Japanese Journal of Ichthyology
Vol. 32, No. 1 1985

魚類学雑誌
32巻1号 1985年

Development of the Bitterling *Rhodeus uyekii* (Cyprinidae), with a Note on Minute Tubercles on the Skin Surface

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(Received July 1, 1984)

Abstract The development of eggs and larvae, and minute tubercles on the skin surface of larvae of *Rhodeus uyekii* were observed. The egg and larval development, and minute tubercles on the skin surface of larvae of this species were relatively similar to those of two subspecies, *R. atremius atremius* and *R. atremius suigensis*. This species in larval development is characterized by having a yolk sac, which is laterally slender and convex downward at the posteriormost portion, and numerous vestigial minute tubercles on the skin surface except for the wing-like yolk projection.

Rhodeus uyekii (Mori) is a common freshwater bitterling in Korea. Though Uchida (1939) described the larvae and juveniles of this species based on field specimens, the details on its embryonic and larval stages are poorly known. On the other hand, it is known that bitterling larvae of *Acheilognathus* and *Pseudoperilampus* have minute scale-like tubercles on the skin surface (Uchida, 1937; Nakamura, 1969). Fukuhara *et al.* (1982) reported the morphology of minute scaly tubercles on the surface of the yolk sac in the larvae of *Acheilognathus*. Recently, Suzuki and Hibiya (1984a) described the morphology and distribution of minute tubercles on the skin surface of larvae in all species of *Rhodeus* from Japan. However, those of larvae in *R. uyekii* are still unknown.

The present paper deals with the development of eggs and larvae, and minute tubercles on the skin surface of the larvae in *R. uyekii*.

Materials and methods

Parental fish of *R. uyekii* were collected from the Naktong River, Korea. Artificial insemination was carried out 5 times during May to June using the same pair (a femal, 42.4 mm TL and a male, 54.3 mm TL). During the period, the ovipositior of female shows cyclic changes and develops to its maximal length (ca. 29.5 mm) at intervals of 4 to 9 days. The number of ripe eggs obtained ranges from 16 to 21 (mean, 18 eggs). Methods of artificial insemination and rearing of eggs and larvae are the same as those of Suzuki and Hibiya (1984b). The development of eggs and larvae were examined under a dissecting microscope. Total length of live larvae was measured with an ocular micrometer.

Specimens used for morphological observations of minute tubercles on the skin surface of larvae are identical to those of larval development in the present study. Five specimens were fixed for 24 hours in cacodylate-buffered 2.5% glutaraldehyde at each stage of larval development and were dehydrated by a graded series of ethanol, and then dried at a critical point with liquid CO_2. The dried specimens were coated with platinum by ion sputtering and examined with a Hitachi S-450 scanning electron microscope.

Egg and larval development of *Rhodeus uyekii*

Embryonic stages. The sequence of embryonic development is illustrated in Fig. 1 as depicted by sixteen stages (A–P) of development. Unfertilized eggs are nearly spindly, opaque yellow in colour, measuring about 3.2 mm in length, about 1.7 mm in breadth (Fig. 1A). The time required for each embryonic stage under controlled water temperature at 22±1°C is shown in Table 1. About 33 hours after insemination, a pair of small yolk projections can be seen just posterior to the head region (Fig. 1M). About 46 hours after insemination, embryos begin to hatch continuing for about 10 consecutive hours. All embryos hatch from the vegetal pole side (Fig. 1P).

