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

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Abstract The development of eggs and larvae and minute tubercles on the skin surface in larvae of *Tanakia tanago* were observed. The eggs began to hatch approximately 52 hours after insemination and the larvae reached free-swimming stage 19 days after hatching at water temperature of 22±1°C. The egg and larval development and minute tubercles on the skin surface in larvae of this species were similar to those of *Acheilognathus lanceolata* and *A. limbata*. However, *T. tanago* was distinguishable in egg and larval development from *A. lanceolata* and *A. limbata* by the following characters: the perivitelline space was narrower, embryonic and larval development was faster, and minute tubercles on the skin surface of the anteriormost parts of the yolk sac, and of the body and head were hemispheric in shape. From these characters, *T. tanago* is considered to be more specialized than *A. lanceolata* and *A. limbata*.

Tanakia tanago (Tanaka), an uncommon bitterling, is a natural monument in Japan. Recently, Oka and Sugo (1984) reported on the artificial breeding of this species. Also, Nakamura (1969) described the larvae and juveniles of this species. However, details of its embryonic and larval stages remain poorly known. It is well known that larvae of bitterlings have minute tubercles on the skin surface (Uchida, 1937; Nakamura, 1969). The morphology and distribution of minute tubercles in larvae of *Acheilognathus*, *Pseudoperilampus* and *Rhodeus* have been reported (Fukuhara *et al.*, 1982; Suzuki and Hibiya, 1984a, 1985a), whereas those found in *T. tanago* are still unknown.

The present paper deals with the development of eggs and larvae and minute tubercles on the skin surface in the larvae of *T. tanago*. In addition, the phylogenetic relationship of this species within acheilognathine fishes is discussed.

Material and methods

Broodstock of *T. tanago* were collected from Gonta Pond, Kanagawa, and reared in an aquarium at Kanagawa Prefectural Freshwater Fisheries Experimental Station. Artificial inseminations were carried out several times from May to July using a single pair (a female, 42.5 mm in TL and a male, 53.4 mm in TL) of breeders. During this period the ovipositor elongated, reaching maximal length (ca. 25.3 mm) at intervals of 3 to 8 days. The number of ripe eggs obtained ranged from 3 to 18 (mean, 8 eggs) per spawning. Methods of artificial insemination and rearing of eggs and larvae followed those of Suzuki and Hibiya (1984b). The development of eggs and larvae was observed under a dissecting microscope. Total length of live larvae was measured with an ocular micrometer.

A single set of specimens was used for morphological observations of minute tubercles on the skin surface of larvae for observations of larval development. In addition, for each stage of larval development, five specimens were fixed for 24 hours at 2°C in cacodylate-buffered 2.5% glutaraldehyde, dehydrated by a graded series of ethanol, dried to critical point by ion sputtering, and then examined with a Hitachi S-450 scanning electron microscope.

Egg and larval development of *Tanakia tanago*

Embryonic stages. Sixteen stages (A–P) were found during embryonic development (Fig. 1). Unfertilized eggs appeared nearly pear-shaped, opaque yellow in color, and measured 2.2 mm in length, 1.5 mm in breadth (Fig. 1A). The time required for each embryonic stage at 22±1°C is shown in Table 1. Thirty minutes after insemination, the chorion separated from the plasma membrane to form a narrow perivitelline space.

