The Adaptation strategy for increasing survival rate of bitterling embryos

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Abstract

Bitterlings are small freshwater with an unusual spawning character with freshwater mussel. They use ovipositors to spawn their eggs in host mussels and display remarkable morphological adaptations to increase larval survival. Their eggs vary in shape, size, and number and four types have been classified: bulb-like, pear-shaped, spindly, and ovoid. Some bitterling have small number of eggs but sticky, which is possible to prevent premature ejection from mussels. The most well-known adaptation structure is the minute tubercle on the skin surface of bitterling larvae. In bitterling groups, the changes in height and shape of minute tubercles of larva can be categorized as six stages: formation, growth, peak, abrupt reduction, reduction, and disappearance stage. The minute tubercles on the larval skin surface start to be grown after hatching and after that it were gradually decreased as the larvae grow and then disappeared at the free swimming stage. The previous study suggested that the minute tubercles are the developmental structures that the larvae have morphologically evolved to prevent ejection from the mussel for increase survival rate of embryos.

Keywords: Acheilognathinae, bitterling, freshwater mussel, minute tubercle, host-parasite relationship.

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Bitterling (Pisces, Cyprinidae)

Bitterlings are small freshwater fish predominantly distributed in Southeast Asia, mainland China, Korea, Japan, and Europe, and have an unusual spawning symbiosis with freshwater mussels. During the spawning season, the female bitterlings have a long ovipositor and spawn on the gill cavity of mussels through the mussels' exhalant siphons. Males have nuptial coloration and defend territories around the living host mussels. They release their sperm that enter the mussels' inhalant siphons, so that fertilization can process in the gill cavity of the host mussels. The embryos develop in the host mussels for one month until the yolk sac have been absorbed and they reach the free-swimming larvae, which then leave the mussels [1].

Mussel (Bivalve: Unionidae)

The life cycle of freshwater mussels comprises a phase in which the mussel larvae act as fish ectoparasites called glochidia. Before being released to find the host fish, the glochidia are incubated in the inner and outer demibranchs of the adult mussel's gills. Although glochidia develop and are incubated within adult mussels for approximately 2-6 weeks to 8 months before being released, over 99% of the glochidia then fail to attach to an appropriate host and thus have a very low survival rate. One of the main advantages of having fish as hosts for the glochidia is that because fish perform upstream colonization, they enable connectivity in the mussel population, which mussels would not normally have because of their low mobility [2].

Relationships between Bitterlings and Mussels

Coevolution is a process that consists of reciprocal evolutionary changes resulting from the relationship between a group of organisms and associated populations. It plays a leading part in the adaptation and speciation of all living organisms. host-parasite, prey-predator, and symbiont relationships are typical examples of coevolution[3]. The relationship between bitterlings and unionid mussels is considered as mutualistic because the bitterlings use the mussels as their spawning sites, while the attachment of mussel glochidia to the bitterlings enables dispersal. However, there is insufficient evidence of this relationship being mutualistic. Bitterling larvae in the mussels' gills may interfere with water circulation, feeding, and breathing; they may also directly pressurize the gills or damage the gill epidermis. Moreover, bitterling eggs in the gill cavity may compete with glochidia because of oxygen and space [4].

Recent studies have reported that the bitterling-mussel relationship is a well-known model of host-parasite interaction. Additionally, the bitterlings make sophisticated oviposition decisions to prevent ejection and have several remarkable physiological, behavioral, and morphological adaptations for spawning on host mussels. These adaptations include a short hatching period, a small number of large eggs, an ethanol pathway to tolerate low oxygen conditions, the development of a cutaneous embryonic respiratory system, different ovipositor lengths, the development of minute tubercles on the skin surface, and the spatial utilization of host mussels [5].

Egg Types and Minute Tubercle

The bitterling eggs with large size ensure that they fit properly into the interlamellar space of the demibranchs in the mussels. The eggs of bitterlings can be divided into four types: bulb-like, pear-shaped, spindly, and ovoid; moreover, some eggs are sticky, which is an adaptive property to prevent premature ejection from mussels (Table1 and Figure1) [6].

Table 1. Comparisons of embryological characters in Acheilognathinae fishes.