Larval development. 1) Immediately after hatching, 4.3–4.5 mm in total length (Fig. 2A). Nineteen to twenty-seven myotomes may be

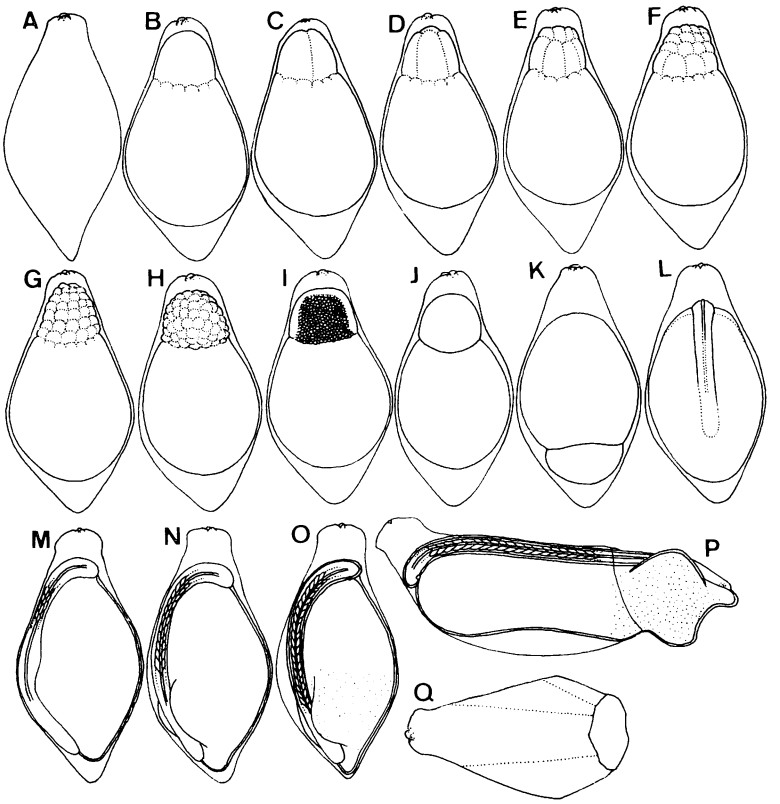

Fig. 1. Egg developmeut of *Rhodeus uyekii* at 22±1°C in water temperature. Time required for each developmental stage is shown in Table 1.

countable. The yolk sac contains a substantial amount of yolk, and is slender laterally and runs downward at the posteriormost part. The fin-fold at the trunk and caudal portion is small. The larvae at this stage are usually motionless.

2) 2 days after hatching, 5.5–5.7 mm in total length (Fig. 2B). The number of myotomes ranges from 30 to 32. The tail elongates backward and the caudal fin-fold slightly develops. The optic cup without lens and the auditory vesicles with two pairs of otoliths are clearly observed. The anteriormost part of the yolk sac develops downward to form a projection.

3) 3 days after hatching, 6.5–6.7 mm in total length (Fig. 2C). The number of myotomes ranges from 30 to 32 (24–26+6). The heart begins to pulsate beneath the head region. A pair of dorsal yolk projections connects with the ventral yolk projection at the mid-yolk sac region. The larvae of this stage begin to move when the primordial fin-fold becomes well-developed. The larvae, however, usually lay their back on the bottom of the petri-dish.

4) 4 days after hatching, 7.1–7.2 mm in total length (Fig. 2D). The number of myotomes ranges from 30 to 32 (21–23+9). The circulatory system is already established. Blood cells become reddish and increase in number. The dorsal and ventral yolk projections gradually elongate posteriorly. The margin of the fin-fold extends. The dorsal part of the head is raised slightly and the brain has undergone further development.

5) 7 days after hatching, 7.6–7.8 mm in total length (Fig. 2E). The number of myotomes ranges from 30 to 34 (20–23+10–11). The lenses are already formed. The optic cup envelops the lens completely; melanin pigments begin to deposit on the optic cup. The notochord starts to flex, the cartilaginous hypural elements begin to differentiate, and the incipient fin-rays are not visible. A pair of dorsal yolk

Table 1. Time required for embryonic stages in *Rhodeus uyekii* at 22±1°C in water temperature.

