) (Uchida 1939), hatch within a shorter time at a lower temperature than summer type spawning cobitids.

Cobitids showed different reproductive strategies in egg size and fecundity depending on the habitat

(Table 1). Cobitids inhabiting the bottom of shallow streams with pebbles and cobbles (*N. delicata*, *C. takatsuensis*, *K. brevifasciata*, and *K. multifasciata*) laid a small number (< 1000) of large eggs (> 1.5 mm in diameter). In contrast, cobitids mainly inhabiting muddy bottoms (*M. anguillicaudatus* and *M. mizolepis*) laid a large number (8500-40000) of small eggs (< 1.1 mm). Most *Cobitis* and *Iksookimia* species, including *I. yongdokensis*, laid an intermediate number of eggs (1000-3000), but showed variations in the size of the egg and in the habitat type. *Cobitis* inhabiting sandy bottoms laid eggs of medium-small size (1.0-1.3 mm), but *Iksookimia* inhabiting the bottom comprising pebbles and cobbles laid eggs of medium-large size (1.27-1.54 mm). In fact, the egg size of *I. yongdokensis* was the largest among the species of the genus *Iksookimia*. Generally, the egg size or ova of polyploids or ova of more than 2n are larger than that of the ovum of n, and the hatching time is also longer (Ko 2009). Interestingly, Kim et al. (1999) reported that *I. yongdokensis* is tetraploid and produces ova of more than 2n. Therefore, the larger egg size and longer (5-10 hours) time to hatching of *I. yongdokensis* compared to other congeneric species could be explained by its polyploidy. To better understand the reproductive strategy of *I. yongdokensis*, more research is needed on egg production, spermatogenesis and reproductive mechanisms.

The size of larvae just after hatching was associated with the size of the mature eggs (Table 1). While the larvae hatched from large sized eggs (*Niwaella delicata*, *C. takatsuensis*, *K. brevifasciata*, and *K. multifasciata*) were 5.4-7.5 mm in total length, the larvae of most *Iksookimia* species that hatched from medium-large sized eggs were 4.7-5.8 mm in length, except for *I. pacifica* (2.9 ± 0.05 mm TL). The larvae of *Cobitis* that hatched from medium-small sized eggs were 4.2-4.6 mm in length, except for *C. choii* (3.6 mm). The larvae of *M. anguillicaudatus*, *M. mizolepis*, *K. naktongensis* that hatched from small eggs were small (2.7-4.0 mm TL). *I. yongdokensis* had the largest egg size (5.8 ± 0.15 mm TL) in its genus.

Among the early life histories of Cobitidae reported to date, external gills always appear in the pre-larval stage except in the case of *N. delicata* (Suzuki 1966) (Table 1). External gills of *I. yongdokensis* hatchlings were observed from one to five DAH, which is similar to the congeneric *I. longicorpa*, *I. koreensis*, *I. pumila*, and *I. hugowolfeldi* (Ko et al. 2009, 2012, 2013, Ko & Bang 2016) and the other three species in the *Cobitis*

genus, *C. hankugensis*, *C. tetralineata*, and *C. lutheri* (Lee et al. 2011, Ko & Park 2012, Ko & Bang 2013, Ko & Won 2015).

In terms of full yolk absorption after hatching, the species of *Iksookimia* and *Cobitis* took five days (Table 1), which is four days longer than the time taken by the *Misgurnus* genus (Uchida 1939, Kim et al. 1987), and shorter than the time taken by the genera of *Koreocobitis* (Song et al. 2009), *Kichulchoia* (Kim & Lee 1995, Ko & Bang 2014), and *Niwaella* (Honjo & Taguchi 1974). In addition, the time to become a juvenile (23 days) in *I. yongdokensis* was similar to that in *I. longicorpa* (Ko et al. 2009), *I. pacifica* (Lee et al. 2011), *C. hankugensis* (Ko & Park 2012), and *M. mizolepis* (Kim et al. 1987).

Among the species of Cobitidae, the genera *Iksookimia* and *Cobitis* exhibit a regular pattern of spots in the middle part of the body flank. The genera *Iksookimia* and *Kichulchoia* show horizontally elongated blotches, while the genus *Cobitis* shows vertical or rounded spots and usually forms Gambetta's zone along the length of the body (Linnaeus 1758, Kawanabe & Mizuno 1989, Kim 2009). In the case of *Iksookimia*, including *I. yongdokensis*, rounded spots appeared on the lateral centre of the body, and then the spots appeared on the upper half of the lateral flank between 20-40 DAH. The central spots became elongated horizontally after 70 DAH (Ko et al. 2009, 2012, 2013, Lee et al. 2011, Ko & Bang 2016). In most Korean *Cobitis* species, melanophores of the lateral centre of the body gather to form Gambetta's zone-4 initially, and then the other lines of Gambetta's zone-1, -2, and -3 sequentially appear along the length of the body between 14-17 DAH. However, it was reported that the rounded spots

of *C. tetralineata* became elongated 80 DAH and merged into a line of the Gambetta's zone-4, which is different from the rounded or elliptical spots of *C. hankugensis* and *C. nalbanti* (Song et al. 2008, Ko & Park 2012, Ko & Bang 2013, Ko & Won 2015). To summarise, the genera *Iksookimia* and *Cobitis* have similarities in which they have similar rounded spots on the lateral centre of the body in the early juvenile period, but they differ in later stages, in which the spots of *Iksookimia* become elongated horizontally but those of *Cobitis* become elongated vertically or are merged into a line.

Taken together, Cobitidae species exhibit substantial variation in early life history as features such as egg size, fecundity, and time to hatching, larval and juvenile periods, differ among them primarily depending on the genus and habitat type. Our study shows that overall, *I. yongdokensis* was most similar in these characteristics to its congeneric species, *I. longicorpa*, *I. hugowolfeldi*, *I. koreensis*, and *I. pumila*, and then it was secondarily similar to other species in different genera, *K. brevifasciata* and *C. takatsuensis*. Interestingly, these seven species commonly prefer a bottom of pebbles and cobbles in the middle to upper streams as their habitat. These findings indicate that although the degree of taxonomic closeness is a primary factor for characteristics of early life history among cobitid species, the habitat type can also have an influence on these characteristics.

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