Species	Egg type	Egg size (mm)
Rhodeus ocellatus	Bulb-like	2.5 ×1.5
R. pseudosericeus	Bulb-like	3.0 × 1.8
R. uyekii	Bulb-like	3.2 ×1.7
R. notatus	Bulb-like	3.5×1.5
Acheilognathus lanceolatus	Spindly	4.5 × 1.5
A. signifer	Pear-shaped	2.3 ×1.8
A. koreensis	Spindly	4.3 × 1.8
A. somjinensis	Pear-shaped	3.5×2.4
A. yamatsutae	Ovoid	1.9 × 1.5
A. majusculus	Ovoid	2.0×1.5
A. rhombeus	Ovoid	2.6×1.7
A. macropterus	Ovoid	1.9 ×1.5

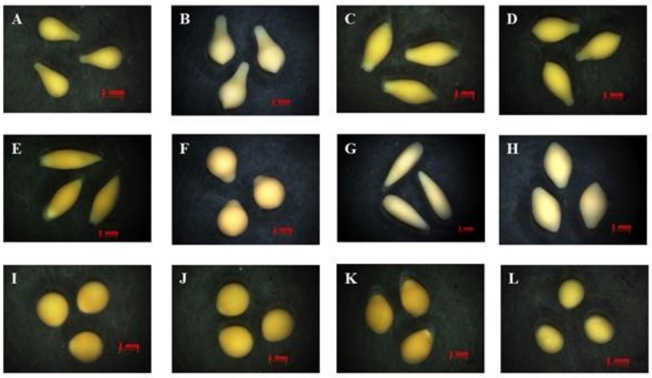

Figure 1. Morphology of fertilized eggs in Acheilognathinae fishes from Korea. *A: Rhodeus ocellatus; B: R. pseudosericeus; C: R. uyekii; D: R. notatus; E: Acheilognathus lanceolatus; F: A. signifer; G: A. koreensis; H: A. somjinensis; I: A. yamatsutae; J: A. majusculus; K: A. rhombeus; L: A. macropterus.*

The bitterling eggs begin to hatch approximately 2-4 days after insemination, depending on the water temperature. The bitterling larvae develop single-celled epidermal cell son their skin surface, called 'minute tubercles', which are considered with main function to prevent the larvae ejection from the gills of host mussel. Minute tubercles are common structure in all developmental embryonic stages of bitterlings, even though their larval morphology is diverse. Previous studies have suggested that minute tubercles are mainly developed in the anterior part and the eyes and head of the larvae, forming a wing-like projection[7].

In *Rhodeus pseudosericeus*, the egg types were bulb-like shape, and minute tubercles on the surface of larvae were observed immediately after hatching. The minute tubercles were grouped in to the following three sites: the anterior regions of yolk sac projection on the eyes and head, the skin surface of the wing-like projection consist of a couple of dorsal and one ventral yolk sac projection, and the posterior regions of the yolk sac projection and most parts of the body ranging over the caudal fin-fold region. These were observed by scanning electron microscopy (SEM) [8].

The changes in height of minute tubercles were classified with the six stages: formation, growth, peak, abrupt reduction, reduction, and disappearance. The height of the minute tubercles gradually increased from day one after hatching (formation stage), with the highest values recorded on the surface on day seven(growth and peak stages). The height of the minute tubercles was abruptly decreased by approximately 50%-60% from 8-10 days(abrupt reduction stage). The height of the minute tubercles continuously decreased from11 days to 26 days (reduction stage). The small size of the minute tubercles was mainly observed around the eyes and head and no confirmation on the skin surface of the larvae from 27 days (disappearance stage) [8] (Figure 2).

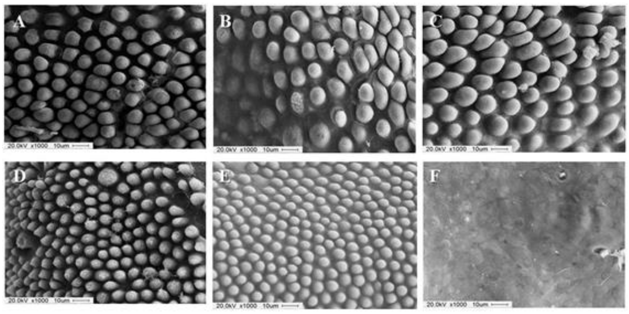

Figure 2. Minute tubercles on the skin surface of anterior and wing-like projection regions of *Rhodeus pseudosericeus* larvae. A: less-developed minute tubercles on anterior yolk projections 1 day after hatching (Formation stage); B: More-developed minute tubercles on wing-like yolk projections 3 days after hatching(Growth stage); C: well developed minute tubercles on the wing-like yolk projections 7 days after hatching (Peak stage); D: abruptly decreased minute tubercles on the wing-like yolk projections 10 days after hatching (Abrupt reduction stage); E: small minute tubercles on the wing-like yolk projections 24 days after hatching (Reduction stage); F: disappeared minute tubercles on the yolk projections 27 days after hatching (Disappearance stage).