Stage*	Time after insemination (hr: min)	Remarks
A	—	Unfertilized egg
B	1: 00	Blastodisc
C	2: 00	Two-celled egg
D	2: 30	Four-celled egg
E	3: 00	Eight-celled egg
F	3: 30	Sixteen-celled egg
G	4: 00	Thirty-two-celled egg
H	4: 30	Early morula
I	5: 00	Post morula
J	6: 00	Blastula
K	28 :00	Blastopore nearly closed
L	30: 00	Neurula
M	33: 00	Four somite formation
N	40 :00	Embryo formed entirely
O	44: 00	The last embryonic stage
P	46: 00	Hatching begins
Q	—	Castoff chorion

* Stages A to Q correspond to those in Fig. 1.

projections is reduced slightly, while the ventral yolk one is still well-developed.

6) 8 days after hatching, 8.6–8.7 mm in total length (Fig. 2F). The number of myotomes ranges from 32 to 34 (18–19+14–15). The mouth sometimes opens and shuts. The part of the fin-fold of the future dorsal and anal fins becomes high. Black pigments are developed on the retinal layers. The optic cups are silvery blue because guanine is distributed diffusely on this organ. Melanophores appear on the dorsal part of both head region and the yolk sac. A pair of small nasal sacs become evident in front of the eye cups. Rudiments of the pectoral fins appear as small membranes beneath the auditory vesicles. The cartilaginous hypural elements differentiate completely, and some rays of the caudal fin are formed. A pair of dorsal yolk projections is considerably reduced in comparison with those of the former stage. At this stage, the larvae sometimes actively swim upside down.

7) 11 days after hatching, 8.7–8.9 mm in total length (Fig. 2G). The number of myotomes ranges from 32 to 34 (17–18+15–16). Eye pigments, both melanin and guanine, are heavily concentrated. The caudal fin-rays are completed in number. About 5 rays are formed in the dorsal and anal fins. Melanophores are observed on the head region, the auditory vesicles, the caudal fin-rays, the dorsal, ventral and lateral parts of the body and the yolk sac. A small gas bladder and a green gall bladder can be easily seen beneath the pectoral fins.

8) 18 days after hatching, 8.8–9.0 mm in total length (Fig. 2H). The upper and lower jaws are approximately equal in size. The posterior margin of the caudal fin changes from a rounded to a truncated shape. The caudal fin-rays begin to fork into two branches. Melanophores increase in number and newly appear on the dorsal and anal fin-rays. Yellow pigments also appear over the melanophore on the head region and on the dorsal part of the body. The pectoral fins become functional. The gas bladder becomes larger without dividing into two lobes. At this stage, the larvae swim with good balanced orientation, however, they cannot continue the vigorous swimming for more than a few seconds.

9) 22–24 days after hatching, 9.2–9.4 mm in total length (Fig. 2I). Free-swimming stage. The gas bladder is divided completely into front and hind lobes. This means that larvae are able to swim actively with good balanced orientation for hours. Guanine is slightly distributed on the surface of the yolk sac. The dorsal and anal fins are completed in number. Yellow pigments are widely distributed on the body. The yolk projections on the breast are so reduced that they are not easily found. Although the yolk still remained, the larvae begin to feed on commercial diets ("Tetramin") for the initial food item.

10) 26 days after hatching, 9.2–9.6 mm in total length (Fig. 2J). Rudiments of the ventral fins appear as small membranes on the breast. Some rays are formed in the pectoral fins. Guanine is slightly distributed on the belly. Melanophores on the dorsal fin-rays are aggregated at the anterior region of the dorsal fin to form a black spot. In this stage, the shape of the larval black spot in *R. uyekii* relatively resembles the scalene spot of *R. ocellatus ocellatus* (Kner) and *R. ocellatus smithi* (Regan), and also is distinguishable from the nearly round spot of *R. atremius atremius** (Jordan et Thompson) and