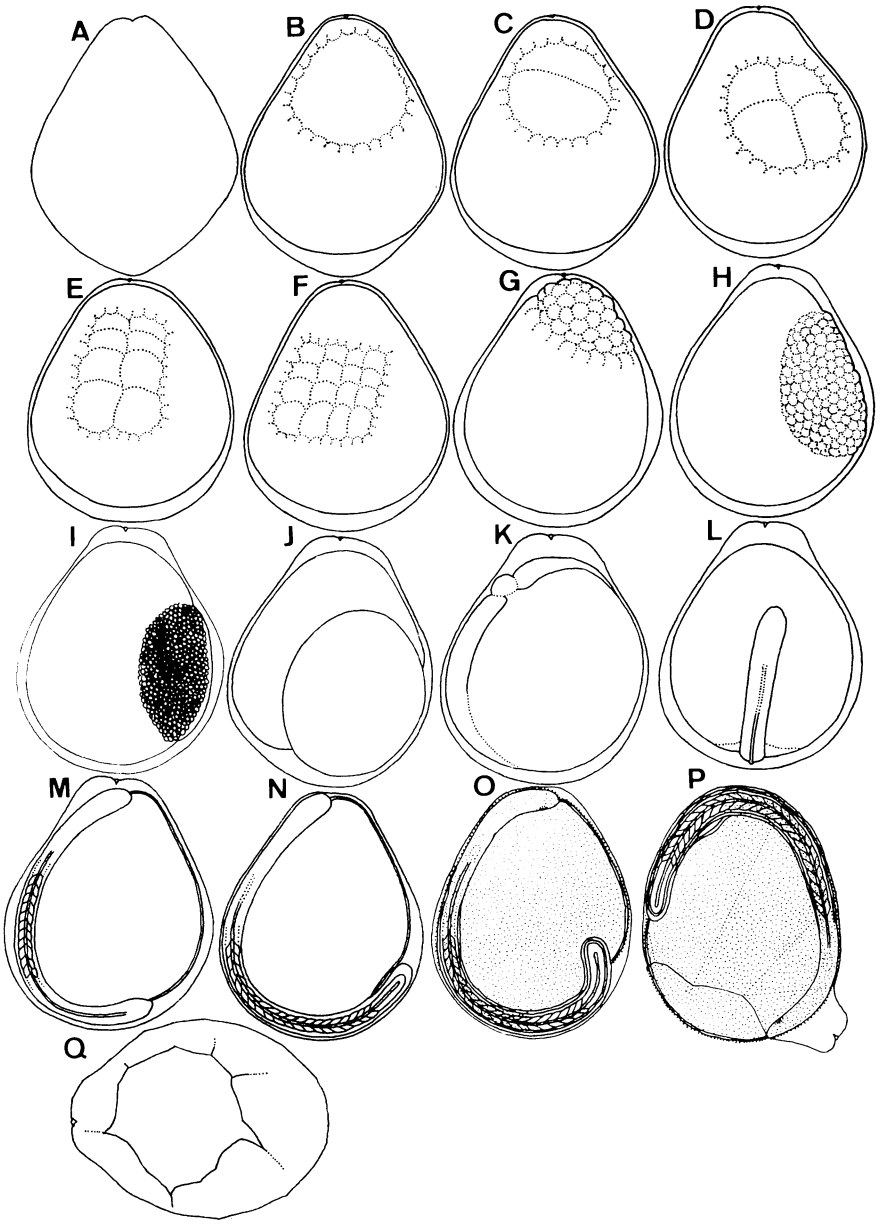

Fig. 1. Egg development of *Tanakia tanago* at 22±1°C water temperature. Time required for each developmental stage is shown in Table 1.

Thirty-nine hours after insemination, the embryonic body became evident, with the posterior tip of the embryo situated at the vegetal pole (Fig. 1O). Fifty-two hours after insemination, most of embryos began to hatch from the animal pole side (Fig. 1P).

Larval development. 1) Immediately after hatching, 4.4–4.6 mm in total length (Fig. 2A). Twenty-nine to thirty-two myotomes were counted. The yolk sac contained a substantial amount of yolk, with the dorsal part of the sac slightly developed to form a pair of hilly projections (Fig. 4A). A few hours after hatching, when the primordial fin-fold at the caudal portion has developed, larvae began to move. However, the larvae usually lay on their trunk on the bottom