The larvae were observed in the interlamellar space of the four gill demibranchs until 10 days after hatching (from formation stage to abrupt reduction stage). However, when the tubercle height became considerably smaller and shorter, the larvae start to move to the suprabranchial cavity after 11 days after hatching (from reduction stage to disappearance stage). At this period, the larvae had a heart rate, migrate to side direction slowly and the caudal fin began to develop[8].

Adaptation of Bitterling for using Host Mussels

Bitterlings display unique physiological, morphological, and behavioral adaptation characters for using the host mussels as the spawning sites, and they offer the valuable model of host-parasite relationship in evolutionary ecology.

Two main factors were affected to the mortality of bitterling larvae. There were premature ejection from the hosts and death in the mussel gill cavity by asphyxiation and nutrient deficiency. In *Rhodeus*, an egg size that is relatively larger than those of other cyprinids and a unique egg-shape are adaptive in providing a proper fit for the egg between the mussel gill lamellae. The bitterlings' long ovipositors allow them to lay eggs deep within the gill cavity of the mussels, which reduces the competition for oxygen and space between larvae. However, water circulation rate and the dissolved oxygen content in the water filtered by mussels may appear higher in the space near the mussels' siphons compared to the positions deeper in the mussel gills; bitterling embryos laid nearer the exhalant siphon in the mussel gills may develop faster and survive better, however they face a greater risk of premature ejection by host mussels [9].

In autumn-spawning bitterlings, the developmental arrest of free-embryos may be a simple physiological response to a decline of water temperature during winter and an ecological adaptation to low water temperatures determined by genetic factors [10]. Many studies have reported that the inner gill of female mussels might have been selected by bitterlings in preference to the outer gills to avoid mortality due to the presence of the glochidia in the outer gill demibranchs, which act as brood pouches. Some researchers have suggested that the dissolved oxygen content in the water leaving the exhalant siphon of mussel is the proximate cue for ovi position choice as spawning sites by the bitterlings. Previous study suggested that density-dependent mortality of bitterling embryos in the mussels arises through the competition for oxygen and space [11].

The minute tubercles on the skin surface of bitterling larvae are made of large unicellular epidermal cells. Previous studies have indicated that minute tubercles have an attachment function that enables them to vegetation and submerged objects. The minute tubercles appear only in developmental larvae stage with no swimming abilities; when the fins begin to develop and consequently have the swimming ability the height of the minute tubercles are abruptly reduced. In case of genera *Acheilognathus* and *Tanakia* bitterlings, it was not have a wing-like projection, the minute tubercles develop most intensively in the anterior regions on eyes and head, and the type of minute tubercles form of the yolk projection is scaly or hilly, which differ to that of *Rhodeus* bitterlings [12]. The height and shape of larger and sharper minute tubercles in genera *Acheilognathus* and *Tanakia* bitterling larvae compared to those of *Rhodeus* larvae (approximately 15-40 μm vs. 3-11 μm) is an adaptation phenotype that inhibits premature ejection from host mussel. Also, minute tubercles on the skin allow the larvae to properly fit in the interlamellar space of the gill demibranchs (Table 2).The change in the height of the minute tubercles of the larvae was related to the position of the larvae in the mussels and may play a leading part in preventing premature ejection by host mussels.

Table 2. Developmental stage of minute tubercles on skin surface by larval growth in Acheilognathinae fishes.

Hatching day	Surface of region	Height of minute tubercle (μm)	Type	Species [references]
1	Most part of the yolk sac projections	15~25	1	*Tanakia tanago* [13]
3	Most part of the yolk sac projections	15~25	1	*Acheilognathus lanceolatus* [14]
2	Most part of the yolk sac projections	15~25	1	*Acheilognathus signifier* [15]
5	Most part of the yolk sac projections	16~20	1	*Acheilognathus somjinensis* [12]
3	Most part of the yolk sac projections	15	2	*Acheilognathus yamatsutae* [16]
2	Most part of the yolk sac projections	20~40	2	*Acanthorhodeus asmussi* [17]
2	Most part of the yolk sac projections	20~40	2	*Acanthorhodeus gracilis* [18]
3	Wing like yolk projections	5~10	3	*Rhodeus uyekii* [19]
1	Wing like yolk projections	3~10	3	*Rhodeus suigensis*[20]
6	Wing like yolk projections	10~11	3	*Rhodeus pseudosericeus* [8]

Conclusion

Bitterlings have a remarkable early life history and a small number of eggs, a variety of egg size, and develop unique skin structures called minute tubercles during the early developmental stages of larvae. Also, they show a fast hatching time and are unique in that they lay eggs in mussels. Approximately 60 species of bitterlings are known worldwide with living mussels, evolved because of various factors: maturation type, development period, spawning type, spawning position, larval migration in mussels, and host selection. In *R. pseudosericeus*, minute tubercles are developed to prevent premature ejection of embryos by their hosts. Thus, this review may enhance our understanding of the evolutionary advantages of the development of minute tubercles and the migration of larvae inside mussels to increase larval survival.