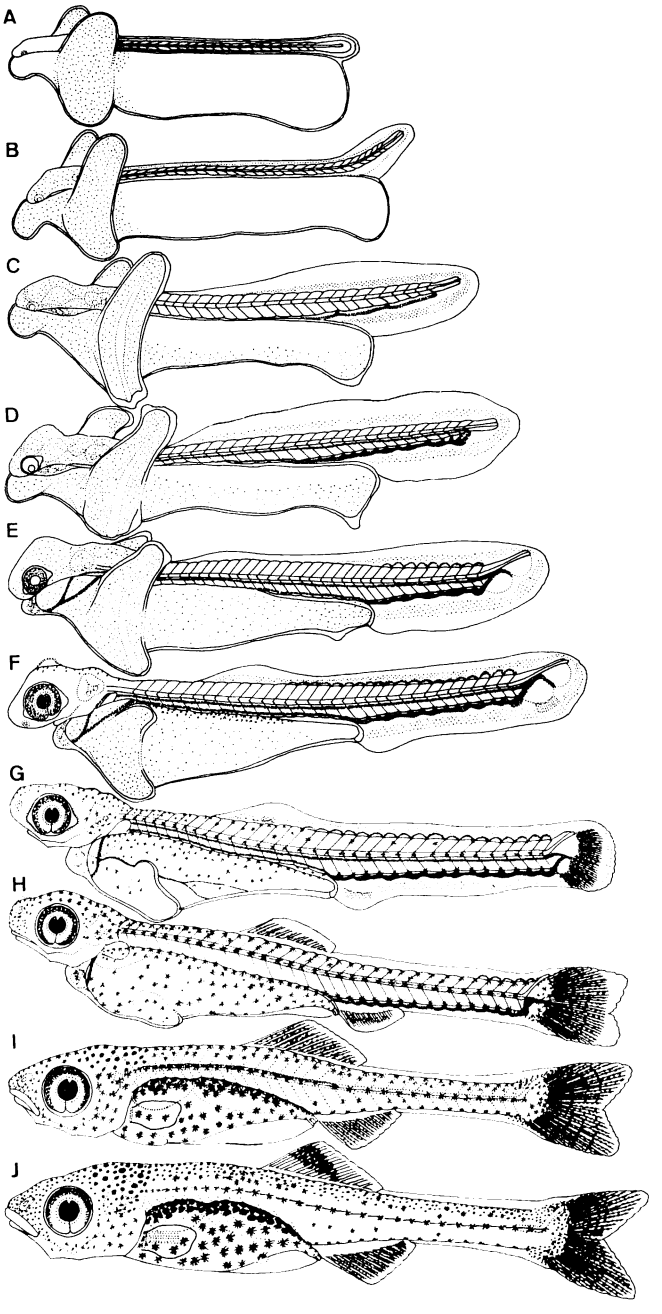

Fig. 2. Larvae of *Rhodeus uyekii*. A, immediately after hatching, 4.3 mm in total length (TL). B, 2 days after hatching, 5.5 mm in TL. C, 3 days after hatching, 6.5 mm in TL. D, 4 days after hatching, 7.1 mm in TL. E, 7 days after hatching, 7.6 mm in TL. F, 9 days after hatching, 8.6 mm in TL. G, 11 days after hatching, 8.9 mm in TL. H, 18 days after hatching, 9.3 mm in TL. I, 22 days after hatching, 9.5 mm in TL. J, 26 days after hatching, 9.5 mm in TL.

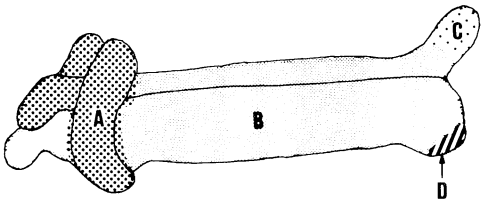

Fig. 3. Diagram of the distribution of minute tubercles on the skin surface of larvae in *Rhodeus uyekii*. Hemispheric minute tubercles are distributed on both parts of A and D, and vestigial minute tubercles on both parts of B and C, respectively. For details with the parts of A to D see text.