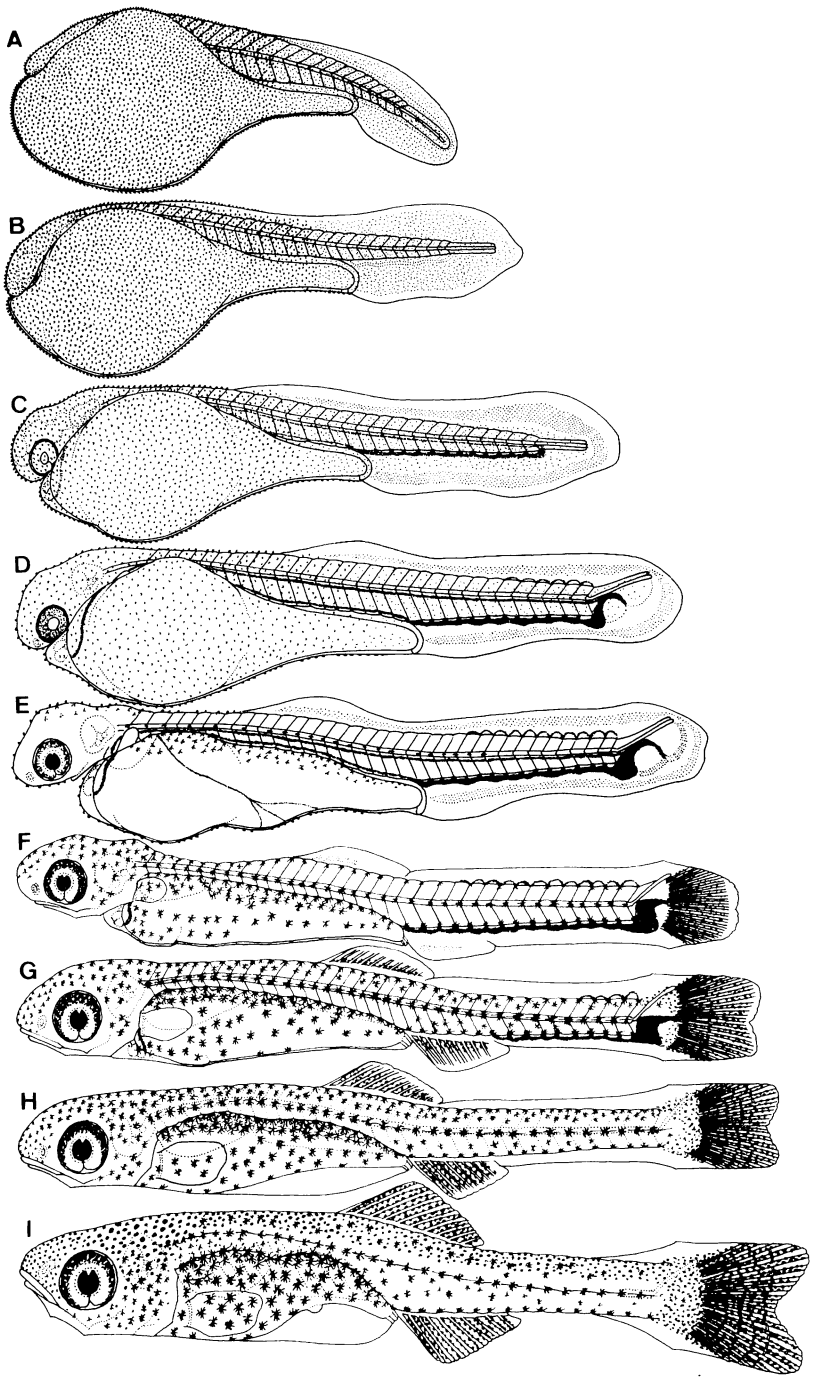

Fig. 2. Larvae of *Tanakia tanago*. A, immediately after hatching, 4.4 mm in total length (TL). B, 2 days after hatching, 5.9 mm in TL. C, 4 days after hatching, 6.7 mm in TL. D, 6 days after hatching, 7.8 mm in TL. E, 10 days after hatching, 8.0 mm in TL. F, 13 days after hatching, 8.3 mm in TL. G, 16 days after hatching, 8.7 mm in TL. H, 19 days after hatching, 9.2 mm in TL. I, 24 days after hatching, 9.8 mm in TL.

of the petri-dish.

2) 2 days after hatching, 5.8–6.0 mm in total length (Fig. 2B). The number of myotomes ranged from 30 to 32 (21–22+9–10). The tail elongated backwards and the caudal fin-fold became well-developed. The optic cup, still without lens, and auditory vesicles possessing two pairs of otoliths were clearly observed. The heart began to pulsate.

3) 4 days after hatching, 6.7–6.9 mm in total length (Fig. 2C). The number of myotomes ranged from 32 to 34 (20–21+12–13). The circulatory system was already established. Blood cells became reddish and increased in number. The pair of hilly projections of yolk gradually expanded toward both sides of the body. At this stage, the larvae sometimes moved actively while laying down on their side.