References

1. Aldridge DC. Development of European bitterling in the gills of fresh water mussels. J Fish Biol.1999; 54: 138-51.
2. Haag WR. The role of fecundity and reproductive effort in defining life-history strategies of North American freshwater mussels. Biol Rev.2013; 88: 745-66.
3. Thompson JN. Coevolution. Oxford, UK: Oxford University Press, 2002; pp. 178-83.
4. Reichard M, Ondrackova M, Przybylski M, et al. The costs and benefits in an unusual symbiosis: Experimental evidence that bitterling fish *Rhodeus sericeus* are parasites of unionid mussels in Europe. J Evol Biol. 2006; 19: 788-96.
5. Smith C, Reichard M, Jurajda P, et al. The reproductive ecology of the European bitterling (*Rhodeus sericeus*).J Zool.2004; 262: 107–24.
6. Kim CH, Lee WO, Lee JH, et al. Reproduction study of Korean endemic species *Acheilognathus koreensis*. Korean J Ichthyol. 2011; 23: 150-57. (in Korean with English abstract)
7. Suzuki N, Jeon SR. Development of the bitterling, *Acanthorhodeus asmussi* (Cyprinidae), with note on minute tubercles on the skin surface. Korean JIchthyol. 1989; 1:73–82.
8. Kim HS. Minute tubercles in bitterling larvae: Developmental dynamic structures to prevent premature ejection by host mussels. Ecol Evol. 2020; 10: 5840-851.
9. Kitamura J. Factors affecting seasonal mortality of rosy bitterling (*Rhodeus ocellatus kurumeus*) embryos on the gills of their host mussel.Popu Ecol. 2005; 47: 41-1
10. Kawamura K, Uehara K. Effects of temperature on free-embryonic diapause in the autumn-spawning bitterling *Acheilognathus rhombeus* (Teleostei: Cyprinidae). JFish Biol. 2005; 67: 684-95
11. Mills SC, Reynolds JD. Mussel ventilation rates as a proximate cue for host selection by bitterling, *Rhodeus sericeus*. Oecologia.2002; 131: 473-78

12. Park JY, Oh MK, Kim, CH, et al. Morphology and distribution of the minute tubercles on the skin surface of larvae in the Korean endemic bitterling, *Acheilognathus somjinensis* (Pisces, Cyprinidae), with its larval growth. Animal cells syst. 2008; 12: 297-04.
13. Suzuki N, Oka A, Sugoh Y. Development of the bitterling,*Tanakia tanago*(Cyprinidae), with a note on minute tubercles on the skin surface. Jpn J Ichthyol. 1986; 33:225–31.
14. Suzuki N, Jeon SR. Development of *Acheilognathus lanceolatus* from Ungchon river, Korea. Korean JIchthyol. 1990; 2:77–87.
15. Suzuki N, Jeon SR. Development of the bitterling, *Acheiloganthus signifer* (Cyprinidae), with a note on minute tubercles on the skin surface. Korean J Limnol. 1988; 21:165–79.
16. Suzuki N, Jeon SR. Development of the bitterling, *Acheilognathus yamatsutae* (Cyprinidae), with a note on minute tubercles on the skin surface and pharyngeal apparatus. Korean J Limnol. 1987; 20:229–41.
17. Suzuki N, Jeon SR. Development of the bitterling, *Acanthorhodeus asmussi* (Cyprinidae) with a note on minute tubercles on the skin surface. Korean JIchthyol. 1989;1:73–82.
18. Suzuki N, Jeon SR. Development of the bitterling, *Acanthorhodeus gracilis* (Cyprinidae), with a note on minute tubercles on the skin surface. Korean JIchthyol. 1990; 2: 169–81.
19. Suzuki N, Akiyama N, HibiyaT. Development of the bitterling *Rhodeus uyekii* (Cyprinidae), with a note on minute tubercles on the skin surface. Jpn J Ichthyol. 1985; 32:28–24.
20. Suzuki N, Jeon SR. Development of egg and larvae of the bitterling, *Rhodeus suigensis* (Cyprinidae) from Korea, with a note on minute tubercles on the skin surface. Korean J Limnol. 1988; 21:231–42.

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