*R. atremius suigensis** (Mori).

Minute tubercles on the skin surface of larvae in *Rhodeus uyekii*

The distribution of minute tubercles is divided into four parts of the body (Fig. 3). Each part of the body is: (A) the wing-like yolk projections composed of a pair of dorsal yolk projections and a ventral yolk projection, and the anterior portion of the body such as eye cups and the head, (B) the yolk sac except for the wing-like yolk projections, i.e. the anteriormost part of yolk sac and most part of the body excluding the posteriormost part, (C) the caudal fin-fold, and (D) the posteriormost part of the yolk sac.

The minute tubercles are differentiated into two types by the following characters. The first includes small hemispheric minute tubercles (ca. 3–10 μm in height) arising from single cells of the free surface of the epidermis (Fig. 4A) and the other includes vestigial minute tubercles distributed mainly on the surface of the yolk sac except for the wing-like yolk projection (Fig. 4B). Their distribution and development altered with growth of the larvae. The larvae, immediately after hatching, have many hemispheric minute tubercles with ca. 3 to 7 μm in height on the parts of A, C and D (Fig. 5 A, B, C) and also some vestigial minute tubercles arising from single cells of the free surface of the epidermis on the part of B (Fig. 5D). In larvae at 3 days after hatching, the hemispheric minute tubercles on both A and D increase in height, ca. 5–10 μm, (Fig. 5 E, F), and the vestigial minute tubercles on B increase in number. The vestigial minute tubercles arise from a corner of the cell borders of the epidermal cells and increase in height, ca. 5 μm, (Fig. 5 G). In larvae at 5 to 8 days after hatching, no

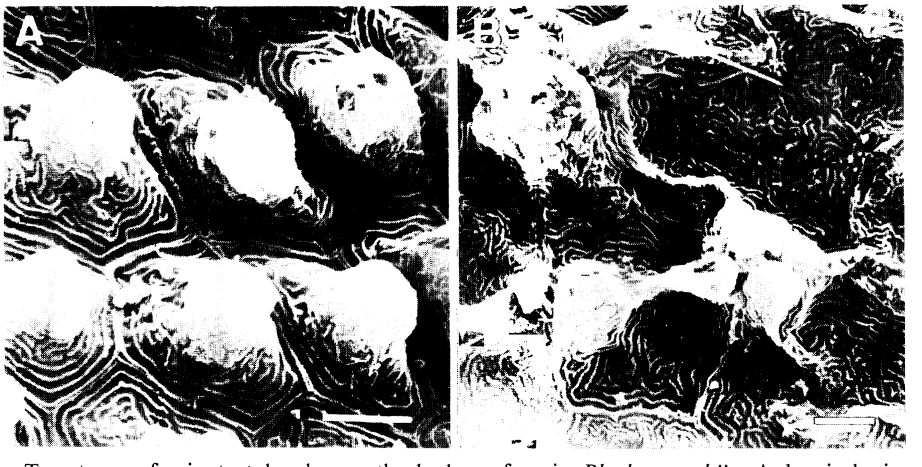

Fig. 4. Two types of minute tubercles on the body surface in *Rhodeus uyekii*. A, hemispheric minute tubercles on the surface of the head region in larva 7 days after hatching, 7.7 mm in TL. The hemispheric minute tubercles arise from single cells of the free surface of the epidermis. B, vestigial minute tubercles of the body surface in larva 8 days after hatching, 8.6 mm in TL. The vestigial minute tubercles arise from a corner of the cell borders of the epidermal cells. Scales indicate 5 μm.

* Suzuki and Hibiya (1984b) considered that *R. suigensis* is a subspecies of *R. atremius* as the result of embryological observation, both species therefore are treated here as *R. atremius atremius* and *R. atremius suigensis*, respectively.