4) 6 days after hatching, 7.6–7.8 mm in total length (Fig. 2D). The lenses became completely developed and melanin pigments began to form on the optic cup. The notochord started to flex and cartilaginous hypural elements began to differentiate. Incipient fin-rays were not visible. The hilly projections of the yolk became slightly reduced in size. A pair of small nasal sacs were evident in front of the eye cups. Rudiments of the pectoral fins appeared as small membranes beneath the auditory vesicles.

Table 1. Time required for each embryonic stage of *Tanakia tanago* at 22±1°C 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	Thiry-two-celled egg
H	5: 00	Early morula
I	6: 00	Post morula
J	8: 00	Blastula
K	32: 00	Blastopore nearly closed
L	35: 00	Neurula
M	36: 00	Eleven somites formation
N	39: 00	Embryo formed entirely
O	42: 00	The last embryonic stage
P	52: 00	Hatching begins
Q	—	Castoff chorion

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

5) 10 days after hatching, 8.0–8.1 mm in total length (Fig. 2E). The number of myotomes ranged from 32 to 34 (17–19+15). The hilly projections of yolk is considerably reduced in size. Some rays of the caudal fin were formed. The part of the fin-fold comprising future dorsal and anal fins bulged considerably. A small gas-bladder and green gall bladder were be easily seen beneath the pectoral fins. Black pigments developed on the retinal layers. The optic cups appeared silvery blue due to the presence of guanine scattered on this organ. Melanophores were observed on the dorsal part of the head region and yolk sac. The mouth occasionally opened and closed.

6) 13 days after hatching, 8.1–8.3 mm in total length (Fig. 2F). Eye pigments, both melanin and guanine, were heavily concentrated. All caudal fin-rays have started to develop. Five to six rays were formed in the dorsal and anal fins. Melanophores were observed on the head region, auditory vesicles, caudal fin-rays, dorsal, ventral and lateral parts of the body and yolk sac. The hilly projections of yolk were so diminished that they were not easily found.

7) 16 days after hatching, 8.7–8.8 mm in total length (Fig. 2G). The upper and lower jaws were approximately equal in size. The posterior margin of the caudal fin changed from a rounded to a truncated shape. Caudal fin-rays began to fork. Melanophores increased in number and began to appear on dorsal and anal fin-rays. Yellow pigments also became visible over the melanophores on the head region and on the dorsal part of the body. The pectoral fins commenced to move. The gas-bladder, which was yet undivided into lobes, grew larger. At this stage, the larvae swam with well balanced orientation. However, they were unable to swim vigorously for more than a few seconds.

8) 18–20 days after hatching, 9.0–9.2 mm in total length (Fig. 2H). This marked the beginning of the free-swimming stage. The gas-bladder divided completely into anterior and posterior lobes. The larvae were now able to swim actively with well balanced orientation for much longer periods. All dorsal and anal fin-rays were completed in number. Yellow pigments were found widely distributed on the body. Although yolk

still remained, larvae began to feed on commercial diets ("Tetramin").

9) 24 days after hatching, 9.6–9.8 mm in total length (Fig. 2I). Rudiments of ventral fins emerged as small membranes on the breast. Guanine was sparsely distributed on the belly. The larvae at this stage became dark in color due to intensive pigmentation by melanophores on both sides of the body. However, unlike in larvae of *Rhodeus*, melanophores on the dorsal fin-rays did not aggregate towards anterior region of the dorsal fin to form a black spot.

Minute tubercles on the skin surface of larvae in *Tanakia tanago*

The skin surface of *T. tanago* larvae was divided into the following four parts (A–D) to facilitate description of the distributional patterns of tubercles (Fig. 3): (A) most part of the yolk, composed of a pair of hilly yolk projections and the mid-yolk sac, and the mid-body region, (B) posterior parts of both the yolk sac and the body, (C) the caudal fin-fold, and (D) anterior parts of both the yolk sac and the body. The larvae of this species possessed minute tubercles on the skin surface of the whole body, which developed from single cells of the free surface of the epidermis (Fig. 4A–G). Their distribution and development altered with growth of larvae. Immediately after hatching, minute scale-like tubercles (ca. 15–25 μm in height), shaped like circular cones that tilt posteriorly, were observed on part A of the body (Fig. 4B). Also, many hemispheric minute tubercles (ca. 5–10 μm in height) on parts B and D (Fig. 4C, E), and vestigial minute tubercles on part C (Fig. 4D) were found. In larvae at 6 days after hatching, the minute scale-like tubercles on part A changed from circular cone shape to hemispheric shape and decreased in height to ca. 10 μm (Fig. 4F). Thereafter, all minute tubercles started to reduce in size as the larvae begin to swim actively, about 10 days after hatching (Fig. 4G). In the free-swimming stage, the minute tubercles on most parts of the body have almost completely diminished except for being sparsely present on the ridges, such as eye cups and dorsal part of the head (Fig. 4H).