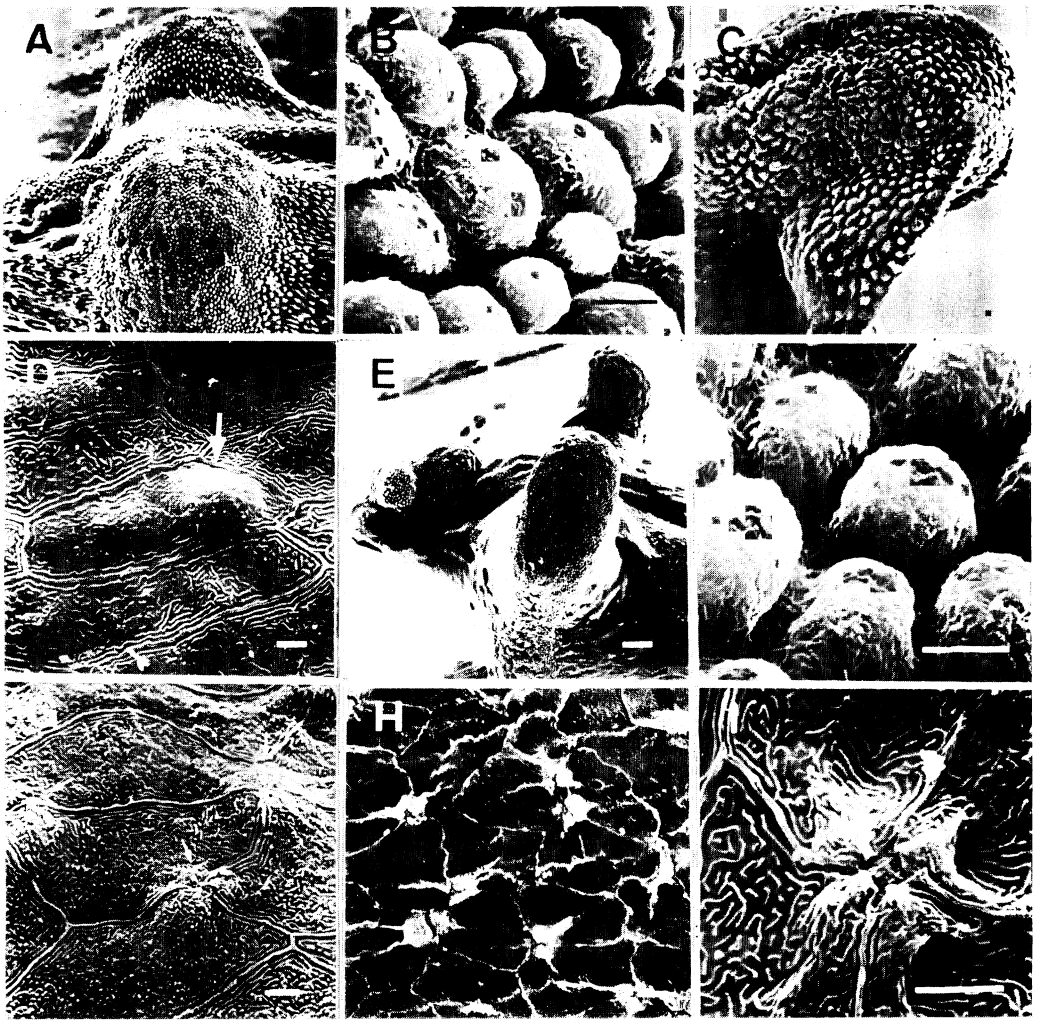

Fig. 5. Hemispheric minute tubercles on the surface of wing-like yolk projections (A–B and E–F) and vestigial minute tubercles on the body surface (C–D and G–I) of larvae in *Rhodeus uyekii*. A–B, immediately after hatching, 4.3 mm in TL. C, minute tubercles on the caudal fin-fold in larvae immediately after hatching, 4.3 mm in TL. D, the tubercles arising from single cells of the free surface of the epidermis (arrow) in larvae 3 days after hatching, 6.5 mm in TL. E–F, 3 days after hatching, 6.5 mm in TL. G, the tubercles arising from a corner of the cell border of the epidermal cells (arrows) in larvae 3 days after hatching, 6.5 mm in TL. H, 7 days after hatching, 7.7 mm in TL. I, 11 days after hatching, 8.9 mm in TL. Scales indicate 5 μm.

pronounced changes of the hemispheric minute tubercles on A can be found in shape and size. On the other hand, the vestigial minute tubercles on B increase in height, ca. 5–8 μm, to the development of the cell borders of the epidermal cells (Fig. 5H). Thereafter, the two types of minute tubercles on each part of the body begin to reduce when the larvae begin to swim actively, about 11 days after hatching, (Fig. 5I). By then, the minute tubercles are bearly present on the ridges such as eye cups, dorsal and hind parts of the head, and ventral and hind parts of the yolk projections *in the* free-swimming stage.

Discussion

R. uyekii and all species of *Rhodeus* from Japan share the wing-like yolk projections and the

hemispheric minute tubercles on the skin surface in their larval development. This fact suggests that these morphological characters are important for phylogenetic studies of *Rhodeus*.

Uchida (1939) stated that unfertilized eggs of *R. uyekii* are distinguishable in shape from those of *R. ocellatus*, but not from those of *R. notatus* (Nichols) which is considered to be closely related to *R. suigensis*. On the other hand, unfertilized eggs of *R. uyekii* are distinguishable in shape from those of two subspecies of *R. atremius*; those of the former are nearly spindly compared with those of two subspecies of the latter (Suzuki and Hibiya, 1984b).

The time required for each embryonic stage in *R. uyekii* is the same as that in *R. atremius suigensis* under controlled water temperature at 22±1°C (Suzuki and Hibiya, 1984b). *R. uyekii* shared the developed anteriormost part of the yolk forming a projection with two subspecies of *R. atremius* in the larval development. Regarding the shape of the larval spot on the dorsal finrays, *R. uyekii* is more similar to *R. ocellatus* than to *R. atremius*.

Uchida (1939) stated that *R. atremius* and *R. uyekii* should not be separated at the species level because they resemble each other in the adult form. However, as regards the larval development, *R. uyekii* is characterized by the following characters which are not shared by *R. atremius* and *R. ocellatus*: (1) the lateral part of the yolk sac is slender and the posteriormost part of the yolk sac forms a downward directed projection, and (2) numerous vestigial minute tubercles arising from a corner of the cell borders of the epidermal cells are distributed on part B in the body (Fig. 3).

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ウエキゼニタナゴの卵発生と仔魚の発育ならびに仔魚の表皮上小突起

鈴木伸洋・秋山信彦・日比谷　京

ウエキゼニタナゴの卵発生と前期仔魚期の発育について経時的に観察し，併せてこれら仔魚の表皮上に存在する小突起についても観察した．22°C の飼育下では受精後約 46 時間から孵化を開始し，浮上期に達するのにほぼ 24 日を要した．この期間の卵発生ならびに仔魚の発育形態はカゼトゲタナゴとスイゲンゼニタナゴのそれに類似したが，卵黄嚢が細長くこの後端が下方へ突出することが近縁種とのよい区別点となる．本種の仔魚は卵黄嚢がよく発達した翼状突起をもち，この表皮上には高さ約 3～10 μm の半球状の小突起を備える。これは鈴木・日比谷 (1984a) が報告した *Rhodeus* 属（ニッポンバラタナゴ・タイリクバラタナゴ・カゼトゲタナゴ・スイゲンゼニタナゴ）の仔魚に共通してみられる形質で，タナゴ類の系統発生上の重要な形質の一つと考えられる。また同時に，上記 *Rhodeus* 属の仔魚には翼状突起と体前部を除く表皮上に退化的な小突起が存在するが，本種の仔魚ではこれが多数存在しかつ高さも 3～5 μm 程度で大きい．

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