Discussion

The larvae of *T. tanago* possessed a pair of hilly projections on the yolk and circular cone-shaped minute and scale-like tubercles on part A and hemispheric minute tubercles on part B, and vestigial minute tubercles on part C of the body as in the larvae of *Acheilognathus lanceolata* (Temminck et Schlegel) and *A. limbata* (Temminck et Schlegel). These facts suggest that they are closely related species, because these characters are the important markers for determining phylogenetic relationships between acheilognathine fishes (Fukuhara *et al.*, 1982; Suzuki and Hibiya, 1984a, 1985a, b).

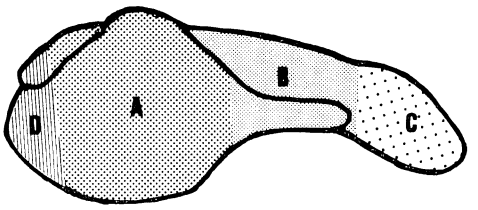

Fig. 3. Diagram showing division of skin surface of larvae to facilitate description of the distribution of minute tubercles in larval *Tanakia tanago*. Minute scale-like tubercles are distributed on part A and hemispheric minute tubercles on parts B and D, and vestigial minute tubercles on part C, respectively. For details on parts A to D, see text.

Tanakia tanago began to hatch about 52 hours after insemination and the larvae reached the free-swimming stage about 19 days after hatching. The time required for embryonic and larval development of this species is faster than in *A. lanceolata* and *A. limbata* (Suzuki and Hibiya, 1985c). The shape of unfertilized eggs of *T. tanago* resembles that of *A. limbata*, but are distinguishable from that of *A. lanceolata* which is closely related to *A. limbata* (Suzuki and Hibiya, 1985b, 1986). On the other hand, the perivitelline space of fertilized eggs in *T. tanago* was narrower than that in *A. limbata*. From these facts, *T. tanago* is considered to be more specialized than both *A. lanceolata* and *A. limbata*.

Tanakia tanago has been treated as belonging to a separate genus from *Acheilognathus*, *Rhodeus* and *Pseudoperilampus* on the basis of incomplete latearl line and the presence of a pair of snout barbeles in adult form (Aoyagi, 1957; Okada, 1960; Nakamura, 1969). On the other hand, *Acheilognathus* is composed of two groups; one including *A. lanceolata* and *A. limbata*, and another including

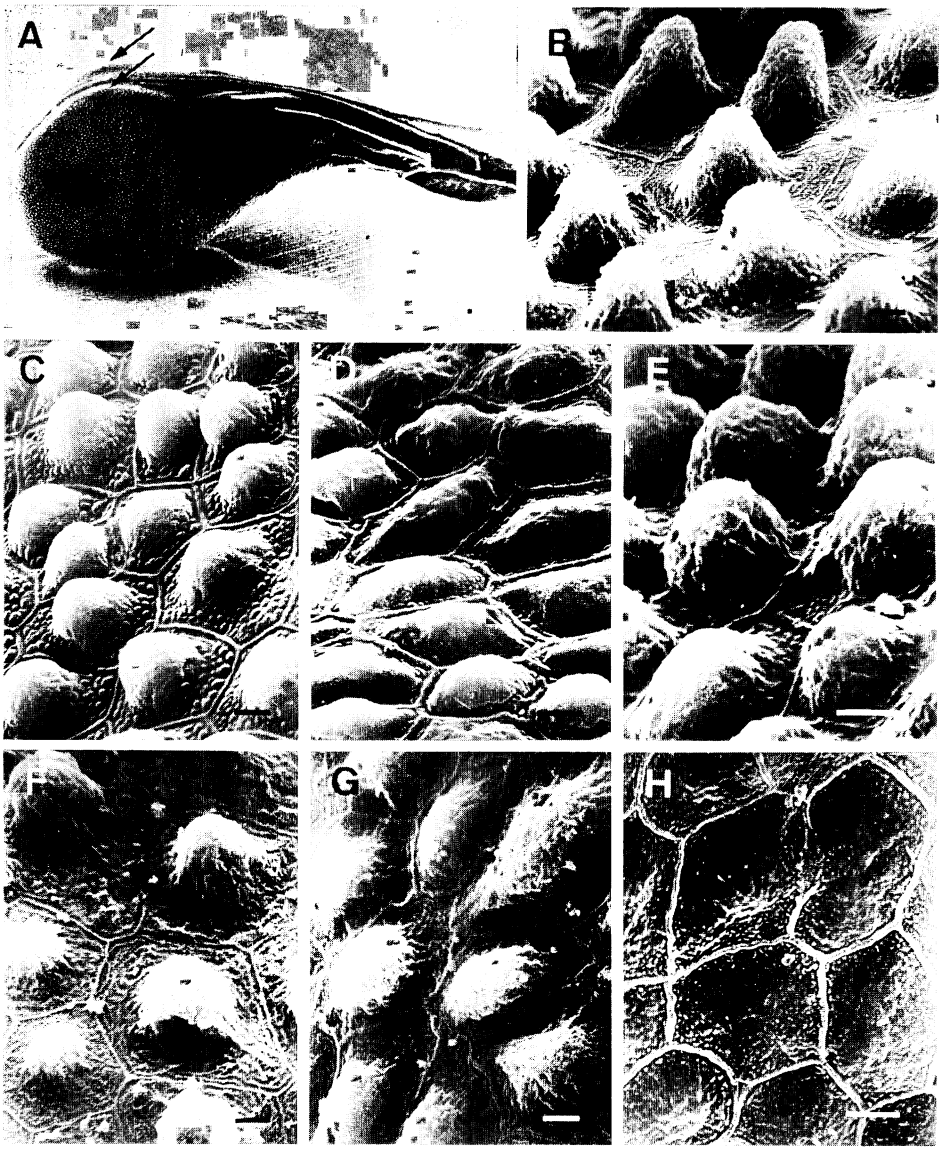

Fig. 4. The hilly yolk projections (arrows) (A), minute scale-like tubercles on part A (B and F–H), hemispheric minute tubercles on parts B and D (C and E), and vestigial minute tubercles on part C (D) on the body of surface larval *Tanakia tanago*. A–E, immediately after hatching, 4.4 mm in TL. F, 6 days after hatching, 7.6 mm in TL. G, 10 days after hatching, 8.1 mm in TL. H, 19 days after hatching, 9.1 mm in TL. Scales indicate 5 μm.

the remaining six species and subspecies, based on karyotypes, dorsal fin-ray formula, embryonic morphology and structure of the pharyngeal apparatus (Arai, 1978, 1982; Suzuki and Hibiya, 1985c). As regards larval development, however, no fundamental differences with phylogenetic implications were found between *T. tanago* and either *A. lanceolata* nor *A. limbata*.

Acknowledgments

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ミヤコタナゴの卵発生と仔魚の発育ならびに仔魚の表皮上突起

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ミヤコ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，これらの仔魚の表皮上に存在する突起の形態も観察した．22℃ の飼育下では受精後約 52 時間から孵化を開始し，浮上期に達するのにほぼ 19 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ヤリタナゴとアブラボテのそれに類似した。本種の仔魚は，孵化直後にすでに尾部仔魚膜鰭（鰭褶）がやや発達していること，孵化後数時間には仔魚が動き始めること，卵黄嚢背面が上方へ低く隆起して1対の丘状突起を形成することならびに卵黄嚢および体の中央部の表皮上には高さ約 15–25 μm の斜円錐状の鱗状突起を卵黄嚢後部および鰭褶の表皮上には高さ約 5–10 μm の半球状の小突起をそれぞれ備えている．これらはヤリタナゴおよびアブラボテの仔魚にも共通してみられる系統発生上重要な形質である．しかし，本種はヤリタナゴとアブラボテに比べて卵発生ならびに仔魚の発育経過が速やかであることや，卵黄嚢の前部および体の前部の表皮上には高さ 5–10 μm 程度の半球状の小突起が多数存在することで両種と区別された。以上のことから，これら3種は近縁な関係にあるものと考えられるが，ミヤコタナゴは両種よりもやや特化しているものと判断された．

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